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A One Year Epidemiological Study of Mechanical Injury Cases brought to SIMS Hospital, Western Uttar Pradesh

Abhishek Sharma¹, Sumit Tellewar², Barkha Gupta³, Vishwajeet Singh⁴, Gurdeep Singh⁵
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Abstract

Aim: To find out causes and factors responsible for the mechanical injuries. Materials & Methodology: This prospective study was conducted at Saraswathi Institute of Medical Sciences, Hapur, from 1st JULY 2017 to 30th JUNE 2018. There were total 306 patients of mechanical injury cases reported to the department of emergency during the study period. Result: Of the total 306 cases 228 (74.51%) cases were male and 78 (25.49%) cases were female. Most susceptible age group was between 21-30 years (30.1%) of age and RTA were the leading cause of death (83.66%).

Key words: Mechanical injuries, Road Traffic accident, Fall from height, Assault

Introduction

India is passing through a major socio-demographic, epidemiological, technological and media transition. The political, economic and social changes have altered the health scenario. In a developing country like India, infectious diseases were the leading cause of mortality in the past decades, but at present, non communicable diseases and traumatic injuries are on the rise. Globally about 5.8 million people die each year as a result of traumatic injuries. [1] Mechanical injuries are injuries produced by mechanical force-blunt, sharp or firearms. The severity and extent of mechanical injuries depend on amount of force delivered to the body- if the weapon deforms or breaks on impact; some energy is spent in deforming or breaking. Lesser energy is thus delivered to the body, injury is less severe. Legal definition of injury- Any harm, whatever illegally, caused to any person in body, mind, reputation or property. [2] A road traffic accident (RTA) can be defined as “an event that occurs on a way or street open to public traffic; resulting in one or more persons being injured or killed where at least one moving vehicle is involved”. [3] Worldwide, every day about 3400 people die due to road traffic accidents (RTA) and predicted to result in death of around 1.9 million people annually by 2020. [4] Among trauma road traffic accidents are one of the major cause of disability and mortality in younger population and projected to the fifth leading contribution to global burden. [5]

Materials & Method

This study was conducted at Saraswathi Institute of Medical Sciences, Hapur, from 1st JULY 2017 to 30th JUNE 2018. There were total 306 patients of mechanical injury cases reported to the department of emergency during the study period. All Mechanical injury cases including Road traffic accidents, assault and fall from height.

Result

In our study, 306 cases of injuries were reported to the Emergency of Saraswathi Institute of Medical sciences (SIMS) Hapur, out of which 74.51% cases were male and 25.49% cases were female. In age group analysis, maximum number of cases was seen in the age group of 21 – 30 years (30.1%), followed by 11 – 20 years (22.9%). Least number of cases was seen in the age group of 0 – 10 years (3.3%) (Table 1). In the present study, majority of cases (68.30%) were from rural background and remaining 31.70% were from urban background (Table 4). In maximum cases the
place of incidence was road comprising of 88.89% cases and least cases of injuries were found happening at work place comprising of 1.96% cases (Table 2). In distribution of nature of injuries, simple injuries were seen in 52.61% of cases and in grievous injuries 47.39% of cases were noted (Table 3). Most of the cases were conscious at the time of arrival (80.39%) (Table 5). Most common type of injury noted was blunt mixed injury (47.71%) (Table 6).

Table 1: Distribution of subjects according to Age, sex and mode of injury

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>RTA</th>
<th>Fall</th>
<th>Assault</th>
<th>RTA</th>
<th>Fall</th>
<th>Assault</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>Male</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1.63</td>
<td>0.00</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1.31</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>11-20</td>
<td>Male</td>
<td>43</td>
<td>3</td>
<td>12</td>
<td>14.05</td>
<td>0.98</td>
<td>3.92</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>3.59</td>
<td>0.00</td>
<td>0.33</td>
</tr>
<tr>
<td>21-30</td>
<td>Male</td>
<td>65</td>
<td>1</td>
<td>11</td>
<td>21.24</td>
<td>0.33</td>
<td>3.59</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>4.58</td>
<td>0.33</td>
<td>0.00</td>
</tr>
<tr>
<td>31-40</td>
<td>Male</td>
<td>34</td>
<td>1</td>
<td>4</td>
<td>11.11</td>
<td>0.33</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>15</td>
<td>3</td>
<td>2</td>
<td>4.90</td>
<td>0.98</td>
<td>0.65</td>
</tr>
<tr>
<td>41-50</td>
<td>Male</td>
<td>33</td>
<td>0</td>
<td>2</td>
<td>10.78</td>
<td>0.00</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>2.29</td>
<td>0.65</td>
<td>0.33</td>
</tr>
<tr>
<td>51-60</td>
<td>Male</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>1.96</td>
<td>0.00</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>2.29</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>&gt;61</td>
<td>Male</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1.63</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>2.29</td>
<td>0.00</td>
<td>0.33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>256</td>
<td>12</td>
<td>38</td>
<td>83.66</td>
<td>3.92</td>
<td>12.42</td>
</tr>
</tbody>
</table>

Table 2: Distribution of subjects according to Place of Incidence

<table>
<thead>
<tr>
<th>Place</th>
<th>Number of case (N = 306)</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home</td>
<td>28</td>
<td>9.15</td>
</tr>
<tr>
<td>Workplace</td>
<td>6</td>
<td>1.96</td>
</tr>
<tr>
<td>Road</td>
<td>272</td>
<td>88.89</td>
</tr>
<tr>
<td>TOTAL</td>
<td>306</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Distribution of subjects according to nature of injury

<table>
<thead>
<tr>
<th>Nature of injury</th>
<th>Number (N=306)</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>161</td>
<td>52.61</td>
</tr>
<tr>
<td>Grievous</td>
<td>145</td>
<td>47.39</td>
</tr>
<tr>
<td>TOTAL</td>
<td>306</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4: Area wise distribution of cases

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of cases (N=306)</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>97</td>
<td>31.70</td>
</tr>
<tr>
<td>Rural</td>
<td>209</td>
<td>68.30</td>
</tr>
<tr>
<td>TOTAL</td>
<td>306</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 5:- Distribution of subjects according to Condition of patient

<table>
<thead>
<tr>
<th>CNS status</th>
<th>Number of cases (N = 306)</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conscious</td>
<td>246</td>
<td>80.39</td>
</tr>
<tr>
<td>Unconscious</td>
<td>41</td>
<td>13.40</td>
</tr>
<tr>
<td>Semi Conscious</td>
<td>19</td>
<td>6.21</td>
</tr>
<tr>
<td>Total</td>
<td>306</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 6:- Distribution of subjects according to types of injury

<table>
<thead>
<tr>
<th>Types of injury</th>
<th>Number of Cases (N=306)</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrasion</td>
<td>82</td>
<td>26.79</td>
</tr>
<tr>
<td>Bruise</td>
<td>22</td>
<td>7.18</td>
</tr>
<tr>
<td>Laceration</td>
<td>42</td>
<td>13.72</td>
</tr>
<tr>
<td>Incised Wound</td>
<td>2</td>
<td>0.65</td>
</tr>
<tr>
<td>Fire arm</td>
<td>1</td>
<td>0.32</td>
</tr>
<tr>
<td>Blunt mixed</td>
<td>156</td>
<td>50.98</td>
</tr>
<tr>
<td>Blunt and Sharp mixed</td>
<td>1</td>
<td>0.32</td>
</tr>
<tr>
<td>Total</td>
<td>306</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

During the study period a total of 306 cases of injuries were reported to the Emergency of Saraswathi Institute of Medical sciences (SIMS) Hapur, out of which 74.51 percent cases were male and 25.49 percent cases were female. In age group analysis, maximum number of cases were seen in the age group of 21 – 30 years (30.1%), followed by 11 – 20 years (22.9%). Least number of cases were seen in the age group of 0 – 10 years (3.3%). Out of 306 cases 83.67 percent cases were of Road traffic accidents, 12.41 percent were cases of Assault and 3.92 percent cases of fall from height. The present study is in agreement with the study of Malik Y et al, where maximum incidence took place on road (51%).

In distribution of nature of injuries, simple injuries were seen in 52.61 percent of cases and in grievous injuries 47.39 percent of cases were noted. In the cases of road traffic accident (n=256), simple injuries were seen in 53.12 percent and grievous injury cases were 46.88 percent. In the assault cases, 57.90 percent of cases had simple injury and grievous injury was seen in 42.10 percent of cases. In this study of fall from height, in majority of cases grievous injury (75%) was seen while Simple injuries were seen in 25.00 percent of the cases. The present study results are consistent with study of Kumar S et al, where majority of cases (62%) suffered from grievous injury. [8]

In the present study, Majority of the patients (80.39%) were Conscious at the time of presenting to emergency. Unconscious was noted in 13.40 percent cases and 6.21 percent cases were in Semi conscious condition. In the cases of Road Traffic Accidents (n=256), blunt mixed injuries were reported in maximum (54.29%) cases, followed by abrasion (26.95%) and laceration in 12.50 percent of cases. In the cases of Assault in this study (n=38), blunt mixed injury (34.21%) was reported in maximum number of cases, followed by abrasion in 26.31 percent of cases. In this study out of total 12 cases of fall from Height (n=12), blunt mixed injury (33.33%) was reported in maximum number of cases, followed by abrasion in 25.00 percent of cases. A study, Chakraborty P N et al, done in road traffic accident cases showed maximum number of injuries were of blunt mixed type (91.83%) followed by lacerations (44.38%) and least were of sharp injuries (4.08%) which is in agreement with the present study. [9] The present study is in disagreement with the study of Jaiswal K et al, done in road traffic accidents, where maximum injuries were laceration (53.46%). [10]

Conclusion

This study shows the workload of medico legal cases reporting to the emergency department of this tertiary care hospital. From this study it is clear that Road Traffic accidents are making huge number to the emergency department and hence putting burden on health care system.

Thus recommends:

• Availability of well trained medical and supporting
staff along with well equipped trauma centre round the clock for prompt treatment.

• To post more number of medical and supporting staff on weekends and during evening hours.

• To be well prepared to tackle the large number of cases reporting at the same time.

Apart from above recommendations, incidence of RTA can be decreased by combined efforts from the community, governmental and non-governmental organizations.

Conflicts of Interest: We declare that there is no conflict of interest.

Source of Funding: None

Ethical Clearance: Taken from Saraswathi institute of medical sciences.

References

9. Chakraborty PN, Sarkar SC. Epidemiological Study of Patterns of Head Injuries in fatal Road Traffic Accidents in Tripura. Indian Journal of Forensic Medicine & Toxicology, January-June 2017, Vol.11, No. 1
Estimation of Age by Modified Gustafson’s Method from Incisor and Canine Teeth

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Background: Estimating age from teeth is generally reliable as they are naturally preserved long after all the tissues and even bones have disintegrated. Aim of the study: The present study was an attempt to estimate the age of an individual by using modified Gustafson’s method from age related alteration in incisors and canines in Karad. Materials and methods: A cross sectional study was conducted in Karad for a period of 4 months from Nov 2018 to Feb 2019. Results: 95 teeth were observed, out of which 59 were incisors and 36 canines. The coefficient of variations among parameters ranged from 22.61 to 29.92%. Conclusion: - The estimated age error was minimized to 1.15 years. The age calculated by this method was found to be more accurate, reliable and reproducible.

Keywords: Age, tooth, incisor, canine, attrition, secondary dentin, cementum apposition.

Introduction

Identification of the person is of paramount importance in forensic practice. Age estimation is one of the important factors employed to establish identity. Estimating age from teeth is generally reliable as they are naturally preserved long after all the tissues and even bones have disintegrated¹⁻⁵. Recent natural disasters like earth quakes in different states of India, world trade center attack etc. has brought in to focus the importance of teeth in determination of age of an individual and thus helping in their identification. Age estimation from the teeth may be the only useful method especially when the skull is only available as skeletal remains⁶.

In children age estimation from the teeth is relatively simple and accurate. For adults, the methods are often based on degenerative modifications, such as attrition, periodontosis, transparency of the root, secondary dentin, cementum apposition and root resorption⁷⁻¹⁰. With the lack of quantification in the Gustafson’s system, modified method which is based on the quantitative evaluation of four parameters: the attrition, the secondary dentine, cementum apposition and the transparency of root was used to estimate age where standard error of ± 1.50 years was claimed⁶, ⁷. In this study incisors and canines were used as these are less affected by pathological conditions such as caries as compared to the molars and premolars.

Aims and objective:

To estimate the age of an individual by using modified Gustafson’s method from age related alteration in incisors and canines in Karad.

Material and Method

A cross sectional study was conducted in department of Forensic medicine and Toxicology, Krishna institute of medical sciences, Karad for a period of 4 months from Nov 2018 to Feb 2019. Criteria for selection of specimen (Incisors and canines) were the tooth should be caries free and should not have either root or crown fracture.

The incisors and canine were collected from known cadavers between the age group of 20 to 70 years dissected by medical students in the department of Anatomy, Krishna institute of medical sciences, Karad. They were cleaned under tap water and then kept in 10% formalin for 24 to 48 hours. The sample bottles were labeled with actual age of the person from whom the specimen was collected and also labeled to the type of
Prior to grinding, incisors and canines were kept in water. Then 1-2 mm sections were prepared to the central axis of each tooth symmetrically from both sides using the rough carborundum stone grinder with supplementation of continuous water flow from an IV set to reduce the heat produced during grinding. Precautions to avoid cracks and cleavages were taken during grinding of tooth.

After grinding the tooth up to 2 mm, the section of 0.1 mm thickness was prepared on the Arkansas stone. The Arkansas stone was kept in enamel tray containing water. The grinding action was only in one direction to avoid irregularities in sections.

Following dehydration, tooth sections were mounted on glass slides with DPX mountant for microscopic observations. The microscopic parameters were observed at the magnification of 0.25 using light microscope.

**Principles:**

The formulae of the various index values for each parameter are as follows.

Each index value of the various parameters undergoing regressive changes were calculated by relating the measured change to a fixed measurement on the tooth. The changes in the various tooth parameters were due to functional changes along with age.

1. **Attrition (A):**

   \[
   [A] = \frac{a}{A} \times 100
   \]

   ‘a’ is width of the tooth in mm at tip showing attrition.

   ‘A’ is the width of the tooth in mm at cervical margin.

2. **Secondary Dentin (D):**

   The index value of secondary dentin was measured as percentile value of secondary dentin deposited and the total length of pulp cavity.

   \[
   [D] = \frac{d}{D} \times 100
   \]

   ‘d’ is length in mm of secondary dentin deposition in the pulp cavity.

   ‘D’ is the length of entire pulp cavity of the tooth.

3. **Translucency of the root (T):**

   \[
   [T] = \frac{t}{T} \times 100
   \]

   ‘t’ is the length in mm of the translucent region of the root.

   ‘T’ is the length in mm of the entire tooth.
Measurement of parameters:

This is known as modified Gustafson’s formula for age determination from tooth which was used in the present study. The data was collected and analysed with SPSS 20 Statistical Software and presented as descriptive statistics. The efficacy of proposed modified Gustafson’s method in relation with actual age was assessed by computing mean, coefficient of correlation and student t test.

The average age was calculated from different index values and it was estimated as

\[
\text{Age} = \frac{(A) + (D) + (T) + (CE)}{4}
\]

Results

Table No. 1: Showing total number of samples of incisors and canines.

<table>
<thead>
<tr>
<th>Teeth</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incisors</td>
<td>59</td>
</tr>
<tr>
<td>Canines</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
</tr>
</tbody>
</table>

Table No. 2: Information of attrition, secondary dentin, root translucency, cementum apposition, actual age and estimated age of 95 cases.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Variable</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Attrition</td>
<td>13.99–57.62</td>
<td>39.28</td>
<td>11.12</td>
<td>28.30%</td>
</tr>
<tr>
<td>2</td>
<td>Secondary dentine</td>
<td>24.13–63.52</td>
<td>47.47</td>
<td>11.19</td>
<td>23.57%</td>
</tr>
<tr>
<td>3</td>
<td>Root translucency</td>
<td>19.05–104.27</td>
<td>75.76</td>
<td>17.47</td>
<td>22.75%</td>
</tr>
<tr>
<td>4</td>
<td>Cementum apposition</td>
<td>10.06–64.13</td>
<td>35.39</td>
<td>10.59</td>
<td>29.92%</td>
</tr>
<tr>
<td>5</td>
<td>Actual age</td>
<td>20.00–70.00</td>
<td>48.94</td>
<td>11.34</td>
<td>23.17</td>
</tr>
<tr>
<td>6</td>
<td>Estimated age</td>
<td>21.53–67.96</td>
<td>49.74</td>
<td>11.25</td>
<td>22.61</td>
</tr>
</tbody>
</table>

Table No. 2 revealed that coefficient of variations among parameters ranged from 22.61 to 29.92%.
Table No. 3: Showing correlation coefficient (r) among actual age (AA), attrition (ATT), secondary dentine (SD), root translucency (T), cementum apposition (CE) and estimated age (EA).

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>ATT</th>
<th>SD</th>
<th>T</th>
<th>CE</th>
<th>EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>ATT</td>
<td>0.9276***</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>SD</td>
<td>0.7777***</td>
<td>0.1407*</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>T</td>
<td>0.6470**</td>
<td>0.2258*</td>
<td>0.6379***</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>CE</td>
<td>0.2089*</td>
<td>0.8812***</td>
<td>0.5210***</td>
<td>0.6321***</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>EA</td>
<td>0.9884***</td>
<td>0.7852***</td>
<td>0.7852***</td>
<td>0.941***</td>
<td>0.820***</td>
<td>--</td>
</tr>
</tbody>
</table>

Correlation is significant at the *** = 0.001 level, ** = 0.01 level and * = 0.05 level.

Table No. 3 gives the information regarding the relationship between the different parameters under investigations. The relationship between these parameters was expressed in the form of correlation coefficient (r).

Table No. 4: t-test for actual age of sample and estimated age.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of samples</th>
<th>Mean</th>
<th>SD</th>
<th>SE of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual age</td>
<td>95</td>
<td>48.94</td>
<td>11.37</td>
<td>1.167</td>
</tr>
<tr>
<td>Estimated age</td>
<td>95</td>
<td>49.74</td>
<td>11.25</td>
<td>1.155</td>
</tr>
</tbody>
</table>

Mean difference = 0.091

T test for equality of means:

<table>
<thead>
<tr>
<th>Variances</th>
<th>t values</th>
<th>df</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal</td>
<td>0.001</td>
<td>188</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table No. 5: Showing index value of attrition, secondary dentine, root translucency and cementum apposition by different workers:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Attrition</th>
<th>Secondary dentine</th>
<th>Root translucency</th>
<th>Cementum apposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.93 (Kambe T)</td>
<td>0.83 (Tore Solheim)</td>
<td>0.67 (Wegener R)</td>
<td>0.38 (Solheim T)</td>
</tr>
<tr>
<td>2</td>
<td>0.607 (Tomaru Y)</td>
<td>0.72 (Kwak KW)</td>
<td>0.60 (Solheim T)</td>
<td>0.251 (Monzavi)</td>
</tr>
<tr>
<td>3</td>
<td>0.630 (Kwak KW)</td>
<td>0.522 (Monzavi)</td>
<td>0.344 (Monzavi)</td>
<td>0.4937 (Sangeetha P)</td>
</tr>
<tr>
<td>4</td>
<td>0.925 (Seth S)</td>
<td>0.609 (Sangeetha P)</td>
<td>0.872 (Sangeetha P)</td>
<td>0.4937 (Sangeetha P)</td>
</tr>
<tr>
<td>5</td>
<td>0.394 (Monzavi)</td>
<td>0.764 (Shrigirirwar M)</td>
<td>0.838 (Shrigirirwar M)</td>
<td>0.58 (Vystreilova)</td>
</tr>
<tr>
<td>6</td>
<td>0.622 (Sangeetha P)</td>
<td>0.664 (Arora J)</td>
<td>0.97 (Narayan VK)</td>
<td>0.58 (Vystreilova)</td>
</tr>
<tr>
<td>7</td>
<td>0.90 (Narayan VK)</td>
<td>0.83 (Vystreilova)</td>
<td>0.75 (Vystreilova)</td>
<td>0.208 (Present study)</td>
</tr>
<tr>
<td>8</td>
<td>0.927 (Present study)</td>
<td>0.777 (Present study)</td>
<td>0.647 (Present study)</td>
<td>0.208 (Present study)</td>
</tr>
</tbody>
</table>
Discussion

In the present study, total of 95 teeth were observed, out of which 59 were incisors and 36 canines. Attrition, secondary dentin, root translucency and cementum apposition were four parameters used in calculating the age of an individual. The coefficient of variations among parameters ranged from 22.61 to 29.92%. The variations are biologically induced variations. They are interrelated with each other. The extent of variation among the observation for actual age and estimated age was almost same.

The relationship between actual age (AA), attrition (ATT), secondary dentin (SD), root translucency (T), cementum apposition (CE) and estimated age (EA) was expressed in the form of correlation of coefficient (r). All the values of (r) obtained were positive indicating the positive correlation between various parameters. There is significant positive correlation between age and index value of all four parameters as shown in table no 3. Both the values of actual age and estimated age showed a similar trend regarding its relationship with attrition, secondary dentin, root translucency, cementum apposition.

The difference between the actual age and estimated age was insignificant from statistical t test (p > 0.05). So on the basis of values obtained from correlation of coefficient and t test it is confirmed that the age of the subject can be safely calculated by the modified Gustafson’s method.

Index value of attrition was ranging from 13.99 to 57.62, with a mean 39.28 and standard deviation ± 11.12. The value of r = 0.927 between actual age and attrition represented a significant correlation coefficient at 0.001 level. Though attrition varies according to functional variation, it can be used as a reliable index in age estimation. From the present study, it can be concluded that root translucency above the age of thirty can be a valuable criterion in age estimation. Root translucency can be used as a sole criterion only when the remains consist of root portion. But more accuracy can be obtained when root translucency is used with other three criteria.

In this study, index value for cementum apposition ranged from 10.06 to 64.13 with mean of 35.39. Value for correlation coefficient among actual age and cementum apposition is (r=0.2089) which shows positive correlation.

Though a number of modifications have been suggested by investigators, the modified Gustafson’s method still remains the most reliable method in determination of age from tooth. The findings of the present study are in accordance with this.

After complete evaluation of this method, it can be stated that the root translucency and secondary dentin can only be used in estimation of age of an individual, because these two criteria show a simultaneous increase as the age advances. Most commonly affected criterion by pathology is the attrition and cementum apposition. So it is stated that, though no one of the four criteria can be used alone, all four are useful if used in combination. The estimation of changes should be evaluated. For example: in the cases of attrition, occlusion and articulation must be taken into consideration. Their variations are in the old age individual depending upon factors, such as periodontal conditions and habits.
5. Estimated age:

Table No. 6 showing comparative data of estimated age by different workers.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the workers</th>
<th>SE of estimated age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bang and Ramm (1970)</td>
<td>7 to 13 years</td>
</tr>
<tr>
<td>2</td>
<td>Johnson (1971)</td>
<td>± 5.16</td>
</tr>
<tr>
<td>3</td>
<td>Kashyap VK (1990)</td>
<td>± 1.59</td>
</tr>
<tr>
<td>4</td>
<td>Amandeep Singh (2003)</td>
<td>± 2.16</td>
</tr>
<tr>
<td>5</td>
<td>Present study</td>
<td>± 1.15</td>
</tr>
</tbody>
</table>

In the present study, the estimated age error was minimized to 1.15 years. Thus the age calculated by this method was found to be more accurate, reliable and reproducible.

Conclusion

The present study showed that the correlation coefficient between actual age and various parameters was found to be significant. The estimated age error was minimized to 1.15 years. Thus the age calculated by this method was found to be more accurate, reliable and reproducible.

Acknowledgement: Authors acknowledge the great help received from the faculty of department of Anatomy, Krishna institute of medical sciences, Karad.

Conflict of Interest: No.

Source of Funding: Self

Ethical Clearance: The ethical clearance was obtained from the Institutional Ethical Committee Krishna institute of medical sciences, Karad.

References

16. Sangeetha P. Age estimation from the physiological changes of teeth using modified Gustafson’s


Study of Demographic Pattern of Poisoning in Patients Presenting to Emergency Department of a Tertiary Care Centre, SIMS, Hapur

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1Post-Graduate, 2Professor, 3Associate Professor, 4Assistant Professor, Department of Forensic Medicine & Toxicology, Saraswathi Institute of Medical Sciences, Hapur, UP, India

Abstract
Poisoning is a common medical emergency and one of the important cause of mortality and morbidity in developing countries like India due to easy availability of poisonous substances and its low cost. This study was carried out on poisoning cases reported to casualty of Saraswathi Institute of Medical Sciences, Hapur, UP in eighteen-months duration from 1st January 2017 to 30th June 2018. In this study, incidence was more among men (58.70%) compared to women (41.30%) and maximum cases were of age group 21 – 30 years (48.91%). More cases from rural area (89.13%) were reported and highest number of poisoning cases were admitted in the month of June 2018 (10.87%) followed by May 2017 (8.70%). Maximum cases (60.87 %) reported to casualty between 8am to 4pm. Aluminium phosphide poisoning (44.26%) constituted the highest number of cases and in maximum cases manner of poisoning was of suicidal in nature (59.78%). Majority of cases 53.26% were discharged after improvement and 3.26% cases died.

Key Words: Poisoning, Mortality, Sociodemographic profile, Agrochemical poisons.

Introduction
The word poison has been evolved from Latin word potion i.e. to drink for health. Poison is defined as any substance (solid, liquid or gaseous) which if introduced in the living body or brought into contact with any part thereof will produce ill-health or death by its constitutional or local effects or both. [1]

National Poisons Information centre (NPIC), New Delhi, received a total of 2719 poison calls over a period of 3 years (April 1999-March 2002). The age of victims ranged from <1 to 70 years, with the highest incidence in the range of 14-40 years, with males (57%) outnumbering females (43%). The most common mode of poisoning was suicidal (53%), followed by accidental (47%). [2]

According to WHO data, in 2012 an estimated 1,93,460 people died worldwide from unintentional poisoning. Of these deaths, 84% occurred in low- and middle-income countries. In the same year, unintentional poisoning caused the loss of over 10.7 million years of healthy life. [3]

Everyday around the world almost 700 people die from the poisoning and several thousands more are affected by poisoning, of this 90% of fatal poisoning occurs in developing countries like southeast Asia particularly among agriculture workers. [4]

Aims and Objective
1. To analyse pattern of poisoning cases in patients presenting to Emergency department of Tertiary health care center, Saraswathi Institute of Medical Sciences, Hapur.

2. To study demographic variables of poisoning cases.

3. To study the nature, pattern & magnitude of the morbidity and mortality due to poisoning.
Material and Method

The study was conducted at Saraswathi Institute of Medical Sciences, Hapur, from 1st January 2017 to 30th June 2018. Total 92 patients of poisoning were reported to the department of emergency during the study period. A detailed history was taken from patient, patient relatives and relevant data of the individual poisoning cases were collected from medicolegal cases register of casualty and case papers from concerned department.

Observation and Results

In our study, out of total 92 poisoning cases 58.70% were males and 41.30% were females. Male female ratio was 1.42: 1. (Table 1)

Maximum incidence was seen in the age group of 21 – 30 years (48.91%). (Table2)

The rural population (89.13%) was affected more both in males and females. (Table 3)

Maximum cases (60.87 %) reported to casualty between 8am to 4pm. (Table 4)

In this study, it was observed that 66.30% of cases gave history of known poisoning while 33.70% gave history of unknown poisoning at the time of admission to casualty. Out of total 61 cases of known poisoning it was observed that Aluminium phosphide (44.26%) constituted the maximum number of cases followed by Rat Poison (14.75%) and Mosquito Poison (14.75%). (Table 5)

More number of suicidal and accidental poisoning was seen in male than female. Homicidal poisoning was only observed in females. (Table 6)

Maximum number of poisoning cases (53.26%) was discharged after improvement. (Table 7)

Table 1: - Sex-wise distribution

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of cases (N = 92)</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>54</td>
<td>58.70</td>
</tr>
<tr>
<td>Female</td>
<td>38</td>
<td>41.30</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: - Age-wise Incidence of poisoning cases

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male</th>
<th>Female</th>
<th>Total (N)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11 – 20</td>
<td>16</td>
<td>14</td>
<td>30</td>
<td>32.61</td>
</tr>
<tr>
<td>21 – 30</td>
<td>25</td>
<td>20</td>
<td>45</td>
<td>48.91</td>
</tr>
<tr>
<td>31 – 40</td>
<td>12</td>
<td>2</td>
<td>14</td>
<td>15.22</td>
</tr>
<tr>
<td>41 – 50</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.09</td>
</tr>
<tr>
<td>51 – 60</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1.09</td>
</tr>
<tr>
<td>&gt;61</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.09</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>38</td>
<td>92</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: - Distribution of area according to sex

<table>
<thead>
<tr>
<th>Residence</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>7(12.96)</td>
<td>3(7.89)</td>
<td>10(10.87)</td>
</tr>
<tr>
<td>Rural</td>
<td>47(87.03)</td>
<td>35(92.10)</td>
<td>82(89.13)</td>
</tr>
<tr>
<td>Total</td>
<td>54(100)</td>
<td>38(100)</td>
<td>92(100)</td>
</tr>
</tbody>
</table>

Table 4: -Time of Incident

<table>
<thead>
<tr>
<th>Time of Incident</th>
<th>Number of cases (N = 92)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>8AM-4PM</td>
<td>56</td>
<td>60.87</td>
</tr>
<tr>
<td>4PM-12AM</td>
<td>28</td>
<td>30.43</td>
</tr>
<tr>
<td>12AM-8AM</td>
<td>8</td>
<td>8.70</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5: - Type of known poison

<table>
<thead>
<tr>
<th>Poison</th>
<th>Number of cases (N = 61)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphorus</td>
<td>3</td>
<td>4.92</td>
</tr>
<tr>
<td>Rat Poison</td>
<td>9</td>
<td>14.75</td>
</tr>
<tr>
<td>Mosquito Poison</td>
<td>9</td>
<td>14.75</td>
</tr>
<tr>
<td>Aluminium phosphide</td>
<td>27</td>
<td>44.26</td>
</tr>
<tr>
<td>Zalim Lotion</td>
<td>1</td>
<td>1.64</td>
</tr>
<tr>
<td>Corrosive</td>
<td>1</td>
<td>1.64</td>
</tr>
<tr>
<td>Alcohol</td>
<td>4</td>
<td>6.56</td>
</tr>
<tr>
<td>Laxman-rekha</td>
<td>1</td>
<td>1.64</td>
</tr>
<tr>
<td>Cockroach killer</td>
<td>1</td>
<td>1.64</td>
</tr>
<tr>
<td>Bhang Ingestion</td>
<td>1</td>
<td>1.64</td>
</tr>
<tr>
<td>Lysol</td>
<td>2</td>
<td>3.28</td>
</tr>
<tr>
<td>Benzene Hexachloride</td>
<td>1</td>
<td>1.64</td>
</tr>
<tr>
<td>Formalin</td>
<td>1</td>
<td>1.64</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table 6: - Distribution of manner according to sex

<table>
<thead>
<tr>
<th>Manner</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suicidal</td>
<td>32(59.25)</td>
<td>23(60.52)</td>
<td>55(59.78%)</td>
</tr>
<tr>
<td>Accidental</td>
<td>22(40.74)</td>
<td>13(34.21)</td>
<td>35(38.04%)</td>
</tr>
<tr>
<td>Homicidal</td>
<td>0(0)</td>
<td>2(5.26)</td>
<td>2(2.17%)</td>
</tr>
<tr>
<td>Total</td>
<td>54(100)</td>
<td>38(100)</td>
<td>92(100)</td>
</tr>
</tbody>
</table>

Table 7: - Outcome of Patient

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of cases (N = 92)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharged</td>
<td>49</td>
<td>53.26</td>
</tr>
<tr>
<td>Referred</td>
<td>14</td>
<td>15.22</td>
</tr>
<tr>
<td>Leave against medical advice</td>
<td>26</td>
<td>28.26</td>
</tr>
<tr>
<td>Death</td>
<td>3</td>
<td>3.26</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

Pesticide poisoning is a major health problem in India. Aluminium Phosphide is an effective fumigant and rodenticide and used extensively in India. Acute poisoning from these poisonous substances is one of the commonest causes of emergency hospital admissions.

During the study period 92 poisoning cases were admitted in the Emergency of SIMS, out of which 58.70% were males and 41.30% were females. This finding in the study is in agreement with the study of Md Ziya Ahmad et al[5] in which more cases were of males as compared to females. The reason for more number of male cases is more exposure to harmful poisonous substance in occupation, more stress and strain due to financial burden of the family.

In present study majority of cases were seen in the age group of 21 – 30 years (48.91%) followed by 11 – 20 years (32.61%). This finding is agreement with the finding of Hareesh. S. Gouda et al[6], in which, incidence was highest in age group 21 – 30 years (38.60%) followed by 11 – 20 years (35.08%). Higher incidence of poisoning in the younger age group of 21 – 30 years is reflective of the social causes like failure in love, failure in examinations, stress of the modern life style and scolding from parents or teachers.

The current study shows that rural population (89.13%) was more affected than urban (10.87%). In female incidence of cases in rural area was more as compared to male. These results are in agreement with study of Md Ziya Ahmad et al[5], Hareesh. S. Gouda et al[6] and Barkha Gupta et al[7] who also show rural population to be more affected than urban population. It can be concluded that the rural areas, having increased farming activities, agricultural and domestic use of insecticidal compounds

In the present study, maximum number of cases 56 (60.87 %) reported to casualty between 8am to 4pm. These results are consistent with study of Vivek Gopinathan et al[8], as time of poisoning in majority of cases (39%) was observed during day.

In the present study, history of known poisoning was noted in 66.30% cases and unknown poisoning in 33.70% cases. Out of total poisoning cases Aluminium phosphide was the leading cause of poisoning. Aluminium phosphide poisoning (44.26%) constituted the highest number of cases followed by Rat poison (14.75%) and Mosquito poison (14.75%). The results of present study are consistent with study of R K Mathur et al[9] as Aluminium phosphide was the most common poison (19.71%), leading to maximum fatalities.

In the present study incidence of suicidal cases (59.78%) were highest followed by accidental (38.04%). The present figure is in agreement with Md Ziya Ahmad et al[5] where majority of patients 79.23% consumed poison with suicidal intent as compared with 20.76% of the patient exposed accidentally. Reasons are inability to face adverse situations in life like unemployment in spite of being graduates, failure in love and examinations, failure of crops, family disputes, poverty, mental instability etc.

The present study reveals that most of patient had a shorter stay in the hospital. 53.26% were discharged after improvement while 15.22% cases were referred to higher centres. In present study mortality rate was 3.26% and all these cases were of Aluminium Phosphide poisoning. This observation is in the agreement with the study of Prashant Gupta et al[10] in which 78.4% cases were discharged after improvement.
Conclusion

The present study includes 92 cases of alleged poisoning during the study period from January 2017 to June 2018, conducted at Emergency department of Saraswathi Institute of Medical Sciences.

- The present study indicates that younger age and males are at higher risk for suicidal poisoning. Poisoning is an important preventable public health problem especially in the developing countries.
- To decrease the incidence, mortality and morbidity of the poisoning cases following precautions and measures should be taken -
  - The farmers should be educated regarding the appropriate measures which needs to be taken in cases of accidental exposure.
  - The face and hands should be thoroughly washed with soap and water after handling toxic agrochemicals.
  - Strict rules and regulations over the sale of insecticides, rodenticides and pesticides should be formulated.

Conflict of Interest: We declare that there is no conflict of interest.

Source of Funding: None

Ethical Clearance: Taken from Saraswathi Institute of Medical Sciences.

References


5. Ahmad Md Ziya, Hussain Tina, Kumar Manoj “Pattern of Poisoning Reported at BC Roy Hospital, Haldia Purba Midnapur, West Bengal” Medico-Legal Update 2012, Vol.12, No.2 P228-230.


Forensic Evaluation of Various Osteometric Parameters of Human Mandible in both Sexes

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1Assistant Professor, 2Ex Resident, Dept. of Forensic Medicine and Toxicology, Gandhi Medical College, Bhopal, Presently Assistant Professor, Late Baliram Kashyap Govt. Medical College, Jagdalpur, Chattisgarh, 3Demonstrator, Dept. of Forensic Medicine and Toxicology, Gandhi Medical College, Bhopal, 4Ex Professor and Head, Dept. of Forensic Medicine and Toxicology, Gandhi Medical College, Bhopal, Presently Additional Professor, Dept. of Forensic Medicine and Toxicology, AIIMS, Bhopal, 5Ex Professor and Head, Dept. of Forensic Medicine and Toxicology and Ex Dean, Gandhi Medical College, Bhopal, Presently Dean, L. N. Medical College, Bhopal

Abstract

Identification of sex could be made on differences in the size and shape of the morphological markers i.e. shapes of chin, size of condyles etc. Morphological traits are more subjective and sex determination depends on experience of the investigator. So, visual methods of sexing mandible are likely to be inaccurate when performed by an inexperienced worker and sometimes produce misleading results. Hence, metric analyses are found to of superior value owing to their objectivity, accuracy, reproducibility and lower incidence of inter and intra observer errors as compared to that of descriptive traits.

The focus on present study is to use morpho-metric analytical method to evaluate the ability of selected parameters to determine sex in forensic sample.

Aims and Objectives: To study the various osteometric parameters of human mandible and evaluate their utility in sex determination

Material and Method: Some measurements of the 200 mandibles were taken using sliding calliper and mandibulometer and statistically analysed.

Findings: On statistical analysis, it is found that height of the symphysis menti, bigonial width, shows a higher index of sexual dimorphism. Hence, these can be used for determination of sex. Other parameters such as bicondylar width, mandibular angle are found to be statistically insignificant in this study.

Conclusion: When the findings of present study were compared with those of past investigators, marked variations in the various osteometric parameters of mandible was noticed, which differed all over the world as well as in different regions of India. Hence, it is suggested, not to apply the findings of study of one region over the samples of other region, to avoid fallacies.

Keywords: symphysis menti, bigonial width, bicondylar width, mandibular angle

Introduction

100 % accuracy in determining sex could be achieved if entire skeleton is available (Krogman1946). But problems arise, when either incomplete or fragmentary remains of skeleton are brought for examination. Then it depends largely on the available parts of the skeleton. A number of literatures have shown sexual dimorphism in
almost every bone of human skeleton. As evident from the past studies, skull is the most dimorphic and easily sexed portion of skeleton after pelvis, providing accuracy up to 92%. But in cases, where intact skull is not found, mandible may play a vital role in sex determination, as it is the most dimorphic bone of the skull.

Identification of sex could be made on differences in the size and shape of the morphological markers i.e. Shape of chin, size of condyles etc. Morphological traits are more subjective and sex determination depends on experience of the investigator, so visual methods of sexing mandible are likely to be inaccurate when performed by an inexperienced worker and sometimes produce misleading results. Hence, metric analyses are found to of superior value owing to their objectivity, accuracy, reproducibility and lower incidence of inter and intra observer errors as compared to that of descriptive traits.

The focus on present study is to use morpho-metric analytical method to investigate the sexual dimorphism and to evaluate the ability of selected parameters to determine sex in forensic context.

**Material & Method**

The present study was undertaken in the Department of Forensic Medicine and Toxicology, Gandhi Medical College Bhopal. 200 mandibles were measured, of which 130 were male and 70 were female. All the pathological, deformed and fractured mandibles were excluded from the study. The numbers of female samples were limited, because all sample were forensic cases and the female skeleton brought for examination are limited.

**For sex determination, following point was taken into consideration** –

1. Size – larger / thicker heavier in male, smaller, thinner lighter in female.
2. Surface – smooth relatively in female and rugged in female.
3. Shape of chin – square shaped in males and pointed or rounded (U shaped) in females.
4. Condyles – bigger and larger in male and smaller in females.

Allotment of mandibles to different sexes was carried out by experts in the department, namely, forensic medicine experts and forensic anthropologists.

**Instruments used for taking measurements** – were sliding caliper and mandibulometer.

Of the various measurements of mandible mentioned in different standard anthropological studies, 4 anthropometric parameters were selected (Table-II). To ensure familiarities of reference and forensic anthropologist alike, landmarks were selected to correspond closely to those common in both traditional and geometric morphometric studies,(Table- I) To minimize the intra observer error, all the measurements are taken with sliding caliper (0.1 mm precision) 3 times and the average value were utilized for the analysis.

**TABLE - I (Landmarks on Mandible)**

<table>
<thead>
<tr>
<th>No.</th>
<th>Landmarks</th>
<th>Definition</th>
<th>Unilateral/ Bilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Condylion superior</td>
<td>The most superior point of the mandibular angle</td>
<td>Bilateral</td>
</tr>
<tr>
<td>2</td>
<td>Condylion laterale</td>
<td>The most lateral point of the mandibular angle</td>
<td>Bilateral</td>
</tr>
<tr>
<td>3</td>
<td>Gonion</td>
<td>The most lateral and external point at the junction of the horizontal end on – rami of the lower jaw.</td>
<td>Bilateral</td>
</tr>
<tr>
<td>4</td>
<td>Infradentate(id)</td>
<td>The mid- point of a line tangent to the outer margin of the cavities of the two mandibular central incisor</td>
<td>Unilateral</td>
</tr>
<tr>
<td>5</td>
<td>Gnathion</td>
<td>The middle point of the lower border of the mandible in the sagittal plane</td>
<td>Unilateral</td>
</tr>
</tbody>
</table>
TABLE – II (Anthropometric Parameters for measurement)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measurement Description</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of symphysis mentii</td>
<td>Direct distance from infradentate to gnathion</td>
<td>Sliding calliper</td>
</tr>
<tr>
<td>Bigonial width</td>
<td>Measured between right and left gonion</td>
<td>Sliding calliper</td>
</tr>
<tr>
<td>Bicondylar width</td>
<td>Measured between the right and left condylion laterale</td>
<td>Sliding calliper</td>
</tr>
<tr>
<td>Mandibular angle</td>
<td>It is the angle formed by the inferior border of the corpus and the posterior border of the ramus</td>
<td>Mandibulometer</td>
</tr>
</tbody>
</table>
Statistical methods used for analysis were as follows:

**Index of Sexual dimorphism (ISD)**

ISD = Mean value of Male / Mean value of female X 100.

It indicates the level of difference between sexes; values close to hundred indicate low level of sexual difference and on the other hand the level of sexual difference increases with the increase of the distance from hundred.

1. **Mean**

Arithmetic mean was calculated by dividing the total number of individual observations by number of cases

\[ \text{Mean} = \frac{\sum x}{n} \]

Where \( \sum x \) = summation of individual observation,

\( n \) = number of cases

2. **Standard Deviation**

It is the square root of the arithmetic average of the square of the difference between the observations and their mean. The formula is as follows:

\[ \text{S.D.} = \sqrt{\frac{\sum (x - x)^2}{n}} \]

Where \( \sum (x-x)^2 \) = Summation of each squared deviation

\( n \) = number of cases

3. **Level of Significance (‘t’ test)**

This test was used to find out any significant difference between the means of different groups. It was calculated by the formula, given below:

\[ \text{S.E.}(d) = \sqrt{\frac{(S.D.1)^2}{n1} + \frac{(S.D.2)^2}{n2}} \]

Where S.E. (d) = Standard error of difference

\( S.D.1 \) & \( S.D.2 \) = Standard deviations of observations in two samples

\( n1 \) & \( n2 \) = No. of cases in the 2 samples

**Probability( P value )**

\( P<0.001 \) = extremely significant

\( P<0.01 \) = very significant

\( P < 0.05 \) = significant

\( P>0.05 \) = not significant
Findings

The data is represented in the master chart, the observation were statistically analysed and the results obtained were compared with the findings mentioned in pre-existing literature.

1. Height of Symphysis Menti

Mean of symphysis menti height for males is found to be 30.1508 with a standard deviation of 4.0136; and that of females is 28.4672 with a standard deviation of 2.4168. P value obtained for the parameter is <0.001 that means the difference between the two variables is extremely significant. Also the ISD is above 100, which shows the significant level of sexual differences between two means. In the study conducted by Fabien and Mpembeni in the year 2002, on 25 male and 25 females; they found this parameter to be statistically significant and a reliable parameter for sex determination. Ionescu et al (2007) in his study on 50 male and 50 female mandibles have also found this parameter to be statistically significant.

Similar finding was observed by Corici et al in 2009 in his study conducted on 80 dry specimen of mandibles.

2. Bigonial Width

The mean for this parameter is 98.5049 with a standard deviation of 5.8765; and for females mean found is 93.3166 with standard deviation of 5.4786. Probability level of P < 0.001 is obtained, showing an extremely significant statistical difference. Also, ISD is above 100, meaning the level of sexual difference for this parameter is significant.

Similarly, in 2002, Fabien and Mpembeni in their study of sexual dimorphism in 25 male and 25 female mandibles of homogenous black population of Tanzania; found this parameter to be statistically insignificant.

But, the finding in the present study is in contradiction with a study carried out by Ionescu et al in the year 2007 on 50 male and 50 female mandibles; that showed the bicondylar width to be a statistically significant parameter.

3. Bicondylar Width

The mean obtained for this parameter in males is 111.4213 with a standard deviation of 7.9183; and that for female is 110.4887 with standard deviation of 6.0136. In this case, p = 0.39, which is insignificant, also ISD obtained is just few decimal points above 100. Hence, it can be concluded by these observations that, this parameter is not that significant statistically.

Also, Ionescu et al in 2007 while studying sexual dimorphism in mandible, using 25 male and 25 female samples, found this parameter to be statistically significant.

Badiu et al in his study on 80 preserved adult skull with matching mandibles in 2010 found that this parameter to be extremely significant statistically and having a higher ISD. He stated that “the bigonial width could be used for sex determination of an unidentified skull.”
4. Mandibular Angle

As observed in the present study, the mean + standard deviation for male on right side is 122.8672 + 4.2976; on left side, 122.7861 + 4.2472. For females, on right side, the mean is 124.7661 with a standard deviation of 4.0974; on left side, the mean and standard deviation are 124.6760 and 4.0471 respectively. Also, ISD is below 100, which gives an indication that the level of sexual difference is insignificant. But, on comparing means of male and female in adult and elderly age group separately, though the difference is statistically significant (p< 0.001), but the ISD calculated is below 100, indicating that the level of sexual difference for this parameter is insignificant.

Similarly, Ionescu et al\(^6\) in the year 2007 in his study on 25 male and 25 female mandibles found this parameter to be insignificant to study sexual dimorphism. This finding is also supported by the study of Oettle et al (2006)\(^10\) who stated that “In the assessment of human remains, the mandibular angle is not very usable in determining sex.” Loth and Henneberg (2000)\(^8\) in their study have shown that the gonial form has a highly heritable component that appears to be associated with overall facial architecture and determining sex using this trait has an accuracy of only 45 %

On the contrary, Acsadi and Nemeskeri (1970)\(^1\) and Novotny et al (1993)\(^9\) stated the gonial eversion to be a sex marker for adults. Same is also considered a male characteristic by Ferembach et al (1980)\(^4\)

When the findings of present study were compared with those of past investigators, marked variations in the various osteometric parameters of mandible was noticed, which differed all over the world as well as in different regions of India. Hence, it is suggested, not to apply the findings of study of one region over the samples of other region, to avoid fallacies. It is also recommended, to carry out more studies incorporating a larger sample; to make the data more precise and useful in terms of anthropological and medico-legal application.

**Conflict of Interest** – Nil

**Source of Funding** - self with assistance from institute

**Ethical Clearance** – The study protocol was approved by Institutional Ethics Committee of Gandhi Medical College, Bhopal

**References**

An Autopsy Study of Drowning Deaths in and around Visakhapatnam

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Abstract

Introduction: According to World Health Organisation, drowning is among the ten leading causes of death for children and young people in every region of the world. Drowning amounts to an estimated incidence of about 5.6 per 100,000 of population worldwide. This study is aimed to know the incidence, manner and epidemiology of deaths due to drowning in and around Visakhapatnam.

Materials and Method: It is a one year prospective post-mortem study of drowning in and around Visakhapatnam. All the deaths due to drowning that were autopsied at the mortuary, KGH, Visakhapatnam were studied based on inquest, post mortem examination findings to know the profile of drowning deaths.

Results and Conclusion: Out of 1675 total autopsies done at our centre during the study period, 79 bodies that are found in water are studied. Males in the age group of 21-30 are the most common victims. Sea water drowning amounted for most cases of drowning in contrary to other studies. About 58% of cases of drowning were during the day. Most number of drowning deaths occurred in the month of October and monsoon season. Accidental drowning is the most common manner of death in males and suicidal manner in female drowning victims. About 32% of drowning victims in the study are from low socio-economic status. Most of the female victims are married while male victims are unmarried.

Keywords: Drowning, Sea water drowning, season, manner of death, Socio economic status.

Introduction

According to the World Health Organization, drowning is defined as “The process of experiencing respiratory impairment from submersion/immersion in liquid”¹. India is a vast country having abundant of water supplies from rivers, ponds, wells and an extensive seacoast. The world incidence of death by drowning is estimated at about 5.6 per 100,000 of population². About 150,000 people die from drowning each year around the world³. In 2013, all India survey made by the National crime record bureau (NCRB) showed 8% of total unnatural deaths are due to drowning⁴. 25% occur in the sea and the rest in inland waters; the majority of victims are young adults and children⁵; two-third is accidental and one-third is suicidal; homicide by drowning is rare⁶. All unattended drowning cases should be presumed homicide until proven otherwise⁷. Drowning and alcohol intoxication may represent accidents or suicides. In suicidal attempts by drowning, the suicidal attempter often leaves behind a letter or a phone call before departing to jump into the water. Homicidal drowning is uncommon and requires either physical disparity between the assailant and the victim or a victim incapacitated by disease, drink or drugs or taken by surprise⁸. Disposal in water may be attempted where the victim has already been killed by other means⁹.

Aim of the Study

To study and analyze the incidence, epidemiology,
manner and the circumstances of drowning deaths reported in and around Visakhapatnam.

**Materials and Method**

In this study, all the autopsies done on persons died due to drowning are studied based on the requisition for conduct of autopsy, the inquest report (panchanama), the post-mortem examination and the clinical record (Hospital data) in certain cases were used to relate, correlate and to substantiate after ruling out other causes of death.

First charts were made for collecting all the information regarding the drowned body entering the details regarding age, sex, marital status, medium of immersion, history from inquest etc.,

Then individual information charts were prepared such as month wise distribution, age, sex, occupation, marital status, medium of drowning, timing and manner of drowning. Out of them the possible number of bar graphs, and pie charts were drawn to show the statistical trends in drowning.

**Observations & Discussion**

In the study year, a total of 1684 autopsies were done. Among these, the drowning cases reported amounts for 79 cases i.e., a percentage of 5 cases of all post-mortem examinations. The number of drowning cases occupy the sixth place among the 10 most common or frequently encountered cases during study period as shown in the bar diagram 1. It amounted to about 35% of all asphyxial deaths.

Diagram 1: A Bar diagram showing Number of different autopsy cases based on cause of death during study period

During the study period, the number of cases of drowning that is reported show a male preponderance. As it is evident that nearly 76% of drowning deaths have occurred in males as opposed to a mere 24% in females. These figures further establish and correspond with the already existing data regarding more number of drowning deaths in male

The study of age highlights that the maximum number of cases were reported in 21-30 years group i.e., 26.5% and closely competed by 31-40 and 11-20 respectively with 23 and 20%. In both males and females, the highest number of victims is from the age group of 21-30 years with 27% and 26% of cases respectively. The study further confirms and more or less matches the already established figures of previous studies in India. The maximum number of cases occurred between 15 and 25 years of age in males there by establishing the exuberance, excitement and eagerness among the youth coupled with zeal for adventure. Whereas in females, most number of cases encountered were aged between 21-35 years. There are very minimal or negligible numbers of cases in age groups 1-10, 61-70 and 71-80.

Diagram 2: A Bar diagram showing Age wise distribution of cases of deaths due to Drowning.

A large number of drowning deaths have occurred in the month of March, August and October i.e., summer month of March and monsoon months of August and October. The summer and monsoon seasons almost matched each other nearing 37% and 43% cases respectively. The summer and monsoon seasons have almost 80% of deaths occurred due to drowning. This phenomenon is denoted by the holiday season in summer, when a large number indulge in adventurous exploits and monsoon draws one towards adventure and is as well occupation related. These findings are similar to those in Phad and Dhawane study.
Table 1: Table showing the month wise incidence of drowning deaths.

<table>
<thead>
<tr>
<th>Month</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>February</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>March</td>
<td>7</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>April</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>May</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>June</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>July</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>August</td>
<td>10</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>September</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>October</td>
<td>11</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>November</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>December</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>19</td>
<td>79</td>
</tr>
</tbody>
</table>

In our study we have found that sea water is the main culprit which amounts to 64%, fresh water 25% and alternate media for 11% of drowning deaths. There is gross disparity between the existing data and our study data where the figures are reversed in relation to sea water over freshwater drowning. The main reason to be attributed to this statistic is the vast and expansive coast, easy accessibility to the sea, dependence on sea as a vocational resource, adventure and related professional pursuits.

Table 2: Table showing cases of drowning in respect to medium of immersion.

<table>
<thead>
<tr>
<th>Medium of immersion</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Sea water</td>
<td>39</td>
<td>12</td>
</tr>
<tr>
<td>Well &amp; Lake</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Tanks</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Pits</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Drainage</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Canal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Septic tank</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Swimming pool</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The manner of death in cases of drowning is of prime importance. In the study, the maximum number of deaths that were reported as drowning are as a result of an accident, next in order is suicides and one case registered as homicide although a handsome number of cases which are listed as undetermined amount to 11 i.e., nearly 20% of sum. The manner at inquest and based on circumstances, drowning deaths show accidental (41 cases i.e., 68%) as most common manner in males and suicidal (10 cases 53%) as most common manner in females. These findings are in contrary to other studies.
Table 3: Manner of death due to drowning based on Inquest & Circumstances.

<table>
<thead>
<tr>
<th></th>
<th>Accidental</th>
<th>Suicidal</th>
<th>Homicidal</th>
<th>Undetermined</th>
<th>Associated natural disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>41</td>
<td>4</td>
<td>1</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Percentage</td>
<td>51.90</td>
<td>5.06</td>
<td>1.26</td>
<td>13.92</td>
<td>3.80</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Percentage</td>
<td>6.33</td>
<td>12.66</td>
<td>0</td>
<td>5.06</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>14</td>
<td>1</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Percentage</td>
<td>58.23</td>
<td>17.72</td>
<td>1.26</td>
<td>18.99</td>
<td>3.80</td>
</tr>
</tbody>
</table>

Another important aspect of our study is to determine the relation of time with drowning deaths. A vast number of cases of drowning were reported during day time, a 15% of cases were reported during night and in about 25% of cases it appeared that the time of occurrence of the incident remained indeterminate upon inquest and circumstances.

Table 4: Table showing time of drowning based on inquest & Circumstances.

<table>
<thead>
<tr>
<th></th>
<th>Day (6am-6 pm)</th>
<th>Night (6pm – 6am)</th>
<th>Not known</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>35</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>Percentage</td>
<td>58.23</td>
<td>15.19</td>
<td>26.58</td>
</tr>
</tbody>
</table>

The occupation and its relation to drowning deaths is studied based on inquest which showed a large number of accidental deaths due to drowning were seen in labourers or coolies is 25 cases i.e., 32%, fishermen is 6 cases i.e., 8% and officials 9 of which 3 cases were a result of accidental drowning at work i.e., 30%. The other indicators (occupations) students, officials, no occupation have been usually suicides and that too the large chunk of the unemployed being women.

The analysis of Table 5 points to almost an equal number of deaths due to drowning in married a well as unmarried males. Coming to the female sex, the number of cases of drowning among married women was reported at 9 cases while those of unmarried women amounts to 4 such cases, similarly owing to the non-establishment of identity i.e., 6 out of 60 in males and 6 out of 19 in females, their marital status could not be assessed. Of the unmarried males, the manner of such deaths was usually accidents and a few suicides, but in the females who were reportedly unmarried most of the deaths could be attributed to suicides.

Diagram 3: A bar diagram showing Occupation of the deceased died of drowning.
Table 5: Table showing marital status of the victims died due to drowning.

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Married</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>Unmarried</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Not known</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

**Conclusion**

From this study it is evident that drowning amounts to significant number of deaths in young males in the age group of 21-30 years. Sea water is the commonest source of drowning medium in this study. Most of the drowning deaths occurred during day time. Most of the victims died accidentally and are labourers which denotes the lack of safety measures in the fishing industry from which they belong and the necessity of improving the safety of fisherman by providing them with safety equipment. All people particularly school going children and college going youth should be made aware of the rules of safety near and while in water and the authorities has to improve the safety at beaches by commissioning the rescue teams after identifying the places with high activity and saving these young victims. Provision of mechanical boats, monitoring systems, systems of surveillance will go a long way in aiding accident struck victims.

**Ethical Clearance:** There were no ethical issues involved as the study was analytical and did not violate any ethical principles.

**Source of Funding:** Nil

**Conflict of Interest:** Nil

**References**

Morphologic and Radiographic Effects of Acids on the Teeth: An in-Vitro Forensic Study

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Abstract

Background: Teeth are generally considered as the most durable structures that resist physical, chemical and biological insults than skeletal tissue. Human identification through dental remains is of prime importance when the remains are found in chemical accidents such as acid attacks or uneventful accident in chemical industries, where soft tissues cannot provide reliable information. Proper examination and analysis of dental remains can provide vital information in forensic context.

Objective: The present study aims to evaluate the morphology and radiographic features of teeth after exposure to highly acidic environment.

Material and Method: A sample of total 80 extracted permanent teeth was used. Teeth were exposed to different acidic conditions like - 35% hydrochloric acid (30ml), 69% nitric acid (30ml), 98% sulphuric acid (30ml), 85% phosphoric acid (30ml) at intervals of 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 16 hr and 24 hr. Before and after exposure to acids, radiographic and stereomicroscopic images were taken.

Results: The effect of acids on teeth depends on various factors such as the type and concentration of the acid, and also the time period. The morphological and radiological changes normally includes - changes in dimensional stability, changes in texture and colour, changes in the appearance of fissures and cracks and also appearance of the fracture and fragmentation of the samples.

Conclusion: Teeth can serve as a tool in identification of the victim when in contact with acidic condition. Hydrochloric acid, nitric acid and sulfuric acid cause changes in the teeth and it is possible to deduce the approximate duration for which a body has been immersed in acid based on the changes observed. The characteristics of teeth may be of assistance in cases of human identification where soft tissue features are unavailable.

Keywords: Acids, stereomicroscope, dental identification, teeth, forensic odontology.

Introduction

Forensic odontology has now evolved among the intriguing branches of forensic sciences over the past few years. Forensic dentistry contributes well by way of reconstruction through dental structures. The forensic odontologist mainly deals with the identification based on recognition of unique features present in an individual’s oral structures.¹

Teeth normally survives in most natural disasters and are a possible tool for personal identification of an otherwise unrecognizable body.² Teeth can survive in extreme climatic condition because enamel is the hardest substance in the body being 96% mineralized, compared to bone which is approximately 70% mineralized.³ The posterior teeth are well protected by soft tissues like the

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tongue, facial musculature, and adipose tissue. Due to its hard structural integrity it survives prolonged immersion, decomposition, desiccation, extensive trauma, and direct heat in excess of 1000°F.

In chemical accidents, the hard tissues including the skeletal structures are destroyed completely and soft tissues cannot provide reliable information, hence thereby rendering a challenge for identification of the individual. Moreover, there is an apparent lack of data on radiographic alterations along with morphologic changes in teeth at various acids which necessitates study in this area.

The purpose of the study was to identify the acid that may be used by criminals for destruction of hard tissue – teeth and to find out the approximate time taken for total destruction of tooth after immersion in an acid. The objective was to observe the stereomicroscopic and radiographic changes.

**Material and Method**

Eighty extracted human natural teeth were obtained for an in-vitro experimental study.

All teeth were non-carious and had been extracted for orthodontic treatment or periodontal reason. Before exposing to various acids, teeth used for the study were rinsed thoroughly in tap water and cleaned with a toothbrush in order to remove any soft tissue and later were disinfected by using 10% formalin. Deposits of calculus were removed using a manual scaler.

Thirty-five percent hydrochloric acid (conc. HCl), 69% nitric acid (conc. HNO3), 98% sulfuric acid (conc. H2SO4) and 85% Phosphoric Acid (conc. H3PO4) are used in this study.

Twenty samples were immersed in 30 ml of each acid solution and were observed for 24 hours. Teeth were retrieved at intervals of 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 16 hr and 24 hours from the acidic environment, washed in distilled water, dried, photographed, and later radiographed. Each of specimen’s morphology was observed under a stereomicroscope at 15X magnifications (Zeiss Stemi DV4 Stereo Zoom Microscope) at time intervals of 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 16 hr and 24 hours. The macroscopic and radiographic changes were well noted.

**Results**

The teeth samples that were subjected in 30 ml of 35% hydrochloric acid (conc. HCl) showed following morphological and radiographic changes. (Table -1) (Figure-1)

<table>
<thead>
<tr>
<th>Time</th>
<th>30 mins</th>
<th>1 hour</th>
<th>2 hours</th>
<th>4 hours</th>
<th>8 hours</th>
<th>16 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphological changes</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
</tr>
<tr>
<td>Radiographic changes</td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Figure-1: Sequential morphological and radiologic changes observed after immersion in 30 ml - 35% hydrochloric acid (conc. HCL)
Table-1: Changes observed in teeth placed in 30 ml - 35% hydrochloric acid (conc. HCL)

<table>
<thead>
<tr>
<th>Time</th>
<th>Morphological changes</th>
<th>Radiographic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mins - 1 hour</td>
<td>Only deterioration of enamel surface was seen.</td>
<td>Deterioration of enamel surface and the curvature appeared smooth.</td>
</tr>
<tr>
<td>2 - 4 hours</td>
<td>Disintegration of the crown with gel like formation on dentin</td>
<td>Presence of small fractured lines in root portion.</td>
</tr>
<tr>
<td>8 - 24 hours</td>
<td>Crown loses its morphology and deterioration is well appreciated in enamel and dentin</td>
<td>Teeth to be showed as start dissolving.</td>
</tr>
</tbody>
</table>

The teeth samples that were subjected in 30 ml of 69% Nitric acid (Conc. HNO₃) showed following morphological and radiographic changes (Table-2) (Figure-2).

<table>
<thead>
<tr>
<th>Time</th>
<th>Morphological changes</th>
<th>Radiographic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>Loss of enamel surface and surface structure become smooth and yellow color residue deposited over the tooth.</td>
<td>Showed no fracture lines in teeth, deterioration of enamel surface was noticed, surface outline of the teeth is smooth.</td>
</tr>
<tr>
<td>1 hour</td>
<td>Further dissipation of enamel is observed and yellow colored residues are seen over the tooth. At this temperature, surface texture becomes sticky in nature</td>
<td>Deterioration of enamel surface, many fracture lines are seen in root portion.</td>
</tr>
<tr>
<td>2 - 8 hours</td>
<td>Teeth shows gel like consistency and sticky over the entire crown portion than compared to the root.</td>
<td>Teeth showed loss of thickness of the root portion and the complete deterioration of the crown.</td>
</tr>
<tr>
<td>16 - 24 hours</td>
<td>The 3-dimensional structure of teeth gets converted into 2-dimensional structure, and later, the total morphology of teeth gets lost.</td>
<td>Teeth lost their complete morphology.</td>
</tr>
</tbody>
</table>

Figure-2: Sequential morphological and radiolographic changes observed after immersion in 30 ml - 69% Nitric acid (Conc. HNO₃)

Table-2: Changes observed in teeth placed in 30 ml - 69% Nitric Acid (conc. HNO₃)

<table>
<thead>
<tr>
<th>Time</th>
<th>Morphological changes</th>
<th>Radiographic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>Loss of enamel surface and surface structure become smooth and yellow color residue deposited over the tooth.</td>
<td>Showed no fracture lines in teeth, deterioration of enamel surface was noticed, surface outline of the teeth is smooth.</td>
</tr>
<tr>
<td>1 hour</td>
<td>Further dissipation of enamel is observed and yellow colored residues are seen over the tooth. At this temperature, surface texture becomes sticky in nature</td>
<td>Deterioration of enamel surface, many fracture lines are seen in root portion.</td>
</tr>
<tr>
<td>2 - 8 hours</td>
<td>Teeth shows gel like consistency and sticky over the entire crown portion than compared to the root.</td>
<td>Teeth showed loss of thickness of the root portion and the complete deterioration of the crown.</td>
</tr>
<tr>
<td>16 - 24 hours</td>
<td>The 3-dimensional structure of teeth gets converted into 2-dimensional structure, and later, the total morphology of teeth gets lost.</td>
<td>Teeth lost their complete morphology.</td>
</tr>
</tbody>
</table>

The teeth samples that were subjected in 30 ml of 98% Sulphuric Acid (conc. H2SO4) showed following morphological and radiographic changes (Table-3) (Figure-3).
Figure-3: Sequential morphological and radiographic changes observed after immersion in 30 ml - 98% Sulphuric Acid (conc. H2SO4)

Table-3: Changes observed in teeth placed in 30 ml - 98% Sulphuric Acid (conc. H2SO4)

<table>
<thead>
<tr>
<th>Time</th>
<th>Morphological changes</th>
<th>Radiographic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 hours</td>
<td>Chalky white appearance seen on the crown portion and slight over the root portion, loss of glossy surface of the crown.</td>
<td>No morphologic were observed.</td>
</tr>
<tr>
<td>4-8 hours</td>
<td>White chalky precipitates seen on crown and root portion, dryness of teeth with loss of glossiness of surface.</td>
<td>Flaking of enamel was observed and fracture line seen in root portion.</td>
</tr>
<tr>
<td>16-24 hours</td>
<td>Chalky white appearance and dissipation of the morphology of the crown portion and irregular lines seen over the surface of the root.</td>
<td>Deterioration of enamel surface and multiple fractures line are seen in root portion.</td>
</tr>
</tbody>
</table>

The teeth samples that were subjected in 30 ml of 85% Phosphoric Acid (conc. H3PO4) showed following morphological and radiographic changes (Table-4) (Figure-4).

Figure-4: Sequential morphological and radiologicraphic changes observed after immersion in 30 ml - 85% Phosphoric Acid (conc. H3PO4)
Table-4: Changes observed in teeth placed in 30 ml - 85% Phosphoric Acid (conc. \( \text{H}_3\text{PO}_4 \))

<table>
<thead>
<tr>
<th>Time</th>
<th>Morphological changes</th>
<th>Radiographic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mins - 2 hours</td>
<td>Teeth loss its glossiness and enamel becomes dry and chalky deterioration of enamel.</td>
<td>No morphologic changes were observed.</td>
</tr>
<tr>
<td>4-8 hours</td>
<td>slight brownish discoloration of teeth is seen with removal of enamel surface</td>
<td>Crown covered by some radioopaque cloudiness no morphologic changes were observed in crown and root portion.</td>
</tr>
<tr>
<td>16-24 hours</td>
<td>Crown portion showed melting of enamel surface by which crown portion look slightly large as compared to pre-exposure teeth and the root surface look dry and shiny.</td>
<td>More radioopaque cloudiness over the crown portion slight deterioration of enamel surface, morphology of the teeth is intact.</td>
</tr>
</tbody>
</table>

**Discussion**

This study justified that the natural teeth are the most durable of all the living tissues as they persist by chemical agents.

Teeth can be used as a source of evidence in cases of human identification, especially those cases where the soft tissues are unavailable as a possible identification tool. 5, 6

In this study, the morphologic and radiographic changes were observed to the teeth exposed to varied acidic medium. From this experimental study, we could conclude that teeth do not react in the same way in all acids.

In case of 35%HCl and 69% HNO₃ the teeth were completely dissolved but in HNO₃ medium, the color change in the form of the yellow colored deposit was mainly because of the nitrate formed in the chemical reaction and in \( \text{H}_2\text{SO}_4 \) and \( \text{H}_3\text{PO}_4 \) there was no dissolution; instead, a white precipitate was formed because of the formation of a non-soluble salt. Thus, there are clear differences in the destructive capacities of the different acids.

Recognizable morphological and radiographical appearances of teeth persisted for 8 hr. in HCl but Raj et al (2013) ⁴, in their study reported that mild to deep cracks developed in tooth in 15 to 30 mins, and the tooth started dissolving in 1 hour, the tooth was completely dissolved in 8 hours, for Nitric acid is same as 8 hr. in HCl where as Jadhav et al (2009)⁷ observed in their study that yellow colour appears in 15 min, disintegration of apical 3rd in 4 hours, splitting of teeth in 8 hours, and completely dissolved in 15 hours. Cope and Dupras note in their study that the teeth immersed in hydrochloric acid took on a “jelly-like appearance” which describes the outer layer translucency observed in this study ⁸, and for Sulphuric and phosphoric acid morphological and radiographical appearances of teeth persisted even after 24 hr Mazza et al (2005)⁹ found in their study that no changes seen till 5 hours..

Thus by seeing the morphological and radiographical feature we can determine the type of acid used and for which duration it can also determine, slight elevation of dimension were observed in Sulphuric and phosphoric acid during 16-24 hr. Thus there are clear differences in the destructive capacities of different acids used in the study.

**Conclusion**

The durability of these teeth may be utilized as a successful identification tool in forensic science. According to this study, the morphological and radiographical changes observed in teeth after particular time interval of immersion in an acid solution can be utilized for identification of the acid used and to deduce the approximate duration of immersion in the acid. However, the final decision on which acid has been used should be based on biochemical tests. It should also be noted that the effect of acids on teeth not only depends on the type of the acid or the concentration of acid or the time period but it also depends upon the size of the teeth or the position of the teeth.

**Conflict of Interest** – None Declared

**Source of Funding** – None

**Ethical Clearance** – Not required (As this article has used only extracted teeth and does not contain any
studies with human participants or animals)

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J Forensic Sci & Criminal Inves 7(5): JFSCI. MS.ID.555723 (2018


Pattern of Abdominal Viscera Injuries in Blunt Abdominal Trauma Deaths in Ahmedabad

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Abstract

In this age of speed and traffic accidents, the incidence of blunt injuries to the abdomen has been at its height due to the development of the modern industrial era with the development of the automobile. This study was conducted to describe the frequency and pattern of intra-abdominal injuries in patients with blunt abdominal trauma.

The present study was undertaken on 693 victims who died due to blunt abdominal injuries which were autopsied at B. J. Medical College mortuary, Ahmedabad during the year 2016-2017. This study shows that most common organ involved in blunt abdominal injuries is liver. Most common associated injuries with this is chest injuries. Highest number of people died due to shock and hemorrhage.

Keywords: Blunt abdominal trauma; Intra-abdominal injuries.

Introduction

Trauma is one of the leading preventable causes of death in developing countries, and is a major health and social problem. Trauma affects generally the young people, and accounts for loss of more years of life, than lost due to cancer and heart diseases put together. Since prehistoric times, the abdomino-pelvic cavity has been looked upon as one of the most vulnerable regions of the body and injuries involving it have always been considered very serious. As early as 460 BC, Hippocrates was aware of the danger to life caused by injury to liver and spleen ¹.

Most of the information about abdominal trauma has been gained through military experiences, as Sir Winston Churchill stated, “War is an epidemic of trauma” ². It is only in the recent time that the automobile became more deadly than the rifle. Deaths are occurring every day, in many different settings, from injuries to the upper abdomen and lower rib cage that produce damage to the liver, spleen and pancreas. The location and severity of the blow and the position of the victim when injured determine which combination of organs is affected.

In open cases of abdominal trauma the clinical manifestations, diagnosis and management will be easier. But closed cases of trauma, offers a great challenge to the treating surgeon. Sometimes it may escape detection or lead to an error in diagnosis from medico-legal point, and the same is often true with autopsy doctor, where in closed cases of abdominal trauma, the autopsy findings may sometimes be trivial or complex and frustrating. It may be difficult to interpret the anatomic abnormalities to understand the mechanism of death, and may require a lengthy explanation. The object of the dissertation is to make a comprehensive study of pattern of blunt abdominal injuries, which are fatal and resulting in death.

Materials and Method

The present study was conducted at B. J. Medical College, Ahmedabad. Total 693 cases of deaths of all age groups and both sex from blunt abdominal trauma during the year 2016-2017 were taken up for study. Death due to head injury, chest injuries where abdominal injuries are minor and badly decomposed bodies where significant injuries were not visible also not included in
this study. Routine information like age, sex, occupation brief facts of the cases collected from the inquest report. Clinical history like time of admission, and deaths and other relevant data was collected from the hospital case sheets and death summaries. Pattern, nature of injuries and other information were obtained from a detailed follow up and study of the autopsy cases and reports. Finally all the data thus collected, complied and presented in the tables.

**Results**

The present study comprised 693 (15.1%) autopsy cases of deaths due to blunt abdominal injuries, out of total 4588 medico-legal autopsies (Table-1). Liver was commonest organ injured in 74% cases. Out of 511 cases, 333 cases were due to injury by motor vehicles, 116 cases were due to fall from height. splenic injury was found in 36.65% cases. Out of 254 cases, 170 cases were due to injury by motor vehicles, 56 case due to fall from height and 16 cases due to railway hit. Injury to intestine was found in 13.85% cases. Out of total 96 cases, 63 cases were due to injury by motor vehicles. Kidney was found injured in 11.11% cases. Out of 77 cases, 49 cases were due to injury by motor vehicles 20 case due to fall from height and 6 cases due to railway hit. Stomach was found injured in 4.76%. Out of 33 cases, 26 cases were due to injury by motor vehicles 5 case due to fall from height. Urinary bladder was found injured in 1.15% cases. All cases were injured due to injury by motor vehicles. Most common associated injuries with blunt abdominal injuries are chest injuries. It is followed by fracture and head injuries. In males out of 549 cases hemorrhage is seen in 460 cases and in females in 117 cases, where as infection cases 77 cases are of males and 25 cases are of females. Multi organ failure is seen in 2.00% of cases.

**Table 1: No. of Cases of Death Due to Blunt Abdominal Injuries**

<table>
<thead>
<tr>
<th>Death due to Blunt Abdominal Injuries</th>
<th>NO. OF CASES</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Autopsies</td>
<td>4588</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2: Abdominal Viscera Involved**

<table>
<thead>
<tr>
<th>Abdominal Viscera Involved</th>
<th>Cases</th>
<th>Percentage</th>
<th>Rta</th>
<th>F/H</th>
<th>Railway Hit</th>
<th>Assault</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>254</td>
<td>36.65</td>
<td>170</td>
<td>56</td>
<td>16</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>511</td>
<td>73.74</td>
<td>333</td>
<td>116</td>
<td>33</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>96</td>
<td>13.85</td>
<td>63</td>
<td>22</td>
<td>7</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Gall Bladder</td>
<td>22</td>
<td>3.17</td>
<td>13</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>77</td>
<td>11.11</td>
<td>49</td>
<td>20</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>33</td>
<td>4.76</td>
<td>26</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Urinary Bladder</td>
<td>8</td>
<td>1.15</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Internal Reproductive Organ</td>
<td>13</td>
<td>1.88</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mysentry</td>
<td>34</td>
<td>4.91</td>
<td>24</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Major Vessels</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>35</td>
<td>5.05</td>
<td>24</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3: Associated Injuries

<table>
<thead>
<tr>
<th>Associated Injuries</th>
<th>Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head Injury</td>
<td>72</td>
<td>22.50</td>
</tr>
<tr>
<td>Chest Injury</td>
<td>134</td>
<td>41.88</td>
</tr>
<tr>
<td>Fracture</td>
<td>114</td>
<td>35.63</td>
</tr>
<tr>
<td>Combined</td>
<td>11</td>
<td>3.44</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Table 4: Mechanism of Death

<table>
<thead>
<tr>
<th>Mechanism of Death</th>
<th>Male</th>
<th>Female</th>
<th>Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock And Haemorrhage</td>
<td>460</td>
<td>117</td>
<td>577</td>
<td>83.26</td>
</tr>
<tr>
<td>Infection</td>
<td>77</td>
<td>25</td>
<td>102</td>
<td>14.72</td>
</tr>
<tr>
<td>Multy Organ Failure</td>
<td>12</td>
<td>2</td>
<td>14</td>
<td>2.02</td>
</tr>
<tr>
<td>Total</td>
<td>549</td>
<td>144</td>
<td>693</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Discussion**

The observations and results of the present study were compared and contrasted with the work of preceding researchers.

As shown in Table 2, Liver was commonest organ injured in 74% cases. It was consistent with the findings of Abdelrahman H and Morales Uribe CH. Out of 511 cases, 333 cases were due to injury by motor vehicles, 116 cases were due to fall from height, 23 cases were due to direct blow on abdomen by lathi, hockey etc., 3 cases were due to fall of roof upon the deceased. Tonge et al had reported 24.9% incidence of liver injury in fatal road accidents. Kaur study showed liver injuries in 16.55% cases of motor cyclists. 22.7% liver injuries were reported by Hussain et al and 15% by Hoyt.

As shown in Table 2, splenic injury was found in 36.65% cases. Tonge et al reported splenic injury in 21% of cases. Another observation derived in the present series shows out of 254 cases, 170 cases were due to injury by motor vehicles, 56 case due to fall from height and 16 cases due to railway hit.

As shown in Table 2, injury to intestine was found in 13.85% cases. This incidence was 6.2% in the observation of Tonge et al. Out of total 96 cases, 63 cases were due to injury by motor vehicles.

As shown in Table 2, Kidney was found injured in 11.11% cases. Out of 77 cases, 49 cases were due to injury by motor vehicles 20 case due to fall from height and 6 cases due to railway hit. It is consistent with the findings of Kaur in which she reported that kidney injury was found more commonly in fatal heavy motor vehicular and two wheeler accidents.

As shown in Table 2, stomach was found injured in 4.76%. Out of 33 cases, 26 cases were due to injury by motor vehicles 5 case due to fall from height. Tonge et al reported the incidence of 0.8% while Bruce et al (1965) reported stomach injury in 2% of cases. Urinary bladder was found injured in 1.15% cases. All cases were injured due to injury by motor vehicles. Incidence of urinary Bladder injury reported by Tonge et al was 4.9%. Kaur in her study of fatal auto vehicular two wheeler accidents reported incidence of 1.37%.

Gall Bladder was found injured in 3.17% cases and pancreas was found injured in 5.05% cases. The incidence of gall bladder & pancreatic injury reported by Tonge et al was 1.7%; the similar explanation had been given by Orr.

The present study showed that parenchymatous visceral injury is common than hollow visceral injury in both sexes (2:1). This is because consistency of parenchymatous viscera and hollow viscera has some
capacity to absorb the force of blow. In most of the cases multiple organ involvement was very common.

Involvement of uterus in female cases was a rare phenomenon as it is strongly protected by pelvic cage. In all cases bladder involvement is associated with fracture pubic symphysis. The most commonly associated part along with abdomen involved is chest due to its close proximity with abdomen. It showed rib fractures and lung injuries. Hemorrhage is more common with parenchymatous injury where death has occurred within 12 hours after sustaining trauma (57.43%).

Infection is more common with hollow visceral injury where death is delayed. The ratio of incidence between hemorrhage and infection is more or less common in both males and females. In males out of 549 cases hemorrhage is seen in 460 cases and in females in 117 cases, where as infection cases 77 cases are of males and 25 cases are of females.

**Conclusion**

The present study was undertaken to focus light upon the pattern of blunt abdominal injuries in relation to various factors in the Ahmedabad. Total No. of 693 cases were studied in the year 2016-2017. The result of the present study is summarized as follows.

1) Liver is the most commonly involved organ in blunt injuries of abdomen followed by spleen, small intestine, kidney, myosentery, stomach, gall bladder, urinary bladder and pancreas in decreasing order respectively.

2) In most of the cases, involvement of more than one viscera is reported.

3) Very high incidences of associated injuries in the form of chest injuries, followed by fractures and head injuries were noted. Chest injury is the most commonly associated injury (41.88%) due to its close proximity with abdomen.

4) Most common cause of death was shock and haemorrhage (83.26%), especially when the person dies within few hours. In those cases where death was delayed, cause of death was infection (14.72%)

**Conflict of Interest** – None declared

**Source of Funding** - self, no other funding sources

**Ethical Clearance** – The study was approved by institutional ethical committee.

**References**

Pattern of Fatal Firearm Injuries Pertaining to Manner of Death: An Original Research Article

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Abstract

The purpose of this study was to collect a data related to, but are not limited to, medico legal aspect, manner of death, type of firearm used, anatomical site involved, range of fire and their direction pertaining to manner of death in fatal firearm injury (FFI) cases, as limited data are available for such fatalities in this region. The direction of the internal bullet path has not been investigated in this area. This study was designed to address that deficiency.

Method- A prospective study over a period of 22 months was carried out on FFI cases brought to the mortuary.

Result –Among 54 FFI cases during study period, homicidal deaths were outnumbered the accidental and suicidal cases. Country made firearm was used in 37.03% FFI cases. More than one entry wound found only in homicidal cases. The typical entry wounds (n-59) in homicide were fired from close or near distance (61.02%), thorax were commonly involved (44.07%) with direction of fire running backward 73.08% (n-19), downward 69.23% (n-18) and towards the right 76.92% (n-20). Entry wound (n-5) in accidental FFI cases were fired from close or near range in 80%, abdomen involved in 80% (n-4) with direction of fire downward 75% (n-3), towards right 75% (n-3) and forward in 50% (n-2),and backward in 50% (n-2). In suicidal cases (n-3) head was involved in 66.67% (n-2) with all direction in backward, upward and towards left side.

Conclusions Country made firearms were involved in majority of cases. Multiple shots were present only in homicidal cases. Non contact entry wounds strongly indicate homicidal death. In homicidal death thorax was commonly involved and the direction of internal bullet path was towards the backward, downward and to the right side (B-D-R) in majority of cases. In all suicidal cases, the direction of fire was backward, upward and to left.

Key words: Firearm, Country made firearm, Site of entry wound, Range of fire, Direction of fire.

Introduction

Stabbing, mechanical asphyxia, blunt head injury and shooting are the most common methods of homicides, with firearm homicides on the increase throughout the world [1]. Indian national statistics reveal extreme variations across states and cities. As compared to other countries, firearm fatality rates in India are not particularly high. In 2008, India officially reported a national firearm murder rate of 0.36 per 100,000 people. Use of firearm for violent death is more common in Uttar Pradesh as compared to other states and firearm fatality rate in this city (Varanasi) was 3.1 per 1,00,000 population [2]. In India, in 2008 most of the victims of firearm murder are killed by unlicensed firearms (86%) [2] and use of unlicensed firearm increased in last
few years. As per national data, firearm were used in approximately 9% of homicidal deaths in India and only this region was accounted for 34.7% in the year 2012 [3]. Few studies have been published in other part of the world regarding internal bullet path pertaining to manner of death [4, 5]. Though in this region a number of studies are published on characteristics of firearm injuries [6, 7, 8], but direction of internal bullet path pertaining to manner of death were not investigated which can be also helpful for investigation of crime and reconstruction of event.

**Material and Method**

This prospective study was carried out on FFI cases brought to the mortuary of the department of Forensic Medicine, IMS, BHU, Varanasi, during period of 1st June 2009 to 30th March 2011. Before the start of the study, ethical clearance was taken from Ethical Committee of IMS, BHU. For the study, relevant questionnaires schedule were prepared.

Data relating to the study were collected by:

1) Examination of inquest report.
2) Interviewing the police personnel accompanying the cases
3) Interviewing the relatives, friends and eyewitnesses
4) Examination of photographs of scene of crime
5) Operative notes in case of hospitalized victims.
6) X-ray examination before autopsy in some cases.
7) Marking each gunshot wound on body diagram.

A large variety of features such as type of firearm, ammunition, number and site of entrance wounds, range of fire and direction of the internal bullet path were recorded.

The wound track was described in relation to planes of body relative to standing position:

(a) Frontal plane: From front to back or back to front.
(b) Sagital plane: From left to right or right to left.
(c) Horizontal plane: From above downwards (caudal) or from below upwards (cephalad).

Results were expressed in percentages. Chi-square and “P” values were calculated wherever applicable. P-value of <0.05 was considered significant.

**Findings**

Total 54 autopsies were studied due to fatal firearm injuries (FFI). Out of 54 case, 44 cases (81.48%) were victims of homicidal attacks, 5 cases (9.25%) were accidental and only 3 cases (5.55%) were suicidal, in 2 cases (3.7%) manner of death was undetermined. Overall unlicensed country made firearms was used in 37.03% FFI cases. Out of 44 homicidal FFI cases, country made firearm was used in 17 (38.64%) cases and was used in 3 out of 5 accidental FFI cases (Table-1). Pistol was preferred in suicidal cases.

A total of 69 entrance wounds were recorded in the 54 fatalities. Single entry wound was present in 79.62% (n=43) cases, two in 14.81% (n=8), three in 3.7% (n=2) and four in 1 case. Out of 44 homicidal cases single entry wound was found in 33 cases (75%) and multiple entry wound was present in 11 cases (25%). Interestingly Multiple entry wounds were present only in homicidal cases where as in accidental and suicidal cases only single entry wound was present (Table-2).

Total 59 entry wounds were recorded in homicidal cases, of which 36 (61.02%) were fired from close or near range and only 2 (3.39%) entry wounds were from contact range (Table-3). In accidental cases, 80% (n=4) was fired from close or near range and only 20% (n=1) was fired from distant range. In all suicidal cases range of fire was contact range (Table-3).

Anatomical site most commonly involved was the thorax (42.03%) followed by head (26.09%) and abdominal (17.39%) region (Table-4).

In homicidal cases, 44.07% of entry wound was present on thorax region followed by head (26.09%) and abdominal (17.39%) region (Table-4).

In accidental cases, 44.07% of entry wound was present on thorax region followed by head, neck and face region (33.9%). Limbs were involved only in homicidal cases. In majority of homicidal cases the direction of entry wound on vital anatomical region (Head, Neck, Face, Thorax and Abdomen) was backward, downward and left to right side (Table-5).

Among the non homicidal cases, 5 cases represent accidental death and 3 cases represent suicidal death. In accidental FFI cases abdomen was involved in 4 cases and thorax involved in a single case. Out of 3 suicidal
cases, head (right temple) was preferred region in 2 cases by using pistol and with long barreled shotgun abdomen was preferred in 1 case. In all suicidal cases, the direction of fire was backward, upward and right to left (Table-6). Direction of fire on vital anatomical regions (Head, Neck, Face, Thorax and Abdomen) involved in homicidal and non homicidal cases showed association ($\chi^2 = 3.8839$, $p$-value = 0.487). Direction of fire towards backward, downward and left to right in FFI cases represents homicidal injuries.

**Table-1: Type of firearm used pertaining to manner of death (n-54)**

<table>
<thead>
<tr>
<th>Type of firearm used</th>
<th>Homicide (n-44) (81.48%)</th>
<th>Accidental (n-5) (9.25%)</th>
<th>Suicide (n-3) (5.55%)</th>
<th>Undetermined (n-2) (3.7%)</th>
<th>Total (n-54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country made</td>
<td>17</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>20 (37.03%)</td>
</tr>
<tr>
<td>Pistol</td>
<td>9</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>11 (20.37%)</td>
</tr>
<tr>
<td>Rifle</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8 (14.81%)</td>
</tr>
<tr>
<td>Shot gun</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>15 (27.77%)</td>
</tr>
</tbody>
</table>

**Table-2: Number of entrance wound pertaining to manner of death.**

<table>
<thead>
<tr>
<th>Number of entry wound</th>
<th>Homicide (n-44)</th>
<th>Accidental (n-5)</th>
<th>Suicide (n-3)</th>
<th>Undetermined (n-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>33</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Two</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Three</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Four</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table-3: Range of fire pertaining to manner of death**

<table>
<thead>
<tr>
<th>Range of fire</th>
<th>Homicide</th>
<th>Accident</th>
<th>Suicide</th>
<th>Undetermined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Close/near</td>
<td>36</td>
<td>4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Distant</td>
<td>21</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total no of entry wound</td>
<td>59</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table-4: Anatomical site and number of entry wound**

<table>
<thead>
<tr>
<th>Anatomical site</th>
<th>Number of entry wound</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>18</td>
<td>26.09%</td>
</tr>
<tr>
<td>Face</td>
<td>1</td>
<td>1.45%</td>
</tr>
<tr>
<td>Neck</td>
<td>3</td>
<td>4.35%</td>
</tr>
<tr>
<td>Thorax</td>
<td>29</td>
<td>42.03%</td>
</tr>
<tr>
<td>Abdomen</td>
<td>12</td>
<td>17.39%</td>
</tr>
<tr>
<td>Upper extremity</td>
<td>3</td>
<td>4.35%</td>
</tr>
<tr>
<td>Lower extremity</td>
<td>3</td>
<td>4.35%</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Head, Neck, Face (n-20) (33.90%)</td>
<td>Thorax (n-26) (44.07%)</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Frontal plane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F to B</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>B to F</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td><strong>Horizontal plane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U to D</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>D to U</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td><strong>Sagital plane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R to L</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>L to R</td>
<td>12</td>
<td>20</td>
</tr>
</tbody>
</table>

F=forward, B=backward, D=downward, U=upward, R=right, L=left

### Table-6: Entry wound on anatomical site and their direction of fire in non homicidal cases.

<table>
<thead>
<tr>
<th></th>
<th>Accidental cases (n-5)</th>
<th>Suicidal cases (n-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thorax (n-1) (20%)</td>
<td>Abdomen (n-4) (80%)</td>
</tr>
<tr>
<td><strong>Frontal plane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F to B</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>B to F</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><strong>Horizontal plane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U to D</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>D to U</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Sagital plane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R to L</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>L to R</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

F=forward, B=backward, D=downward, U=upward, R=right, L=left

**Discussion and Conclusions**

**Manner of death and type of firearm:**

In our study, total 54 fatal firearm injury (FFI) cases were studied and this account for 1.54% of total autopsy cases. It was observed that maximum number of death were homicidal (81.48%) and rarely used for suicidal purpose (5.55%), this observation coincides with other studies conducted in India, similar result was reported in a study carried out in Edirne, Turkey, Egypt. However, some other studies conducted in developed countries like, Brescia (Northern Italy), Sweden, New Zealand reported that suicides accounted for the vast majority of firearm fatalities. We observed that unlicensed country made firearms were used in 37.03% FFI cases which is consistent with other studies conducted in India and other developing
countries. Firearms mainly used for homicides in developing countries, the reason could be easy and cheap availability of unlicensed country made firearms and they typically cannot be traced to any owner or by ballistic fingerprinting. These features make unlicensed firearms ideal for criminal use. In this country people rarely use firearm for suicide because people prefer more cheaper and more easily available methods like poisoning and hanging[15, 16]

The number of entry wound and manner of death:

We observed that in majority of cases (79.62%) single shot was fired. Similar observation was also found in Saudi Arabia by Magdy et al. [17], Druid H [5], A Kohli [9]. In 75%(n-33) of homicidal cases single entry wound was present as compared to 26-55% in other studies [5,10]. Interestingly we found in all non homicidal cases only single shot was present and multiple shots were present only in homicidal cases. In all accidental cases single gunshot injury was exclusively present in study by B.Karger et al [19] Myint Sithu et al [20] study also observed multiple entrance wound was common in homicidal cases. However some studies recorded multiple entry wounds in suicidal cases [12, 21]

Range of fire and manner of death:

The present study underlines that vast majority of homicide have close, near and distant range wounds, this consistent with other studies [4, 22]. In accidental cases contact wound was absent. In all suicidal cases only single contact wound was present and this findings are consistent with other studies in which contact or near contact wounds were present in more than 97% [22,23]. Only two cases of homicide were found to be discharged from contact range. Manner of death and range of fire showed significant association in our study. Non contact entry wounds strongly indicate homicidal death.

Anatomical site involved and direction of fire pertaining to manner of death:

Anatomical site preferred and direction of fire in homicidal, accidental and suicidal deaths is different. In homicidal cases, generally the victims are in motion either due to arguing with or opposing the assailant and the assailant generally prefer the large, vital and easy to target anatomical area to shoot. Contrary to this in suicidal cases, the person prefers comfortable position and most vital anatomical site for immediate death. While in accidental cases, the involvement of anatomical site and direction cannot be predicted and these cases totally depend on history of incidence.

In the current study, most common anatomical site of entrance wounds was thorax (n- 29, 42.03%), this result was consistent with other studies [10, 11]. Out of 59 entrance wound recorded in homicide, thorax (n-26, 44.07%) was most common targeted region followed by head, neck and face region (n-20, 33.90%) with the direction of internal bullet path towards backward, downward and to the right side (B-D-R) in majority of cases.

Out of 3 suicidal cases, head (right temple) was preferred region in 2 cases by using pistol because both persons were right handed, and in one case abdomen was preferred with long barrelled shotgun by using right great toe to pull a trigger. In all suicidal cases, the direction of fire was backward, upward and right to left (B-U-L). Similar observation was found by other studies [4, 5]

Acknowledgement: I would like to thanks to former Head of Department Dr.(Prof) C.B.Tripathi for his valuable guidance for this study and also thanks to Dr. S.K Tripathi, Prof. Dept. of Forensic Medicine, IMS, Banaras Hindu University, for his kind support.

Conflict of Interest: None

Source of Funding: Self.

Ethical Clearance: Yes, From Ethical Committee of Institute of Medical Sciences ,Banaras Hindu University, Varanasi (as a thesis for M.D., Forensic Medicine course).

References


Epidemiological Profile of Snake Bites over 1-Year Period from Tertiary Care Centre in Maval Region of Maharashtra, India: Original Research Article

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²Assistant Professor, Dept. of Pharmacology, MIMER Medical College, Talegaon (D), Pune

Abstract

Estimated death rate due to snake bites are more than 46,000 annually in our country. Ninety-seven to ninety eight percent bites occur in rural or remote areas. Snake bite is a one of common problem faced in rural India. It takes a high toll of lives mainly of working male of the family. The current prospective observational study was carried out to study the clinical and management by systematic approach of snake bites. The 245 cases of snake bite were hospitalized to Rural Hospital in a period of July 2017 to June 2018.

The present data showed that majority (91.43%) of cases were encountered from rural area, in the age group ranging from 21 to 30 years (24.90%). Majority were male patients (62.04%). Most affected were the agricultural laborers and farmers (56.33%). 60.82% of the cases were presented with bite over lower extremities. Most of the snake bite (75.97%) occurred during day time. July to October was the period in which most snake bite cases were observed. It was seen that syndromic or systematic approach was more effective in early diagnosis and treatment of snake bite. The average requirement of anti-serum venom (ASV) required for cobra bite was 16, krait bite 22, Russell's viper bite 12 and saw scaled viper bite 10. The total duration of treatment in hospital for cobra bite were 6, krait bite 8, Russell’s viper bite 4 and saw scaled viper 4 days.

Key words: Epidemiology, Snake bite, Anti-snake venom, Syndromic approach

Introduction

Snake bite is one of common medical emergency and an occupational hazard, more so in tropical region of India, where farming is a main or major source of earning. India has remained well known for its venomous snakes and the effect of their bites. Almost every year, 50,000 Indians died in 2,50,000 cases of snake bites, in spite of that India is not home for the largest number of venomous snakes in the globe, nor is there a shortage of anti-snake venom in the country. In the Maharashtra number of snake bite cases are highest i.e. 70 bites per 100,000 people and mortality of 2.4 per 100,000 per year. Main reason for high mortality and morbidity in any part of rural India is ignorance or negligence of the people. The initial approach to snake bite and treatment given by traditional village healers or non specific options, lack of transport facilities in rural India and sleeping habits of the villagers, difficult to access or unavailability of the doctor at Primary Health Centers most of the time, time taken by the doctors to identify the snakes, the signs and symptoms of envenomation in the patient. Important to note is that these are all preventable causes. In addition to the same is unavailability of ASV and endotracheal intubation and ventilation in neurotoxic envenoming. There are very few studies available from the Maval region of Maharashtra regarding the same.

Materials and Method

The current observational study was carried out in an Intensive Care Unit of Rural Hospital in Maval region of Maharashtra, India, in a period of July 2017 to June 2018. The aim of the study was to find out clinical profile, types of snakes and reasons to reduced decreased mortality rates at this center which may helpful to take
appropriate management approach and prompt treatment options or care be rendered in a rest of Maval region as well.

A total of 245 cases of snakebite were admitted during the said period. A written consent from patient or relative was taken, data was collected on a pre-designed proforma by interviewing the patient or close relatives and thorough clinical examination was done. Detailed information regarding the demographic and epidemiological parameters such as age, sex, occupation, site of bite, place of bite was noted. The type of snake was identified, if the snake was brought dead or identified by Syndromic approach.

Following syndromes were identified:

Syndrome 1 - Local envenomation (pain, swelling, ecchymosis); Bleeding/ clotting disturbances (haematuria, positive whole blood clotting test) = Russell’s viper and saw- scaled viper.

Syndrome 2 - Neuroparalytic symptom (ptosis, ophthalmoplegia, dysarthria); minimal haematuria and local swelling = Russell’s viper.

Syndrome 3 - Local envenomation (pain, swelling, blebs); Neuroparalytic symptom (ptosis, dysarthria); rapid progress towards respiratory arrest = Cobra.

Syndrome 4 - Neuroparalytic symptom (ptosis, dysarthria, dysphagia with respiratory arrest); no local swelling; history of severe abdominal pain and sleeping on floor = Krait.

Syndrome 5 - History of snake bite; no development of local or systemic symptoms; bite mark and mild burning present; observe till 12-24 hours; if asymptomatic = Nonvenomous snakebite(Rat snake, wolf snake; green keelback, trinket) orit may be a dry bite(Venomous snake). Subsequent data was collected i.e. total number of average ASV requirement, total duration in a hospital.

Inclusion Criteria

Patients of any age, sex and any locality of Maval region admitted with suspected or confirmed snake bite.

Exclusion Criteria

Patients brought dead with history of snakebite.

Patient with other insect bite

Observations and Results

A total of 245 cases were admitted in our hospital. Among them 152 (62.04%) were men and 93(37.96%) were women (Table No.1). The high incidence of snakebite was observed in the age group of 21-50 years (n=155, 63.27%) and low in the age group ≥51 years (n=38, 15.51%) (Table No.1). Most of the cases were from the rural area (91.42%, n=224) and rest of cases (8.58%, n=21) were from urban area (Table No.1). The most of the cases were bitten in the farms or field (n=171, 69.80%), followed by 61 (24.90%) in the houses or residences and 13 (5.30%) in public or common areas (Table No.2). Their occupational status 138 subjects (56.32%) were farm laborers, 42(17.14%) were farmers and 65(26.54%) were housewives, students (Table No.1). Large number of cases 149(60.82%) had site of bite on lower extremity followed by 87(35.51%) on upper extremity, 5(2.04%) on trunk and 4(1.63%) on head, neck and face (Table No.2). Regarding activities at the time of bite, 24 were sleeping on floor, 57 were handling debris or animal waste, 124 were working in the field or farms and 40 were doing or performing other field related activities (Table No.2). Majority of the patient of krait bite were sleeping on floor. The snake bites i.e. 186 (75.92%) occurred during daytime and 59 cases during night time. The common species was krait. Because of the seasonal variation maximum snake bite 127 (51.84%) were seen during rainy season (Table No.3). On further analysis of 245 snake bite cases, 153 cases were bitten by venomous snakes found in this region (The big four) and 92 cases were bitten by non venomous snakes. Of the venomous snakes 60 (24.48%) were Cobra bites, 41 (17.74%) were Krait bites, 45 (18.36%) were Russell's viper bite, 7 (2.86%) were bitten by Saw Scaled viper (Table No. 2). Regarding the identification of snake, 153 snakes were poisonous and they were identifies when they were brought dead or alive by the relatives or the snake friend (Sarpamitra) and by the Syndromic approach as per WHO guidelines while 92 snakes were non venomous snakes (Table No.2). In 245 cases of snake bite maximum patients had fear 240 (98%) as the prominent sign or symptom, 218 (89.04%) had pain at the same site followed by swelling in 186 (76%) and 32 (13%) of the patient had difficulty to open eyes and 39 (16%) had difficulty in breathing or respiratory distress, 44 (18 %) had bleeding from the site 29 (12%) had pain in abdomen and 22 (9%) had nausea and vomiting, on local examination bite marks, blister formation and necrosis were observed in 205 (83.46%), 23 (9.42%) and 12 (5.06%) cases respectively (Figure
Maximum cases causing rapid cellulitis were of Russell’s viper where as blister and necrosis were caused by Cobra, absence of bite marks was an important feature of Krait. In case of Russell’s viper 23.4 % cases had both neurotoxic as well as vasculotoxic symptoms. 10 patients had mild alteration or derangements of renal function test (acute renal failure) 16 % cases of cobra bite developed ulcers, all of which healed completely by regular dressings (Table No.5). The basic or first aid treatment given before admission in most of the cases was in the form of application of tourniquet (78.36%) proximal to the site of bite. Average ASV required for the cobra bite was 16, krait bite was 22, Russell’s viper bite was 12, and Saw Scaled viper bite was 10. Average total hospital stay for Cobra bite patients was 6 days, for krait bite patient was 8 days and Russell’s viper bite patients and saw scaled viper bite patient required total 4 days (Table No: 4). Overall mortality in 245 cases, one patient who died because of neurotoxic envenomation the patient was obese, diabetic and the other dying of hemotoxic envenomation had severe hypothyroidism with poor compliance.

Table No. 1: Socio-demographic factors of study subjects

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Total number n=245(100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤20</td>
<td>52 (21.22%)</td>
</tr>
<tr>
<td>21-50</td>
<td>155 (63.27%)</td>
</tr>
<tr>
<td>≥51</td>
<td>38 (15.51%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>152 (62.04%)</td>
</tr>
<tr>
<td>Female</td>
<td>93 (37.96%)</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>224 (91.42%)</td>
</tr>
<tr>
<td>Urban</td>
<td>21 (8.58%)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
</tr>
<tr>
<td>Farm laborers</td>
<td>138 (56.32%)</td>
</tr>
<tr>
<td>Farmers</td>
<td>42 (17.14%)</td>
</tr>
<tr>
<td>Others</td>
<td>65 (26.54%)</td>
</tr>
</tbody>
</table>

Table No.2: Snake bite related factors

<table>
<thead>
<tr>
<th>Place of bite</th>
<th>Total number n=245(100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm</td>
<td>171 (69.80%)</td>
</tr>
<tr>
<td>House</td>
<td>61 (24.90%)</td>
</tr>
<tr>
<td>Public Place</td>
<td>13 (5.30%)</td>
</tr>
<tr>
<td>Activity during bite</td>
<td></td>
</tr>
<tr>
<td>Sleeping on floor</td>
<td>24 (9.80%)</td>
</tr>
<tr>
<td>Handling debris</td>
<td>57 (23.26%)</td>
</tr>
<tr>
<td>Farming</td>
<td>124 (50.62%)</td>
</tr>
<tr>
<td>Other</td>
<td>40 (16.32%)</td>
</tr>
<tr>
<td>Type of snake</td>
<td></td>
</tr>
<tr>
<td>Cobra</td>
<td>60 (24.48%)</td>
</tr>
<tr>
<td>Krait</td>
<td>41 (16.74%)</td>
</tr>
<tr>
<td>Russell’s Viper</td>
<td>45 (18.36%)</td>
</tr>
<tr>
<td>Saw Scaled Viper</td>
<td>07 (2.86%)</td>
</tr>
<tr>
<td>Non-venomous</td>
<td>92 (37.56%)</td>
</tr>
<tr>
<td>Anatomical site of bite</td>
<td></td>
</tr>
<tr>
<td>Head, neck &amp; face</td>
<td>04 (1.63%)</td>
</tr>
<tr>
<td>Upper limb</td>
<td>87 (35.51%)</td>
</tr>
<tr>
<td>Lower limb</td>
<td>149 (60.82%)</td>
</tr>
<tr>
<td>Trunk</td>
<td>05 (2.04%)</td>
</tr>
<tr>
<td>Identification of snake (brought dead/alive or by syndromic approach)</td>
<td></td>
</tr>
<tr>
<td>Poisonous</td>
<td>153 (62.44%)</td>
</tr>
<tr>
<td>Non-poisonous</td>
<td>92 (37.56%)</td>
</tr>
</tbody>
</table>
Table No.3: Environment related factors related to bite

<table>
<thead>
<tr>
<th>Seasonal variation</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter (Nov – Feb)</td>
<td>52 (21.22%)</td>
</tr>
<tr>
<td>Summer (Mar-Jun)</td>
<td>66 (26.94%)</td>
</tr>
<tr>
<td>Rainy (Jul- Oct)</td>
<td>127 (51.84%)</td>
</tr>
</tbody>
</table>

Table 4: Average ASV requirement & Hospital stay

<table>
<thead>
<tr>
<th>Type of snake</th>
<th>Average vials of ASV required</th>
<th>Average Hospital stay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobra</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Krait</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Russell’s viper</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Saw scaled viper</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 5: Complications

<table>
<thead>
<tr>
<th>Complication</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute renal failure</td>
<td>10</td>
</tr>
<tr>
<td>Respiratory paralysis</td>
<td>24</td>
</tr>
<tr>
<td>Shock</td>
<td>3</td>
</tr>
<tr>
<td>Infection</td>
<td>1</td>
</tr>
<tr>
<td>Gangrene/limb loss</td>
<td>1</td>
</tr>
<tr>
<td>Chronic ulcer</td>
<td>12</td>
</tr>
</tbody>
</table>

Discussion

In the present study, most of the snakebite cases were in the age group of 21-50 years and from rural areas (91.42%) these factors have great impact on the psychosocial and economical status of the family. Loss of the earning family member, most commonly male (62.04%) has long lasting effect on the family status. Majority of cases were reported from the farms during their routine work. Similar observations were reported in earlier studies.5,6,7,8. It was noted that 62.44% cases were bitten by venomous snakes, found in this area. The venomous snakes identified were – Cobra, Krait, Russell’s viper and Saw scaled viper. Syndromic approach was found to be useful in identification of species of snake. Regional signs and symptoms or syndromes should be identified and syndromic approach should be applied. In this study, we discussed with physicians, intesivists, resident doctors and paramedical staffs about identification of snake and Syndromic approach. It was helped in early recognition and prompt management. The most common presenting symptoms include fear (98%), pain (89.04%), swelling (76%), difficulty to open eyes (13%), difficulty in breathing (16%), bleeding from the site (18%), pain in abdomen (12%) and vomiting (9%) of the observed cases. Bite marks were observed in 83.46% of the cases and bite marks were absent in Krait. Blister formation was seen in 9.42% cases, all of which were cobra bites. Tissue necrosis was found in 5.06% of the cases. The Most common site was lower extremities followed by the upper extremities this is consistent with the occupational exposure. The krait bites occurred during mid night and
while sleeping over floor. The preventive measures were discussed with people about the mode of bite of these poisonous snakes and recommended to sleep on a bed and avoid sleeping on the floor. Use of mosquito nets is an effective preventive measure for krait bites. Average ASV required were 16 for cobra bite cases, 22 for krait bites cases, 12 for Russell’s viper and 10 for saw scaled viper cases. The total hospital duration for cobra was observed to be 6 days, 8 days for krait and Russel’s viper and saw scaled viper to be 4 days. Similar findings were made by Kulkarni and Annes. The overall mortality was 2 in 245 total snake bite cases (0.81%). One patient who died because of neurotoxic envenomation the patient was obese, diabetic and the other dying of hemotoxic envenomation had severe hypothyroidism with poor compliance. Higher rates of mortality were observed by Kulkarni and Annes, Hansdak et al, and Punde. Lower mortality at our hospital could be attributed to the dedicated Intensive Care unit, trained staff and early recognition of envenomations by Syndromic Approach and early endotracheal intubation.

Conclusions

Snake bite is a one of common problem in rural areas of India, taking a high toll of lives mainly of working male of the family. In the present study 245 cases of snake bite were analysed who were admitted to Medical Intensive Care unit of Rural Hospital. The data showed that majority (91.42%) of cases were belong to rural area, in the age group of 21 to 50 (63.27%), out of total cases, majority were male patient (62.04%). Most commonly affected was the agricultural laborers (56.32%) and 60.82% of the cases had bite over lower extremities. Most of the cases (75.97%) occurred during day time in the month of July to October. It was observed that Syndromic approach was very effective in early diagnosis and treatment of snake bite. Average number of ASV required for cobra bite cases was 16, krait bite 22, Russell’s viper bite 12 and saw scaled viper bite 10. Average hospital stay in days for cobra bite was 6, krait bite was 8, russell’s viper bite was 4 and saw scaled viper was 4. All cause mortality was 1.67% for neurotoxic and 2.43% for vasculotoxic snakes.

Recommendations

1. It is strongly recommended to make snakebite a specific notifiable disease and should be formally recognized as an important occupational disease in Maval region.
2. As most snakebite occurred during the agricultural work, strict protective measures should be practiced by the farmers.
3. Sleeping on floor should be discouraged to prevent specially krait bite.
4. Anti-venom alone cannot be relied upon to prevent early death from respiratory paralysis. Artificial ventilation is essential in such cases. In this region more doctors should be trained to carry out endotracheal intubations, as it was found to decrease the mortality rate in neurotoxic envenomations.
5. Syndromic Approach should be followed for management of snakebite, when snake is not brought.
6. Training of Physicians, resident doctors and paramedical staff regarding Syndromic Approach is necessary.
7. Establishment of dedicated snakebite management units, at least at every district hospital, where the incidence of snake bite is high, as dedicated snakebite management units have positive impact on mortality rates.
8. Community education about snakebite is strongly recommended as the method most likely to succeed in preventing bites. Use of flip charts or videos related to awareness about symptoms of snake bite and first aid measures in schools and colleges will definitely help in sensitizing the people in rural area.

Conflict of Interest: There is no conflict of interest; the author does not have financial or other relationship with other people or organization that may inappropriately influence the author’s work.

Ethical Clearance: Research was approved by the institutional ethical committee.

References


4. Bawaskar HS, Bawaskar PH Profile of snake bite envenoming in western Maharashtra, India. Transactions of the Royal Society of Tropical Medicine and Hygiene 2002;96(1)79-84.


Informed Consent in Clinical Practice and Research and Its Awareness among Under Graduate Medical Students

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Abstract

Consent denotes voluntary agreement, permission or compliance. Informed consent in medical practice is the grant of permission by a patient for an act to be carried out by a doctor, such as a diagnostic, surgical or therapeutic procedure. Informed Consent in research is the voluntary agreement to participate in the study.

The study was undertaken with the objective to assess the level of knowledge regarding informed consent among Second MBBS undergraduate medical students at the beginning of the class by a pretest and to demonstrate measurable gain in knowledge at the end of the class by a posttest using the same questionnaire.

This quasi-experimental study conducted in the Department of Forensic Medicine, Govt.T.D Medical College, Alappuzha, among 118 students using a structured and close ended, pretested questionnaire containing 13 multiple choice questions.

Statistical analysis done on average pre and posttest score and on difference in pre and posttest responses to individual questions; revealed measurable gain in knowledge at the end of the class, since p value was < 0.05.

Key words: informed consent, clinical practice, research, pre and posttest, gain in knowledge.

Introduction and Background

Consent denotes voluntary agreement, permission or compliance. It should be free, voluntary, informed, clear and direct. According to Indian Contract Act, 1872 two or more persons are said to consent when they agree upon the same thing in the same sense¹. As per Indian Medical Council (Professional conduct, Etiquette and Ethics) Regulations, 2002 that a physician, performing an operation without written informed consent, shall constitute professional misconduct rendering him/her liable for disciplinary action by MCI².

Informed Consent must be obtained for all types of human subjects research including; diagnostic, therapeutic, interventional, social and behavioral studies, and for research conducted domestically or abroad³. A doctor must give a patient adequate information to understand the various aspects of the proposed treatment such as: The nature and procedure of the treatment, purpose and benefits, likely effects and complications, alternatives if available, substantial risks and adverse consequences of refusing treatment².

Types of Consent⁴,⁵.

Implied consent: Seen in routine medical practice. Here the consent is implied in the mere fact that the patient comes to the physician with a problem or when a patient holds out his arm for an injection.

Expressed consent: May be written or oral. Any procedure beyond the routine physical examination, like operation, collection of blood, blood transfusion etc. needs expressed consent. Consent should be taken before the proposed act and not at the time of admission to the hospital.
Informed consent: In medical practice anything beyond routine would require informed written consent.

Blanket consent: It is a consent taken on a printed form that covers (like a blanket) almost everything a doctor or a hospital might do to a patient, without mentioning anything specifically. Blanket consent is legally inadequate for any procedure that has risks or alternatives.

The concept of informed consent is embedded in the principles of Nuremberg Code, The Declaration of Helsinki and The Belmont Report. Consent to participate in a research should be understood as a process rather than an event. In order for participants to give meaningful consent, they should be able to understand the intent of the research, be clear about what they are being asked to do and if any risks are involved, and know how their information will be used.

Informed Consent Document (ICD)

Before requesting an individual’s consent to participate in research, the researcher must provide the individual with detailed information and discuss her/his queries about the research in the language she/he is able to understand. ICD has two parts –

- Patient/participant information sheet (PIS)
- Informed consent form (ICF).

Information on known facts about the research, which has relevance to participation, is included in the PIS. This is followed by the ICF in which the participant acknowledges that she/he has understood the information given in the PIS and is volunteering to be included in that research. Adequate time should be given to the participant to read the consent form.

Special situations

1. Waiver of consent: The researcher can apply to the Ethics committee (EC) for a waiver of consent if the research involves less than minimal risk to participants and the waiver will not adversely affect the rights and welfare of the participants.

2. Re-consent or fresh consent: Re-consent is required in situations where new information pertaining to the study becomes available, a research participant who is unconscious regains consciousness, a child becomes an adult during the course of the study, research requires a long-term follow-up or requires extension, there is a change in treatment modality, procedures, site visits, data collection methods or tenure, and when there is possibility of disclosure of identity.

3. Assent: Assent is defined as a child’s affirmative agreement to participate in research. The assent process should take into account the children’s developmental level and capability of understanding and should be obtained from children of 7 to 18 years of age.

4. Electronic consent: Electronic media can be used to provide information as in the written informed consent. Hon’ble Supreme Court has issued direction that in all clinical trials, in addition to the requirement of obtaining written informed consent, audio-visual recording of the informed consent process also should be done. This is applicable to the new subjects to be enrolled in all clinical trials including Global Clinical Trials.

5. Gatekeepers: Permission of the gatekeepers, that is, the head/leader of the group or culturally appropriate authorities, may be obtained in writing or audio/video recorded on behalf of the group and should be witnessed.

6. Community consent: There may be situations when individual consent cannot be obtained as it will change the behaviour of the individual. In such situations community consent is required. When permission is obtained from an organization that represents the community, the quorum required for such a committee must be met. Individual consent is required even if the community gives permission.

7. Consent from vulnerable groups: Vulnerable persons are those individuals who are relatively or absolutely incapable of protecting their own interests and providing valid informed consent.

Informed consent is probably the most important concept flowing from the doctrine of autonomy. In the Indian context, informed consent was practically nonexistent till the Consumer Protection Act was made applicable to the medical profession. Now, both doctors and patients are becoming more aware about this concept, and patients are better informed of their rights.

This study was undertaken with the objective to assess the level of awareness regarding informed consent among Second MBBS undergraduate medical students at the beginning of the class by giving a pretest and to
demonstrate measurable gain in knowledge at the end of the class by a posttest using the same questionnaire.

**Materials and Method**

This quasi-experimental study was conducted in the Dept. of Forensic Medicine, Govt. T D Medical College, Alappuzha for one month period. One Hundred and Eighteen (118) Second professional MBBS students willing to participate in the study were included. Students who were absent on the day of assessment, did not participate both in pre and posttest and have given partly filled questionnaire were excluded. Domains included definition, types and components of consent, informed consent in medical and medicolegal practice, informed consent in research and special situations.

Study tool was structured and close ended, pilot tested questionnaire based on informed consent in clinical practice and research containing 13 multiple choice questions with five options and single correct response, covering key points. After obtaining informed consent, pretest questionnaire was given, then a lecture pertaining to the topic was delivered for about 40 minutes and the same questionnaire was given as posttest. For pre and posttest 10 minutes each was given.

Data entered in Microsoft Excel worksheet and analysis done using SPSS version 18.0. Correct answer was given score 1 and wrong answers were given score 0. Maximum achievable score was 13. Average score and standard deviation (SD) for pre and posttest were calculated and analysis done using Wilcoxon signed rank test for paired data. Responses to individual questions were also expressed as number and percentage and difference in responses were subjected to statistical analysis using McNemar’s test for paired samples. Gender wise distribution of students and its relation with score obtained was also studied.

**Findings**

Gender wise distribution of the students and score gained:

Among the students participated, 39 (33.1%) were males and 79 (66.9%) were females. Pre and posttest scores were categorised into two groups, those who scored ≤ 6 and who scored ≥7. These groups were cross tabulated with gender. Majority of male students scored less than or equal to 6 (19.5%) for pretest and more than or equal to 7 in posttest (28.0%). Majority of female students scored more than or equal to 7 in both (38.1% and 64.4% respectively). Only posttest values showed significant association with gender (p = 0.026 i.e. <0.05) (Table 1).

**Table 1. Showing association of gender of students and test score.**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Pretest group Number (%)</th>
<th>Posttest group Number (%)</th>
<th>Total Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score ≤6</td>
<td>Score ≥7</td>
<td>Score ≤6</td>
</tr>
<tr>
<td>Male</td>
<td>23(19.5%)</td>
<td>16(13.6%)</td>
<td>6(5.1%)</td>
</tr>
<tr>
<td>Female</td>
<td>34(28.8%)</td>
<td>45(38.1%)</td>
<td>3(2.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>57(48.3%)</td>
<td>61(51.7%)</td>
<td>9(7.6%)</td>
</tr>
<tr>
<td>Significance(Chi-square)</td>
<td>P=0.103 (p&gt;0.05)</td>
<td>P=0.026 (p&lt;0.05)</td>
<td>-</td>
</tr>
</tbody>
</table>

Analysis for paired data:

Mean and standard deviation (SD) calculated for pre and posttest samples for all questions (1 to 13). Mean pretest score was 6.32 with SD 1.871 and mean posttest score was 9.57 with SD 2.154. Wilcoxon signed rank test for paired data revealed highly significant p value i.e. p=0.000 (p<0.05), so there is statistically significant difference between pre and posttest score (Table 2).

Domains were classified into informed consent in clinical practice including its essential elements, application in medical and medicolegal practices (questions 1 to 6) and informed consent in research
including informed consent document and special situations (questions 7 to 13). Mean and SD calculated for these sub groups also. For questions 1to 6, mean pretest score was 3.60 with SD 1.269 and mean posttest score was 4.74 with SD 0.910 and for questions 7 to 13, mean pretest score was 2.72 with SD 1.161 and mean posttest score was 4.83 with SD 1.635. Wilcoxon signed rank test revealed statistically significant difference between pre and posttest sub group values (for questions 1to6, p=0.000 and for 7 to 13, p=0.000) (Table 2).

Table 2. Showing Mean, Standard Deviation (SD), Median and Wilcoxon Signed Rank Test

<table>
<thead>
<tr>
<th>Score</th>
<th>Question numbers 1 to6</th>
<th>Question numbers 7to13</th>
<th>Total (1to13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Posttest</td>
<td>Pretest</td>
</tr>
<tr>
<td>Mean</td>
<td>3.6</td>
<td>4.74</td>
<td>2.72</td>
</tr>
<tr>
<td>SD</td>
<td>1.269</td>
<td>0.910</td>
<td>1.161</td>
</tr>
<tr>
<td>Median</td>
<td>4.0</td>
<td>5.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Wilcoxon Signed Rank Test

<table>
<thead>
<tr>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-6.900a</td>
<td>0.000</td>
</tr>
<tr>
<td>-7.875a</td>
<td>0.000</td>
</tr>
<tr>
<td>-8.493a</td>
<td>0.000</td>
</tr>
</tbody>
</table>

a. Based on negative ranks.

Analysis based on response to individual questions:

Individual questions were analysed and difference in pre and posttest responses were expressed as number and percentage. Statistical analysis was also done using McNemar’s test, for comparison of responses in paired samples. All questions except 2 and 10 showed increase in frequency of correct response and statistically significant p value (Table 3).

Table 3. Distribution of correct response for individual questions and p value of McNemar’s test for paired samples.

<table>
<thead>
<tr>
<th>Question No.</th>
<th>Questions</th>
<th>Correct response (Pretest)</th>
<th>Correct response (Posttest)</th>
<th>McNemar’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
</tr>
<tr>
<td>1</td>
<td>Informed consent in medical practice refers to</td>
<td>89</td>
<td>75.4%</td>
<td>109</td>
</tr>
<tr>
<td>2</td>
<td>The type of consent required for medicolegal examination is</td>
<td>75</td>
<td>63.6%</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>Minimum age for giving valid consent for surgery is</td>
<td>93</td>
<td>78.8%</td>
<td>110</td>
</tr>
<tr>
<td>4</td>
<td>Consent is not required as per law in the following situations, EXCEPT.</td>
<td>44</td>
<td>37.3%</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>A person can give consent for physical examination if he is above</td>
<td>24</td>
<td>20.3%</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Essential component/s of consent includes</td>
<td>99</td>
<td>83.9%</td>
<td>118</td>
</tr>
</tbody>
</table>
Parts of informed consent documents are

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Audio-visual recording of consent process has been made mandatory in

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Waiver of consent may be granted by the ethics committee in following situations, EXCEPT

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Reconsent or fresh consent is required in situation/s

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In some situations, consent has to be obtained from organisations that represents the community. This type of consent is termed as

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Fundamental ethical principles of autonomy is ensured through the practice of

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Assent is obtained from

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Discussion

Didactic lecture is one of the most widely accepted methods among teaching and learning methodology. Because of time restriction and vast syllabus to be covered through lectures, feedback knowledge before and after the lectures to assess the extent of knowledge of learners gained provides the platform for feedback.

The pretest can be used as a feasible tool to get useful information about the knowledge of students, to shape group specific education programs, to use as a diagnostic tool for obtaining early feedback on the need for assistance and also to provide a benchmark for assessing teaching effectiveness.

Pre-post testing is valuable to teachers because it allows for real-time progress monitoring. Multiple posttest can be administered throughout a student’s enrollment, and thus educational gains can be monitored and instruction can be adjusted appropriately. In pre and posttest based learning method, the student will be actively involved in education. Pre and posttest can also be used as a powerful diagnostic tool to identify weak students, strongest students, topics students already know, topics students don’t know and the topics students have not leaned.

Disadvantages of pre and posttest includes, students may absorb knowledge just from taking the test and may attend more readily to the content and there may be a tendency to teach to the posttest.

A pre and posttest study conducted to evaluate whether formal communications skills training on informed consent improves the quality of written informed consent among untrained Post-Graduate Residents in the Department of Obstetrics and Gynaecology at a Medical College Hospital, Maharashtra revealed that the intervention had increased the mean scores in post-test (5.17 to 9.33) and the paired t test was highly significant with $t = -13.61$, $< 0.0001$. In the present study also statistical analysis done on average pre and posttest score and on difference in pre and posttest responses to individual questions; revealed measurable gain in knowledge at the end of the class, since $p < 0.05$.

Conclusion

Knowledge regarding informed consent is indispensable in medical practice and research and it is an integral part of medical curriculum. Measuring academic progress through appropriately administered pre-post tests can be a powerful tool in providing teachers feedback about how to better meet students’ academic needs.

Acknowledgement: Express sincere gratitude to the students participated in the study. Owe special thanks.
to Smt. Ancy, Lecturer, Statistics and Demography, for her valuable suggestions and help. Also grateful to Faculty Members, Residents and Staff members of the Department for their help and support.

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Ethical Clearance: The study was conducted after getting clearance from Institutional Ethics Committee.

References

Sexual Dimorphism in Mandibular Canine Crown Dimensions in Early Adolescents: A Hospital based Study

Kanak Priya¹, Poonam Agrawal², Dinesh Kumar Bagga³, Hiten Kalra⁴, Disha Sirohi⁵, Ritika Gupta⁵

¹Post graduate Student, ²Professor, ³Professor and Head, ⁴Professor, ⁵Senior Lecturer, Department of Orthodontics and Dentofacial Orthopaedics, School of Dental Sciences, Sharda University, Greater Noida, UP

Abstract

Determination of sex is valuable in forensic investigations. Forensic odontology plays a major role due to the hardness and chemical stability of teeth. This study was done to find out the utility of mandibular canine crown dimensions as a tool for sex determination in North Indian population. Crown length and mesiodistal width of right and left mandibular canines were measured on the 60 casts and analyzed to assess sex difference using Students ‘t’ test. The canine crown parameters as measured for males and females when compared were found to be statistically significant. However, when these parameters were compared between left and right sides for the same sex, they were found to be statistically insignificant.

Key words: Sexual dimorphism, canine, crown dimensions.

Introduction

“Sexual dimorphism” is variations in morphological features between males and females. Sex determination is important in the identification of unknown individuals especially when information relating to the person is unavailable. Sex determination can be done either by morphological or molecular analysis. Most anatomical methods for sex determination are based on pelvic and cranio-facial morphology. However, these bones are sometimes recovered as fragments, rendering sex estimation difficult especially in juvenile or sub-adult remains since dimorphic traits become apparent only after puberty. Morphology of teeth is potentially useful in sex determination because both primary and permanent sets of teeth develop before puberty. Mandibular canines are considered as the ‘key teeth’ for personal identification because they are least affected from periodontal diseases and are the last to be extracted. Nair et al (1999) found that the mandibular canines exhibit greatest sexual dimorphism among all teeth. The aim of this study was to find out the utility of mandibular canine crown dimensions as a tool for sex determination in North Indian population and to compare the crown dimensions of right and left mandibular canines.

Material and Method

A retrospective, hospital-based study was conducted in post graduate department of orthodontics and dentofacial orthopaedics, School of Dental Sciences, Sharda University, Greater Noida. Total 60 casts of completely erupted and caries free dentition of 12-15 yrs age group subjects (30 male and 30 female) were studied. Cast with partially erupted, restored, and attrited canine were excluded from the study. Type of malocclusion present was not taken into consideration. Sexual dimorphism was studied on the basis of comparison of mandibular canine crown length, mesiodistal width, and length:width ratio. The crown length and mesiodistal width of right and left mandibular canines were measured using a digital Vernier caliper. The measurements were recorded and statistical analysis using Students ‘t’ test was done to assess sexual dimorphism.

- Following measurements were taken in all the casts on an anatomically sound basis using a digital Vernier Caliper with a measuring range of 0–150 mm/0–6 inch, resolution: 0.01mm/0.0005 inch, repeatability: 0.01mm/0.0005 inch, accuracy: + 0.02 mm/0.001 inch. (<100 mm), + 0.03 mm/0.001 inch. (>100 – 150 mm), maximum measurement speed: 1m/s.

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e mail: drpoonamajai@yahoo.co.in
Crown length of the mandibular canines on each side was recorded as the greatest distance on labial surface from the incisal line to cervical line parallel to the occlusal plane.

Width of the mandibular canines taken as the greatest mesiodistal width between the contact points of teeth on either side of jaw.

- Sexual Dimorphism in right and left mandibular canines was calculated using formula given by Garn & Lens (1967) i.e., Sexual dimorphism = (xm/xf) x 100 (where xm is mean value of male and xf is mean value of female).

**Results**

- The readings obtained were subjected to statistical analysis to derive conclusions and the results have been depicted in the tables 1-6. From the findings, it can be interpreted that the left canine is found to exhibit greater sexual dimorphism. The sexual dimorphism as computed for measurements on casts has been observed as follows.
  - Length
    - Sexual dimorphism in Right canine = 1.016%
    - Sexual dimorphism in Left canine = 1.022%
  - Width
    - Sexual dimorphism in Right canine = 1.059%
    - Sexual dimorphism in Left canine = 1.077%

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Mean</th>
<th>S.D.</th>
<th>Coefficient of Variation</th>
<th>Standard Error</th>
<th>t' Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Canine Length</td>
<td>Male</td>
<td>8.233</td>
<td>0.28</td>
<td>3.87</td>
<td>0.169</td>
<td>0.523</td>
<td>0.007**</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7.62</td>
<td>0.256</td>
<td>3.83</td>
<td>0.191</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Canine Length</td>
<td>Male</td>
<td>8.317</td>
<td>0.01</td>
<td>4</td>
<td>0.186</td>
<td>0.691</td>
<td>0.006**</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7.711</td>
<td>0.01</td>
<td>4.83</td>
<td>0.189</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*p>0.05: not significant, **p≤0.05: significant, ***p≤0.001: highly significant)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>Coefficient of Variation</th>
<th>Standard Error</th>
<th>t' Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>8.233</td>
<td>0.926</td>
<td>0.111</td>
<td>0.169</td>
<td>-0.331</td>
<td>0.371*</td>
</tr>
<tr>
<td>Male</td>
<td>8.317</td>
<td>1.021</td>
<td>0.121</td>
<td>0.186</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Right</td>
<td>8.100</td>
<td>1.046</td>
<td>0.127</td>
<td>0.191</td>
<td>-0.124</td>
<td>0.451*</td>
</tr>
<tr>
<td>Female Left</td>
<td>8.133</td>
<td>1.033</td>
<td>0.125</td>
<td>0.189</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*p>0.05: not significant, **p≤0.05: significant, ***p≤0.001: highly significant)
### Table 3: Crown width of mandibular canines in male vs female

<table>
<thead>
<tr>
<th>Sex</th>
<th>Mean</th>
<th>S.D.</th>
<th>Coefficient of Variation</th>
<th>Standard Error</th>
<th>t' Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right Canine Width</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6.99</td>
<td>0.44</td>
<td>3.89</td>
<td>0.19</td>
<td>7.869</td>
<td>0.001***</td>
</tr>
<tr>
<td>Female</td>
<td>6.384</td>
<td>0.37</td>
<td>3.55</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Left Canine Width</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6.98</td>
<td>0.61</td>
<td>3.53</td>
<td>0.27</td>
<td>8.368</td>
<td>0.001***</td>
</tr>
<tr>
<td>Female</td>
<td>6.374</td>
<td>0.32</td>
<td>4.82</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p>0.05: not significant, **p≤0.05: significant, ***p≤0.001: highly significant*

### Table 4: Crown width of mandibular canines in Right vs Left

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>Coefficient of Variation</th>
<th>Standard Error</th>
<th>t' Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>6.99</td>
<td>0.43</td>
<td>0.062</td>
<td>0.196</td>
<td>0.029</td>
<td>0.488*</td>
</tr>
<tr>
<td>Left</td>
<td>6.98</td>
<td>0.61</td>
<td>0.088</td>
<td>0.276</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>6.6</td>
<td>0.374</td>
<td>0.056</td>
<td>0.167</td>
<td>0.569</td>
<td>0.292*</td>
</tr>
<tr>
<td>Left</td>
<td>6.47</td>
<td>0.323</td>
<td>0.049</td>
<td>0.144</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p>0.05: not significant, **p≤0.05: significant, ***p≤0.001: highly significant*

### Table 5: Crown length : width ratio of mandibular canines male vs female

<table>
<thead>
<tr>
<th>Sex</th>
<th>Mean</th>
<th>S.D.</th>
<th>Coefficient of Variation</th>
<th>Standard Error</th>
<th>t' Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right Canine Length: Width Ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.158</td>
<td>0.073</td>
<td>0.063</td>
<td>0.03</td>
<td>2.07</td>
<td>0.035**</td>
</tr>
<tr>
<td>Female</td>
<td>1.326</td>
<td>0.165</td>
<td>0.124</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Left Canine Length: Width Ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.15</td>
<td>0.095</td>
<td>0.083</td>
<td>0.04</td>
<td>2.07</td>
<td>0.035**</td>
</tr>
<tr>
<td>Female</td>
<td>1.332</td>
<td>0.171</td>
<td>0.128</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p>0.05: not significant, **p≤0.05: significant, ***p≤0.001: highly significant*
Table 6: Crown length : width ratio of mandibular canines Right vs left

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>Coefficient of Variation</th>
<th>Standard Error</th>
<th>t' Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>1.15</td>
<td>0.073</td>
<td>0.063</td>
<td>0.032</td>
<td>0.148</td>
<td>0.443*</td>
</tr>
<tr>
<td>Male</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>1.15</td>
<td>0.095</td>
<td>0.083</td>
<td>0.042</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>1.32</td>
<td>0.165</td>
<td>0.124</td>
<td>0.07</td>
<td>0.126</td>
<td>0.451*</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>1.34</td>
<td>0.184</td>
<td>0.137</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*p>0.05: not significant, **p≤0.05: significant, ***p≤0.001: highly significant)

Discussion

The present study establishes the existence of statistically significant sexual dimorphism in mandibular canines. The results are in consistence with a study on Saudi males and females of 13-20 year age group population by Hashim and Murshid (1993) who found that only the canines in both jaws exhibited a significant sexual difference. Lew and Keng (1991) obtained similar results in their study on ethnic Chinese population with normal occlusions. Garn & Lewis (1967) and Lysell & Myrberg (1986) established that the mandibular canine with 6.4% and 5.7%, respectively demonstrates the greatest sexual dimorphism amongst all teeth. Nair et al (1999) in their study on South Indian subjects concluded that the left mandibular canine (7.7%) shows the maximum sexual dimorphism followed by the right mandibular canine (6.2%). In the present study also, the left mandibular canine was found to exhibit greater sexual dimorphism.

In contrast, Mohd. Abdulla has reported a low degree of sexual dimorphism that is not statistically significant in Saudi population. Similarly insignificant sexual dimorphism was reported by Al Rifaiy et al, in Saudi Arabian population. A study of human fossil excavated at Ra’s Al-Hamra, Eastern Arabian Coast also showed statistically insignificant sexual dimorphism of mandibular canine teeth. Gabriel (1958) has stated that any measurement of teeth unaccompanied by age, race and sex must be treated with great reserve. Apart from sex the other significant findings that can be obtained from teeth are age, race, customs and habits.

This study concludes that human teeth are sexually dimorphic and although males and females exhibit overlapping dimensions, there are significant differences in mean values. Sexual dimorphism has been observed in both deciduous and permanent dentition, with more dimorphism exhibited by the permanent teeth. On an average, male teeth are slightly larger than female teeth, with canine showing the greatest difference. Microtomographic scans of the internal dental tissues have also shown significantly greater quantities of dentine in male teeth than in female. Researchers have attempted to use statistical techniques such as discriminant functions or logistic regression equations based on these sex differences to estimate sex, but the usefulness of such formulae is unsure as sexual dimorphism in teeth might vary between populations.

Since the present study has been conducted on both sexes in a definite age group in the North Indian population, it establishes the morphometric criteria of canine size for the North Indian population. The emerging field of forensic odontology in India relies a lot on inexpensive and easy means of identification of persons from fragmented jaws and dental remains. The present study measured by only linear dimensions because of its simplicity and low expenses. The practicing dentists and the dental students should be made aware of the available technologies and its use in forensic dentistry.
New researches have to be encouraged in the field of forensic dentistry which will pave way for incorporating newer technologies in establishing the human identity.

**Conclusion**

This study concludes that canine crown length, width, and length:width ratio as measured for males and females when compared were found to be statistically significant. However, these parameters for the same sex both in male and female, when compared between left and right, were found to be statistically insignificant. Further, the left canine is found to exhibit greater sexual dimorphism.

**Ethical Clearance:** Not required in an in-vitro study.

**Source of Funding:** Self

**Conflict of Interest:** None

**References**


Rationale of Evaluation of Potency in Medicolegal Cases of Sexual Assault by Penile Color Doppler with Injection of Papaverine

Shilpa Domkundwar¹, Varsha V Jadhav², Sonal Khandelwal³

¹Professor & Head of Dept of Radiodiagnosis, Grant Govt Medical College and JJ Groups of Hospitals, Byculla, Mumbai, ²Associate Professor at Dept of Radiodiagnosis, Grant Govt Medical College and JJ gr of Hospitals, Byculla, Mumbai, ³Senior Resident at Dept of Radiodiagnosis, Grant Govt Medical College and JJ gr of Hospitals, Byculla, Mumbai

Abstract

Introduction: Impotency in male is inability to develop or maintain a sufficient penile erection to conclude the act of intercourse to orgasm and ejaculation. The question of potency arises in many lawsuits in civil and criminal courts. In India, potency testing which includes penile color doppler with injection papaverine is done for all accused of sexual assault as a blanket rule. Penile color doppler with injection papaverine has its own limitations and side effects. Our study therefore aims to analyze and ascertain the justification of subjecting each accused of sexual assault to penile color doppler with injection papaverine.

Aims and Objectives:

1. To analyse whether evaluating potency in every medico legal case of sexual assault referred to our department using color doppler with injection papaverine is justified.

2. To recommend changes based on our study.

Method: This study is a retrospective analysis of data of 166 accused of sexual assault, between the period of May 2015-April 2018. Baseline ultrasound and penile color doppler assessment of erection following papaverine injection was done.

Results: Procedure was performed on 166 accused of sexual assault, of whom the, 3 (1.80%) had arterial insufficiency. 6 (3.61%) accused on whom the procedure was performed developed priapism as complication. The rate of complications was found to be double than the positive result of the procedure.

Conclusion: We conclude from our study that, due to limited role and complications of penile doppler and change in the definition of rape and laws related to it, overburdened health infrastructure of our country, every accused of sexual assault should not be subjected to penile color doppler study.

Keyword: Erectile dysfunction(ED), Potency, Color Doppler, Papaverine

Introduction

Erectile dysfunction is described as inconsistent ability to generate or maintain an erection of sufficient rigidity for sexual intercourse.

A male is said to be impotent when there is inability to develop or maintain a sufficient penile erection to conclude the act of intercourse to orgasm and ejaculation.
In civil courts the question of potency arises in cases like adoption, disputed paternity, nullity of marriage and divorce. In criminal courts the potency is examined in cases of crime against women and children like rape, sexual assault, molestation, sodomy.

A number of medico-legal cases assert impotency in plea. In India, potency testing for all accused of sexual assault is done as a blanket rule despite of the fact whether or not the accused claims it. Such accused are subjected to penile color doppler with injection papaverine for establishing the potency. Penile color doppler with injection papaverine, although a minimally invasive tests has its own limitations and side effects.

There are many other screening tests available to screen for erectile dysfunction.

With the change in the current legislation, the new amendment of Section 375 IPC, implies, peno-vaginal intercourse is not necessary to commit rape.

Our study therefore aims to systematically analyze and ascertain the justification of subjecting each accused of sexual assault to penile color Doppler with injection papaverine.

Aims and Objectives

3. To analyse whether evaluating potency in every medico legal case of sexual assault referred to our department using color doppler with injection papaverine is justified.

4. To recommend changes based on our study.

Materials and Method

This study is a retrospective analysis of data of 278 accused (sec 375,377,376 IPC) referred to the Department of Radio-diagnosis, Sir JJ group of hospitals, Mumbai between the period of May 2015-April 2018. They were in the age group of 15-77 years. All those who consented for the procedure were included. In case of minors, informed consent was taken from the accompanying guardians.

Any accused who did not consent for the procedure were excluded from the study (110 cases). 2 of the accused had e/o local inflammation and hence the procedure was not carried out. Hence procedure was performed on 166 accused.

A base line study was performed initially. Patient was placed in supine position, preferably in a quiet cool place with penis in its anatomical position.

The penis was scanned in longitudinal and cross-sectional views using B mode high frequency linear transducer (7.5-9.0 MHz).

Cavernosa and tunica albuginea were analysed. The local area is cleaned and draped using all aseptic precautions.

Pre injection baseline diameters of the cavernosal arteries are recorded.

60 mg of papaverine (2 mL ampoule of 30 mg/mL diluted in 2 ml of distilled water) was injected intra-cavernosally using an insulin syringe under USG guidance avoiding the cavernosal artery. Precaution taken to avoid spill.

Post-injection measurements were taken at 5, 10, 15 and 20 minutes. The inner diameter of cavernosal artery, peak systolic velocity, end diastolic velocity, visual tumescence and erection were monitored.

Assessment of erection following papaverine injection:

Phase 1: sudden increase in both systolic and diastolic flow velocity in cavernosal artery with minimal tumescence

Phase 2: with further increase in intra-cavernosal pressure, there is a decrease in diastolic flow with a classical ‘dicrotic’ notch

Phase 3: as the intra-cavernosal pressure increases, diastolic flow reaches zero with further increase in tumescence

Phase 4: diastolic flow reversal occurs with maximum systolic velocity; this is associated with penile rigidity

Phase 5: decrease in the systolic flow velocity occurs; this is usually 15 minutes post-injection which is associated with reduction in tumescence and rigidity

Interpretation: In flaccid state, monophasic flow is seen with absent/minimal diastolic flow. With onset of erection, systolic and diastolic flow both increases. With further increase in pressure, ‘dicrotic notch’ appears with dip in diastolic flow. End-diastolic flow may go...
down to zero or reversal may be seen. Then monophasic flow is seen with sharp systolic peak.

Table 1: Penile Doppler parameters and diagnostic criteria

<table>
<thead>
<tr>
<th>Doppler parameter</th>
<th>Diagnostic criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak systolic velocity</td>
<td>Indicator of arterial influx</td>
</tr>
<tr>
<td>of CA</td>
<td>Normal &gt;35 cm/sec</td>
</tr>
<tr>
<td></td>
<td>Gray zone 25-35 cm/sec</td>
</tr>
<tr>
<td></td>
<td>Abnormal &lt;25 cm/sec</td>
</tr>
<tr>
<td>End diastolic velocity</td>
<td>Normal &lt;3-5 cm/sec</td>
</tr>
<tr>
<td>of CA</td>
<td>Venous leak : &gt;5 cm/sec</td>
</tr>
<tr>
<td></td>
<td>Diastolic flow reversal is indicator of intact veno occlusive mechanism</td>
</tr>
<tr>
<td>Resistive index of CA</td>
<td>Normal &gt;0.9</td>
</tr>
<tr>
<td></td>
<td>Venous leak &lt;0.75</td>
</tr>
<tr>
<td>Arterial compliance of</td>
<td>60%-75% increase in diameter , Evident pulsation</td>
</tr>
<tr>
<td>the CA</td>
<td>CA, cavernosal artery.</td>
</tr>
</tbody>
</table>

Observations and Results

Out of the 278 accused of sexual assault, 110 did not consent for the procedure. 3 had Peyronies disease, out of which one had normal penile doppler study and in other two procedure was not performed due to local inflammation. Procedure was performed on 166 accused of whom the, 3 (1.80%) had arterial insufficiency. 6 (3.61%) accused on whom the procedure was performed developed priapism as complication.

The rate of complications was found to be double than the positive result of the procedure.

Fig:1. Total number of patients consenting for the procedure

Fig:2. The results of the procedure{positive:3(1.8%), complications:6(3.61%)}

Review of Literature

Physiologic process of normal erection begins with psychological factors (mental impulse) which cause transmission of parasympathetic motor impulses to the penis. This causes relaxation of smooth muscles in wall of cavernosal sinusoids, allowing sinusoids to expand and decreasing resistance to incoming blood flow. At the same time, cavernosal arteries dilate and carry increased blood flow into the penis. As the sinusoidal spaces start filling, the corporal veno-occlusive mechanism activates, and the fibrous tunica albuginea compresses the draining veins of the corpora, and rigid erection is achieved.2

Normal erectile function requires normal psychological health, normal endocrine balance, intact innervation to penis, normal cavernosal sinusoids, adequate arterial supply and normal venous occlusion with erection. Abnormalities of any of these systems may lead to erectile dysfunction.2

Erectile dysfunction is described as inconsistent ability to generate or maintain an erection of sufficient rigidity for sexual intercourse.

Etiology of erectile dysfunction:

I) Psychogenic- 10%

II) Organic-Neurogenic, Arteriogenic-30%, Venogenic-15%, Morphological, Drugs

To correctly diagnose/rule out impotency other than general physical examination, following Tests/investigations are needed to be performed-11

I) Blood test:

a. Serum testosterone and sex hormone binding globulin- to exclude endocrine causes.
b. Fasting glucose and glycated hemoglobin (HbA1c)- to exclude latent diabetes mellitus

c. Lipid profile- for deranged lipid profile

d. Thyroid hormones- for hypogonadal patients.

e. FSH- for patients with special clinical conditions.

II) Nocturnal penile tumescence.

III) Duplex ultrasonography.

IV) Intracavernosal testing with vasoactive substance.

V) Cavernosometry – used to test for venous leaks.

VI) Cavernosography – it is radiographic visualization of the cavernous penile tissues using contrast material in order to exclude structural abnormalities within the cavernous tissues. VII) Penile arteriography.

VIII) Spongiosography.

Priapism (an erection that lasts for more than 4 hours) is the most serious complication of the procedure which may lead to penile fibrosis. Other complications of penile Doppler with vasoactive stimulation include hypotension, pain, and hematoma.

Current legislation

Excerpts from section 375 IPC (Criminal Law (Amendment) ACT, 2013)  

A man is said to commit “rape” if he—

A.

Penetrates his penis, to any extent, into the vagina, mouth, urethra or anus of a woman or makes her to do so with him or any other person; or

B.

Inserts, to any extent, any object or a part of the body, not being the penis, into the vagina, the urethra or anus of a woman or makes her to do so with him or any other person; or

C.

manipulates any part of the body of a woman so as to cause penetration into the vagina, urethra, anus or any part of body of such woman or makes her to do so with him or any other person; or

D.

Applies his mouth to the vagina, anus, and urethra of a woman or makes her to do so with him or any other person, under the circumstances falling under any of the following seven descriptions.

Discussion

Vincenzo Mirone et al 4 in his study had proposed two tiered examination for evaluating erectile dysfunction. First level diagnostic tests included anamnesis, validated questionnaire, routine laboratory tests and hormonal profile. These tests were enough to make an etiological diagnosis of erectile dysfunction and to identify and remove any erectile dysfunction risk factors in most cases. First level tests should be performed, so that urologists can accurately diagnose erectile dysfunction and prescribe relevant treatment. Second level diagnostic evaluation included specialist instrumental exams helpful for accurate etiological diagnosis of erectile dysfunction. These exams, included Penile dynamic color-duplex Doppler ultrasonography, nocturnal penile tumescence recording, cavernosometry/cavernosography and neurological investigation. However, they proposed that these tests should be performed when first level diagnostic assessment was not clear, or an underlying organic pathology needed to be excluded in young patients with persistent ED, when veno-occlusive or neurogenic ED was suspected.

Martins FE et al 5 determined the role and validity of visual erotic stimulation as a more physiological and cost-effective diagnostic modality for primary noninvasive screening of psychogenic versus organic impotence. He reported that a positive response to visual erotic stimulation is strongly indicative of a predominantly psychogenic cause of erectile dysfunction and can be performed as an initial, minimally invasive test for cost-effective screening of psychogenic impotence.

There are other ways of screening like physical examination, psychometric evaluation, nocturnal penile tumescence, serum hormone levels blood chemistry and newer methods like visual erotic stimulation, which have no test related complications.

There are complications related to color Doppler study with intracavernous papaverine injection, most important is priapism. Penile Doppler with
intracavernous injection of papaverine induced priapism rate in different previous studies has been compared with our study in table 2.

**Table 2: Penile Doppler with intracavernous injection of papaverine induced priapism rate in different studies**

<table>
<thead>
<tr>
<th>STUDY</th>
<th>Cases of intracavernous injection of papaverine induced priapism(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our study</td>
<td>3.61%</td>
</tr>
<tr>
<td>Kilic M et al. 6</td>
<td>2.68 %</td>
</tr>
<tr>
<td>Metawea B et al 7</td>
<td>10%</td>
</tr>
<tr>
<td>Shamloul R et al 8</td>
<td>4.7%</td>
</tr>
<tr>
<td>Sönmez M G et al 9</td>
<td>12.9%</td>
</tr>
<tr>
<td>Yang Y et al.10</td>
<td>3.6%</td>
</tr>
</tbody>
</table>

Although maximum systolic velocity fairly correlates with arterial function of the penis, there are limitations to this diagnostic method. Patient’s anxiety may diminish the arterial response to vasoactive pharmacological agents to the point that maximum velocities fall below the normal range despite normal arterial function.²

A study conducted by Raheel M S et al.¹¹ concludes that since a person is assumed to be innocent by law unless proved otherwise, should be treated with dignity. They also oppose the trend of subjecting each and every accused of sexual assault to potency test without any rationale.

A similar study conducted by Ozkara H et al.¹² indicated that lawyers may abuse the assertion of erectile dysfunction in lawsuits for divorce and rape.

Although penile doppler ultrasound is extremely helpful in accurate estimation of erectile dysfunction it only caters to detection of arterial/venous/combined/morphological causes of erectile dysfunction. The psychogenic causes not being acknowledged. Potency unlike sterility is not permanent and depends on various factors. Person may be potent at the time of alleged rape but may be impotent at the time of medical examination or vice versa. This is mainly relevant, when accusation is brought up after considerable time gap. Person who is impotent towards one person may be potent to other person. Person impotent to one gender may be potent to other gender. So even after having normal result of Doppler study, which excludes organic cause, person may still be impotent at any particular situation or vice versa.

The new amendment of Section 375 IPC ¹, implies, peno-vaginal intercourse is not necessary to commit rape. This broadens the definition of abuse beyond the organic causes and hence further reduces the role of an invasive test as relevance in the accusation.

There is also involvement of a lot of specialised manpower (urologist, radiologist and para medical staff) who not only have to do the examination but also attend court summons in future. Also, the cost of the procedure is borne by the government. In India health sector already struggles lack of funds and manpower. There is no justification of diverting so many resources to one such issue when the study will not even have much impact on fate of the case.

Hence accused who is not asserting impotency should not be subjected to penile color doppler study and Penile color doppler with papaverine injection should be done in cases found impotent on screening.

**Conclusion**

We conclude from our study that, due to
- Limited role of penile Doppler in evaluation of causes of erectile dysfunction.
- Complications like priapism of the procedure.
- Change in the definition of rape and laws related to it.
- overburdened health infrastructure of India

Every accused of sexual assault should not be subjected to penile color doppler study

Based on our study we would also like to offer some suggestions:

1) Not all accused of sexual assault should be subjected to potency tests- only the accused asserting impotency should undergo potency test and color Doppler ultrasound.

2) Proper medical history with lab investigations
should be done.

3) Screening methods for impotency like visual erotic stimulation, NPTR (nocturnal penile tumescence and rigidity) should be done. Penile color doppler with papaverine injection should be done in cases found impotent on screening.

Conflict of Interest: None declared

Source of Funding: None

Ethical Clearance: None declared

References


Determination of Stature from Anthropometric Measurements of Thyroid Cartilage in the Population of Punjab

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Abstract

The astonishing task of determining the identity of an individual has been simplified, with the innovation of scientific technologies like DNA fingerprinting in the developed countries. Yet the application of such advanced technologies in developing countries is still a daunting task due to its complexity. Anthropometric analysis for the identification of unknown bodies is reasonably productive owing to its inexpensiveness. The aim of this study was to correlate the anthropometric measurements of the thyroid cartilage with the stature of the individual. Thyroid cartilages from 300 post mortem cases done in native population of Punjab were studied anthropometrically. A total of seventeen anthropometric measurements of selected parameters of thyroid cartilage were defined, measured, and statistically correlated with the body length of the individual. The variables which were significantly correlating with the body length were further analysed by regression analysis for derivation of a regression equation.

Key words: Anthropometric measurements, Regression analysis, Stature determination, Thyroid cartilage.

Introduction

One of the most important duties of any forensic expert is to determine the identity of an unknown deceased and establishing the same from any dismembered part of body. Stature of an individual is an essential requirement for establishing the identity of the individual. Most of the previous studies have relied on the anthropometric measurements of long bones for identifying the stature of an individual. The various well defined anatomical features of thyroid cartilage are not only quantifiable as anthropometric measurements (¹) but it also has an added advantage of being in a superficial anatomical location that negates the tedious removal and preparation process that must be done when utilising long bones for stature estimation. There are a few literatures (²-⁶) that estimates the stature of individual using anthropometric measurements of thyroid cartilage, which are done exclusively within a target native population. The aim of this study was to determine the stature from the anthropometric measurements of the thyroid cartilage in native population of Punjab.

Objectives:

1. To establish correlation between the anthropometric measurements of thyroid cartilage and the body length of the deceased.

2. To derive regression equation for estimation of stature from significantly correlating anthropometric measurements of thyroid cartilage.

Materials and Method

Materials used:

Portable anthropometer, Vernier caliper and Goniometer were used for the present study.

Method:
It is a prospective analytical study conducted from July 2012 to December 2013 on 300 cases autopsied in the department of Forensic Medicine of a tertiary care hospital in Chandigarh. Written informed consent from the legal heirs of the deceased were taken.

**Inclusion criteria**

- All cases above the age of 18 years.
- Residents of Punjab having at least two generation of ancestors from Punjab.

**Exclusion criteria**

- Cases with injury to thyroid cartilage.
- Known cases of Kyphosis
- Known cases of Scoliosis
- Acromegaly.
- Gigantism.
- Cretinism.
- Dwarfism.
- Congenital abnormalities of neck.
- Cases with advanced decomposition changes.

The recumbent length of the deceased was measured with the help of portable anthropometer after extending the neck, spine, and the lower limbs to overcome flexion due to rigor mortis. The body length was measured from vertex to the heel of the foot. To avoid inter observer error, the measurements were measured by three different observers and the mean value was taken. The thyroid cartilage was dissected out during autopsy, with great care as not to damage the superior and inferior horns in the process. The entire thyroid cartilage was then soaked in warm caustic soda solution until all muscular and ligamentous attachments sloughed off. After removal of all attachments, the dimensions of the thyroid cartilage were measured with the help of a thread, vernier caliper and goniometer. The recumbent body length and 17 parameters including the thyroid angle of thyroid cartilage were measured as per Table 1. All the parameters were measured by three observers and the mean value was taken as the final measurement to avoid inter observer error of measurement. All the analyses were carried out with the help of IBM SPSS Statistics package (version 20.0).

**Results**

A total of 300 samples of thyroid cartilages of the deceased belonging to Punjab population were collected and studied. Of these 300 samples, 238 were males and 62 were females. The length of body varied from 146 cm to 195 cm in males with a mean of 169.95 cm, mean standard deviation of 7.16 cm and standard error of 0.46. In females, body length ranged from 144 cm to 184 cm with a mean of 156.59 cm, mean standard deviation of 8.06 cm and standard error of 1.02. The length of body was compared and correlated with each of the seventeen variables using bivariate correlation analysis. A significant positive correlation was obtained between the body length of male and female with the length of both the sides of thyroid lamina with Pearson correlation value of \( r > 0.8 \) as shown in the table 2A, 2B, 2C.

In males:

- L RT L was correlating with the length of body with Pearson correlation of \( r = 0.908 \).
- L LT L was correlating with the length of body with Pearson correlation of \( r = 0.895 \).

In females:

- L RT L was correlating with the length of body with Pearson correlation of \( r = 0.959 \).
- L LT L was correlating with the length of body with Pearson correlation of \( r = 0.953 \).

Regression equation for prediction of body length of male and female was obtained from length of right and left thyroid lamina with standard error of estimate of 2.87 (male) and 2.89 (female)

**MALE:** Body length = 97.087 +7.481(L RT L) - 4.822(L LT L)

**FEMALE:** Body length = 93.119 +7.851(L RT L) - 4.919(L LT L)
<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Points of measurements</th>
<th>Units of measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Recumbent body length</td>
<td>Vertex of head to heel of feet</td>
<td>Centimetre</td>
</tr>
<tr>
<td>2</td>
<td>Length of right thyroid lamina (L RTL)</td>
<td>Midpoint of upper border of right thyroid lamina to Midpoint of lower border of right thyroid lamina</td>
<td>Millimetre</td>
</tr>
<tr>
<td>3</td>
<td>Length of left thyroid lamina (L LT L)</td>
<td>Midpoint of upper border of left thyroid lamina to Midpoint of lower border of left thyroid lamina</td>
<td>Millimetre</td>
</tr>
<tr>
<td>4</td>
<td>Breadth of right thyroid lamina (B RT L)</td>
<td>Anterior thyroid prominence to Midpoint of posterior border of right thyroid lamina</td>
<td>Millimetre</td>
</tr>
<tr>
<td>5</td>
<td>Breadth of left thyroid lamina (B LT L)</td>
<td>Anterior thyroid prominence to Midpoint of posterior border of left thyroid lamina</td>
<td>Millimetre</td>
</tr>
<tr>
<td>6</td>
<td>Ventral thyroid height (V HT)</td>
<td>Deepest point of Superior thyroid incisura to Prominent point of Inferior thyroid incisura</td>
<td>Millimetre</td>
</tr>
<tr>
<td>7</td>
<td>Dorsal right thyroid height (DR RT HT)</td>
<td>Tip of right superior horn to Tip of right inferior horn</td>
<td>Millimetre</td>
</tr>
<tr>
<td>8</td>
<td>Dorsal left thyroid height (DR LT HT)</td>
<td>Tip of left superior horn to Tip of left inferior horn</td>
<td>Millimetre</td>
</tr>
<tr>
<td>9</td>
<td>Upper thyroid breadth (U BR)</td>
<td>Outermost point of base of right superior thyroid horn to Outermost point of base of left superior thyroid horn</td>
<td>Millimetre</td>
</tr>
<tr>
<td>10</td>
<td>Lower thyroid breadth (L BR)</td>
<td>Outermost point of base of right inferior thyroid horn to Outermost point of base of left inferior thyroid horn</td>
<td>Millimetre</td>
</tr>
<tr>
<td>11</td>
<td>Maximum thyroid Breadth at superior thyroid tubercle. (B S TB)</td>
<td>Outermost prominent point of right superior tubercle to Outermost prominent point of left superior tubercle</td>
<td>Millimetre</td>
</tr>
<tr>
<td>12</td>
<td>Maximum thyroid Breadth at inferior thyroid tubercle. (B I TB)</td>
<td>Outermost prominent point of right inferior tubercle to Outermost prominent point of left inferior tubercle</td>
<td>Millimetre</td>
</tr>
<tr>
<td>13</td>
<td>Length of right superior horn (RTS HR)</td>
<td>Tip of right superior horn to Base of right superior horn</td>
<td>Millimetre</td>
</tr>
<tr>
<td>14</td>
<td>Length of left superior horn (LT S HR)</td>
<td>Tip of left superior horn to Base of left superior horn</td>
<td>Millimetre</td>
</tr>
<tr>
<td>15</td>
<td>Length of right inferior horn (RT I HR)</td>
<td>Tip of right inferior horn to Base of right inferior horn</td>
<td>Millimetre</td>
</tr>
<tr>
<td>16</td>
<td>Length of left inferior horn (LT I HR)</td>
<td>Tip of left inferior horn to Base of left inferior horn</td>
<td>Millimetre</td>
</tr>
<tr>
<td>17</td>
<td>Depth of superior thyroid notch (DEPTH)</td>
<td>Highest level of thyroid lamina to Deepest point of superior thyroid notch</td>
<td>Millimetre</td>
</tr>
<tr>
<td>18</td>
<td>Angle of thyroid (ANGLE)</td>
<td>Posterior surface of right lamina to Posterior surface of left lamina</td>
<td>Degree</td>
</tr>
</tbody>
</table>
Table 2 A: Mean, SD, correlation of body length with thyroid cartilage measurements.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Body Length (cm)</th>
<th>L RT L (mm)</th>
<th>L LT L (mm)</th>
<th>B RT L (mm)</th>
<th>B LT L (mm)</th>
<th>V HT (mm)</th>
<th>DR RT HT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>238</td>
<td>39.24</td>
<td>169.95</td>
<td>27.42</td>
<td>27.43</td>
<td>36.50</td>
<td>36.48</td>
<td>16.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±13.63</td>
<td>±7.16</td>
<td>±2.47</td>
<td>±2.49</td>
<td>±7.56</td>
<td>±7.49</td>
<td>±2.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Correlation with body length, r</td>
<td>0.908 **</td>
<td>0.895 **</td>
<td>-0.007ns</td>
<td>-0.008ns</td>
<td>0.220**</td>
<td>-0.005ns</td>
</tr>
<tr>
<td>Female</td>
<td>62</td>
<td>40.95</td>
<td>156.59</td>
<td>21.58</td>
<td>21.58</td>
<td>32.44</td>
<td>32.52</td>
<td>14.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±16.35</td>
<td>±8.06</td>
<td>±2.75</td>
<td>±2.82</td>
<td>±8.18</td>
<td>±8.21</td>
<td>±2.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Correlation with body length, r</td>
<td>0.959 **</td>
<td>0.953 **</td>
<td>0.173**</td>
<td>0.170**</td>
<td>0.445**</td>
<td>0.05ns</td>
</tr>
</tbody>
</table>

**p value <0.01, *p value <0.05, ns - p value >0.05

Table 2 B: Mean, SD, correlation of body length with thyroid cartilage measurements.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Body Length (cm)</th>
<th>DR LT HT (mm)</th>
<th>U BR (mm)</th>
<th>L BR (mm)</th>
<th>B S TB (mm)</th>
<th>B I TB (mm)</th>
<th>RT S HR (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>238</td>
<td>39.24</td>
<td>169.95</td>
<td>34.87</td>
<td>45.76</td>
<td>38.70</td>
<td>45.74</td>
<td>29.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±13.63</td>
<td>±7.16</td>
<td>±7.66</td>
<td>±7.514</td>
<td>±5.96</td>
<td>±7.61</td>
<td>±5.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Correlation with body length, r</td>
<td>-0.012ns</td>
<td>-0.04ns</td>
<td>0.022ns</td>
<td>0.024ns</td>
<td>0.015ns</td>
<td>-0.023ns</td>
</tr>
<tr>
<td>Female</td>
<td>62</td>
<td>40.95</td>
<td>156.59</td>
<td>34.58</td>
<td>45.39</td>
<td>38.54</td>
<td>45.37</td>
<td>29.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±16.35</td>
<td>±8.06</td>
<td>±8.61</td>
<td>±8.64</td>
<td>±7.77</td>
<td>±8.68</td>
<td>±6.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Correlation with body length, r</td>
<td>0.042ns</td>
<td>-0.010ns</td>
<td>0.013ns</td>
<td>-0.001ns</td>
<td>0.016ns</td>
<td>-0.004ns</td>
</tr>
</tbody>
</table>

ns - p value >0.05

Table 2 C: Mean, SD, correlation of body length with thyroid cartilage measurements.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Body Length (cm)</th>
<th>LT S HR (mm)</th>
<th>RT I HR (mm)</th>
<th>LT I HR (mm)</th>
<th>DEPTH (mm)</th>
<th>ANGLE (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>238</td>
<td>39.24</td>
<td>169.95</td>
<td>16.62</td>
<td>10.37</td>
<td>10.38</td>
<td>10.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±13.63</td>
<td>±7.16</td>
<td>±4.70</td>
<td>±2.67</td>
<td>±2.59</td>
<td>±3.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Correlation with body length, r</td>
<td>-0.0210ns</td>
<td>-0.040ns</td>
<td>-0.030ns</td>
<td>0.011ns</td>
<td>0.006ns</td>
</tr>
<tr>
<td>Female</td>
<td>62</td>
<td>40.95</td>
<td>156.59</td>
<td>16.00</td>
<td>9.80</td>
<td>9.79</td>
<td>10.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±16.35</td>
<td>±8.06</td>
<td>±4.52</td>
<td>±2.47</td>
<td>±2.42</td>
<td>±2.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Correlation with body length, r</td>
<td>0.000ns</td>
<td>0.012ns</td>
<td>0.025ns</td>
<td>-0.005ns</td>
<td>-0.133*</td>
</tr>
</tbody>
</table>

*p value <0.05 ns - p value >0.05
Discussion
Various studies, which analysed the anthropometric measurements of thyroid cartilage of males and females, found a significant difference between them and established that sex can be determined from anthropometric measurements of thyroid cartilage (7-15). In the present study, the body length of the deceased was compared with various parameters of thyroid cartilage and it was found to be significantly correlating with length of right and left thyroid lamina for both gender. This finding was consistent with the studies conducted by Monica and Dhall (2) and Pierre Fayoux et al (3). The study conducted by Monica and Dhall (2) also found a significant correlation between body length of individual with height of arch of cricoid cartilage and transverse diameter of cricoid cartilage. In the present study those anthropometric parameters were not measured. The study conducted by Pierre Fayoux et al (3) done on 300 human larynxes also found significant correlation with thyroid alar width, and median anterior thyroid height. Whereas the present study didn’t find any correlation of body length with thyroid alar width and median anterior thyroid height. Too-Chung and Green (4) conducted a study in paediatric age group with 67 samples. They reported a linear correlation between body length with coronal and sagittal diameter at lower border of cricoid cartilage whereas the present study, the anthropometric parameters like coronal and sagittal diameter of lower border of cricoid cartilages were not evaluated. The study conducted by Schild (5) was conducted on human infant larynxes and found a linear correlation between crown rump length and the ventral thyroid height, but in the present study the parameter ventral thyroid height was not correlating with the body length. The study conducted by Ajmani et al (6) didn’t find any correlation between various anthropometric measurements of thyroid cartilage and the body length of the individual. The deviations of the above study results from the present study might be due to variation in the definition of parameters studied, differences in the number of samples and age range of samples studied from the present study.

Conclusion
Significant correlations were found between length of both sides of thyroid lamina and body length in both gender. Regression equation for prediction of body length from length of right and left thyroid lamina was derived for each sex. With a sample of thyroid cartilage of known gender, one can estimate the body length of the individual using the regression formula we have derived. This regression formula uses only the major parameters of thyroid cartilage namely length of right and left thyroid lamina, which are less prone to be damaged in the process of recovery from the body compared to other parts of thyroid cartilage. Further studies need to be conducted in different population to derive a standard regression equation for estimation of stature from anthropometric measurements of thyroid cartilage for a native population.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: The ethical clearance was taken for the present study from the institute ethical committee.

References
5. Schild JA. Relationship of laryngeal dimensions to body size and gestational age in premature neonates and small infants. Laryngoscope. 1984; 94:1284-92
Study of Patterns of Injuries by Road Traffic Accidents

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Associate Professor, Department of Forensic Medicine and Toxicology, IQ Medical College, Durgapur, West Bengal

Abstract

210 autopsies (176 males and 34 females) aged between 2 to 70 years were studied. 12(6.81%) males, 8(23.5%) females total 20(9.52) were aged between 2-10 years 32 (18.1%) males, 4(11.7%) females total 36(17.1%) were aged between 11-20 years. 52(29.5%) males, 12(35.2%) female total 64(30.4%) were aged between 21-30 years. 31(17.6%) males, 3(8.82%) females total 34(16.1%) aged between 31-40 years 21(11.9%) males, 2(5.81%) females, total 23(10.9%) were aged between 41-50 years 15(8.5%) males, 3(8.82%) females, total 18(8.57%) were aged between 51-60 years 13(7.3%) males, 2(5.88%) female total 15(7.14%) aged between 61-70 years. The types of riders were, 17(8.09%) pedestrian 29(13.8%) cyclist, 103(49%) were riding two wheelers. 49(23.3%) travelling in car, 12(5.71%) were Lorry drivers / bus travelers. The types of injuries were 72(34.2%) had head injury 22(10.4%) had neck injury, 31(14.7%) had injuries to lower limb, 21(10%) abdominal injuries, 34(16.1%) had injuries to thorax, 13(6.19%) had injuries to upper limb 17(8.09%) had injuries to face. This pragmatic study will be quite useful to medico legal expert to assess the age group, region wise injury, types of riders because accident is an unexpected mishap which claims life, who are in hurry and disobey the traffic rules.

Key words – Accidents, injuries, Autopsies, Telangana

Introduction

Globally, the growth of transport system has been growing continuously and become a key element in economic development Due to industrialization and urbanization the transport system has been increased at leaps and bounds But in India. Bad roads, ignorant of traffic rules. Corruptive administration, law and orders ultimately leads to road accidents and deaths Every year Road traffic Accidents (RTA) claim 600000 and fifteen million get injure in RTA.(1)

The term accident has been defined as an occurrence in the sequence of events which usually produces unintended injury death or property damage(2) and majority of death occurs in age group between 5-44 was reported globally(3) It is surprise to note that, a person is killed or injured in every 4 minutes in Delhi traffic accidents in India(4) Hence attempt was made to study deaths in both sexes at different age, and different vehicles of Telangana state highways.

Material and Method

210 Autopsies (176 males and 34 females) aged between 2-70 years brought to osmania government collage hospital Hyderabad which is attached to Bhaskera medical college Yennukapally, Moinabhad Hyderabad

Information of age riding vehicles, or pedestrian were collected from police records. Majorities of autopsies blood / viscera traces of alcohol was found. Most of the accident took place during night time. The age group in both sexes, types of riders / pedestrian Region wise external injuries were classified with number and percentage

Duration of the study was 2010-2014 (four years)

Observation and Results

Table-1 classification age and sex in patterns of Road accidental deaths- 12(6.8%) males, 8(23.5%) of females total 20(9.52%) were aged between 2-10 years. 32 (18.1%) males 4(11.7%) females total 36(17.1%) aged between 11-20 years. 52(29.5%) males, 12(35.2%) female total 64(30.4%) were aged between 21-30 years. 31(17.6%) males, 3(8.82%) female, total 34(16.1%) were aged between 31-40 years. 21(11.9%) males,
2(5.88%) females, total 23(10-9%) were aged between 41-50 years. 15(8.5%) males, 3(8.82%) female total 18(8.57%) were aged between 51-60 years 13(7.31%) 2(5.88%) females total number 23(7.14%) were aged between 61-70 years.

Table-2 study of types of riders in patterns of injuries 17(8.09%) were pedestrian, 29(13.8%) cyclist, 103(49%) were two wheelers. 49(23.3%) were travelling in car, 12(5.71%) were lorry drivers / travelling in Bus

Table-3 study of injuries in road accidents. 72(34.2%) had head injury 22(10.4%) had neck injuries 31(14.7%) had lower limbs injuries, 21(10%) had abdominal injuries, 34(16.1%) had injury to thorax, 13(6.19%) had injuries to upper limb, 17(8.09%) had injuries on face.

### Table-1: Classification of age and sex in pattern of Road Injuries

(Total No deaths 210)

<table>
<thead>
<tr>
<th>Age-group</th>
<th>Males (176)</th>
<th>% Percentage</th>
<th>Females (34)</th>
<th>% Percentage</th>
<th>Total no of Deaths</th>
<th>% Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-10 years</td>
<td>12</td>
<td>6.81</td>
<td>8</td>
<td>23.5</td>
<td>20</td>
<td>9.52</td>
</tr>
<tr>
<td>11-20</td>
<td>32</td>
<td>18.1</td>
<td>4</td>
<td>11.7</td>
<td>36</td>
<td>17.1</td>
</tr>
<tr>
<td>21-30</td>
<td>52</td>
<td>29.5</td>
<td>12</td>
<td>35.2</td>
<td>64</td>
<td>30.4</td>
</tr>
<tr>
<td>31-40</td>
<td>31</td>
<td>17.6</td>
<td>3</td>
<td>8.82</td>
<td>34</td>
<td>16.1</td>
</tr>
<tr>
<td>41-50</td>
<td>21</td>
<td>11.9</td>
<td>2</td>
<td>5.88</td>
<td>23</td>
<td>10.9</td>
</tr>
<tr>
<td>51-60</td>
<td>15</td>
<td>8.5</td>
<td>3</td>
<td>8.82</td>
<td>18</td>
<td>8.57</td>
</tr>
<tr>
<td>61-70</td>
<td>13</td>
<td>7.3</td>
<td>2</td>
<td>5.88</td>
<td>15</td>
<td>7.14</td>
</tr>
</tbody>
</table>

### Table-2: Study of types of Riders in the patterns of Injuries

(No of death - 210)

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Types of Riders</th>
<th>No of Riders</th>
<th>% Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pedestrian</td>
<td>17</td>
<td>8.09</td>
</tr>
<tr>
<td>2</td>
<td>Cyclist</td>
<td>29</td>
<td>13.8</td>
</tr>
<tr>
<td>3</td>
<td>Two wheelers</td>
<td>103</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>Car / jeep</td>
<td>49</td>
<td>23.3</td>
</tr>
<tr>
<td>5</td>
<td>Lorry / bus</td>
<td>12</td>
<td>5.71</td>
</tr>
</tbody>
</table>

### Table-3: Injuries in the traffic Road accidental deaths

(Total No -210)

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Region of body</th>
<th>No of persons</th>
<th>% Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Head</td>
<td>72</td>
<td>34.2</td>
</tr>
<tr>
<td>2</td>
<td>Neck</td>
<td>22</td>
<td>10.4</td>
</tr>
<tr>
<td>3</td>
<td>Lower limb</td>
<td>31</td>
<td>14.7</td>
</tr>
<tr>
<td>4</td>
<td>Abdomen</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Thorax</td>
<td>34</td>
<td>16.1</td>
</tr>
<tr>
<td>6</td>
<td>Upper limb</td>
<td>13</td>
<td>6.19</td>
</tr>
<tr>
<td>7</td>
<td>Face</td>
<td>17</td>
<td>8.09</td>
</tr>
</tbody>
</table>
Discussion

In the present study of patterns of injuries by traffic accidents in Telangana population. Classification of age and sex was 12(6.81%) in males, 8(23.5%) in female total 20(9.52%) in the age between 2- 10 years 32(18.1%) in males 4(11.7%) in females total 36(17.1%) in the age between 11-20 years 52(29.5%) in males 12(35.2%) in females total 64(30.4%) in the age between 21-30, 31(17.6%) males 3(8.82%) in females total 34(16.1%) in the age group of 31-4 years. 21(11.9%) males, 2(5.88%) female total 23(10.9%) ware in the age group between 41-50 years, 15(8.5%) males, 3(8.82%) female total 18(8.57%) were aged between 51-60, 13(7.3%) males 2(5.88%) females total 15(7.14%) were aged between 61-70 years. (Table-1) In the study of types of riders 17(8.09%) were pedestrian, 29(13.8%) were cyclist 103(49%) two wheelers, 49(23.3%) were travelling in car, / Jeep, 12(5.71%) were al- lorry / Bus (Table-2) In the evaluation injuries

During the autopsies 72(34.2%) had head injuries, 22(10.4%) had neck injury, 31(14.7%) had injuries to lower extremities, 21(10%) had injuries to abdomen, 34(16%) had injuries to thorax, 13(6.19%) had injuries to upper limb, 17(8.09%) had injuries to face. (Table-3). These different findings were more or less in agreement with previous studies(5)(6)(7)

Head injury includes, scalp injury in the form of contusions, lacerations, under scalp hematoma intracranial hemorrhages, comprising skull fractures(8) the tempo- regional injury was quite common. The neck injury include compression of spinalcord followed by fracture of vertebrae, fracture of larynx, laryngeal cartilages, laceration of common carotid and Jugalar vein.(9) Injury to thorx included fracture of ribs, with lung and heart injury was also noted. Injuries to upper limb and lower limb mainly included fracture of long bones followed by laceration blood vessels and musculature in addition to joint fractures. Moreover abdominal injury includes injury to vital organs rapture of spleen, intestinal perforation and bladder rapture(10) injury to spinal column was also noted. Most of the deaths due to severe bleeding injury to respiratory centres of brain, and involvement of nervous system and respiratory organ (cardio-vascular)

Summary and Conclusion

The present study of pattern of injuries in road traffic accidents in Telangana population is quite useful to medico legal expert, police and judiciary officials, Death or accidents in RTA is not only a Indian problem rather it is problem of the whole world where transport is functioning. The reasons are riders are un-aware or ignorant of traffic rules and consequent punishment. Disobedience of traffic rules and fearless consequences of punishments. Hence stringent law should be imposed and awareness of traffic rules in illiterates. Apart from this journey in night and during rainy seasons must be discouraged. Use of alcohol, non usage of helmet and belts in cars. Pedestrian should take particular caution when crossing the roads and look both ways before crossing walking on the road must be discouraged. Wide and neat roads, ban on old vehicles can prevent the major accidents. Above all, early slow driving self confidence will save our life and others too.

This research paper was approved by ethical committee of Bhaskara medical college yennukapally. Moinabad- Hyderabad (Telangana)

No Conflict of Interest

No Funding

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Socio-Demographic Study of Deaths Due to Poisoning in Autopsies Conducted at KIMS Hospital, Bangalore

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Abstract

Poisoning is a common day to day problem. Profile of poisoning in an area depends upon a variety of factors, ranging from access to and availability of poison, socio-economic status of the individual, cultural and religious influences, etc. A prospective study was carried out in the Department of Forensic medicine, Kempegowda Institute of Medical Sciences and Hospital Bangalore to study the socio-demographic profiling of deaths due to poisoning. Out of the 100 cases studied, incidence of poisoning was found to be more amongst the age group of 21 to 30 years in both sexes with mean age being 25.4 years and 25.4 years amongst males and females respectively. Poisoning is more in males (68%) as compared to females. In both the sexes, married (68%) outnumbered the unmarried in rural and urban community. This study shows that maximum number of poisoning cases were noticed among Upper Lower socio economic Class and in Nuclear families(61%). It was observed that majority of the victims were Graduate (28%). Financial constraints(22%) were the most common motive behind poisoning deaths.

Keywords: Poisoning Death, Autopsy, Socio-demographic profile, Motive

Introduction

Indiscriminate use of pesticides in agriculture, introduction of a variety of newer drugs for treatment, exposure to hazardous chemical products due to rapid industrialization, unhealthy dietary habits and increase in alcohol consumption have led to a wide spectrum of toxic products to which people are exposed nowadays. Profile of poisoning in an area depends upon a variety of factors, ranging from access to and availability of poison, socio-economic status of the individual, cultural and religious influences, etc.

Both suicidal and homicidal cases of poisoning are more common in India than in Western countries owing to the easy availability of poisonous substances on account of non stringent application of the laws of the land. Accidental poisoning is also on an increase because of the greater use of chemicals for agro industry and domestic purposes.

It is very difficult to draw a common report to say which kind of poisoning is more frequent as the nature of poisoning varies from one region to another depending upon the poison availability and the knowledge of local population regarding the properties of poisons. So this study has been aimed to determine the various parameters of poisoning such as mode of poisoning, type of poison, relation to occupation, marital status, religion status, motivation, vulnerable age group in South Bangalore.

Materials and Method

The present study has been carried out in the Department of Forensic medicine, Kempegowda Institute of Medical Sciences and Hospital Bangalore, during the period of Nov-2011 – May-2013. All the cases brought to the department for medicolegal autopsy with history of poisoning and cases that were diagnosed as poisoning after post mortem examination were selected. A sum total of 100 cases were selected by simple random sampling for this prospective study.

Ethical Clearance: Ethical clearance for the
present study was obtained from the institutions ethical committee, Kempegowda Institute of Medical Sciences, Bangalore.

**Method of Collection of Data:**

All cases with history of poisoning autopsied at Kempegowda Institute of Medical Sciences and Research Centre (KIMS), Bangalore were included in the study.

Detailed information of the deceased pertaining to the case was collected from the concerned police and relatives of the deceased by a questionnaire, Post mortem findings were analyzed with the chemical analysis reports. In treated cases information was acquired by perusal of hospital records. In cases of allegations, information was supplemented by either visit to the scene of crime or from the photographs of the scene of crime.

**Results**

In our study, the maximum number of poisoning in the study population are seen in the age group of 21 to 30 years (43%). In males it amounted to (42.65%) followed by 31 to 40 years (26.47%). Least number was noticed in the age group of 0 to 10 years and 11 to 20 years, accounting to (1.47%) each. In females, maximum number of poisoning occurred in the age group of 21 to 30 years accounting to (43.75%) followed by 31 to 40 years and 51-60 years of age group (15.63%) each. Least number was noticed in the age group of 0 to 10 years and 71 to 80 years, accounting to (3.13%) each.

Males outnumbered the females in the study population. In males it amounted to 68% and in females 32%. Poisoning is seen more among married people (68%) as compared to unmarried (32%). It is observed that poisoning cases were predominantly seen in urban areas (92%) as compared to rural areas (8%).

According to Kuppuswamy’s Socio-Economic classification, it is observed that the maximum number of poisoning cases were noticed among Upper Lower Class, followed by Upper Middle Class. Least is seen among the Upper Class group. Majority of victims were Hindus (86%) (n =86) followed by Muslim (10%) (n = 10) and Least were seen amongst the Christians (4%) (n=4). Incidence of poisoning is more among the Nuclear Family (61%) as compared to the Joint families (39%) in the present study population.

In the present study, it is observed that the incidence of poisoning were seen more amongst the Students comprising (25%), followed by House wives (16%), Domestic works (14%), Office work (13%), Professional (11), Unemployed and Salesperson (6%) each and Others (5%) who comprised of transport workers, retired persons and those whose profession were not clear. Least were seen amongst the Agriculture works (4%). It is also observed that majority of the victims were Graduate (28%) followed by High school certified (24%) and least were among profession (2%).

With respect to motive, most victims had financial constraints (22%) followed by Love Failures (15%), Ill Health (12%), Marital problems (10%), Educational problems and others comprised of (9%) each [others included death of dear ones, etc.], Family problems (7%) and the least accounted for Unemployment problems (6%). In (10%) of cases motive could not be ascertained.

**Discussion**

In our study it is observed that maximum number of poisoning is seen in the age group of 21 to 30 years (43%). In males it amounted to 21 to 30 years (42.65%) followed by 31 to 40 years (26.47%). Least number was noticed in the age group of 0 to 10 years and 11 to 20 years, accounting to (1.47%) each. In females, maximum number of poisoning occurred in the age group of 21 to 30 years accounting to (43.75%) followed by 31 to 20 years and 51-60 years of age group (15.63%) each. Least number was noticed in the age group of 0 to 10 years and 71 to 80 years, accounting to (3.13%) each. Similar findings were observed in the studies conducted by Adarsh Kumar1, Lan Zhou2, M. Shoaib Zaheer3, Kartik Prajapati4, Tejas Prajapati5, J.Gargi6, Shetty AK7 and Murari Atul8. It is not in accordance with studies by conducted by Margaret Warner9 and Deepak Pokhrel10.

Sex wise distribution of cases in this study showed the maximum number of cases were among males. Males outnumbered the females in this study. In males it amounted to 68% and in females 32%. Males being the main breadwinner in the family and the burden of earning for a livelihood are on them and the male ratio being more than the female, are the reasons for the incidence of poisoning more in males. Similar findings were observed in the studies conducted by Adarsh Kumar1, Kiran N11, M. Shoaib Zaheer3, Margaret Warner9, Heethal Jaiprakash12, Kartik Prajapati4, Chataut J13, Mizanur Rahman14, Tejas praajapati5, J.Gargi6, Shetty
AK and Murari Atul. It is not in accordance with studies by conducted by Deepak Pokhrel.

In the present study, poisoning is seen more among married people (68%) as compared to unmarried (32%). Similar findings were observed in the studies conducted by Kiran N, Heethal Jaiprakash and Kartik Prajapati.

In this study, it is observed that poisoning cases were predominantly seen in urban areas (92%) as compared to rural areas (8%). Similar findings were observed in the studies conducted by Kartik Prajapati and B. Maharani.

According to Kuppuswamy’s classification of Socio-Economic Status, this study shows that maximum number of poisoning cases were noticed among Upper Lower Class, followed by Upper Middle Class. Least is seen among the Upper Class group. Since the study involved the subjects residing more in urban setup, the annual income of the Upper Lower class could not suffice to meet the basic amenities resulting in disillusionment in life amongst them. Similar findings were observed in the studies conducted by Chataut J and Maharani.

Incidence of poisoning is more among the Nuclear Family (61%) as compared to the Joint families (39%) in the present study. Similar findings were observed in the studies conducted by Kartik Prajapati.

In the present study, it is observed that incidence of poisoning were seen more amongst the Students comprising (25%), followed by House wives (16%), Domestic works (14%), Office work (13%), Professional (11), Unemployed and Salesperson (6%) each and Others (5%) who comprised of transport workers, retired persons and those whose profession were not clear. Least were seen amongst the Agriculture works (4%). Similar findings were observed in the studies conducted by Adarsh Kumar and Kartik Prajapati.

In the present study it is observed that incidence of poisoning were seen more amongst the Graduates (28%) followed by High school graduate (24%) and least were among profession (2%). Similar findings were observed in the studies conducted by Adarsh Kumar and Kartik Prajapati. It is not in accordance with studies by conducted by Heethal Jaiprakash, Chataut J, Maharani and Tejas prajapati.

It is seen from the above study that financial constraints (22%) followed by Love Failures (15%), Ill Health (12%), Marital problems(10%) and Educational problems and others comprised of 9% each [others included death of dear ones, etc.], Family problems (7%) and the least accounted for Unemployment problems (6%). In (10%) of cases motive could not be ascertained. Among financial constraints the reasons were excessive debts, poverty, not able to pay the loan, extravagant lifestyle, engaging in activities in an urge to achieve instant richness were the prominent financial causes noticed. Among the ill health, in majority of cases evidence of chronic illness like gastrointestinal disorders, bronchial asthma, tuberculosis, diabetes, hypertension, and gynecological problems as procured through the history and hospital records, on autopsy corroborated with autopsy finding. Similar findings were observed in the studies conducted by Adarsh Kumar.

Conclusion

- Incidence of poisoning is more among the age group of 21 to 30 years in both sexes with mean age being 25.4 years and 25.4 years amongst males and females respectively.
- Poisoning is more in males as compared to females.
- In both the sexes, married outnumbered the unmarried in rural and urban community.
- Majority of the victims were Graduates (28%).
- Socio economic status of the present study among the deceased belongs to upper lower class and the least is among upper class.

Conflict of Intrest: Nil

Source of Funding: Self Funding.

Ethical Clearance: Ethical clearance from the Institutional Ethical Committee obtained for the study.

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Lifelines as a Tool to Predict Death

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Abstract

The study of the relative positions of astronomical objects and their movements is called Astrology. It is used as a means for deriving information about terrestrial planets and affairs of humans. The fear of the unknown is the primary reason for the current fascination with horoscopes. The beliefs are different among different cultures and every country has their own belief systems. Some of the beliefs and superstitions of Indians are seen to have been passed on from generation to generation. The Aim of the study is to co-relate the death with life line. Plotting the data against the age for correlation in scattered diagram it was observed that it did not reflect the life span of the individuals. Though people have belief in different aspects of astrology, this study proves that there is no scientific basis to the claims of palmistry.

Key Words: Astrology, Palmistry, Life line, Death

Introduction

In India 50% of the population is below the age of 25 years and the expected average age of an Indian will be 29 years by 2020[1]. India stands 165th in the life expectancy of the citizens with 69.09 years.[2] Hence the loss of life would affect the productivity of the state. This study is imminent, as it analyses the lifeline that could be used in predicting the death.

The study of the relative positions of astronomical objects and their movements is called Astrology. It is used as a means for deriving information about terrestrial planets and affairs of humans.[3] The origin of the word Astrology is from ‘Astrologia’ which is a Latin word ‘that means the account of the stars. Astrology is a language that symbolizes a form of art and ‘clairvoyance’. Astrology, all throughout the history of astrology, has been closely related to medicine, astronomy, thaumaturgy and meteorology.[4] The scientific value of astrology was questioned with the onset of the scientific revolution and it has been challenged successfully both on theoretical and experimental grounds. Certain studies have shown it not to have any scientific validity.[5] Thus Astrology is said to have lost its academic and conceptual standing, and the belief in it of people has seen a decline.[6]

The fear of the unknown is the primary reason for the current fascination with horoscopes. Some of the beliefs and superstitions of Indian are seen to have been passed on from generation to generation. The faith in Astrology stems from the objective that predictions given will help to protect them from evil spirits.[7]

Astrology is an integral part of Indian culture. In today’s time also, people prefer to start auspicious things such as taking the first step into a newly made home (Gruhapravesha), buying a new vehicle, its number, getting a marriage proposal fixed, selecting a marriage date, to begin a new business etc, according to their beliefs in astrology. Superstitions are seen not only in India, but it is present all over the world. The beliefs are different among different cultures and every country has their own belief systems. Though some believe these superstitions and follow them blindly, there are others, who do not believe in them and just ignore them.[7]

Palmistry is believed to have been originated from India, is more than 3000 years old. It includes studying
various aspects of the hand of an individual such as the shape and size of the hand, configurations and contours of the palm, finger shape, size and also features of the fingernails. It is said that at least a hundred signs or marks are there that could help predict qualities, events or supposed relations. The basic assumption is that every minute detail of the hand has information that could help predict the individual’s present or future.\textsuperscript{[8]}

The closest relationship that palmistry bears to science as conceived by many is the branch of dermatoglyphics, defined as the study of fingerprints. It has helped discover genetic correlation between particular patterns of fingerprint and certain human diseases.

Palmistry is said to have some branches such as

1) “Medical” palmistry, states that there is an association between the hand pattern and the emotional or physical states of the individuals.

2) “Psychotherapeutic palmistry” associates the hands, character, and personality giving the positive and negative aspects of the individual.

The lines of the palms are divided in two categories – the major lines and the minor lines. There are a minimum of 14 major lines that include the lifeline, head line, heart line, and lines of marriage, children, intuition, fortune, fate, intuition and spirituality. The lines are also studied in terms of their size, shape, length, so on and so forth. The lifeline is a line that indicates the constitution and physical well-being of the individual. In general it gives the vitality of the individual while its length indicates natural life expectancy\textsuperscript{[9]}

**Aim of the Study**

1. To measure the length of the life line in both hands of those who died

2. To co-relate the death with life line (palmistry)

**Materials and Method**

This cross-sectional study was undertaken between April 2017 and September 2018 at the Department of Forensic Medicine & Toxicology, SRMC & RI, and Chennai. Socio demographic and other data was collected from the deceased relatives, friends and Police/Investigating officer who were present during the autopsy of the deceased using standardized proforma developed for the purpose.

The lifeline was correlated with the age at time of death (longevity) obtained from the inquest report /FIR

Lifeline length was measured by a single observer in 100 cases brought to the Department of Forensic Medicine for autopsy. The lifeline lengths in both hands were measured by laying a stretched piece of thread or string along the line and then measuring its length using a ruler.

Cheiro was probably the first man to identify palmistry as seen in his work ‘Undisputed Doyen of Palmistry’. From his work on lifeline it is understood that it runs from a place at the meeting point of the radial end of the hand and distal end point of lifeline to the junction of proximal end of lifeline and palmar crease. This maximum lifeline length called the maximum potential lifeline was also measured as a control to account for the difference in hand sizes. The corrected life line ratio was thus calculated as the ratio of actual lifeline length to its potential maximum.

**Results**

In the theory of palmistry the length of life line in hand refers to longevity of a person. The life line extends from the corner of the palm above the thumb and travels towards the wrist in an arc. This lifeline in both hands were measured in all ages and compared with the actual life span of the deceased. The length of the lifeline in the left hand divided by the maximum length of left hand gave the left hand ratio. Plotting the data against the age for correlation in scattered diagram it was observed that it did not reflect the life span of the individuals. In the similar manner the right hand was analysed, which showed similar results.

**Left hand life line ratio: Chart– 1**
Discussion

From the time, Palmistry has been quoted in Indian and Chinese manuscripts, some 3000 years ago; the scientific validity of it has always been questioned. It gained peak popularity with intensive work done by society palmist Louis Hamon, known by his false name of Cheiro.\[10\]

Theoretically the length of life line in hand is related with the longevity of an individual in palmistry. The length of the life line is measured in both hands and it is compared with the actual life span of the deceased. The length of the lifeline in the left hand divided by the maximum length of left hand gave the left hand ratio. Plotting the data against the age for correlation in scattered diagram it was observed. In the similar manner the right hand was analyzed, in many cases which were studied had the same life line ratio but were of different ages and it was seen that left hand life line ratio and right hand life line ratio individually also had no correlation with longevity of a person.

This is contradicting the observations made by P G Newrick et al in their study which states that a strong statistical correlation exists between the life line length and the age up to which a person lived. This was particularly true for the correlation of the age at death and the right hand lifeline length.\[11\]

Conclusion

Though people have belief in different aspects of astrology, this study proves that the there is no scientific basis to the claims of palmistry.

In palmistry aspect the lifeline ratio of the subjects was found to have no correlation with the longevity of a person. This clearly disproves the claims in the palmistry.

Conflict of Interest: Nil

Source of Funding: Self Funded

Ethical Clearance: Ethical clearance was obtained from the Institutional Ethics Committee

References

Agar-Paraffin Double Embedding Over Conventional Embedding for Minute Oral Biopsies- Cohort Study

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1 Postgraduate, 2 Professor and Head of the department, 3 Professor, 4 Professor, 5 Reader, 6 Senior Lecturer; Dept. of Oral Pathology, Saveetha Dental College & Hospitals, Saveetha University

Abstract

Agar-Paraffin double embedding technique is a simple technique that combines the advantages of both the embedding material. It preserves the minute tissue biopsies in orientation and holds them together from getting lost. Oral biopsies, being most commonly small incisional tissues, have to be preserved all through the processing and embedding to ensure optimal visualization of all the mucosal layers without compromise. In the present study, samples were divided into two groups: Group A: Agar-paraffin double embedding (APE) and Group B: conventional paraffin embedding (PE). Tissue samples collected were sectioned into two approximately equal sized bits. Both the bits were simultaneously processed, embedded in two different techniques (APE and PE method). Sections obtained were scored by an observer and analyzed using independent sample t-test (SPSS software version 21) to evaluate the efficacy of agar-paraffin double embedding technique in comparison to the conventional paraffin embedding technique. Agar paraffin embedded tissue was found to be well processed, firm and well preserved. Orientation was comparatively easier and the blocks yielded sections of good quality. They showed no interference with staining and cell morphology was of good clarity. Thus Agar-paraffin embedding technique represents a simple, reliable method that can greatly improve the quality of diagnostic information.

Key words: Agar-Paraffin, double embedding, oral biopsies

Introduction

Agar-Paraffin double embedding refers to the technique whereby the tissue is first impregnated with agar and subsequently blocked in Paraffin to combine the advantages of both the embedding media [1]. Use of Agar-Paraffin embedding for small specimens in routine surgical pathology was first reported in 1959, which was developed further by Herbert Z Lund in 1961 [2]. Agar-formalin solution to aggregate fragments of tissues prior to paraffin embedding was described by Cook and Hotchkiss in 1977 [3].

Oral mucosal biopsies, being the small incisional ones, in common instances poses frequent problem in appropriate orientation. Proper visualization of mucosal layers is most necessary to accurate diagnosis. Although Agar-Paraffin double embedding holds numerous advantages, it remains little used technique in oral pathology laboratory.

The present study aims to assess the efficacy of Agar-paraffin double embedding technique in comparison with conventional paraffin technique to emphasize the usefulness of the technique in handling small oral specimens.

Materials and Method

Ethical approval was obtained from the institution standard review board prior to the commencement of the study and oral mucosal biopsies were prospectively collected during gingivectomy and operculectomy procedures (Prospective experimental study). Each
specimen obtained was sectioned into two approximately equal sized bits (Figure 1b). One bit was given to Group A- agar-paraffin embedding group, APE and the other bit to Group B - conventional paraffin embedding group PE. Group A and B specimens were processed together and subsequently embedded in paraffin. Except for the difference that Group A specimens (eight samples) were embedded in 3% agar (type 1 bacteriological agar) prior to processing. Both the tissue sections were scored and analyzed by an observer who is blind to the choice of the procedure. All the data obtained were tabulated and statistically analyzed using independent sample t-test (software: IBM SPSS Statistics version 21).

Preparation of Agar-Paraffin (APE) blocks

3% Agar solution was prepared by adding 3gms of Type I agar (extra pure bacteriological grade Type I agar, GRM666 - HIMEDIA research lab, Mumbai, India) to 100 ml of distilled water in a conical flask and bringing to a boil on continuous stirring. Stirring was kept until the solution appeared clarified and no particle of agar was visible (Figure 1). The agar solution was slowly cooled to room temperature. Then using a dropper/ micropipette the solution was added slowly in the L-mold containing the fixed/unfixed minute oral specimen in correct orientation to cover the entire specimen. After cooling and solidifying the agar block (Figure 2) was (put for fixation if unfixed) processed in the usual way and subsequently embedded with paraffin. It was made sure at least 3mm of agar surrounds the tissue in all directions. The remaining agar are stored in a beaker and maintained in the laboratory refrigerator. As needed, sufficient quantities are re-liquefied by heating.

Fixation, Processing, Sectioning & Staining

Both the Group A and Group B tissue samples were fixed by leaving the tissues in 10% neutral buffered formalin overnight.

These samples were simultaneously processed without any difference in dehydrant (isopropyl alcohol), clearing (2 changes of xylene), infiltrated in molten paraffin wax and subsequently embedded in paraffin. 2-3 \( \mu m \) thick section of each block were taken, stained with routine hematoxylin and eosin and cover slipped with DPX mounting medium.

Results

Conventional oral biopsies as small as 0.2 to 0.5 cm were processed by this technique. These biopsies are further reduced in tissue volume and surface area to nearly half during formalin fixation and paraffin processing making it difficult to identify embedding surface. Agar paraffin Embedding technique has helped to overcome this problem as the fresh tissue has already been embedded in agar before processing. The colorless Agar block was easily viewed in liquid and gel forms, orientation of the biopsy was easily monitored. Agar had not interfered with the penetration of fixative or processing chemical reagents, thus all the APE tissues were well processed.

There were eight sections in each group [Group A-Agar paraffin embedded sections (APE) and Group B- Paraffin embedded sections (PE)]. The sections obtained from each group (Figure 4) were scored in observer score sheet based on quality of section, staining properties, optimal orientation and cell morphology. The results are summarized in the Table 1.

The mean score for section quality of APE sections was 1.63±.744 whereas those for PE sections was 1.50±.535 (p= 0.228). The mean score for staining clarity of APE sections was 1.88±.354 whereas those for PE sections was 1.38±.744 (p= 0.052). Both the APE & PE sections showed a mean score of 0.88±.354 (p= 0.052) for orientation. The clarity of the cell morphological characteristics of APE and PE sections were with average score of 1.5±0.535 and 1.38±0.518 respectively (p= 0.744).

Table 1: T-test for parameters comparing agar-paraffin double embedding and Paraffin embedding methods.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Sub-criteria</th>
<th>Agar-paraffin section (total 8)</th>
<th>Paraffin sections (total 8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality of sections</td>
<td>Torn</td>
<td>1 (12.5%)</td>
<td>0</td>
<td>.228</td>
</tr>
<tr>
<td></td>
<td>Wrinkled</td>
<td>1(12.5%)</td>
<td>4 (50%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>6 (75%)</td>
<td>4 (50%)</td>
<td></td>
</tr>
</tbody>
</table>
**Table 1: T-test for parameters comparing agar-paraffin double embedding and Paraffin embedding methods.**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Agar embedding with Unfixed specimen (total 4)</th>
<th>Agar embedding with Formalin fixed specimen (total 4)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality of sections</td>
<td>2.00</td>
<td>1.25±.957</td>
<td>0.080</td>
</tr>
<tr>
<td>Clarity of stain</td>
<td>2.00</td>
<td>1.75±.500</td>
<td>0.006</td>
</tr>
<tr>
<td>Orientation</td>
<td>.75±.5</td>
<td>1.00</td>
<td>0.058</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>1.25±.5</td>
<td>1.75±.5</td>
<td>0.006</td>
</tr>
</tbody>
</table>

**Table 2: T-test for Agar embedding with unfixed and fixed specimens**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Agar embedding with Unfixed specimen (total 4)</th>
<th>Agar embedding with Formalin fixed specimen (total 4)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity of stain</td>
<td>2.00</td>
<td>1.75±.500</td>
<td>0.006</td>
</tr>
<tr>
<td>Orientation</td>
<td>.75±.5</td>
<td>1.00</td>
<td>0.058</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>1.25±.5</td>
<td>1.75±.5</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Figure 1: a. Preparation of 3% agar solution, b. sectioning of oral biopsy specimen to equal sized bits, c. orientation of specimen bit held in L-mold around which molten agar poured.

Figure 2: Prepared Agar pre-embedded block along with the corresponding bit for conventional embedding.
Embedding involves enclosing properly processed, correctly oriented specimens in a support medium that provides external support during microscopy. Agar stabilizes the specimen by penetrating and replacing the intercellular fluid between the cells of the mucosa. It forms the cheapest source of embedding medium.

Agar is good choice for tissue embedding because of its property of hysteresis as it remains solid at 36°C±1.5°C, which remains firm even at 60-65°C thus holding the tissue firm and oriented in molten paraffin wax. Agar melts only at 87°C±1.5°C, a temperature range which tissue processing never reaches. Being a product of cell wall polysaccharides, Agarose and Agaropectin has an easy melting and good gel stability properties even at high temperatures. It is a common gelling agent used in various industries.

Improper choice of embedding medium can cause difficulty in section cutting resulting in poor quality of tissue sections. Among the faults in section cutting, crumbling of sections into fragments, wrinkling or folds are common. We found that Agar-Paraffin sections showed 25% more good quality sections compared to paraffin sections. This could be attributed to the strong penetration of tissue by dual materials, agar followed by paraffin, forming a homogeneous block from which sections could be cut easily. Improved cohesion of tissue layers and plasticity given by agar with facility of better ribboning characteristics offered by paraffin are combined to produce sections without inclusion of much of artifacts.

Staining processes are used to give tissue contrast and color before they are examined with the microscope. Presence and absence of embedding medium during staining not only manipulate sections but also result in coloration of the embedding media. The staining characteristics of APE were better than the PE sections by 37.5%. This data proves that the agar was better cleared away from the tissues unaffecting the senility and specificity of the stain used. Moreover agar does not generally stain with hematoxylin and eosin unlike media like gelatin.

In our study, we had used acrylic color paints (Faber-Castell 6 Fabric colors Acrylic paint PC: 1410501, Mumbai, India) to mark the margins of the specimens and found that inking on the specimens were well preserved all through out the processing by agar embedding whereas the inking materials over the specimen margins was lost during conventional processing as the specimen is passed through successive processing solutions. Thus it adds on the credit of using even the cheapest and easiest mode of inking, which can be preserved by the Agar- Paraffin embedding technique.

Improper embedding due to change in shape of the tissue during the processing can result in tangential sectioning which may interfere with reading or cause incorrect critical measurement such as thickness of the tumor. Often difficulty of identifying embedding surface on the minute oral specimens (~0.2-0.4cm) is produced due to shrinkage produced by formalin fixation and paraffin embedding. In the study, Agar held the specimen intact through the processing and since the orientation is also maintained which is accordance with previous studies, it yielded better quality of sections apart from saving the time for the pathology
technicians. Although, we found no significant difference between the tissue orientation achieved by Agar – Paraffin and conventional paraffin embedding, ease of orientation had been better with pre-embedding in agar.

The tissue processing procedure should preserve the underlying tissue architecture giving more comprehensive view of disease and its effect on tissues [4]. APE sections produced sections with well-preserved, discernable cell features- 12.5% more than that achieved by PE sections. Mechanical processing can distort the shape of the specimen due to action of the processing chemicals, which was prevented by agar block.

In the study, we had subdivided group A specimens (8 nos.) into two subgroups A1, A2 (4 no each). A1 group specimens were first embedded in agar followed which they were fixed in 10% neutral buffered formalin for a period of 24 hours whereas A2 group specimens were fixed (24 hours) prior to agar embedding. While the A1 group produced good quality sections with better staining characteristics, orientation and cellular morphology was better with A2 group [Table 2]. It was evident from the A1 sections that formalin had penetrated and fixed the tissue through the pores present in the agar. Cumulative intercellular tissue stabilization, removal with ease during de-waxing procedure and other physical characteristics of agar was responsible for section quality and staining character. The cellular morphology could have been compromised in A1 compared to A2 group due to probable imbibition of water from aqueous formalin solution or due to autolytic changes produced by the delay in the fixation during the process of agar embedding.

Certain slides of Agar-Paraffin showed edematous appearance of the cells showing the imbibition of water by agar. Agar blocks tend to hold more water even at high concentrations (due to imbibing action of agar). Increased duration in the dehydrating and infiltrating medium while tissue processing rectified the problem.

**Conclusion**

The Agar-paraffin embedding technique represents a simple, reliable user-friendly method that can greatly improve the quality of diagnostic information one can obtain from minute biopsies by improving tissue orientation, quality of sections and shortened turn around time. This study is the first comparative study to prove the efficacy of Agar-Paraffin double embedding over conventional Paraffin embedding.

**Conflicts of Interest** – Nil

**Source of Funding** – Self-funded

**Ethical Clearance**- Taken from Institutional standard review board (Approval no: SRB /SDMDS16 OMP/02).

**References**

Severity Grading, Management, Outcome and Epidemiological Profiling of Snakebite Victims in Tertiary Care Hospital in South India: 5 Years Retrospective Study

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Abstract

Objective: We conducted this study to do epidemiological and clinical profiling of the patients with snake bite in a tertiary care referral centre, situated in suburban area of Dakshin Kannada district, Karnataka, south India.

Materials and Method: This retrospective, record based study was done by analysing medical records of all the patients of snake bite admitted over 5 years from June 2015 to July 2019. It included demographic details and clinical profiling. Based on presenting complaint, clinical sign/symptoms and laboratory reports snake envenomation patients were divided into neurotoxic and hemotoxic group. The severity grading, amount of ASV used and outcome assessed in both the groups.

Results: Total 108 patient records were analysed, maximum of which were males (64.8%) in age group of 15-30 years (46.2%). In majority of the cases the species of snake was not identified (67.5%). Dry bites were reported in 40.7% cases, among envenomated cases, 68.7% presented with hemotoxic syndrome, 29.7% with neurotoxic syndrome and 0.9% had both the features. In all envenomated patients with increasing bite to needle time, the grade of severity, amount of used ASV, duration of hospital stay and complications were found to increase.

Conclusion: Snake bite is a neglected life threatening condition, which is a preventable disease. Tourniquet is still very commonly used first aid technique, which can be deleterious. The lack of education and awareness about snake bite management was evident and active mass education is clearly needed.

Key words: Snake envenomation, severity grading, Clinical profile and outcome

Introduction

There are almost 216 species of snakes in India and out of these only 60 are considered as poisonous. In India, there are mainly four species of snake majorly poisonous; cobras or Naja naja, krait or Bungarus caeruleus, Russell’s viper or Daboia russelli, and saw-scaled viper or Echis carinatus. All snake bite does not lead to envenomation, 70% of all snakes are non poisonous and poisonous snakes envenomate only in 50% of cases. Snake envenomation is a medical emergency. In 2009 WHO enlisted snake bite as neglected tropical condition. According to WHO report, maximum number of snake bite has been reported from South Asia, Southeast Asia, and sub-Saharan Africa. Every year highest number of mortality due to snake envenomation occurs in India, which ranges from 5,000 to 13,000 cases annually. The exact mortality due to snake bite may be even higher in India as many deaths go unreported in rural areas.
Clinico - toxicologically, nature of snake envenomation is categorized into hemotoxic, neurotoxic, and myotoxic syndromes\(^7\). We undertook this research for clinico-epidemiological profiling, and to find the risk factors which affects the outcome of different snake envenomation.

**Methodology**

The present retrospective, record based study was done in Srinivas Institute of Medical Science & Research Centre (SIMS&RC), on all the patients of snake bite admitted over a period of 5 years, from July 2015 to June 2019. It is the only tertiary care health facility in 20 km area, serving population of Mangalore city, suburban outskirts of Mangalore city, rural population of Udupi district, adjoining districts of coastal Karnataka and northern districts of Kerala.

Recorded information was entered in a pre-coded proforma which included demographic and clinical data. The data related to part of the body which was bitten, any first aid/ traditional treatment received, time to reach hospital after snake bite and time in receiving ASV after bite (bite to needle time) were collected for the entire patient. If snake species was identified at time of bite, that was also noted.

It is the hospital policy to admit all snakebite patients to Intensive care unit (ICU) and get done complete blood count, coagulation profile, renal function test, serum electrolytes including sodium, potassium and chloride. Patients were followed for 24 hours for “dry bite” confirmation and discharged after a day. In ICU, the 20 WBCT (whole blood clotting test) was done to assess venom induced defibrinogenation\(^8\) and used as a guide for further dosing of ASV in hemotoxic patients. Patients with signs of envenomation were administered ASV, which is known to cause allergic reaction, shock, anaphylaxis and serum sickness, these were also looked in the records.

Cobras and krait both belongs to elapid family and their envenomation leads to neurotoxic and cardiototoxic features\(^8\), local effects are supposed to be more common with cobra envenomation but by medical record evaluation it was difficult to differentiate both species. Russell’s viper and saw-scaled viper venoms are vasculotoxic\(^8\) and produce severe necrotising local effects. So for evaluation, all the patients of envenomation were majorly divided into two groups, neurotoxic group and hemotoxic group. According to the presenting complaints we tried to grade the severity of snake bite from grade 1 (mild) to grade 4 (very severe) in both hemotoxic and neurotoxic group, based on Kumar et al study\(^9\). The amount of ASV received according to the grade of severity also noted.

**Results**

A total of 108 patients of snake bite were admitted to the hospital during the study period. The maximum numbers of snake bite patients were reported in month of July-September and none in the months of January and February. Most of the patients were bitten during 6-11pm (77, 71.29%), at residence and roadside while walking (76, 70.37%) and in lower extremity (67, 62.03%).

**Table 1: Demographic details**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>70 (64.81%)</td>
</tr>
<tr>
<td>Female</td>
<td>38 (35.18%)</td>
</tr>
<tr>
<td>Age distribution (years)</td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>12 (11.1%)</td>
</tr>
<tr>
<td>15-30</td>
<td>50 (46.29%)</td>
</tr>
<tr>
<td>30-45</td>
<td>19 (17.59%)</td>
</tr>
<tr>
<td>45-60</td>
<td>20 (18.51%)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>7 (6.48%)</td>
</tr>
<tr>
<td>Months</td>
<td></td>
</tr>
<tr>
<td>January-March</td>
<td>10 (9.25%)</td>
</tr>
<tr>
<td>April- June</td>
<td>28 (25.92%)</td>
</tr>
<tr>
<td>July-September</td>
<td>40 (37.03%)</td>
</tr>
<tr>
<td>October-December</td>
<td>30 (27.77%)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
</tr>
<tr>
<td>Students</td>
<td>42 (38.88%)</td>
</tr>
<tr>
<td>House wife</td>
<td>30 (27.77%)</td>
</tr>
<tr>
<td>Private job</td>
<td>25 (23.14%)</td>
</tr>
<tr>
<td>Plantation worker</td>
<td>11 (10.18%)</td>
</tr>
<tr>
<td>Educational status</td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>18 (16.66%)</td>
</tr>
<tr>
<td>Students</td>
<td>29 (26.85%)</td>
</tr>
<tr>
<td>Completed primary education</td>
<td>61 (56.48%)</td>
</tr>
<tr>
<td>Alternative treatment</td>
<td></td>
</tr>
<tr>
<td>No first aid</td>
<td>73 (67.59%)</td>
</tr>
<tr>
<td>Tourniquet</td>
<td>32 (29.62%)</td>
</tr>
<tr>
<td>Others( lemon juice application)</td>
<td>1 (0.92%)</td>
</tr>
</tbody>
</table>
One patient had reached hospital after 3 days due to cellulitis at the site of bite; she had earlier received ASV in some other centre, as all the information was present in the medical record so she was not excluded from the study. In majority of the cases the cause of delay was because of not considering it dangerous. Maximum patients did not receive any first aid and 2 (1.8%) patients arrived with immobilisation.

Table 2: Snake species identification and site of bite details

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species of snake</td>
<td></td>
</tr>
<tr>
<td>Rusell’s viper</td>
<td>22(20.37%)</td>
</tr>
<tr>
<td>Krait</td>
<td>10(9.25%)</td>
</tr>
<tr>
<td>Non poisonous snake</td>
<td>2(1.85%)</td>
</tr>
<tr>
<td>Sea snake</td>
<td>1(0.92%)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>73(67.59%)</td>
</tr>
<tr>
<td>Fang marks</td>
<td></td>
</tr>
<tr>
<td>Two</td>
<td>65(60.1%)</td>
</tr>
<tr>
<td>Scratches</td>
<td>21(19.4%)</td>
</tr>
<tr>
<td>Four</td>
<td>1(0.9%)</td>
</tr>
<tr>
<td>No definitive mark</td>
<td>21(19.4%)</td>
</tr>
</tbody>
</table>

Dry bite was confirmed in 44 (40.74%) patients. Out of 64 cases of snake envenomation, 44(68.75%) were categorised as hemotoxic and 19(29.7%) were categorised as neurotoxic syndrome. One patient had presented within half an hour of Krait bite in hand which was sustained while cutting tree, two fang marks were present, profusely bleeding from the site of bite, tourniquet was tied, drooping of eyelid, difficulty in speaking, blurred vision and gait abnormality were present. On investigation, coagulopathy was present (INR 7.8). He left against medical advice in view of financial constrains.

Severity grading of hemotoxic envenomation, management and complications

Pain was found to be the most common presenting complaint in 41(93.1%) patients, followed by swelling in 29(65.9%), discolouration in 28(63.63%), local rise of temperature in 12(27.27%) and blisters in 4(9.09%) patients respectively. Bleeding from the fang wounds, hematemesis and hematuria were present in 5(11.36%), 4(9.09%) and 5(11.36%) patients respectively. Coagulopathy and cellulitis were reported to be the most common complication in 39(88.63%) and 24(54.54%) patients. Ulceration in the limb, renal failure, sepsis, necrotising fascitis and compartment syndrome were other complications noted in 10(22.72%), 8(18.18%), 8(18.18%), 5(11.36%) and 4(9.09%) patients respectively. All the cases of renal failure managed conservatively. Surgical interventions were needed in 11(25%) patients. Blood products were transfused in 27(61.36%) patients. No mortality recorded.

Table 3: Severity grading of hemotoxic group

<table>
<thead>
<tr>
<th>Grade</th>
<th>Total number of patients</th>
<th>Mean ASV vials used (range of vials)</th>
<th>Requirement of ventilator support</th>
<th>Duration of hospital stay in days, mean(range)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>5</td>
<td>11(10-15)</td>
<td>0</td>
<td>4.2(3-5)</td>
<td>0</td>
</tr>
<tr>
<td>Grade 2</td>
<td>14</td>
<td>15.71(10-20)</td>
<td>0</td>
<td>5.71(5-9)</td>
<td>0</td>
</tr>
<tr>
<td>Grade 3</td>
<td>15</td>
<td>24(10-40)</td>
<td>0</td>
<td>6.3(5-9)</td>
<td>0</td>
</tr>
<tr>
<td>Grade 4</td>
<td>10</td>
<td>33(30-45)</td>
<td>0</td>
<td>7.8(5-28)</td>
<td>0</td>
</tr>
</tbody>
</table>

Severity grading of neurotoxic envenomation, management and complications

Ptosis was found to be the most common presenting complaint in 18(94.73%) patients, followed by external ophthalmoplegia in 11(57.89%), pain in 6(42.85%), swelling in 6(42.65%), abdominal pain in 4(21.05%), vomiting in 3(15.78%), discolouration in 3(15.78%), blisters in 3(15.78%), local rise of temperature in 3(15.78%), bulbar weakness in 3(15.78%) and gait
abnormality in 2(10.52%) patients respectively. Significant respiratory weakness was found in 2 patients, they were managed by endotracheal intubation. They improved after ASV administration and extubated after a day. Renal failure, cellulitis and gangrene were other reported complications in 4(21.05%), 4(21.05%) and 1(0.95%) patients respectively.

**Table 4: Severity grading of neurotoxic group**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Total number of patients</th>
<th>Mean ASV vials used (range of vials)</th>
<th>Requirement of ventilator support</th>
<th>Duration of hospital stay in days, mean(range)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>7</td>
<td>12.85(10-15)</td>
<td>0</td>
<td>4.85(4-5)</td>
<td>0</td>
</tr>
<tr>
<td>Grade 2</td>
<td>6</td>
<td>17.5(15-25)</td>
<td>0</td>
<td>6.5(4-12)</td>
<td>0</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grade 4</td>
<td>6</td>
<td>34.16(25-40)</td>
<td>2</td>
<td>7(5-10)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 5: Correlation of bite to needle time (time to receive ASV after snake bite) and severity grading**

<table>
<thead>
<tr>
<th>Bite to needle time</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6 hours</td>
<td>12</td>
<td>18</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>6-24 hours</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>&gt;24 hours</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 6: Amount of ASV used in hemotoxic and neurotoxic group in relation to tourniquet**

<table>
<thead>
<tr>
<th>Hemotoxic group</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of ASV used(mean) in patients with tourniquet</td>
<td>15 (n=1)</td>
<td>20(n=5)</td>
<td>38(n=5)</td>
<td>45(n=2)</td>
<td>29.5</td>
</tr>
<tr>
<td>Amount of ASV used(mean) in patients without tourniquet</td>
<td>10(n=4)</td>
<td>13.3(n=9)</td>
<td>17(n=10)</td>
<td>35(n=8)</td>
<td>18.82</td>
</tr>
</tbody>
</table>

Neurotoxic group

| Amount of ASV used(mean) in patients with tourniquet | 15(n=1) | 20(n=2) | 40(n=3) | 25 |
| Amount of ASV used(mean) in patients without tourniquet | 12.5(n=6) | 16.25(n=4) | 28.3(n=3) | 19.01 |
The mean dose of ASV which was given for the neurotoxic snake bites was 21.5 vials (range 10-40 vials) and 20.92 vials (range 10-45) were given for the hemotoxic bites.

Allergic reactions to ASV were noted in 8 (12.63%) cases (pyrexial reaction in 6 cases, and urticarial reactions in 2 cases).

**Risk factor assessment**

Delay in presentation to hospital lead in increase in grade of severity. Application of tourniquet lead to increased requirement of ASV.

**Discussion**

The study was conducted in SIMS&RC, which is situated in suburban area of Mangalore, Dakshin Kannada district, Karnataka, South India. The Dakshin Kannada district is sheltered by Western Ghats on the east, which is the only remaining rain forest in India and home to several species of reptiles.

We explored medical records of 108 snakebite victims and found that mostly young males in age group of 15-30 years were victims bitten by the snake in the lower limb, which may be because ambulant population are at higher risk of snake bite. The maximum bites reported in months of July – September, which is the monsoon season and activity of snake increases during that season, which was in close agreement to previous studies. Most of the patients were bitten during the time of dusk and night at residence and roadside while walking, which was due to diminished vision and accidentally stepping on the snakes. Occupationally most of the victims were students and house wives, which were unlike other studies where agriculture was most common occupation. It may be the result of occupational difference in the population. Agriculture is not a major occupation of people in Dakshin Kannada.

Contrary to the major believe that all snake bite results in envenomation, we found that 40.7% patients were either bitten by non poisonous snakes or there was no envenomation (dry bite) in case of poisonous snake bites. The studies conducted in other states of India, like Kerala found 66% dry bite cases and in Himachal Pradesh it was found to be 43%.

In current study, maximum patients reported to hospital within 6 hours of bite and early arrival lead to lesser degree of severity. The amount of ASV used was found to be higher in severe grades and duration of stay was also longer due to development of complications. The direct correlation of early presentation and better outcome has earlier been demonstrated in other studies. There was no mortality reported in our study which could be because of early presentation of the patient to the hospital.

In current study, 29.6% patients came with tourniquet, in other studies conducted in South India almost 30-50% patients came with tourniquet application. The tourniquet is known to be potentially dangerous in snake bite cases as it leads to gangrene, increased fibrinolysis, bleeding in the occluded limb, peripheral nerve palsies and intensification of local envenomation. In current study it was found that the amount of ASV used in same grade of severity was more in the patients with tourniquet application in both hemotoxic and neurotoxic groups.

Maximum patients (73, 67.59%) did not receive any first aid. The knowledge to immobilise the bitten part of the body, as muscle contraction promotes venom absorption, was majorly lacking. Only 2 patients arrived with splint. The need of mass education about first aid of snake bite was obvious.

Hemotoxic syndrome was more common among envenomated patients that neurotoxic syndrome, which could be the result of more prevalence of Viper snakes in the region. This finding was in close accordance to studies conducted in neighbouring regions. The average ASV used was more in neurotoxic group, which may be because the elapid venom are small molecules, gets rapidly absorbed in the blood stream and needs higher dose of ASV for neutralisation.

In hemotoxic group, coagulopathy was most common complication followed by cellulitis, ulceration in the limb, renal failure, sepsis, necrotising fasciitis and compartment syndrome. These results were almost similar to other studies conducted on viper snake bite patients. In neurotoxic group; ptosis, external ophthalmoplegia, pain, swelling at the site of bite, abdominal pain, vomiting, discolouration, blisters, local rise of temperature, bulbar weakness and gait abnormality were presenting features, which were comparable to other studies done earlier.

To summarise, India is capital of snake bites so we need to focus on mass education about first aid and importance of early arrival in management of snake bite.
The amount of venom absorption can be decreased by immobilisation and early administration of ASV can cure the disease. The alternative treatment in any form can be deleterious and can make the prognosis poor.

**Limitation:**

It was a retrospective study so few data may be missing. Snake species identification was limited to very few cases so species specific severity grading could not be done. As neuroparalytic features were common to both Cobra and Krait, it was difficult to differentiate both the species based on medical record data evaluation, so both analysed together.

**Financial support and sponsorship:** Nil.

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

**Ethical Approval:** The study was approved by the ethics committee of Srinivas Institute of Medical Sciences & Research Centre, Mangalore.

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Study of Organo-Phosphorous Compound Poisonings with Blood Sugar Levels in Telangana Population

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Duragapur, (West Bengal)

Abstract
85 adult patients aged between 23 to 60 years of age were admitted at emergency wards were studied. These patients were known diabetic since 2 to 5 years, The clinical manifestation were 6(7.0%) had anxiety, 7(8.23%) had giddiness, 5(5.88%) had emotional liability, 7(8.23%) had slurred speech, 4(4.70%) had ataxia, 9(10.5%) had drowsiness, 5(5.88%) had confusion, 6(7.0%) had headache, 8(9.41%) had coma, 6(7.0%) had absence of reflexes, 12(14.1%) had dyspnea, 10(11.7%) had hypotension. The random blood sugar level in 48(56.4%) had 60-200 mg% and 37(43.5%) had > 200 mg%. This pragmatic approach to patients of oregano phosphorus patients who were diabetic and under anti diabetic treatment will be quite useful to physician and medico-legal expert to treat efficiently to avoid morbidity and mortality in Diabetic patients because diabetic mellitus is a silent killer and challenge to the clinicians globally.

Key words - Op = Organo-phosphorous compound DM = Diabetes Mellitus, Blood sugar, Telangana.

Introduction
Organo phosphorus (OP) Compounds have been widely used in agriculture for crop protection and pest control since few decades. Some of these compounds also has been used in the medical treatment of myasthenia gravis. These are DFP (diisopropyl phosphorofluoridate), TEPP (tetraethyl pyrophosphotetramide). Some of OP esters are also used to treat glaucoma (Ecothiopate). In addition to this veterinary medicine OP compounds such as tabun, sarin, soman and VX are used as nerve gases in chemical warfare. They are also used as stabilizers in lubricating and hydraulic oils flame retardants, and gasoline additives.

Acute poisonings with OP compound is quite common in India, generally with intention of suicidal ingestion. Because it is widely available for different uses. Mortality due to OP compound ranges from 4 to 38% in Indian studies. But in survival patients also suffer with health hazards. Hence attempt was made to study the OP compound poisonings in known diabetic patients.

Material and Method
85 patients aged between 23 to 60 years were admitted at emergency ward of Osmania government hospital which was attached to Bhaskera medical college, Yennukapally, Moinabad, Hyderabad. Due to consumption of organo phosphorous compound poisoning, as witnessed by the attenders. The history collected from the relatives (attendants) was that, they were under treatment of anti diabetic. As they had known history since more than 2 to 5 years.

Their physical examination was done, Ryle’s tube aspiration followed by ET tube with stomach wash was given. Before starting specific treatment with antidote atropine, oximes and I V fluids, Blood was drawn for random blood sugar levels, and calculated by glucose oxidase GOD-POD end point caloric metric method.

The patients consumed alcohol other poisons and under treatment of anti-psychiatric were excluded from the study. The ratio of male and female patients were 3:1.
The duration of the study was about 4 years (2010-2014)

**Observation and Results**

Table-1 clinical manifestations of patients consumed OP compounds poison – 6(7.0%) had anxiety, 7(8.23%) had giddiness, 5(5.88%) had emotional liability, 7(8.23%) had slurred speech, 4(4.70%) had ataxia, 9(10.5%) had drowsiness. 5(5.88%) had confusion. 6(7.0%) had headache, 8(9.41%) had coma, 6(7.0%) had absence of reflexes 12(14.1%) had dyspnea 10(11.7%) had Hypotension.

Table-2 Random sugar levels 48(56.4%) had 60-200 mg% 37(43.5%) had > 200 mg%

**Discussion**

In the present study OP compound poisoning with blood sugar levels in diabetic patients in Telangana population. 6(7.0%) had anxiety 7(8.23%) had giddiness, 5(5.88%) had emotional liability 7(8.23%) had slurred speech, 4(4.70%) had ataxia, 9(10.5%) had drowsiness – 85, 5(5.88%) had confusion, 6(7.0%) had headache, 8(9.41%) were in coma state, 6(7.0%) had absence of reflexes, 12(14.1%) had dyspnea, 10(11.7%) had hypotension (Table-1) Random blood sugar levels, 48(56.4%) had 60-200 mg% and 37(43.5%) had > 200 mg% (table-2) these findings were more or less in agreement with previous studies.\(^{(6)(7)(8)}\)

due to potent and appropriate antidotes and proper stomach wash and adequate ventilations no patient could develop ketoacidosis because it was reported that, post OP poisoning there was keto- acidosis in previous studies because hypoglycemia was confirmed in previous studies because of acute pancreatitis can occur with OP intoxication.\(^{(11)}\). It was also observed that, majority of the patients had mental- illness and depression which abated them to consume OP poison hence such patients were referred to psychiatric counseling.

**Summary and Conclusion**

The present study OP compound poisoning in diabetes will be concluded that, proper dosage of antidote, adequate ventilation, proper stomach wash can prevent the rise in blood glucose level and avoid further complication which may end in to mortality of the patients. Moreover diabetics patients having insomnia, depression must consult psychiatrist which might avoid such undue complication because exact complications of D.M is still un-clear

This research work was approved by ethical committee of Bhaskera medical college, Yennukappally Moinabad, Hyderabad- (Telangana)

**Table-1: Clinical manifestation of patients consumed organo phosphorus compound poisoning**

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Particulars</th>
<th>No of patients</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anxiety</td>
<td>6</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>Giddiness</td>
<td>7</td>
<td>8.23</td>
</tr>
<tr>
<td>3</td>
<td>Emotional liability</td>
<td>5</td>
<td>5.88</td>
</tr>
<tr>
<td>4</td>
<td>Slurred speech</td>
<td>7</td>
<td>8.23</td>
</tr>
<tr>
<td>5</td>
<td>Ataxia</td>
<td>4</td>
<td>4.70</td>
</tr>
<tr>
<td>6</td>
<td>Drowsiness</td>
<td>9</td>
<td>10.5</td>
</tr>
<tr>
<td>7</td>
<td>Confusion</td>
<td>5</td>
<td>5.88</td>
</tr>
<tr>
<td>8</td>
<td>Headache</td>
<td>6</td>
<td>7.0</td>
</tr>
<tr>
<td>9</td>
<td>Coma</td>
<td>8</td>
<td>9.41</td>
</tr>
<tr>
<td>10</td>
<td>Absence of reflexes</td>
<td>6</td>
<td>7.0</td>
</tr>
<tr>
<td>11</td>
<td>Dyspnea</td>
<td>12</td>
<td>14.1</td>
</tr>
<tr>
<td>12</td>
<td>Hypotension</td>
<td>10</td>
<td>11.7</td>
</tr>
</tbody>
</table>
Table-2: Study of Random blood sugar levels in organo- phosphorus compound in poisoning patients

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Random Blood sugar level</th>
<th>No of patients</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60-200 mg%</td>
<td>48</td>
<td>56.4</td>
</tr>
<tr>
<td>2</td>
<td>200 mg %</td>
<td>37</td>
<td>43.5</td>
</tr>
</tbody>
</table>

No Conflict of Interest

No Funding.

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Correlation and Comparison of Cheiloscopy, Dactyloscopy and Palatoscopy with Blood Groups among Dental Students From Western Maharashtra

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¹BDS Student, School of Dental Sciences, ²Senior Lecturer, Department of Oral Pathology & Microbiology, School of Dental Sciences, ³Associate Professor, Department of Conservative & Endodontics, School of Dental Sciences, ⁴Associate Professor, Department of Oral Pathology & Microbiology, School of Dental Sciences, KIMS “Deemed to be University”, Karad

Abstract

Personal identification is of paramount importance in the forensic odontology for ethical, humanitarian and criminal investigations. In forensic odontology, scientific methods using DNA is considered to be the gold standard method of identification; however, it’s expensive and time-consuming approach have circumvented the need for less expensive and faster methods of identification. The latest inexpensive approach of quantifying evidences for identification of suspect in crime is using lip print, fingerprint, palatal rugae & blood grouping and this has gained tremendous importance in the present days. Hence, the aim of the present study is to correlate and compare Cheiloscopy, Dactyloscopy and Palatoscopy with blood group and Rh factor. Method: A total of 200 dental students between the age group18 to 25 years were included in the study. Lip print, finger print and palatal rugae patterns with ABO blood groups were collected and compared using Chi square test. The data was analysed using SPSS version 10.0 statistical package. Results & Conclusion: Statistically significant correlation existed between lip print and ABO-Rh factors (χ²statistic = 37.56, p value <0.05) and between finger prints and ABO-Rh factors (χ² statistic = 30.6, p value < 0.05). No association was found between palatal rugae with ABO-Rhesus blood groups (χ² statistic = 17.71, p value >0.05).

Key words: ABO Blood groups, Cheiloscopy, Dactyloscopy, Palatoscopy, Personal identification, Rh factor.

Introduction

Proper identification enacts an important step in forensic odontology that is required for ethical, humanitarian and criminal investigations as well as in archaeology and paleo demographic support research studies.¹,²,³ Scientific methods using DNA are considered to be expensive and time-consuming approach, thus initiating the need for using less expensive and faster methods of identification. The methods using lip print, finger print and palatal rugae are considered to be unique to individuals and have gained tremendous importance in forensic odontology these days. Lip prints being unique to individuals, are formed by numerous elevations and depressions on the part of the lining mucosa called labial mucosa.³ The study of these lip prints is called as Cheiloscopy and was first noted in countries like Poland and USA.⁴ Fingerprint, on the other hand, once formed remain unchanged from birth till death. These raised portions of epidermis that are differentiated during 3rd or 4th month of embryonic life are best seen on palmer planter surface of human hands.⁵ Cummins in 1926 coined the term Dactyloscopy for study of these entities. ⁶ One more latest approach used for quantifying evidences of suspect in crime is studying the palatal rugae patterns present on the anterolateral aspect of hard palate. Due to its internal location in the head, these patterns usually, do not undergo any changes in one’s life, the only exception being its length. Yet another biological record that remains unchanged during the life time of a person is blood grouping. Considering all these points, we decided to conduct a study to correlate and compare Cheiloscopy, Dactyloscopy and Palatoscopy
with blood group and Rh factor.

**Material and Method**

A cross sectional study design was conducted among 200 dental students during the period of December 2015 to March 2017. The study was initiated after obtaining the ethical clearance committee approval from Krishna institute of medical sciences “deemed to be university”, Karad with ref no. KIMSDU/IEC/03/2015 dated 10/12/2015. All the participants belonging to age group of 18-25 years were included in the study design. The exclusion criteria were subjects with deformities of palate like cleft palate, traumatic injury on fingers, palate and lips, allergy to alginate impression material. Informed verbal consent was taken from the included participants. Convenience method of sampling was used for the purpose of randomisation to avoid any selection or performance bias.

For lip print

The lips of the subjects were cleaned and lipstick was applied on the lips. Lip imprints were recorded with usual rest position on A4 sheets (Royal Executive Bond, 85 gsm, premium white) and visualized using the magnifying glass (TAG 3TM, 50mm double reading glass optical graded lens with 5x and 10x magnifying capacity). The lip prints were classified according to Suzuki K and Tsuchihashi Y as type I, I’, II, III, IV, V. [7]

For finger print

The same subjects were asked to place his/her thumb over the stamp pad (CAMLIN COMPANY of size 157x 96mm). The finger prints were recorded on the A4 sheet by rolling their finger gently. Immediately after recording, the finger prints were interpreted using magnifying glass, based on Michael and Kucken’s finger print classification. [8]

For palatal rugae

Alginate (dentsply) impressions of maxillary arch was obtained and casts were made using dental stone (dentstone). The rugae patterns were traced on these casts using a sharp HP graphite pencil and patterns were explored on these casts using magnifying lens. The palatal rugae were classified by Kapali S et al as curved, straight, wavy and circular. [9] All the participants with known blood group were included for the study. Statistical comparison between lip prints, finger prints and palatal rugae impressions are as shown in Figure 1.

**Results**

Amongst, the total sample of 200 dental students the female (72.5%) participants were more in number than the males (27.5%). Majority of the participants belonged to the blood group B (40%). About 96% of individuals were Rh positive and only 4% were Rh negative.

Comparison between lip print and ABO blood group:

In the present study, vertical lip print was the most predominant pattern (37%) followed by intersecting (22%), branched (21.5%), reticular (11.5%) and undetermined (8%). The percentage distribution of most prevalent lip print pattern with ABO blood groups is as shown in Figure 2. There was no significant association between lip prints and ABO blood groups is as shown in Figure 2. There was no significant association between lip prints and ABO blood group (p > 0.05) (χ² statistic = 18.84, p = 0.093).

Comparison between lip print and Rhesus factor:

There was no significant association between lip prints and Rhesus blood group as shown in Table 1 (p > 0.05) (χ² statistic = 6.758, p = 0.149).

Comparison between lip print and ABO-Rhesus blood group:

There was significant association between lip print patterns and ABO-Rhesus blood group as shown in Table 1 (P < 0.05) (χ² statistic = 37.56, p = 0.0107).

Comparison between finger print and ABO blood group:

The highest incidence of occurrence of finger print pattern among the participants was loops (62.5%) followed by whorl (29%) and the least being arch type (8.5%). Composite type was not found in our study. The percentage distribution of most prevalent finger print pattern with ABO blood groups is as shown in Figure 3. There was significant association between finger prints and ABO blood group (χ² statistic = 23.02, p = 0.001). The highest incidence of occurrence of finger print pattern among the participants was loops (62.5%) followed by whorl (29%) and the least being arch type (8.5%). Composite type was not found in our study. The percentage distribution of most prevalent finger print pattern with ABO blood groups is as shown in Figure 3. There was significant association between finger prints and ABO blood group (χ² statistic = 23.02, p = 0.001).

Comparison between finger print and Rhesus factor:

There was no significant association between finger prints and Rhesus blood group as shown in Table 1 (P >
0.05) ($\chi^2$ statistic = 0.963, $p = 0.618$).

Comparison between finger print and ABO-Rhesus blood group:

There was significant association between finger prints and ABO-Rhesus blood group as shown in Table 1 ($P < 0.05$) ($\chi^2$ statistic = 30.6, $p = 0.006$).

Comparison between palatal rugae and ABO blood group:

The most predominant was wavy type (46%) followed by curved (38.5%) and straight type (15.5%). Circular type was not found in our study. The percentage distribution of most prevalent palatal rugae pattern with ABO blood groups is as shown in Figure 4. There was no significant association between palatal rugae and ABO blood group ($\chi^2$ statistic = 6.13, $p = 0.409$).

Comparison between palatal rugae and Rhesus factor:

There was no significant association between palatal rugae and Rhesus factor as shown in Table 1 ($P > 0.05$) ($\chi^2$ statistic = 0.466, $p = 0.792$).

Comparison between palatal rugae and ABO-Rhesus blood group:

There was no significant association found between palatal rugae and ABO-Rhesus blood groups as shown in Table 1 ($\chi^2$ statistic = 17.71, $p = 0.22$).

Comparison of finger print, lip print and palatal rugae with ABO blood group and Rh factor

In the present study, finger print, lip print and palatal rugae were compared with the ABO-Rhesus blood group as shown in Table 1 (Lip print: $\chi^2$ statistic = 37.56, $p = 0.10$, df = 28; Finger print: $\chi^2$ statistic = 30.6, $p = 0.006$, df = 14; Palatal Rugae: $\chi^2$ statistic = 17.71, $p = 0.22$, df = 14).

Figure 1 - Lip print, finger print, palatal rugae impressions

Figure 2 - Graph showing relation between lip print and blood group

Figure 3 - Graph showing relation between finger print and blood group

Figure 4 - Graph showing relation between palatal rugae and blood group
Table 1: Distribution of lip print, finger print and palatal rugae within ABO-Rhesus blood groups

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Finger Print</th>
<th>Palatal Rugae</th>
<th>Lip Print</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arch</td>
<td>Loop</td>
<td>Whorl</td>
</tr>
<tr>
<td>A+ve</td>
<td>8(17.4)</td>
<td>23(50)</td>
<td>15(32.6)</td>
</tr>
<tr>
<td>A-ve</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>2(100.0)</td>
</tr>
<tr>
<td>AB+ve</td>
<td>0(0.0)</td>
<td>14(87.5)</td>
<td>2(12.5)</td>
</tr>
<tr>
<td>AB-ve</td>
<td>0(0.0)</td>
<td>2(100.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>B+ve</td>
<td>2(2.6)</td>
<td>58(74.4)</td>
<td>18(23.1)</td>
</tr>
<tr>
<td>B-ve</td>
<td>0(0.0)</td>
<td>2(100.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>O+ve</td>
<td>7(13.5)</td>
<td>24(46.1)</td>
<td>21(40.4)</td>
</tr>
<tr>
<td>O-ve</td>
<td>0(0.0)</td>
<td>2(100.0)</td>
<td>0(0.0)</td>
</tr>
</tbody>
</table>

Finger print: χ² statistic = 30.6, p = 0.006, df = 14
Palatal Rugae: χ² statistic = 17.71, p = 0.22, df = 14
Lip print: χ² statistic = 37.56, p = 0.10, df = 28

Discussion

In forensic identification, compilation of data from lip print, bite marks, fingerprint, blood groups in crime scenarios have played a benevolent role in identification of suspect in crime. However, no studies that compared and correlated all these parameters with blood groups. Hence, the present study is an attempt made to compare and correlate lip print, finger print and palatal rugae with blood group.

Lip print and blood group

Lip prints are unique to individuals and are present on the lining mucosa. The present study considered the basic pattern of lip prints given by Suzuki K and Tsuchihashi Y in 1971 as I, I’, II, III, IV, V. [7] In the present study, I and I’ are categorized as vertical lip print (type I); in accordance with study done by Nagasupriya A et al [10], where both partial and full vertical lip patterns were included under one category as vertical lip print (Type I). The present study showed that vertical pattern (37%) was the most predominant. These findings were in accordance with the study conducted by Patel S et al. [11] Karim B et al. [12] in 2013 among 122 subjects found that type II was predominant in A-ve blood group and type III with A+ve blood group which was in concordance with the present study. Verma P et al. [13] showed branched pattern was the dominant pattern, which was not similar to the results obtained in the present study. So, studying in depth and establishing further facts and truth in lip print will certainly help us, as useful evidence in forensic science. [14]

Fingerprint and blood group

The epidermal ridges of the fingers and palms as well as the facial structures like the lip, alveolus, teeth and palate are formed from the same embryonic tissues (ectoderm) during the same embryonic period. [15] In our study the finger prints were recorded based on Michael and Kucken’s finger print classification. [8] In the present study loop pattern showed the highest incidence of occurrence, followed by whorl and arch. These results were in accordance with the findings noticed by Mehta AA et al. [16] Sudikshya KC [17] conducted a study among 300 Nepalese, the study results revealed that whorls were more common in blood group B-ve which showed varied results when compared to the present study. In present study there was significant association between finger print patterns and ABO-Rhesus blood group (p<0.05).

Palatal rugae and blood group

Palatal rugae due to their unique features have been widely used in personal identification. The classification of palatal rugae was given by Kapali S et al. [9], based on the shape. Study conducted by Hunasgi S et al. on palatal rugae in two different population and their
correlation with the sex of the individual showed that the wavy and curved patterns were predominant in Karnataka population compared to Kerala. [18] Saraf A et al. conducted a similar study where curved pattern was more common in males than females. [19] Since, varying results were obtained, the present study was done to correlate palatal rugae with blood group and Rh. In the present study wavy palatal rugae pattern is most dominant pattern followed by curved that was in accordance with the findings reported by Abdellatif AM et al. [20] Our attempt to correlate all the three parameters with blood group is to assess their usefulness in the forensic science such as in mass disaster scenarios were identifying unknown victims becomes challenging. To authenticate these results, equivalent studies should be conducted by using these parameters.

Conclusion

The present study was conducted to correlate and compare Cheiloscopy, Dactyloscopy and Palatoscopy with blood group and Rh factor. Statistically significant association of lip prints and finger prints with ABO-Rh blood groups were found in the present study. Furthermore, we would like to conclude that future studies should be conducted with larger sample size to determine the validity of the results of present study.

Conflict of Interest: Nil

Source of Funding: Krishna institute of medical sciences “deemed to be university”, Karad

Ethical Clearance: KIMSDU/IEC/03/2015 dated 10/12/2015.

References

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Unnatural Female Deaths within Seven Years of Marital Life: An Autopsy based Study

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¹Assistant Professor; ²Professor & Head, Department of Forensic Medicine & Toxicology, Great Eastern Medical College, Srikakulam, Andhra Pradesh

Abstract

Unnatural female deaths pose a major challenge for society. The study was conducted on 644 unnatural female deaths during the calendar years from 2008 to 2012, in the Department of Forensic Medicine, Andhra Medical College. Women less than 29 years of age group, in the first three years of their marital life were the major victims in our study. Improving women literacy may give employment and thereby economical independency of women.

Key words: - Unnatural female death, Suicide, Burns.

Introduction

The complexity of “Unnatural female deaths within seven years of marital life” is so deep, as it involves study of human societies and cultures, marriage systems, customs of different religions of Indian society, civilization evolving through decades and psychology of Indian women. Population explosion, illiteracy and unemployment leading to poor socio economic status, fast urbanization with unequal opportunities for female and social dependency of women may be causing more women deaths after marriage. Sizeable sections of population still believe in the die-hard traditions of social customs in regards to dowry and caste. The statement made by Carl Marx “All the human relations are economical”¹ appears to be wise in respect to Indian marriages. In Indian society, ill adaptation of women to the changing social, economical and ethical environments increase the incidence of unnatural deaths when a female gets married and goes to her in-laws house. Failure to adapt to the husband, illegal affairs of spouses, new surroundings, and harassment from the in-laws often leads a married woman to extreme steps.

In Indian society the common methods adopted by women to commit suicide are burning by pouring inflammable liquids on the body, poisoning and hanging².

The incidence of unnatural married female deaths varied from place to place, time to time, season to season, one religion to other religion, in different circumstances and age groups with duration of married life. For the first time during the decade 2001 to 2011 that of the total of 217.70 million literates added, women (110.07million) outnumbered men (107.63million) (source: Ministry of Women and Child Development Government of India (XII th Five Year Plan)).³

From Manual labour stage, the twentieth century opened up new professional avenues for women entering into the Indian Administrative Services, soft ware and other educational services in increasing numbers.⁴ Even though educational and occupational opportunities are providing women with new roles outside the home, their social position in the family remains largely unchanged. They are still subordinate to husbands. Legal proceedings against the husband may mean her social ostracism as public opinion still places the man in the position of “LORD” for a wife.

The reports, statistical data, existing laws create an appearance that the position of women is better in India today. On close scrutiny one can find subordination and inequality of women still prevailing in society. The laws provide protection to women against injustice,
exploitation, dowry and discrimination. But in practice, in cases where the female deaths have not occurred, the crimes are being diluted in local scenario itself. An economical dependency and traditions bound wife can hardly take resource to law even if she has a valid ground for divorce. With the advent of new technology and development of personalized mobile phones, the privacy and intimacy between two persons has lead to the development of unlawful relations between male and female causing disturbance to the family life and leading to female deaths.

**Materials and Method**

A total of 8521 Medico legal Post Mortem Examinations were conducted during calendar years from 2008 to 2012, in the Department of Forensic Medicine, Andhra Medical College, Visakhapatnam. Out of 8521 cases, 644 cases were unnatural women deaths within 7 years after marriage. The statistics of these unnatural deaths were analyzed with available data like inquest report, autopsy report, suicide notes, panchanama report and dying declaration.

Age of the person, incident time, date, month, and period of survival, cause of incident, cause, manner and place of death were studied.

<p>| Table 1: Number of Unnatural female deaths among the autopsies done from 2008-2012 |
|-----------------------------------------|----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Year</th>
<th>Total Number of Post Mortems</th>
<th>Total number of unnatural female deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>2008</td>
<td>1614</td>
<td>112</td>
</tr>
<tr>
<td>02</td>
<td>2009</td>
<td>1685</td>
<td>158</td>
</tr>
<tr>
<td>03</td>
<td>2010</td>
<td>1772</td>
<td>159</td>
</tr>
<tr>
<td>04</td>
<td>2011</td>
<td>1670</td>
<td>109</td>
</tr>
<tr>
<td>05</td>
<td>2012</td>
<td>1780</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>5 years</td>
<td>8521</td>
<td>644</td>
</tr>
</tbody>
</table>

<p>| Table 2: Different age groups of Unnatural female deaths |
|-----------------------------------------|----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Age groups</th>
<th>No of deaths(n=)</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>&lt;20Yrs</td>
<td>166</td>
<td>25.77%</td>
</tr>
<tr>
<td>02</td>
<td>20-29 Yrs</td>
<td>441</td>
<td>68.47%</td>
</tr>
<tr>
<td>03</td>
<td>30-39 Yrs</td>
<td>37</td>
<td>5.74%</td>
</tr>
<tr>
<td>04</td>
<td>&gt;40Yrs</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

<p>| Table 3: Causes of Unnatural female death |
|-----------------------------------------|----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>S.No</th>
<th>COD</th>
<th>No of deaths (n=)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Burns</td>
<td>477</td>
<td>74.06</td>
</tr>
<tr>
<td>02</td>
<td>Poisoning</td>
<td>106</td>
<td>16.45</td>
</tr>
<tr>
<td>03</td>
<td>Mechanical asphyxia</td>
<td>55</td>
<td>8.54</td>
</tr>
<tr>
<td>04</td>
<td>RTA</td>
<td>03</td>
<td>0.46</td>
</tr>
<tr>
<td>05</td>
<td>Stab injury</td>
<td>03</td>
<td>0.46</td>
</tr>
</tbody>
</table>
Table 4: Factors related to Unnatural female deaths

<table>
<thead>
<tr>
<th>S.No</th>
<th>Factors</th>
<th>No of deaths (n=)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Bad kitchen material</td>
<td>277</td>
<td>43.01%</td>
</tr>
<tr>
<td>02</td>
<td>Husband and in-laws harassment</td>
<td>203</td>
<td>31.52%</td>
</tr>
<tr>
<td>03</td>
<td>Ill health</td>
<td>87</td>
<td>13.50%</td>
</tr>
<tr>
<td>04</td>
<td>Forced marriage</td>
<td>77</td>
<td>11.95%</td>
</tr>
</tbody>
</table>

Table 5: Number of deaths occurred from 1st to 7th year of married life

<table>
<thead>
<tr>
<th>S.No</th>
<th>Year after marriage</th>
<th>No of deaths (n=)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Within 1st year</td>
<td>111</td>
<td>17.23%</td>
</tr>
<tr>
<td>02</td>
<td>Within 2nd year</td>
<td>135</td>
<td>20.96%</td>
</tr>
<tr>
<td>03</td>
<td>Within 3rd year</td>
<td>83</td>
<td>12.88%</td>
</tr>
<tr>
<td>04</td>
<td>Within 4th year</td>
<td>75</td>
<td>11.64%</td>
</tr>
<tr>
<td>05</td>
<td>Within 5th year</td>
<td>103</td>
<td>15.99%</td>
</tr>
<tr>
<td>06</td>
<td>Within 6th year</td>
<td>56</td>
<td>8.69%</td>
</tr>
<tr>
<td>07</td>
<td>Within 7th year</td>
<td>81</td>
<td>12.57%</td>
</tr>
</tbody>
</table>

Survival period after the incident – Figure 1

Observations and Discussion

A total number of 8521 postmortems were conducted in the department of Forensic Medicine Andhra Medical College, Visakhapatnam. Among these, 644 cases of unnatural female deaths were recorded basing on the history given by the inquest report.

More number of unnatural female deaths were observed in the age group of <29 years (94.25%), among which 441 deaths were recorded in the age group of 20-29 yrs (68.47%) followed by <20 years (25.77%) and 30-39 years (5.74%). Post marital life pressure, financial problems; illegal affairs of either spouse in house may be responsible for more deaths in the age group of 20-29 years. Less number of deaths in 30-39 years can be explained on basis of adjustment of relation between husband and wife, better environment in in-laws house with time. Our study correlates with Naresh karukutla\textsuperscript{5} and Sandhya arora\textsuperscript{6} studies.

More number of unnatural female deaths occurred in in-laws and husbands place (86.18%) followed by victims parents residence (11.64%). As per Indian tradition staying with husband and in in-laws house is more common and coming back to victim’s parent’s residence is less. Our study correlates with Naresh karukutla\textsuperscript{5} study.

Fire, while cooking or lighting kerosene lamps accounted more deaths (43.01%) followed by Husband and in law’s harassment (31.52%). The other common predisposing causes were forced marriage (11.95%) and ill health (13.5%). This study correlates with Sandhya arora\textsuperscript{6} and contrasts with Rajesh kumar varma etal\textsuperscript{7}, Radhika R.H\textsuperscript{8}.

Death in most of the cases was during day time from 6am to 6pm (58.53%). After quarreling, husband may go out on work and house wife staying alone are vulnerable to unnatural deaths in day time which correlates with Madar ramchadra sane\textsuperscript{9} study.

In summer season more number of deaths were recorded (37.57%) followed by winter season (36.80%).
Among summer, May month recorded highest number (n=86) of deaths. More number of deaths in summer can be explained on basis of increased temperature causing more exhaustion, irritation, more family unions due to holidays, more friction in families due to discussion about dowry and financial issues.

Most of the victims (n=375) died within 72 hrs including 99 spot deaths. More fatality in early hours can be explained on basis of severity of injuries, disease and lack of proper health care facilities at village /mandal level implying the need to focus on patient care at early hours. This study correlates with Naresh karukutla etal 5 study.

Majority of the victims (81.52%) died after admission in to the hospitals followed by deaths at crime scene (16.30%). As explained earlier, Burns were the common cause of death where fatality is more common even in hospitals also. Services like 108 ambulances, arogyasri, are helpful to admit the victims in hospitals there by increased number of hospital admission and deaths. This study correlates with Naresh karukutla etal 5.

Maximum number of deaths occurred within first 3 years of marriage (51.08%). More deaths in first three years can be explained on basis of lack of awareness about marriage life, misunderstanding, financial problems, husband and in-laws harassment for dowry, ill adjustment in in-laws house, and extramarital affairs in early married life. Staying of newly married couple in female’s house for first 3 years may decrease the unnatural female deaths. Deaths in 4 to 7 years (n=315) of married life may be due to problems of infertility and husband bad behavior, forced female feticide. Decreased number of victims in 4-7 years (n=315) may be due to better adjustment of couple, for the sake of children and emotional bonding of family members .This study correlates with Naresh karukutla et al, 5 Rajesh Kumar varma et al 7, Radhika R.H et al 8, and contrasts with Rajesh c.dere 10 where more deaths occurred at 6-7 years of married life.

Most of the victims died from Burns (74.06%) followed by Poisoning (16.45%). Burns as the common cause of death, can be explained on the basis of daily cooking, easy availability of explosive materials like gas cylinders and kerosene. More fatality in burns can be ascertained to loss of skin resulting in decreased resistance of the body to the micro organisms leading to septicemia, shock, multiple organ failure etc., which explains burns as predominant cause of death. This study correlates with Naresh 5, Sandhya arora 6 Rajesh c dere 10, Kailash u zine 11, However M Ramachadra study 9 differs which states that Hanging was the most common cause of unnatural female deaths. Accidental Burns were common (56.18%) followed by suicides (39.20%).

Suicide is the most common manner of death (n=324) followed by accidental deaths (n=274).

Conclusions

1. <29 yrs of Age group is more vulnerable for unnatural female deaths.
2. Most of deaths occurred during the day time between 6:00am to 6:00 pm.
3. Summer recorded highest number of deaths, with peak incidence in May Month.
4. Deaths occurred mostly in first 3 years of marital life.
5. Majority of deaths occurred at husband and in-laws home.
6. Most victims died in hospital, after admission with period of survival <72 hours.
7. Burns were the major cause of death.
8. Commonest manner of death is suicide.

Suggestions and Recommendations:

1. Women protection cells have to be established in all mandal /district headquarters. They should collect the statistical data of all the new couples of their region and counsel the couple in immediate post marital life problems.
2. All marriages have to be registered mandatorily either at panchayath, municipal or at registrars’ offices wherever possible.
3. The society may be educated through audio-visual aids like T.V., Radio, Daily newspaper, Advertisements to prevent unnatural female deaths.
4. The Government should take personal protection measures for women to supply safety cooking devices, especially for low socio economical families.
5. Selling of pesticides and dangerous drugs should be stopped to women.
6. There is a great need for opening suicide prevention centers for prevention of suicides.

7. Improving women literacy may give employment and thereby economical independency of women.

8. Parents showing large gap of disparity between daughters and sons should be counseled to realize their responsibilities.

9. Educate the parents and counsel them to stop early marriages in girls.

Acknowledgement: I am thankful to Dr M Taquiuddin Khan, Professor and Head, Department of Forensic Medicine and Toxicology, Andhra Medical College, and other faculty for giving valuable suggestions during the study period.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: Taken from Ethical committee, Andhra medical college.

References

Study of Dimorphism of Humerus in Maharashtra Population

Mohammad Abdul Mateen
Associate Professor, Department of Forensic, Medicine and Toxicology, Jiu’s Indian Institute of Medical Science Warudi Badnapur (Taluk) Jalna (District) (Maharashtra)

Abstract

45 male and 45 females right and left dried, non pathological humerus were measured with vernier caliper and weighed with digital weighing machine. The vertical diameter of superior articular surface, transverse diameter of superior articular surface, circumference of superior articular surface, circumference of mid shaft in males and females of right and left humerus were studied and found highly significant P values in both sexes and right left humerus (P<0.01). These parameters will be quite useful to medico-legal expert, anthropologist because morphometric values of mesoderm are uncertain.

Key words:- vertical, transverse, circumference, articular, diameter vernier caliper

Introduction

humerus bone is named after sense of humours feels, when medial epicondyle is touched because it is crossed or in contract with ulna nerve, humerus is long bone of the arm forms should or joint and elbow joint. A biological profile which includes age, race or ethnicity and stature, sex when determined from the various human remains require certain parameter to determine with accuracy (1)(2). Many criteria are available to determine sexual dimorphism of human skeleton(appendicular and axial) (3)(4). But least data is available for sexual dimorphism of humerus. Hence attempt was made to differentiate male bones of humerus with females humerus with morphometric parameters in Maharashtra population, because skeleton of a particular individual or ethnic group is able to adopt to its owners life.

Material and Method

45 male and 45 females dried non-pathological humerus were selected for study humerus were available at Anatomy department and forensic department of IIMSC and R.C warudi– Jalna (Maharashtra) In addition to this, anatomy Department of Government Medical College Aurangabad.

Each bone was put in anatomical position and its metrical study was carried out by vernier caliper and weight of bones was carried by digital weighting machine. The obtained values were calculated by 2007 Microsoft computer (values of the males bones were compared with females bones)

The pathological, fractured bones were excluded from the study. The duration of the study was about three years.

Observation and Results

Parameters of right humerus

Table-1 – Comparison of vertical diameters of superior articular surface in male and females – Mean value of male humerus was 4.45cm(SD±0.12) and females was 3.53 Cms (SD±0.08) and t test was 42 (P<0.01) p value was highly significant

Table-2 – comparison circumference of superior articular surface of humerus Mean value of male was 3.53 Cms (SD±0.08) and t test was 42 (P<0.01) p value was highly significant

Table-3 - Comparison of mid shaft circumference of humerus mean value of male was 6.22(SD±0.20) and
females was 5.77 (SD±0.12) t test value was 13, and P value was highly significant (P<0.01)

Parameters of left humerus

Table-4 - Comparison of vertical diameters of superior articular surface. Mean value of male was 3.83 (SD±0.45) and females was 3.16(SD±0.20) t test value was 09 and P value was highly significant (SD±0.01)

Table-5 - Comparison of circumference superior articular surface. Mean value of male was 12.5 (SD±0.10) t test was 26 and P value was highly significant (SD±0.01)

Table-6 - comparison of mid-shaft circumference of humerus mean value was 5.73(SD±0.01) and female was 5.12 (SD±0.01) t test was 29 and P value was highly significant (P<0.01)

Discussion

In the present study of sexual dimorphism of humerus in Maharashtra population. In the study of right humerus the comparison of vertical diameter of superior articular surface. Mean value of male was 4.45 (SD±0.12) and females was 3.53 (SD±0.08) t test 42, p value was highly significant (P<0.01) (Table-1). Comparison of circumference of superior articular surface. The mean value of male was 13.8 (SD±0.37) and females was 12.4 (SD±0.13) t test was 25 and P value was highly significant (p<0.01)(Table-2). Comparison of mid-shaft circumference the mean value of male was 6.22 (SD±0.20), females was 5.77 (SD±0.12) t test was 13, p value was highly significant (P<0.01)(Table-3).

The parameter of left humerus (cms) comparison of vertical diameter of superior articular surface. The mean value of male was 3.83(SD±0.45) and females was 3.16 (SD±0.20) t test was 09, p value was highly significant (P<0.01)(Table-4). Comparison of circumference of superior articular surface of humerus. The mean value of male was 12.5 (SD±0.10) and females was 11.80 (SD±0.17) t test was 26, P value was highly significant (P<0.01)(Table-5). Comparison of mid-shaft value of male was 5.73 (SD±0.01) and female was 5.12 (SD±0.01) t test 29, P value was highly significant (P<0.01)(Table-6) These finding were more or less in agreement with previous studies(5)(6)(7).

These difference could be attributed a more variable life style and differential patterns of physical labour expected of males versus females (8). Moreover early maturity of females than males, giving two or more addition years to develop physically to males (9).

It has been also suggested that sexual dimorphism changes have strong genetic bases. It has been noted that population consuming very low or very high protein of demonstrated least amount of sexual dimorphism(11). Hence nutritional status also play vital role in sexual dimorphism

Summary and Conclusion

The present study of sexual dimorphism of humerus in Maharashtra population. Will be quite useful to medico- legal expert, anthropologist and anatomist. But this study demands further embryological, genetic nutritional, hormonal, biomechanical study because exact mechanism factors and duration of ossification is still unclear

This research paper was approved by ethical committee of jiui’a Indian Institute of medical science and research Warudi-Badnapur (Taluq) Jalna (Dist) (Maharashtra)

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.45</td>
</tr>
<tr>
<td>SD</td>
<td>0.12</td>
</tr>
<tr>
<td>Test statistic</td>
<td>t = 42, p&lt;0.01</td>
</tr>
</tbody>
</table>

Vertical diameter of the superior articular surface in right humerus is significantly more in male than in female(p<0.01).

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>13.87</td>
</tr>
<tr>
<td>SD</td>
<td>0.37</td>
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<tr>
<td>Test statistic</td>
<td>t = 25, p&lt;0.01</td>
</tr>
</tbody>
</table>

Circumference of the superior articular surface of humerus is significantly more in male than in
female (p < 0.01).

Table-3: Comparison of Mid-shaft Circumference of right humerus in male and female

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>6.22</td>
<td>5.77</td>
</tr>
<tr>
<td>SD</td>
<td>0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>Test statistic</td>
<td>t = 13, p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Mid-shaft Circumference of right humerus is significantly more in male than in female (p < 0.01).

Table-4: Comparison of Vertical diameter of superior articular surface in male and female left humerus

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.83</td>
<td>3.16</td>
</tr>
<tr>
<td>SD</td>
<td>0.45</td>
<td>0.20</td>
</tr>
<tr>
<td>Test statistic</td>
<td>t = 09, p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Vertical diameter of superior articular surface is significantly more in male than in female (p < 0.01).

Table-5: Comparison of Circumference of superior articular surface of left humerus in male and female

<table>
<thead>
<tr>
<th></th>
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<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>12.55</td>
<td>11.80</td>
</tr>
<tr>
<td>SD</td>
<td>0.10</td>
<td>0.17</td>
</tr>
<tr>
<td>Test statistic</td>
<td>t = 26, p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Circumference of superior articular surface of humerus is significantly more in male than in female (p < 0.01).

Table-6: Comparison of Mid-shaft Circumference in male and female of left humerus

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.73</td>
<td>5.12</td>
</tr>
<tr>
<td>SD</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Test statistic</td>
<td>t = 29, p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Mid-shaft Circumference is significantly more in male than in female (p < 0.01).

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Perception of Students Regarding Gender based Violence

Muzaffar A1, Shenoy VK2, Vaswani VR3, Badiadika KK4
1Postgraduate Final Year, 2Assistant Professor, 3Professor and Head, 4Associate Professor,
Department of Forensic Medicine, Yenepoya Medical College, Mangalore, India

Abstract

Objectives: The study is undertaken to explore perception of students regarding violence against women with specific reference to domestic violence and sexual violence.

Methods: This is a descriptive cross-sectional questionnaire based exploratory study conducted in Mangalore, Karnataka, India. 220 participants consisting of age group between 18 to 25 years were included. Responses were obtained from the study participants through open and close ended questionnaires. Results: More than half (60%) of medical students and (56%) of engineering students disagree that in modern India women are enjoying equal rights to men. Three forth (77% male, 82% female) of students believe and disagree that it is alright for a man to beat his wife in order to make her obedient. Two forth (61%) of medical students and (63%) of engineering students agree that throwing acid on a woman to cause disfiguration is an example of gender based violence. Conclusion: The perception of medical and engineering students regarding gender based violence is quite similar. This study brought out issues of increased violence against women. A significant number still felt that dressing of a woman may be a factor for sexual assault. Women are still facing gender inequality and are not getting equal rights to men. Even though incidence of harassment, domestic violence are on the rise, women are not ready to report about their suffering because of social and family pressure. There is also need for awareness to be bought in gender mainstreaming for educating all sectors of the society.

Key Word: Sexual harassment, Crime against women, Domestic violence.

Introduction

Women are important part of our society and play vital roles in all aspects of life but their identity is restricted to being mother, sister, wife, or daughter1. In ancient Indian time’s as mentioned in Rigveda and other scriptures women held the most prestigious and honoured place in society2. The status of women in society over the period of time seems to be controversial, violence against women has been increasing and a universal concern too as men hold themselves superior and believes that women are mediocre. Women are given low status at work place and home. In last few decades there has been increase in the incidence of violence against women3. Globally violence against women has increased, it has been reported that more the one in three women (35.6%) have been subjected to sexual or physical violence by their friends or relatives and 38% of all murders are committed by their spouse4. 05% of murder of men is committed by their spouse. A woman has been subjected from verbal abuse, emotional abuse, physical and sexual exploitation leading to femicide. Worldwide 5000 murders in the name of honour killing and 25000 in the name of dowry death have been reported every year. There are also reports that the women are victim of sexual violence in wars and mostly prone to human right violation5. Unwanted pregnancy, child birth and child abuse may be a sign of violence against women. India is the second densely inhabited democratic country and seventh largest country in the world with 1.3 billion people, 48.4% are females and 51.6% are males6. Developing India is emerging as a global power but still women have to struggle for their position and status in the society. Women are subjected to violence irrespective of their cast, educational status, class and their subjugation adds to their suffering by physical, emotional, mental and economical deprivation7,8.

In India women have been subjected to violence and they are suffering from various causes such as
cheating, rape, torture, abduction, violent pornography, immoral trafficking and so on. A total of 3,27,394 cases of crime against women were reported in year 2015, 29.9% of sexual harassments, 10.4% physical assault, 7.6% stalking, 1.0% voyeurism, and 51.8% other cases. Human trafficking has increased from 0.4% in year 2014 to 0.5% in year 2015. According to NCBR “head wise percentage of crime against women” during year 2015 there were 34.6% cases of domestic violence, 25.2% cases of assault on women with intend to outrage her modesty, 18.1% cases of kidnapping and abduction of women, 10.6% of rape cases, 3.05% cases of dowry prohibition, 2.35% cases of dowry death, 2.7% cases of insult of modesty of women, 1.4% cases of attempt to rape, 1.2% cases of abetment of suicide by women, 0.7% cases of immoral trafficking of women, 0.25% other crimes.

Women have to suffer from gender violence throughout their life. As a child her father is in charge of her, as an adult her husband is in charge of her and after death of husband, her son takes over charge of her.

Rafique vs state of U.P 1980, Justice Krishna Iyer said “when a woman is raped, what is inflicted is not mere physical injury but a sense of some deathless shame” and judiciary response could not be muted to such grave human right violation. The implementation of preliminary measures gives a perception that the present criminal justice system India is not robust to protect women.

Women who suffer from sexual assault have physical and mental issues, poor sleep, irritable bowel, diabetes, post-traumatic stress disorder and various studies has shown that they were benefited with screening and discussing sexual assault with health care provider and all this helped in their recovery.

Gender based violence is a serious issue all the students undergoing medical education should be taught about the importance of the issue and how to address such cases.

The study was undertaken with the objective to explore perception of students regarding violence against women with specific reference to domestic violence and sexual violence.

Material and Method

This is a descriptive cross-sectional questionnaire based exploratory study conducted in Engineering and Medical Colleges of Mangalore, Karnataka, India. Questionnaire was externally validated by two subject experts. After obtaining institutional ethical clearance and due necessary permissions study was conducted and data were collected from the study area. The study was conducted in October 2018 to November 2018. 220 participants between ages 18 to 25 years were included. Responses were obtained from the study participants through close and open ended 25 items questionnaires. The data was analysed using SPSS version 20.0 software. Chi – square test was applied.

Result

In the present study, out of 220 participants, 110 (50%) were Medical students and 110 (50%) were engineering students of which 119 males and 101 females. There is no significant difference noted on various issues amongst the students from medical and engineering streams. 59.1% of medical students and 55.4% of engineering students did not agree that in modern India women are enjoying equal rights as men. Nearly half of medical and engineering students agreed that domestic violence in women is common and wide spread issue. More than 80% of medical and engineering students disagree that women feel safe while travelling alone and going out at night. More than 70% of medical and engineering students agree that vulnerable group such as minors and women with disabilities are more prone for sexual violence.

More than 70% of medical and engineering students agree that most of women hesitate to report about crime against them because of social and family pressure. Nearly 30% of male and 20% of females agreed that man can beat his wife to make her disciplined.

51% of medical students and 50% of engineering students (24% of male and 19 % female) believe that dressing style of a woman is be a factor for sexual assaults. More than 60% of medical engineering students agree that throwing acid on a woman to cause disfiguration is an example of gender based violence. 59.1% of medical students and 69.1% of engineering students agree that if a person with political background or influence is named as perpetrator of sexual violence, they are protected by investigating officer. 77.31% of medical students and 80% of engineering students agree that there is a stigma regarding sexual assault survivor in India. 78.2%
of medical students and 91.1% of engineering students agree that domestic violence is preventable.

Medical students perceive that percentage of perpetrators on violence against women are strangers 46.4%, family 30.9%, relatives 21.8%, friends 0.9% and engineering students report that perpetrators of violence on women are strangers 69.1%, relatives 20.9%, family 8.2% friends 1.8%. Medical students perceive that the place where women is commonly assaulted are public transport 44.55%, work place 30.0%, home 15.5%, market place 10%. Engineering students report that the place where women are commonly assaulted were public transport 60.9%, work place 30%, market place 6.4% and home 2.7%.

Medical and engineering students report that information on violence against women comes largely from Internet more than 50%, TV and Radio more than 40%, newspaper more than 10%, and others.

Medical students report that place where a victim of sexual assault more likely to visit is first to Primary health centre, second to Community health centre, then so on Secondary Hospital, District Hospital, Tertiary Hospital and engineering students report that place where a victim of sexual assault more likely to visit first to Primary health centre, second to Community health centre, then so on Secondary Hospital, Tertiary Hospital, District Hospital.

More than 90% of medical and engineering students agree that workshop and continued hands on training in Forensic Medicine is important to deal with cases in rural areas. 78.2% of medical students and 89.1% of engineering students agree that the attitude of people in India can be changed regarding sexual assault victim.

Discussion

In the present study, results did not indicate that Medical and Engineering stream students significantly differed in their perceptions regarding gender based violence. Mangali RN et al conducted a study in year 2007, women are considered lower compared then men in society and women suffer from gender discrimination in all aspects of life. Similarly, in the present study It was observed that more than half of total number of participants (m=60, e=56) disagree that in modern India women are getting equal rights to men.

Watts C et al in year 2002 reported that child sexual abuse is common and under reported because most of times they are threatened or the perpetrators is family member. Similarly, in the present study three forth of students (73%) believe that vulnerable group such as minors are more prone for sexual violence.

Imran M et al 2010, has reported crime against women is increasing, sexual violence cases are often under reported because of stigma and shame related to the survivor of sexual assault. Similarly, three forth of students (79%) believe that there is a stigma regarding sexual assault survivor in India. In the same study Imran in 2010 has reported that, domestic violence so common in our society, that six out of every ten husbands have beaten their wives. Sexual harassment is often not reported due to fear of family and fear of isolation from society. Similarly, in the present study majority of students (85%) believe that domestic violence is a wide spread issue and at least, one fourth of the students (26%) still believe that it is alright for a man to beat his wife in order to make her obedient. This is not only a perception prevalent in male (25%) but also shared by females (20%). This is a concerning fact as violence against women cannot be stopped if the belief by disciplining a woman by physical force continues. Similarly, if this is deep rooted in females, the inflicted violence on self gets justified and she may not access the health care or report to police.

Naskar G et al in year 2015, reports of wife battering and violence against widows are also on rise and legal measures should be taken for elimination of violence against women. The Indian cultural system is not open for wife reporting to the police of having been battered by her husband. Similarly, in the present study three forth (79%) of students believe that women hesitate to report about crime against them because of social and family pressure.

Nagindrappa M et al 2013, reports that women in India are not given equal rights to men irrespective of their cast, class, educational status. They are physically, socially, mentally and economically exploited since long. Bhawana D et al reported that various crime against women that occur in India such as sexual assault, kidnapping, abduction, dowry death, torture, molestation has been increased 59% since year 2011. Similarly, in present study three forth (78%) of students believe that violence against women in our society is common and
is on rise.

Waghamoda R.H 2013, conducted a study on domestic violence where women were the victims which is similar to the study by Badiadka 2017 where much of the public assault was on males and domestic violence in females. The World Human Rights Conference in Vienna, recognised gender based violence as human right violence in 1993. Article 14, 15 and 21 of Indian Constitution provides protection of women from domestic violence (domestic violence Act, 2005). There is cultural gender inequality, economic dependence on men, political under representation, less education and awareness among women and they are facing domestic violence by men because of all these reasons. Present study students perceived that domestic violence is preventable (85%).

T.K.K Naidu et al 2007, reported that Forensic Medicine has an important role in detection and help in administration of justice, forensic training must be given to Medical and Para Medical students to deal with cases in rural and semi urban areas. Proper Medicolegal investigation will help in solving of cases so that cases will not be undetected and unconvicted. Similarly, in present study majority (95%) of students reported there is requirement of specialized nursing training “Forensic nursing” as it is in developed countries and majority (93%) reported that workshop and continue hands on training in Forensic Medicine is important to deal with cases in rural areas.

Hossain N 2015, reported that gender based violence is a serious issue all the students undergoing medical education should be taught about the importance of the issue and how to address such cases.

Watts C 2002, reported that some of the most common and severe forms of violence against women by intimate partner are physical abuse, psychological abuse, acid throwing, sexual abuse, trafficking for sex, forced prostitution, exploitation of labour. Similarly, in the present study more than half (61%) of students agree that throwing acid on a woman to cause disfiguration is an example of gender based violence.

In the present study more than half of students believe that the perpetrators of violence on women are mostly strangers (58%). This is contrary to the study done by Madeswarana et al 2013, majority of cases perpetrators of violence on women were acquaintances such as family member, friends and relatives.

**Conclusion:** The perception of medical and engineering students regarding gender based violence is quite similar. This study brought out issues of increased violence against women. One third of the students still believe that it is alright for a man to beat his wife to make her obedient. More awareness programs should be conducted in gender mainstreaming. Women are still facing gender inequality and are not getting equal rights to men. Even though incidence of domestic violence is on the rise, it may not get reported because of social and family pressure. There should be sensitization program for every stream of students on gender related issues. Registered medical practitioner and Forensic nursing should be available so that when a woman accesses a health centre at any level, be it primary or tertiary, she gets appropriate care and medicolegal service. Investigating agencies, medical and paramedical staff should be sensitized and trained in this regard.

**Limitations of study:** Only medical and engineering students from select colleges are considered for study. They do not represent population of India.

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

**Ethical approval:** Institutional ethical committee (centre of ethics) approval has been obtained (Reference no-YEC198/2018)

**Source of Funding:** Self.

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Legal & Intellectual Property Dimension of Health & Access to Medicines in India

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Abstract

Trade-Related Aspects of Intellectual Property Rights (TRIPS) Agreement, 1995 established synergy with human rights laws in realization of right to health, access to medicine and sustainable development. The Doha Declaration on Public Health, 2001; Sustainable Development Goals, 2015-2030 and United Nations Secretary-General’s High-Level Panel on Access to Medicines Report, 2016 promote innovation of health technologies in developing countries. It is estimated that 75 per cent of the world’s population is health deficient and medicine starved due to patenting requirement of pharmaceutical industries. India passed Patents (Amendment) Act, 2005 dealing with exclusive marketing right, product patent and process patent to protect the interest of the generic drugs in compliance of TRIPS Agreement, 1995 under public interest. In post Patents (Amendment) Act, 2005 phase India faced formidable challenge of the Swiss drug maker Novartis’ patent application for Gleevec in Madras High Court, 2006; Intellectual Property Appellate Board (IPAB) in 2009 and Supreme Court in 2013. The judicial exuberance struck a balance between patent right, health right and access to medicine in Indian socio-economic context. It is followed by spelling out of the National Intellectual Property Right (IPR) Policy, 2016 focused on enhancing access to healthcare as human right.

Key Words: TRIPS Agreement, Public Health, Access to Medicines, Pharmaceutical Industries, Exclusive Marketing Right, Novartis Judgment, IPR& Health Policy.

Introduction

Globally innovations in health technologies and access to medicine has dramatically improved and brought commendable health improvements but the majority of patients in developing countries constituting around 75 per cent of the world’s population are denied access to medicines due to patent by the pharmaceutical industry. The investment in research and development (R&D) of health technologies is ill-equipped to respond to diseases, such as Ebola and Zika, neglected tropical diseases (DTNs) and Antiretroviral (ARV) drugs related to HIV/AIDS.1 The World Health Organization Statistic, 2016 documented that prevention and treatment of AIDS, tuberculosis, malaria hepatitis, communicable diseases and neglected tropical diseases have not received the adequate attention.2 The legal and intellectual property dimension of health and access to medicines in India is governed by the TRIPS Agreement, 19953 and the Patents (Amendment) Act, 1970, Patents (Amendment) Act, 1999, Patents (Amendment) Act, 2002 and Patents (Amendment) Act, 2005.4 The innovation of health technologies in post TRIPS Agreement, 1995 and Indian slew of reform desiderates human rights to health and its links with intellectual property rights.5 The TRIPS Agreement,1995 flexibilities under the Doha Declaration on Public Health, 2001 addressed meaningfully to access to medicines as public health. This is supplemented by United Nations Sustainable Development Goals 2015-2030 which ensures healthy lives and well-being of all people of all ages.6

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Access To Medicines: Promoting Innovation & Access To Health Technologies Which Review And Assess The Situations of Health Technologies Report, 2016 reveals ground realities asserting the millions of people lack of access to health technologies. Thus the legal and intellectual property dimensions needs a serious engagement to foster realization of access to medicine and human right to health in India.

Material & Method

The material and methods applied for the study include analytical method of legal research by undertaking the legislative survey and scrutiny of health and intellectual property laws at international and national levels. The comparative law methods applied for the statutory interpretation of Universal Declaration on Human Rights, 1948, International Covenant on Economic, Social and Cultural Rights, 1966 TRIPS Agreement, 1995, Doha Declaration on Public Health, 2001 and United Nations Panel On Access To Medicines and Access To Health Technologies Report, 2016. The Indian Patents Act, 1970, Patents (Amendment) Act, 1999, Patents (Amendment) Act, 2002 and Patents (Amendment) Act, 2005 is examined for harmonious construction of health and intellectual property legal framework. This study has analyzed the corpus of legal materials on international and national health and IP laws in the framework of Maxwell’s qualitative research design.7

Findings

The Universal Declaration on Human Rights, 1948 which mandates that everyone has the right to standard of living adequate for the health, medical care and wellbeing. This is buttressed by International Covenant on Economic, Social and Cultural Rights, 1966 by conferring enjoyment of the highest attainable standard of physical and mental health.8 The Inter-Ministerial Conference of the WTO adopted Doha Declaration on Public Health, 2001 on access to medicines in the context of the TRIPS Agreement, 1995.

WTO & TRIPS Agreement, 1995: The adoption of the WTO and TRIPS Agreement, 1995 ushered intellectual property norms and enforcement. Article 7 strikes the balance between the intellectual property rights holders and broader social interests and common good. Article 8 adopts measures to protect public health and nutrition, as well as to promote the public interest in sectors of vital importance to socio-economic and technological development.9 Article 27.2 restrict the patentability of inventions to save human life and health without prejudice to environment.10 Article 30 permits states to limit the exclusive privilege granted through patent rights in the interest of third parties, such as people suffering from HIV/AIDS diseases. It is further supplemented by Article 31, which sets out a regulatory framework for compulsory licensing with number of conditions. One of the strategies which have been debated in recent times is based on the implicit provision in the TRIPS Agreement, 1995 on the issue of parallel imports or the principle of exhaustion of rights of the patent holder under Articles 6 as a means of reduced prices and increased basket of access. However, negotiators included safeguards and flexibilities within the TRIPS Agreement, 1995 that could be used by signatories to tailor national intellectual property regimes so that countries could fulfill their human rights and public health obligations.11

Doha Declaration & Access to Medicines: The Doha Declaration, 2001 recognizes that the patent rights in health sector to protect the public health as obligations of WTO members to interpret the TRIPS Agreement, 1995 in public interest of medicinal access and drugs. It recommended exporting patented products and utilizing their compulsory licenses to needed drugs at affordable costs. It further enunciates that public health issues will supersede private interests in view of life threatening diseases such as tuberculosis, malaria and HIV/AIDS be issued under Article 31 of TRIPS Agreement, 1995.12 The Doha Declaration on Public Health, 2001 reaffirmed Article 31 of TRIPS Agreement, 1995 by empowering WTO members to utilize flexibilities available for the right to health and public health. However on the ground we find that the Median availability of essential generic medicines in developing countries generally reveals that only countries 37.7% in public sector whereas the in Private sector 70% found to be disturbingly very high.13 Despite these pronouncements, the sovereign right to issue compulsory licenses provided for by TRIPS Agreement, 1995 has been often stymied by threats of retaliation from governments and corporations.14

United Nations Health Technologies Report, 2016: The UN Secretary-General convened a High-Level panel to review and assess proposals and recommend solutions for remedying the policy incoherence between the justifiable rights of inventors, international human rights law, trade rules and public health in the context of health technologies in 2015. The Panel called for proposals that promote research, development, innovation and
increased access to medicines, vaccines, diagnostics and related health technologies to improve the health, wellbeing and sustainable development. The United Nations Health Technologies Report, 2016 penultimately replicated WHO Model List of Essential Medicines as a tool to advocate for access to priority medicines in all countries. It also calls for more transparency about the patent status of essential medicines and build domestic capacity to procure generic copies and develop Medicines Patent Pool to all disease.\textsuperscript{15}

**Health & Sustainable Development (SDG) Goal 2015-2030:** The SDG Agenda demonstrates unprecedented scope and ambition in the context of the current intellectual property system has created situations where new treatments are launched at increasingly high prices. The SDG Agenda creates affordability and sustainability issues for health systems even in high-income countries. The third SDG Agenda enunciates to ensure healthy lives and promote well-being for all at all ages in its own right. It institutionalizes the fact that the health significantly contributes to environment and sustainable development goals as reliable indicator.\textsuperscript{16} To usher these objectives the SDG Agenda enjoins WHO to play a strategic role public health, innovation and intellectual property.\textsuperscript{17} It also mandates to build capacities by providing an appropriate balance between affordability and maintaining incentives for investment in R&D. These endeavor’s will result in downsizing of rising burden of chronic non-communicable diseases (NCDs) and removal of barriers to access to medicines deficits.\textsuperscript{18}

**Discussion**

India’s obligation to faithfully comply with its commitments under international treaties and resulted to the status of the pharmacy of the world. It protects the interest of the generic manufacturers who have been manufacturing certain drugs patented elsewhere by providing the compulsory licensing regime. This mechanism encourages innovations as well as inventions in India to secure the effective working on a commercial scale. The combined impact of these attempts enlarged the ambit and scope of the public policy in health sector in the existing legal and intellectual property framework.

**Access To Medicines In India:** The Patents (Amendment) Act, 1970, Patents (Amendment) Act, 1999, Patents (Amendment) Act, 2002 and Patents (Amendment) Act, 2005 are to be seen in continuum of TRIPS Agreement, 1995 and Doha Declaration on Public Health, 2001 and the most progressive patent laws in terms of diagnostic, surgical and therapeutic methods of treatments. India extracted benefit of TRIPS Agreement, 1995 and introduced product protection on pharmaceuticals by Patents (Amendment) Act, 2005. By product patent protection India created supply of low-cost active pharmaceutical ingredients and investment in R&D. The shift in public policy dimensions under legal and intellectual property framework is evidenced an exponential growth in pharmaceutical R&D in post Patents (Amendment) Act, 2005 phase.\textsuperscript{19} Indian pharmaceutical companies increased the share of its revenues by focusing on building on their strengths by launching generic versions of big-selling drugs in the from the developed world. In 2003, India was granted 72 pharmaceutical patents in the United States. Although this is a small proportion of the total, it makes India the eleventh largest foreign source of United States patents in that category.\textsuperscript{20} Thus the new patent regime not only enabled better access to medicines at more affordable prices but foster the pharmaceutical industry R&D an upswing trend.

**Health & Public Policy:** The National IPR Policy, 2016 provides strong and effective IPR laws, which balance the interests of rights owners with larger public interest. The policy take away is a vibrant Intellectual Property ecosystem imbibed in public welfare of health care within the framework of the Doha Declaration on Public Health, 2001.\textsuperscript{21} The mission statement of the policy is to stimulate a balanced intellectual property system in India to foster innovation entrepreneurship and enhance access to healthcare as sectors of vital importance.\textsuperscript{22} A perusal of seven broad objectives of policy reveals the desire and direction of the legislative space and flexibilities available in international treaties to engage constructively in the negotiation of human right healthcare, access to medicine and drug as an inalienable entitlement. The National Health Policy, 2017 also expresses a firm commitment to Intellectual Property protection establishing a direct correlation between IPR and healthcare access. It has been observed that economies with the strongest IP protections are 60 percent more likely to provide environments conducive to innovation. And economies with IP protection in life sciences see an average of 13 times more biomedical investment than those lacking IP protection.\textsuperscript{23} The target of universal healthcare can sustain in a cohesive scientific, economic and policy ecosystem of medical
innovation.

**Novartis Judgment:** The impact of legal and intellectual property policies reached to culmination in Novartis Judgment when the patent application for Gleevec was rejected by the Indian Patent Office in 2006. It appealed to IPAB in June 2009 and Madras High Court challenging the denial of patent under Section 3(d) of the Patent Act, 1970. The Madras High Court rejected these appeals on the ground that ‘the domestic courts could not be asked to give an opinion regarding international treaties and obligations and that Novartis should take its complaint to the disputes settlement mechanism in the WTO.’ Novartis further appealed to Supreme Court through special leave petition in 2012. The Supreme Court merged three petitions in one popularly known as Novartis AG and observed that:

The Court was urged to strike a balance between the need to promote research and development in science and technology and to keep private monopoly (called an ‘aberration’ under our Constitutional scheme) at the minimum. The Court was reminded of its duty to uphold the rights granted by the statute, and the Court was also reminded that an error of judgment by it will put life-saving drugs beyond the reach of the multitude of ailing humanity not only in this country but in many developing and under-developed countries, dependent on generic drugs from India.

The Novartis judgment fortified the right to health and access to medicine in public interest than that of commercial interest. The Indian courts played seminal role in enforcing the right to life encompassing health access and affordability of medicine in to national interest.

**Conclusion**

With the adoption of TRIPS Agreement, 1995 and the introduction of pharmaceutical patents in developing countries to the development of Anti retroviral (ARV) drugs related to HIV/AIDS and other tropical diseases have become matters of great concern and posing continuous challenge. The intellectual property rights and human rights auditing demands that access to affordable drugs for realizing agenda of sustainable development goals. The TRIPS Agreement, 1995 flexibilities as approved by Doha Declaration on Public Health, 2001 and TRIPS Agreement, 1995- plus should foster public funded research prioritizing public health objectives among the pharmaceutical companies. The regime which denies product patentability in the field of health naturally leads to reduced access to drugs and derogation of human right norms. This is illustrated by the fact that one-third of the world’s population does not have access to basic drugs and pharmaceutical patents are hitting hard to disadvantaged people. It calls for human rights to place over intellectual property laws and all countries must freely be able to use flexibilities granted under TRIPS Agreement, 1995 to access affordable medicines. There is need to create cohesive legislative and policy framework for access to medicine and health care system.

**Conflict of Interest:** No

**Source of Funding:** Self

**Ethical Clearance:** Not Required

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Assessment of the Awareness of Knowledge of Dental Records in Forensic Dentistry among Undergraduate Dental Students

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Abstract

Aim & Objectives: To assess the awareness of patient’s dental record maintenance among BDS students and to evaluate their knowledge regarding the potential use of their maintained records in forensic identification.

Materials and Method: Data was collected by means of a self administered questionnaire consisting of 24 closed ended questions, addressed on the awareness about patient’s dental record maintenance and its importance in forensic needs among ³rd, ⁴th year BDS students and interns of a private dental college.

Results: 73.1% of the respondents were aware that dentists were legally bound to maintain records in India and 83.5% were aware that these could contribute towards forensic investigations. Most of the students were aware of how to maintain dental records in an institution. 67.4% of the study population felt the need to store records for a minimum of 5 years. 65-70% had knowledge regarding denture markers and role of implants in forensic identification. The most perceived barrier to good dental record keeping was lack of time according to most of the students.

Conclusion: The survey revealed awareness on the importance of dental records in forensic identification among BDS students. Few areas showed inadequate knowledge, thus mandating an utmost need to train dental students in this field. Newer methods like denture marking and Aadhar number in corporeartion in prostheses should be given importance.

Key Words: Aadhar number, Denture markers, Dental records, Forensic Odontology, Undergraduate dental students.

Introduction

Personal identification is of paramount importance in forensic investigations, and this can be done on the basis of the theory that “all individuals are unique and can be identified on the basis of individual characteristics.”¹ Variety of methods can be used to corroborate forensic identity, but the reliability of each of these methods varies and moreover, cannot be used in all sorts of cases. DNA profiling, dermatoglyphics, anthropometric data, use of dental records are the standard methods employed.²³ Since dental remains are usually the last ones to decimate post mortem, these can be of utmost importance in personal identification in mass disasters, natural or man-made. In addition to this, dental records can also help in identification of suspects in criminal investigations and in medico legal cases.⁴
Dental records comprise of clinical notes, radiographs, plaster models, treatment done including serial numbers of prosthesis. These records encompass an in-depth and continual documentation of the patient’s conditions and treatment, hence helping in diagnosis and patient care. These records also serve research, administrative, quality assurance, education purposes and can also be used as evidence in legal proceedings.

Thus, generating detailed accurate patient records, maintaining, storing and retrieving them should be a crucial part of practice and this habit should be inculcated during the professional training of dental students. Keeping all these facts in mind, this study was conducted to assess the awareness of patient’s dental record maintenance among BDS students, and also to evaluate their knowledge regarding the potential use of the maintained dental records in forensic human identification.

Materials and Method

This was a cross sectional study using descriptive qualitative methodology. The study population comprised of 3rd, 4th year students and interns of a private dental college in Bhubaneswar, Odisha. Data was collected by means of a self-administered questionnaire consisting of 24 relevant closed-ended questions addressed on the awareness about patient’s dental record maintenance and its importance in forensic needs. All the students were explained about the nature and purpose of the study and consent was obtained. The study protocol was approved by the Institutional Ethics committee.

Statistical Analysis

Pearson’s Chi-Square test was used to evaluate the association between interns, final and third BDS students regarding awareness on dental record maintenance and its forensic implications. P value of less than 0.05 was considered statistically significant.

Results

A total of 242 students responded to the survey, in which 82 were from 3rd year, 77 from 4th year and 83 were interns. Data regarding awareness of respondents about the medico-legal aspect of dental records and its contribution towards forensic investigations is charted in Table I. It also shows comparative results of the most effective and fastest method of forensic investigation among the students.

Table II shows awareness and knowledge of students regarding how to maintain dental records, how long records should be stored in institutions and the preferred method of maintaining and storing them. Table III displays knowledge of students regarding details to be included in personal information of patients, use of diagnostic aids and reports in patients file and their use as ante-mortem records.

Interns had better knowledge about recording treatment details, materials used and their use in identification of the deceased. Regarding denture markers and role of implants in forensic identification, percentage of knowledge ranged between 65-70% and did not show any statistical significant result. Awareness regarding use of records, dental anomalies, intraoral camera captures and photographs was found to be satisfactory among the three groups, wherein 72.3% of students believed dental anomalies could contribute towards personal identification. Around 70% of the study population believed that incorporation of “Aadhar Number” in dental records and prosthesis would help in identification of deceased individuals.

Table IV shows the most perceived barriers to good dental record keeping, students perception on attending CDE programs and workshops on Forensic odontology and their take on relevance of forensic odontology for a successful clinical practice.

<table>
<thead>
<tr>
<th>Is dental record medico-legally important</th>
<th>Intern</th>
<th>Final Year BDS</th>
<th>III year BDS</th>
<th>%</th>
<th>P-value</th>
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</tbody>
</table>
Cont... Table I: Table showing knowledge regarding importance of dental record maintenance, its medico-legal aspects and its use in forensic investigations.

<table>
<thead>
<tr>
<th>Is dentist legally bound to maintain records in India</th>
<th>YES</th>
<th>NO</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>66</td>
<td>15</td>
<td>0.014*</td>
</tr>
<tr>
<td>Can patient Dental record contribute for forensic investigations</td>
<td>YES</td>
<td>NO</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>12</td>
<td>0.680</td>
</tr>
<tr>
<td>Fastest, cost-effective &amp; scientific method of forensic identification</td>
<td>DNA Profiling</td>
<td>49</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Dental record comparison</td>
<td>34</td>
<td>37</td>
</tr>
</tbody>
</table>

Table II: Table showing awareness of students regarding maintenance of dental records, their duration and preferred method of maintenance.

<table>
<thead>
<tr>
<th>Awareness about how to maintain records</th>
<th>Intern BDS</th>
<th>Final Year BDS</th>
<th>III year BDS</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>72</td>
<td>59</td>
<td>57</td>
<td>77.7</td>
<td>0.028*</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>18</td>
<td>25</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>Knowledge about how long the records should be stored</td>
<td>≤ 5 years</td>
<td>64</td>
<td>48</td>
<td>51</td>
<td>67.4</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 years</td>
<td>18</td>
<td>29</td>
<td>31</td>
<td>32.6</td>
</tr>
<tr>
<td>Preferred method of maintaining and storing Dental Record</td>
<td>Manually</td>
<td>12</td>
<td>7</td>
<td>30</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>Electronic</td>
<td>28</td>
<td>31</td>
<td>31</td>
<td>37.2</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>43</td>
<td>39</td>
<td>21</td>
<td>42.6</td>
</tr>
<tr>
<td>Which method will allow faster accessibility &amp; easy retrieval</td>
<td>Manually maintained Records</td>
<td>21</td>
<td>17</td>
<td>52</td>
<td>37.6%</td>
</tr>
<tr>
<td></td>
<td>Electronic Dental Records</td>
<td>61</td>
<td>60</td>
<td>30</td>
<td>62.4%</td>
</tr>
</tbody>
</table>
### Table III : Table showing knowledge of students regarding contents of dental records including personal details and diagnostic aids.

<table>
<thead>
<tr>
<th>Which contributed towards personal details of the patient</th>
<th>Intern</th>
<th>Final Year BDS</th>
<th>III year BDS</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Name</td>
<td>17</td>
<td>10</td>
<td>24</td>
<td>24.1%</td>
<td></td>
</tr>
<tr>
<td>Date of Birth &amp; Age</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>9.9%</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>6.6%</td>
<td>0.000*</td>
</tr>
<tr>
<td>Contact details</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.4%</td>
<td></td>
</tr>
<tr>
<td>Complete postal address</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>4.5%</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1.7%</td>
<td></td>
</tr>
<tr>
<td>All of the above</td>
<td>53</td>
<td>43</td>
<td>39</td>
<td>55.8%</td>
<td></td>
</tr>
</tbody>
</table>

Should diagnostic aids & their reports be recorded in patient’s file

<table>
<thead>
<tr>
<th>Should diagnostic aids &amp; their reports be recorded in patient’s file</th>
<th>Intern</th>
<th>Final Year BDS</th>
<th>III year BDS</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>71</td>
<td>59</td>
<td>52</td>
<td>75.2</td>
<td>0.004*</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>18</td>
<td>30</td>
<td>24.8</td>
<td></td>
</tr>
</tbody>
</table>

Can reports of diagnostic aids be useful as an ante-mortem record

<table>
<thead>
<tr>
<th>Can reports of diagnostic aids be useful as an ante-mortem record</th>
<th>Intern</th>
<th>Final Year BDS</th>
<th>III year BDS</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>73</td>
<td>53</td>
<td>60</td>
<td>76.9</td>
<td>0.010*</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>24</td>
<td>22</td>
<td>23.1</td>
<td></td>
</tr>
</tbody>
</table>

### Table IV : Table showing the most perceived barriers to good dental record keeping, students perception on attending CDE programs and workshops on Forensic odontology and their take on relevance of forensic odontology for a successful clinical practice.

<table>
<thead>
<tr>
<th>Most perceived barrier to good dental record keeping</th>
<th>Intern</th>
<th>Final Year BDS</th>
<th>III year BDS</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of time</td>
<td>24</td>
<td>17</td>
<td>38</td>
<td>32.6</td>
<td>0.040*</td>
</tr>
<tr>
<td>Lack of experience</td>
<td>18</td>
<td>22</td>
<td>16</td>
<td>23.2</td>
<td></td>
</tr>
<tr>
<td>Lack of training program/CDE</td>
<td>24</td>
<td>26</td>
<td>19</td>
<td>28.5</td>
<td></td>
</tr>
<tr>
<td>Increased work load</td>
<td>17</td>
<td>12</td>
<td>9</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>Attended any CDE programs/Workshops regarding Forensic Odontology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Cont... Table IV: Table showing the most perceived barriers to good dental record keeping, students perception on attending CDE programs and workshops on Forensic odontology and their take on relevance of forensic odontology for a successful clinical practice.

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>40</th>
<th>63</th>
<th>57%</th>
<th>0.040*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you think you need to know Forensic Odontology in detail for your clinical practice</td>
<td>No</td>
<td>48</td>
<td>37</td>
<td>19</td>
<td>43%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>70</th>
<th>63</th>
<th>76%</th>
<th>0.027*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>13</td>
<td>26</td>
<td>19</td>
<td>24%</td>
</tr>
</tbody>
</table>

Discussion

Production, maintenance and release of accurate, legible, comprehensive, organized records is the ethical and legal obligation of a dentist, and hence this practice must be inculcated during the period of professional training of students. Very few studies have been done to evaluate the awareness of importance of dental records among undergraduate dental students, and hence this study was undertaken to do the same.

Hannah et al performed a cross-sectional institution based survey among undergraduate dental students and evaluated their knowledge, attitude and practice of forensic odontology. They reported more than 80% of students had good knowledge about forensic odontology, 82% of their participants pointed their source of knowledge to be from lectures and workshops in college and 83% of their students were interested to undergo formal training in the field. On the contrary, 50.8% of our study population believed that they did not have adequate knowledge regarding the subject and its various methods. 57% of our study population had attended CDE and workshops on the subject, whilst 76% of our students believed they needed to know forensic odontology in detail to run an authentic clinical practice. Ignorance of forensic odontology during the undergraduate level may be responsible for students having comparatively lesser knowledge in this aspect.

Kutesa et al conducted a qualitative cross sectional study among 4th and 5th year undergraduate dental students to obtain information on the factors responsible for unsatisfactory state of dental records at the students clinic. Poorly designed clerking forms, inadequate storage space and poor maintenance of records were the factors attributed to this deficit, and according to them electronic system was deemed to be the ultimate solution to this problem. According to our study, lack of time was the most perceived barrier to good dental record keeping. In addition, factors like lack of experience, lack of training programme/CDE and increased work load were also responsible for this inadequacy. Our study also showed that interns and final years believed electronic dental records to allow faster accessibility and easy retrieval and hence had better understanding of dental record retrieval, a finding in accordance with the study of Kutesa et al.

Several studies have been done to assess the awareness of dental record maintenance among dental practitioners in India. Astekar et al conducted a cross sectional survey among practising dentists in Rajasthan, India and reported only 38% of the surveyed population maintained records. Preethi et al analyzed the knowledge, attitude and practice of forensic odontology among dental practitioners in Chennai and reported 21% of the practitioners did not maintain records in their clinics, while only 12% maintained complete records in which 93% did not maintain them for more than seven years, thus concluding inadequate knowledge, poor attitude and lack of practice of forensic odontology among the practitioners. Similar results were also reported by Harchandani et al and Sengupta et al from Pune and Ghaziabad respectively. On the contrary, Rahman et al reported an adequate level of knowledge, awareness and practice regarding forensic odontology among dental surgeons in Bhubaneswar, Odisha. Ramesh et al from their cross sectional questionnaire survey concluded that knowledge of forensic odontology among dental practitioners in Kanpur to be adequate, while lacking in the attitude and application of that knowledge.
undergraduate students go for clinical practice after their graduation, they should have adequate knowledge and right attitude to maintain dental records and thus should be inculcated during their training period.

Kannan et al assessed the knowledge and awareness on the application of prosthetics in forensic dentistry among dental practitioners in Chennai, and reported awareness amongst 62% of surveyed dentists. However, the acceptance and willingness was found to be very low. Our study revealed the percentage of knowledge regarding denture markers and role of implants to range between 65-70%. The field of forensic science could also benefit from the advent and incorporation of “Aadhar number - the unique identification number of Indian citizens”, in various prosthesis used in patient’s body and thus, help in the identification of an individual. Motivation and education on these grounds right from the training period of students are hence mandatory to promote their awareness, acceptance and application. Ours is the first study to explore the students knowledge in this context.

73.1% of the respondents in our study were aware that dentists were legally bound to maintain records in India, which could help in protection against any commercial, legal or medicolegal litigation. On the contrary, Astekar et al reported that none of the dentists in their study population were aware that it was legally mandatory to maintain records in India. However, the Indian Constitution under Article 51A(h) mandates the maintenance and preservation of medical, medicolegal and legal documents in the best interest of social and professional justice.

Patient records must formally document patient details, clinical findings, reports and treatment given, and these should be kept securely. Indian Law necessitates preservation of official records and documentation for a minimum of 8 years to avoid attracting penalties under Section 271 of Income Tax Act,1961. For legal suits, judicial records should be safeguarded for minimum 2 years in consumer cases, 3 years in civil cases and no time limit in criminal cases. The Indian Dental Association recommends that for practicability, a doctor may maintain records up to a minimum of 5 years to satisfy consumers and the judiciary, for protection against medical negligence and complications. According to 67.4% of our study population, dental records should be stored for a maximum of 5 years. But the Dental Council of India has not set any specific set of rules.

Conclusion

The result of the survey revealed the awareness on the importance of dental records in forensic human identification among BDS students. Few areas showed inadequate knowledge. Thus, there is an utmost need to train dental students to maintain good and adequate dental records as they are the future dental practitioners. Adequate time and guidance should be given to students for proper maintenance of records, so that the habit and attitude can be ingrained, which would prove to be beneficial in the longer run. Newer methods like denture marking and incorporation of Aadhar number in prostheses should be given importance.

Conflict of Interest: NIL

Source of Funding: Self

Ethical Clearance: Taken from the Institutional Ethics Committee.

References


A Study to Compare the Stress Patterns in the Edentulous Mandibular Bone around the Two Implant Retained Over Denture and the Prosthesis Restored with All-On-Four Concept Using the Three Dimensional Finite Element Analysis

Puneeth Hegde¹, Satish Shenoy², Shobha Rodrigues³, Thilak Shetty⁴, Umesh Pai¹, Sharon Saldhana¹, Mahesh M¹

¹Associate Professor, Department of Prosthodontics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal; ²Professor & Head, Department of Aeronautical Engineering, Manipal Institute of Technology, Manipal, Manipal Academy of Higher Education, Karnataka, India; ³Professor & Head, ⁴Professor, Department of Prosthodontics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal

Abstract

When we consider the rehabilitation of patients with the mandibular implant supported over denture, the influence of the implant number and the cantilever design on the stress distribution on the bone needs to be assessed precisely.

Purpose: The purpose of the simulation study was to compare the biomechanical behavior of the two implant supported over denture design with the All-On-Four Concept using the three dimensional finite element method thereby evaluating the Von Misses stresses induced on the implant under different loading simulations.

Materials & Method: Three dimensional models representing mandible restored with two implant supported prosthesis was compared with the models that were restored based on the ‘All-On-Four’ concept. The models were then subjected to four different loading simulations (full mouth biting, canine disclusion, load on cantilever, and load in the absence of cantilever). The maximum von Mises stresses were localized and quantified for comparison.

Results: Among the models, under all loading simulations, the maximum stress concentrations were along the neck of the implant. The stress levels for full mouth loading simulation was highest for two implant supported overdenture design and the least for All-On-Four overdenture design. In all the designs, the least stress was when the implants were loaded in a lateral direction. The stress levels for cantilever and non-cantilevered designs were nearly the same for all the simulated designs.

Conclusion: When tested under different loading simulations, both models showed similar location and distribution of stress patterns. Thus from the study it can be concluded that the All-On-Four Concept is a clinically applicable treatment option for the atrophic edentulous ridges and induces least amount of stresses on the edentulous ridges. Therefore the overall longevity of the prosthesis is greatly enhanced.

Key words: atrophic mandible, biomechanics, finite element analysis, implants supported prosthesis, tilted implants.

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Mangalore, Karnataka - 575001, India.

Introduction

Recent advancements in the field of oral implantology has greatly improved the options available for rehabilitation of edentulous patients with highly resorbed ridges. Other than enhanced retention and
stability the implant supported over denture design demonstrates lot of advantages like preservation of the alveolar ridges, increased comfort, chewing efficiency and overall improved quality of life when compared to the conventional complete dentures. A new concept named All-On-Four is now widely being applied for rehabilitation of completely edentulous arches with implant supported prosthesis.1,2,3,6.

According to this technique, the use of tilted implant permits cantilever reduction while warding off important anatomical structures. Poor planning and designing of the implant supported prosthesis can result in stress concentration and peri-implant bone loss. This eventually would lead to complete loss of implant. Hence an indepth understanding regarding the regions of stress concentration encountered by the implant and the supporting bone is essential for the long term success of the implant and the prosthesis.3,5,6.

Aim

To compare the stress patterns in the edentulous mandibular bone around two implant retained overdenture and the prosthesis restored with All-On-Four Concept using finite element analysis.

Objectives

To compare the biomechanical behaviour of the prosthesis restored with All-On-Four Concept with that of two implant supported mandibular overdenture using three dimensional finite element analysis.

To localize and quantify the Von Mises stresses induced on the implants under different loading simulations.

Material & Method

Armamentarium used for the study

- CT Scan of edentulous mandible
- Replace Select Tapered TiU NP 3.5 x 13mm (Nobel Biocare)
- The Profile Projector (METZ- 801)
- Cylindrical Retainer of 4mm diameter.
- ANSYS - 11 Workbench Software.

Preparation of FEM model of the Edentulous Mandible.

The computerized tomographic (CT) image of the human edentulous mandible was introduced into the Computer Aided Design (CAD) software. The ANSYS software was then used to simplify the CT image of the mandible into an arch shaped bone block with dimensions of 7.5mm thick and 15mm high. To simulate the type III bone which is commonly found in the mandibular posterior region, 1mm cortical bone layer was established overlying the entire mandible whereas the trabecular bone was used in the internal structure. After obtaining the computerized 3-Dimensional model, incorporation of the implant design into the model was planned.

Preparation of the FEM implant model

For a precise and more accurate analysis, the contour and design of the threaded implant is very important. However the shape and depth of the threads could not be evaluated and reproduced in the 3 dimensional model with the help of computerized tomography. Hence an instrument called ‘Profile Projector Optical System’ was used in the study. The values thus obtained were then used to design an accurate 3-D model of the threaded implant along with the retainer.

Profile Projector (METZ -801) Optical System

The profile projectors have the ability to display a two dimensional projection of a part rather than just simple linear dimension as in case of other gauging devices. The images are displayed in a viewing screen as an aid to precise determination of the dimension, form and physical characteristics.

This instrument creates work piece image on the projection screen at desired magnifications (10x, 20x, 50x) to provide accurate dimensional measurement as well as inspection of the contour and surface condition of the work piece. The accuracy of this instrument is known to be 0.001mm.

Preparation of the working model

Three dimensional working models were constructed using 3D computer aided design software (ANSYS). The models represented the mandible restored with 2 - implant supported prosthetic design and the design restored with the All On Four Concept. A rigid type III
For the 3-Dimensional two implant supported prosthesis model, the threaded implants were strategically placed vertically in the region of lateral incisors bilaterally.

For the 3- dimensional ‘All-On-Four’ model, two anterior implants were placed vertically in the position of the lateral incisors and two implants were placed bilaterally in the position of second premolars and tilted distally to 30º angle.

To evaluate and compare the distribution of stresses on the implant on the three models, four loading situations were simulated in each model using load values similar to those of functional bite movements from patients with implant supported prostheses.

- Loading 1: Full mouth biting – bilateral and simultaneous vertical static loads of
  - 200 N was applied on the occlusal surface of the first molars (Cantilevers)
  - 150 N on the occlusal surface of second premolars
  - 150 N on the occlusal surface of first premolars
  - 100 N on the distal of canines

- Loading 2: Lateral Load – Unilateral static load of 50 N applied in the region of left canine.

- Loading 3: Cantilever Load – Unilateral vertical static load of 200 N was applied on the left cantilever.

- Loading 4: Load without the cantilever - Unilateral vertical static load of 200 N was applied in the region adjacent to the left second premolar, simulating absence of cantilever.

The results of the numerical analysis are shown in Table - 2 for Von Mises stresses occurring for the FEM models.

The Graph 1 represents the biomechanical behavior of the two implant supported overdenture FEM modes when subjected to different loading simulations. The graph depicts maximum stress levels during full mouth loading simulation which was 2226.7 Mpa followed by cantilever loading simulation which was 813.09 Mpa and load without cantilever shown as 531.39 Mpa. The least stress for this model was found during the lateral loading simulation which was 64.76 Mpa.

Graph 2 illustrates the graphical representation of the biomechanical behavior of the FEM model simulating the prosthesis restored with the All-On-Four Concept. The maximum stress in this simulation was found during full mouth loading which was 253.37 Mpa followed by load simulating lateral load which was 88.01 Mpa and load simulating the cantilever load which was 85.22 Mpa. The least stress was found when load without cantilever was simulated which was 60.21 Mpa.

From the graphs it can be inferred that among the two models, the stress levels for full mouth loading simulation was highest for two implant supported overdenture design and the least for All-on-four overdenture design. For both designs, the least stress was when the implants were loaded in a lateral direction. The stress levels for cantilever and non-cantilevered designs were nearly the same for all the simulated designs.

Table – 1: Representing young’s modulus and poisson’s ratio.

<table>
<thead>
<tr>
<th>Material</th>
<th>Young’s Modulus (Mpa)</th>
<th>Poisson’s Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical Bone</td>
<td>13.7</td>
<td>0.30</td>
</tr>
<tr>
<td>Trabecular Bone</td>
<td>1.37</td>
<td>0.30</td>
</tr>
<tr>
<td>Titanium</td>
<td>115</td>
<td>0.35</td>
</tr>
<tr>
<td>Type III Gold</td>
<td>100</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Table – 2 : Representing peak stress values under different loading conditions.

<table>
<thead>
<tr>
<th></th>
<th>Two Implant (Mpa)</th>
<th>All-On-Four</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2226.7</td>
<td>253.37</td>
</tr>
<tr>
<td>B</td>
<td>64.76</td>
<td>88.01</td>
</tr>
<tr>
<td>C</td>
<td>813.09</td>
<td>85.22</td>
</tr>
<tr>
<td>D</td>
<td>531.39</td>
<td>60.21</td>
</tr>
</tbody>
</table>

Discussion

Bone remodeling is a constant process and continues throughout the life of an individual. However, despite having progressive medical and technological advancements, as age advances, the bone resorption pattern is faster and aggressive. This leads to a severely resorbed ridge thus compromising the function and esthetics when a conventional denture is delivered. Implant supported denture could be a good option but in patients with resorbed ridges, the amount of bone support might be compromised to assist in osseointegration and withstand masticatory load.

Among several techniques being used in rehabilitation of completely edentulous patients with implant supported prosthesis, the All-On-Four technique is considered to be the most successful and rewarding technique. A proper understanding of the load transfer and stress concentration under implant supported prosthesis is very essential before planning an implant supported prosthesis.

The finite element analysis is a very effective technique to obtain a detailed stress strain analysis at any location. The three dimensional finite element analysis has been used to evaluate the stresses around the various implant systems.

Keeping in mind the consequences of unwanted stresses, this study was an attempt to compare the Von Mises Stresses around the implant by different loading conditions, on two different finite element models. The models were simulated on the basis of implant number, position, angulation and the type of prosthesis which is a Type III gold bar.

References

6. Dorothy E Eger et al, Comparison of angled and standard abutments and their effect on clinical outcome,
Prosthetic Rehabilitation of Patient with Mixed Connective Tissue Disease Using Glove Type Silicone Finger and Toe Prosthesis

Thilak Shetty¹, Abhinav Sharma², Shobha J Rodrigues³, Vidya K Shenoy⁴, Umesh Pai⁵, Sharon Saldanha⁵, Mahesh M⁵

¹Professor, ²Postgraduate Student, ³Professor & Head, Department of Prosthodontics, MCODS, Mangalore, India, ⁴Professor, Department of Prosthodontics, A J Institute of Memorial Dental Sciences, Mangalore, India, ⁵Associate Professor, Department of Prosthodontics, MCODS, Mangalore, India

Abstract

The hand is a powerful tool and its loss causes a severe psychological and physical drawback. A considerable number of people each year suffer finger or partial finger amputations as a result of malignant disease, trauma, and congenital deformity.

Prosthetic restoration is usually considered difficult for the whole or a part of the finger and it worsens if multiple fingers are involved. Additionally in patients with mixed connective tissue disorders with overlapping clinical features of scleroderma and arthritis any undue pressure from the artificial rings or vacuum fit of the prosthesis might cause further vasoconstriction that could worsen blood flow to extremities. This clinical report describes a simple and economical method for prosthetic rehabilitation of a patient with mixed connective tissue disease following digital loss using a custom made prosthesis with medical grade tissue adhesive as retentive aid.

Keywords. Finger prosthesis, silicone, mixed connective tissue disorder

Introduction

Mixed connective tissue disease (MCTD) is a systemic disease of unknown etiology affecting as many as 1 person in 40, often with a predilection for the female sex and seen in a group of patients with overlapping clinical features of lupus, scleroderma, polymyositis, and rheumatoid arthritis.¹ A defining feature of MCTD is the presence of antibodies against the U1-ribonucleoprotein (U1-RNP) complex.¹ The prognosis for patients varies from a benign course to severe progressive disease.

In approximately one third of patients the clinical symptoms go into long-term remission. One third of patients have a severe, progressive disease course with the most severe clinical manifestation being pulmonary hypertension contributing to premature death.² Although in the benign variety most patients are unlikely to have life-threatening consequences, 25% report with digital necrosis which may be extremely debilitating and distressing, significantly reducing quality of life for the patient and his or her family.² Rehabilitation of patients with loss of digital substance can be accomplished either prosthetically or by plastic surgery. The method of rehabilitation depends upon the site, size, severity, age, and the patient’s wishes. However, age, general medical condition of the patient, anatomic complexity, appearance of the area to be rehabilitated, complexity of the surgical procedure, and the patient’s refusal to undergo further surgery may contraindicate surgical reconstruction, resulting in a visible defect. Prosthetic rehabilitation of such patients then has considerable

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advantages, in that prosthesis offers the clinician and the patient the means to observe the health of the tissues, esthetic improvement, technical simplicity and inexpensive care.

For several decades, a number of biomaterials and techniques have been used in the fabrication of finger prostheses. Each material has its advantages and shortcomings. Methyl methacrylate resin has been used as a maxillofacial material because it is easy to work with, hygienic, durable, and economical. Also, it can be satisfactorily colored to match individual skin tone. However, its use is limited by its rigidity. Silicones are generally the preferred materials for fabrication because of its lightweight and life-like appearance. They are synthetic polymeric chains where silicone atoms bond to organic groups typically methyl groups.\(^3\) Silicones are grouped as Class I-IV according to their applications,\(^4\) with the Class II medical grade silicone commonly used for prosthetic fabrication. However, silicone materials fall short of an ideal maxillofacial prosthetic material as adhesives do not work well with silicones, they are difficult to polish, have low tear resistance, and have microbial growth-promoting characteristics. Their use is limited in transcarpal residual limbs, unstable residual limb volume with fluctuations of more than 5%, unhealed residual limb wounds and when the finger joint/wrist and residual limb end that are unable to bear weight.\(^5\) Although attempts have been made to greatly improve the properties of various maxillofacial materials, there is still no ideal material that resembles or duplicates human skin.\(^4,6\)

Success of the prosthesis depends both on the appearance and retention, active grasp with the prosthesis being a key determinant in patient acceptance. Finger prosthesis are challenging in terms of retention. The various methods to increase the retention of the prosthesis are well documented which include customized artificial rings, medical adhesives, creating vacuum fit, etc.\(^7-11\) Leow et al\(^11\) studied optimal circumference reduction of finger models and suggested that 5–7% circumference reduction in the finger showed superior results as compared to 1–3% and 8–9% of reduction.

Conventional retention aids should not be used in patients suffering from Raynaud’s phenomenon as any undue pressure from the artificial rings or vacuum fit might cause further vasoconstriction that could worsen blood flow to extremities.

Medical grade adhesives when used as retentive aids can provide excellent retention. However, disadvantage of medical adhesives is its maintenance. The prosthesis should be removed everyday so that underlying tissues can rest overnight to maintain the health of the tissues. Everyday removal of residual adhesive from under surface of prosthesis and skin can be cumbersome for some patients. In addition, in the post fitting review patients must be advised to clean their prosthesis with soap and water on a daily basis. Abrasives and bleaching agents need to be avoided and the delicate feather edge margins of the prosthesis must be dealt with care.

Technological advances have made available highly dexterous hand and I LIMB\(^{12-14}\) as well as digital technology to accurately replicate patient’s finger characteristics. However the cost of these advances limits their use in most patients.

This clinical report describes a simple and economical method for prosthetic rehabilitation of a patient with mixed connective tissue disease following digital loss using a custom made glove type of prosthesis with medical grade tissue adhesive as retentive aid.

**Case Report**

A 42-year-old female patient diagnosed with MCTD and symptoms of arthritis and Raynaud’s phenomenon reported to the Department of Prosthodontics, of this Institution, for the fabrication of a Fixed Partial Denture involving the left maxillary first premolar. On general examination, limited mouth opening was noted. In addition, the left index, ring, right index finger and right big toe was malformed and the left little finger was missing (Figure 1, 2). The patient was counseled to attend to this digital loss in addition to the missing teeth. Various modalities of prosthetic reconstruction were discussed with the patient and following informed consent, glove type silicone finger prosthesis was planned for the malformed fingers and the expectations of this prosthesis were discussed with the patient. The left little finger was not restored since there was no residual finger stump for adequate retention of glove type prosthesis. Implant supported finger prosthesis was also contraindicated due to the progressive arthritis that the patient was suffering from. Conventional retention aids were not used in this case considering the patient was suffering from Raynaud’s phenomenon. Any undue pressure from the artificial rings or vacuum fit might...
cause further vasoconstriction that could worsen blood flow to extremities. Hence, medical adhesive was used for retention of prosthesis.

The defective and donor hand and foot were lubricated with a thin layer of petroleum jelly and its boundary was boxed with modelling wax to confine the impression material. The impression was obtained in resting state with irreversible hydrocolloid (Imprint; Dental Products of India Ltd). In addition the impression of the individual defective fingers were also obtained and were all poured in Type III dental stone (Dentstone; Pankaj Industries). The impression of the donor appendages, however, was poured in modelling wax and used for characterization of their respective stump mold. The prosthesis was waxed to form and verified at the trial insertion appointment for acceptability. The wax pattern along with the mold stump was indexed, relieved of undercuts and invested in such a manner such that the dorsal and palmar surfaces of the fingers were separable. The wax was eliminated and the mold space (was packed in the presence of the patient with medical grade silicone (Silicone for Prosthesis; M P Sai Enterprises). The silicone was proportioned based on the skin tone at various regions of the finger. Basic skin tone color was formulated using intrinsic coloring pigments to the closest accuracy possible. The respective proportions of the silicone shades were layered on the regions of ventral surfaces and dorsal surfaces. The material was processed overnight as recommended by the manufacturer. The prosthesis was recovered after polymerization trimmed and finished using sharp scissors and finishing burs. The fit and shade was evaluated on the patient. The fingernail was processed using autopolymerising acrylic resin (DPI-RR; Dental Products of India Ltd) and pigmented to match the patient nails to further enhance aesthetics and to break the monochromatic appearance final coloration was achieved by extrinsic staining procedure. Retention in this case was obtained by using medical grade skin adhesive (M.D. Enterprises, Mumbai, India) and patient was educated about its daily use and cleaning technique. The prosthesis was inserted (Figure 3, 4) and the patient instructed on maintenance and home care.

The patient was scheduled for the first post-insertion adjustment 3 days after the insertion. At the first post-insertion appointment the malformed fingers were observed to ensure health of the tissues, to relieve the prosthesis for pressure areas on the tissues, to compensate for processing changes, and to emphasize hygiene and home care. In the post fitting review patients was advised to clean their prosthesis with mild antibacterial soap and water on a daily basis and to avoid wearing the prosthesis in case of inflammation or during sleep.[15] The patient was satisfied with prosthesis and was placed on a 3-month recall for evaluation and observation.

Figure 1 and 2. Preoperative view of the patient.
Figure 3 & 4. Post-treatment view of patient.

Figure 1 & 2 : Preoperative view of the patient

Figure 3 & 4 : Postoperative view of the patient

Summary

A convenient and affordable method of prosthetic rehabilitation of an amputated finger with room temperature vulcanizing silicone material has been presented. The custom made glove type finger prosthesis is esthetically acceptable and comfortable in patients with amputated or malformed fingers, resulting in psychological
improvement and personality.

**Conflict of Interest :** Nil

**Source of Funding :** Self

**References**


Perception of Students Regarding Gender based Violence

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Abstract

Objectives: The study is undertaken to explore perception of students regarding violence against women with specific reference to domestic violence and sexual violence.

Method: This is a descriptive cross-sectional questionnaire based exploratory study conducted in Mangalore, Karnataka, India. 220 participants consisting of age group between 18 to 25 years were included. Responses were obtained from the study participants through open and close ended questionnaires. Results: More than half (60%) of medical students and (56%) of engineering students disagree that in modern India women are enjoying equal rights to men. Three forth (77% male, 82% female) of students believe and disagree that it is alright for a man to beat his wife in order to make her obedient. Two forth (61%) of medical students and (63%) of engineering students agree that throwing acid on a woman to cause disfiguration is an example of gender based violence. Conclusion: The perception of medical and engineering students regarding gender based violence is quite similar. This study brought out issues of increased violence against women. A significant number still felt that dressing of a woman may be a factor for sexual assault. Women are still facing gender inequality and are not getting equal rights to men. Even though incidence of harassment, domestic violence are on the rise, women are not ready to report about their suffering because of social and family pressure. There is also need for awareness to be bought in gender mainstreaming for educating all sectors of the society.

Key Word: Sexual harassment, Crime against women, Domestic violence.

Introduction

Women are important part of our society and play vital roles in all aspects of life but their identity is restricted to being mother, sister, wife, or daughter1. In ancient Indian time’s as mentioned in Rigveda and other scriptures women held the most prestigious and honoured place in society2. The status of women in society over the period of time seems to be controversial, violence against women has been increasing and a universal concern too as men hold themselves superior and believes that women are mediocre. Women are given low status at work place and home. In last few decades there has been increase in the incidence of violence against women3. Globally violence against women has increased, it has been reported that more the one in three women (35.6%) have been subjected to sexual or physical violence by their friends or relatives and 38% of all murders are committed by their spouse4,1. 05% of murder of men is committed by their spouse. A woman has been subjected from verbal abuse, emotional abuse, physical and sexual exploitation leading to femicide. Worldwide 5000 murders in the name of honour killing and 25000 in the name of dowry death have been reported every year. There are also reports that the women are victim of sexual violence in wars and mostly prone to human right violation5. Unwanted pregnancy, child birth and child abuse may be a sign of violence against women. India is the second densely inhabited democratic country and seventh largest country in the world with 1.3 billion people, 48.4% are females and 51.6% are males6. Developing India is emerging as a global power but still women have to struggle for their position and status in the society. Women are subjected to violence irrespective of their cast, educational status, class and

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their subjugation adds to their suffering by physical, emotional, mental and economical deprivation\textsuperscript{7,8}.

In India women have been subjected to violence and they are suffering from various causes such as cheating, rape, torture, abduction, violent pornography, immoral trafficking and so on. A total of 3,27,394 cases of crime against women were reported in year 2015, 29.9\% of sexual harassments, 10.4\% physical assault, 7.6\% stalking, 1.0\% voyeurism, and 51.8\% other cases. Human trafficking has increased from 0.4\% in year 2014 to 0.5\% in year 2015. According to NCBR “head wise percentage of crime against women” during year 2015 there were 34.6\% cases of domestic violence, 25.2\% cases of assault on women with intent to outrage her modesty, 18.1\% cases of kidnapping and abduction of women, 10.6\% of rape cases, 3.05\% cases of dowry prohibition, 2.35\% cases of dowry death, 2.7\% cases of insult of modesty of women, 1.4\% cases of attempt to rape, 1.2\% cases of abetment of suicide by women, 0.7\% cases of immoral trafficking of women, 0.25\% other crimes.\textsuperscript{8}

Women have to suffer from gender violence throughout their life. As a child her father is in charge of her, as an adult her husband is in charge of her and after death of husband, her son takes over charge of her.\textsuperscript{9}

Rafique vs state of U.P 1980, Justice Krishna Iyer said “when a woman is raped, what is inflicted is not mere physical injury but a sense of some deathless shame” and judiciary response could not be muted to such grave human right violation\textsuperscript{10}. The implementation of preliminary measures gives a perception that the present criminal justice system India is not robust to protect women.\textsuperscript{10}

Women who suffer from sexual assault have physical and mental issues, poor sleep, irritable bowel, diabetes, post-traumatic stress disorder and various studies has shown that they were benefited with screening and discussing sexual assault with health care provider and all this helped in their recovery.\textsuperscript{12}

Gender based violence is a serious issue all the students undergoing medical education should be taught about the importance of the issue and how to address such cases\textsuperscript{13}.

The study was undertaken with the objective to explore perception of students regarding violence against women with specific reference to domestic violence and sexual violence.

**Material and Method**

This is a descriptive cross-sectional questionnaire based exploratory study conducted in Engineering and Medical Colleges of Mangalore, Karnataka, India. Questionnaire was externally validated by two subject experts. After obtaining institutional ethical clearance and due necessary permissions study was conducted and data were collected from the study area. The study was conducted in October 2018 to November 2018. 220 participants between ages 18 to 25 years were included. Responses were obtained from the study participants through close and open ended 25 items questionnaires. The data was analysed using SPSS version 20.0 software. Chi – square test was applied.

**Result**

In the present study, out of 220 participants, 110 (50\%) were Medical students and 110 (50\%) were engineering students of which 119 males and 101 females. There is no significant difference noted on various issues amongst the students from medical and engineering streams. 59.1\% of medical students and 55.4\% of engineering students did not agree that in modern India women are enjoying equal rights as men. Nearly half of medical and engineering students agreed that domestic violence in women is common and widespread issue. More than 80\% of medical and engineering students disagree that women feel safe while travelling alone and going out at night. More than 70\% of medical and engineering students agree that vulnerable group such as minors and women with disabilities are more prone for sexual violence.

More than 70\% of medical and engineering students agree that most of women hesitate to report about crime against them because of social and family pressure. Nearly 30\% of male and 20\% of females agreed that man can beat his wife to make her disciplined. 51\% of medical students and 50\% of engineering students (24\% of male and 19 \% female) believe that dressing style of a woman is be a factor for sexual assaults. More than 60\% of medical engineering students agree that throwing acid on a woman to cause disfiguration is an example of gender based violence. 59.1\% of medical students and 69.1\% of engineering students agree that if a person with political background or influence is named as perpetrator of sexual violence, they are protected.
by investigating officer. 77.31% of medical students and 80% of engineering students agree that there is a stigma regarding sexual assault survivor in India. 78.2% of medical students and 91.1% of engineering students agree that domestic violence is preventable.

Medical students perceive that percentage of perpetrators on violence against women are strangers 46.4%, family 30.9%, relatives 21.8%, friends 0.9% and engineering students report that perpetrators of violence on women are strangers 69.1%, relatives 20.9%, family 8.2% friends 1.8%. Medical students perceive that the place where women is commonly assaulted are public transport 44.55%, work place 30.0%, home 15.5%, market place 10%. Engineering students report that the place where women are commonly assaulted were public transport 60.9%, work place 30%, market place 6.4% and home 2.7%.

Medical and engineering students report that information on violence against women comes largely from Internet more than 50%, TV and Radio more than 40%, newspaper more than 10%, and others.

Medical students report that place where a victim of sexual assault more likely to visit is first to Primary health centre, second to Community health centre, then so on Secondary Hospital, District Hospital, Tertiary Hospital and engineering students report that place where a victim of sexual assault more likely to visit first to Primary health centre, second to Community health centre, then so on Secondary Hospital, Tertiary Hospital, District Hospital.

More than 90% of medical and engineering students agree that workshop and continued hands on training in Forensic Medicine is important to deal with cases in rural areas. 78.2% of medical students and 89.1% of engineering students agree that the attitude of people in India can be changed regarding sexual assault victim.

Discussion

In the present study, results did not indicate that Medical and Engineering stream students significantly differed in their perceptions regarding gender based violence. Mangali RN et al conducted a study in year 2007, women are considered lower compared then men in society and women suffer from gender discrimination in all aspects of life17. Similarly, in the present study It was observed that more than half of total number of participants (m=60, e=56) disagree that in modern India women are getting equal rights to men.

Watts C et al in year 2002 reported that child sexual abuse is common and under reported because most of times they are threatened or the perpetrators is family member16. Similarly, in the present study three forth of students (73%) believe that vulnerable group such as minors are more prone for sexual assault.

Imran M et al 2010, has reported crime against women is increasing, sexual violence cases are often under reported because of stigma and shame related to the survivor of sexual assault19. Similarly, three forth of students (79%) believe that there is a stigma regarding sexual assault survivor in India. In the same study Imran in 2010 has reported that, domestic violence so common in our society, that six out of every ten husbands have beaten their wives. Sexual harassment is often not reported due to fear of family and fear of isolation from society18. Similarly, in the present study majority of students (85%) believe that domestic violence is a wide spread issue and at least, one fourth of the students (26%) still believe that it is alright for a man to beat his wife in order to make her obedient. This is not only a perception prevalent in male (25%) but also shared by females (20%). This is a concerning fact as violence against women cannot be stopped if the belief by disciplining a woman by physical force continues. Similarly, if this is deep rooted in females, the inflicted violence on self gets justified and she may not access the health care or report to police.

Naskar G et al in year 2015, reports of wife battering and violence against widows are also on rise and legal measures should be taken for elimination of violence against women. The Indian cultural system is not open for wife reporting to the police of having been battered by her husband. Similarly, in the present study three forth (79%) of students believe that women hesitate to report about crime against them because of social and family pressure.

Nagindrappa M et al 2013, reports that women in India are not given equal rights to men irrespective of their cast, class, educational status. They are physically, socially, mentally and economically exploited since long7. Bhawana D et al reported that various crime against women that occur in India such as sexual assault, kidnapping, abduction, dowry death, torture, molestation has been increased 59% since year 20112. Similarly, in present study three forth (78%) of students believe that
violence against women in our society is common and is on rise.

Waghamoda R.H 2013, conducted a study on domestic violence where women were the victims which is similar to the study by Badiadka 2017 where much of the public assault was on males and domestic violence in females14. The World Human Rights Conference in Vienna, recognised gender based violence as human right violence in 1993. Article 14, 15 and 21 of Indian Constitution provides protection of women from domestic violence (domestic violence Act, 2005). There is cultural gender inequality, economic dependence on men, political under representation, less education and awareness among women and they are facing domestic violence by men because of all these reasons3. Present study students perceived that domestic violence is preventable (85%).

T.K.K Naidu et al 2007, reported that Forensic Medicine has an important role in detection and help in administration of justice, forensic training must be given to Medical and Para Medical students to deal with cases in rural and semi urban areas. Proper Medicolegal investigation will help in solving of cases so that cases will not be undetected and unconvicted15. Similarly, in present study majority (95%) of students reported there is requirement of specialized nursing training “Forensic nursing” as it is in developed countries and majority (93%) reported that workshop and continue hands on training in Forensic Medicine is important to deal with cases in rural areas.

Hossain N 2015, reported that gender based violence is a serious issue all the students undergoing medical education should be taught about the importance of the issue and how to address such cases13.

Watts C 2002, reported that some of the most common and severe forms of violence against women by intimate partner are physical abuse, psychological abuse, acid throwing, sexual abuse, trafficking for sex, forced prostitution, exploitation of labour17. Similarly, in the present study more than half (61%) of students agree that throwing acid on a woman to cause disfiguration is an example of gender based violence.

In the present study more than half of students believe that the perpetrators of violence on women are mostly strangers (58%). This is contrary to the study done by Madeswarana et al 2013, majority of cases perpetrators of violence on women were acquaintances such as family member, friends and relatives20.

Conclusion

The perception of medical and engineering students regarding gender based violence is quite similar. This study brought out issues of increased violence against women. One third of the students still believe that it is alright for a man to beat his wife to make her obedient. More awareness programs should be conducted in gender mainstreaming. Women are still facing gender inequality and are not getting equal rights to men. Even though incidence of domestic violence is on the rise, it may not get reported because of social and family pressure. There should be sensitzation program for every stream of students on gender related issues. Registered medical practitioner and Forensic nursing should be available so that when a woman accesses a health centre at any level, be it primary or tertiary, she gets appropriate care and medicolegal service. Investigating agencies, medical and paramedical staff should be sensitized and trained in this regard.

Limitations of Study: Only medical and engineering students from select colleges are considered for study. They do not represent population of India.

Conflict of Interest: There is no conflict of interest.

Ethical approval: Institutional ethical committee (centre of ethics) approval has been obtained (Reference no-YEC198/2018

Source of Funding: Self.

References


Evaluation for Correlation of Cheiloscopy and Dermatoglyphics in Gender Determination

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Abstract

Background: Identification of the suspect, living or dead with the help of physical characteristics play a vital role in forensic science. Improved methods of gender determination and personal identification are important in solving criminal cases. Cheiloscopy and dactyloscopy have been proved to be of use in personal identification. The present study aims to evaluate correlation of Cheiloscopy and Dermatoglyphics in gender determination.

Materials and Method: The study was carried out from January 2017 to January 2019 on 2112 individuals (1056 males and 1056 females) aged from 15 to 60 years in KMCT Dental College, Calicut. For lip print analysis Suzuki and Tsuchihashi classification was followed. For finger print analysis impression of all the fingers were taken. For analysis, the images were cropped and abode photoshop software was used.

Results: Type 1 and 1’ lip groove patterns were found to be predominant among males. Type 4 and Type 5 lip groove patterns were found to be predominant among females. Loop finger print patterns were found to be predominant among males. Whorl finger print patterns were found to be predominant among females. Positive correlation (+0.80) was found between loop finger print pattern and Type 1’ lip print pattern in males which is statistically significant (p=0.003). Positive correlation (+0.75) was found between whorl finger print pattern and Type IV lip groove pattern in females which is statistically significant (p=0.005).

Conclusion: There was definite correlation of Cheiloscopy and Dermatoglyphics in gender determination.

Keywords: Cheiloscopy, Dermatoglyphics, Gender determination.

Introduction

Forensic science plays a vital role in the identification of the suspected victim or victimizer of a crime, with the help of physical characteristics. Physical evidence is important to solve criminal cases. Improved methods of gender determination and personal identification are gaining importance in recent years.[¹,²]

Lip patterns and finger print patterns remain the same throughout life and are uninfluenced by environmental changes and diseases. Cheiloscopy is a forensic investigation technique that deals with identification of humans based on lip traces. A lip print at a crime scene would throw light on many areas of the investigation like the number of people involved, the gender and the behavior pattern of the crime. Edmond Locard was the first scientist to recommend the use of lip prints in personal identification.[¹]

Dermatoglyphics is the term specified to the scientific study of fingerprints. The term “fingerprint” predominantly means an impression of the epidermal ridges of the fleshy distal portion of a finger. It was first given by Harold Cummins in the year 1926.[³]

Cheiloscopy and dactyloscopy are used in personal identification.[⁴,⁵] The present study aims to evaluate
the uniqueness of lip prints and thumb print patterns and to establish a correlation between them for gender determination.

**Methodology**

The study was carried out from January 2017 to January 2019 on 2112 individuals (1056 males and 1056 females) aged from 15 to 60 years in KMCT Dental College, Calicut. The study protocol and objectives were thoroughly explained to the participants and informed consent was taken from them. The ethical approval was obtained from Institutional Ethical Committee of KMCT Dental College.

Individuals with normal transition zone between the mucosa and skin of both lips and fingers were selected. The Excluded criteria were persons with lip scar, Lip congenital deformities, active lesions on fingers or lips and persons with hypersensitivity to lip sticks.

Materials used to record the lip groove patterns and finger prints were: red coloured lip stick (Lakme, India), cellophane tape, ink (Kores, extra dark pad), white paper and magnifying lens. Costa and Caldas technique was used to record the lip groove pattern, Kucken technique was used for the finger print pattern.

The individuals’ lips were cleaned with wet tissue before starting the procedure. The lip stick was gently applied to the upper and lower lips and the individual was asked to roll the lips in a uniform manner from centre to the corner of lips.

The individual was asked to stop moving the lips during the procedure and to keep the lips in relaxed state. The lip groove patterns were lifted by cellophane scotch tape on upper lip from one side to another; following which the tape was pasted on a white bond paper as a permanent record. The same process was repeated for lower lips. The obtained lip groove patterns were carefully examined under the magnifying lens.

Finger print recording was done by asking the subjects to ink all their finger pads, both right and left hands, and the impression was recorded on a white bond paper. Only one impression was made to avoid artifacts. Then the finger print pattern was studied with the help of magnifying lens and the predominant pattern of 10 finger pads (in each subject) was quantified tabulated and analysed statistically.

Lip groove patterns were categorized into four regions predominantly by dropping a perpendicular from the philtrum of lips; Upper Right (UR) region, Upper Left (UL) region, Lower Right (LR) region and Lower Left (LL) region. The analysis was done as per the Suzuki and Tsuchihashi classification. The classification is as follows: Type I: clear cut grooves running vertically across the lips; Type I’: grooves are straight but disappear halfway instead of covering the entire breadth of the lip; Type II: grooves fork in their course; Type III: grooves intersect; Type IV: grooves are reticular; Type V: grooves do not fall into any of the Type I to IV and cannot be differentiated morphologically.\(^6,^7\) (fig.1)

The finger prints were categorized as follows: Ridge count of thumb, index, middle, ring and little fingers of both hands, Right hand total finger ridge count (TFRC), Left hand total finger ridge count (TFRC), Absolute finger ridge count (AFRC). The finger prints were qualitatively categorized as Arch (simple arch, tented arch), Loop (ulnar loop and radial loop) and whorl (simple, spiral, double pocket, composite) patterns.\(^3\) (fig 2)

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**Fig 1: Armamentarium: Cheiloscopy**

**Fig 2: Armamentarium: Dermatoglyphics**
Statistical analysis

Obtained data was tabulated and statistically evaluated using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Normality of the data was assessed by Kolmogrov-Smirnov Test. If data follows normal distribution, Independent ‘t’ test was used. If data follows non-normal distribution, Mann-Whitney ‘U’ test was used. Chi square test: for association of qualitative variables. Pearson’s Correlation was used for assessing the correlation between cheiloscopy and dermatoglyphics.

Results

Type 1 and 1’ lip groove patterns were found to be predominant among males. Type 4 and Type 5 lip groove patterns were found to be predominant among females (Table 1 and 2).

Loop finger print patterns were found to be predominant among males. Whorl finger print patterns were found to be predominant among females (Table 3-6).

Positive correlation (+0.80) was found between loop finger print pattern and Type 1’ lip print pattern in males which is statistically significant (p=0.003). Positive correlation (+0.75) was found between whorl finger print pattern and Type IV lip groove pattern in females which is statistically significant (p=0.005)(table 7).

Table 1: Lip Groove patterns among males. A total of 2112 lip regions among 1056 males were assessed for the lip groove patterns and the variables of upper lip and lower lip are compared.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Upper Lip</th>
<th>Lower Lip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lip groove patterns</td>
<td>Number (n) (Percentage)</td>
</tr>
<tr>
<td>1</td>
<td>Type I</td>
<td>1984(93.9)</td>
</tr>
<tr>
<td>2</td>
<td>Type I’</td>
<td>2008(95.1)</td>
</tr>
<tr>
<td>3</td>
<td>Type II</td>
<td>970(45.9)</td>
</tr>
<tr>
<td>4</td>
<td>Type III</td>
<td>872(41.3)</td>
</tr>
<tr>
<td>5</td>
<td>Type IV</td>
<td>376(17.8)</td>
</tr>
<tr>
<td>6</td>
<td>Type V</td>
<td>796(37.7)</td>
</tr>
</tbody>
</table>
Table 2: A total of 2112 lip regions among 1056 females were assessed for the lip groove patterns and the variables of upper lip and lower lip are compared.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Upper Lip</th>
<th>Lower Lip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lip groove patterns</td>
<td>Number (n) (Percentage)</td>
</tr>
<tr>
<td>1</td>
<td>Type I</td>
<td>576(27.3)</td>
</tr>
<tr>
<td>2</td>
<td>Type I'</td>
<td>492(23.3)</td>
</tr>
<tr>
<td>3</td>
<td>Type II</td>
<td>684(32.4)</td>
</tr>
<tr>
<td>4</td>
<td>Type III</td>
<td>596(28.2)</td>
</tr>
<tr>
<td>5</td>
<td>Type IV</td>
<td>1978(93.7)</td>
</tr>
<tr>
<td>6</td>
<td>Type V</td>
<td>2022(95.7)</td>
</tr>
</tbody>
</table>

Table 3: Finger print patterns of right hands of 1056 males were recorded. L- Loop, RL- Radial Loop, UL- Ulnar Loop, A- Arch, PA- Plain Arch, TA- Tented Arch, SW- Single Whorl, DW- Double Whorl. N- Total number of finger print patterns of right hands of males (1056 males x 5 right hand fingers = 5280).

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Finger Print Patterns</th>
<th>Number (n) (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loop (RL +UL)</td>
<td>3874 (73.4)</td>
</tr>
<tr>
<td>2</td>
<td>Arch (PA + TA)</td>
<td>348 (6.6)</td>
</tr>
<tr>
<td>3</td>
<td>Whorl (SW + DW)</td>
<td>1058 (20.0)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5280 (100)</td>
</tr>
</tbody>
</table>

Table 4: Finger print patterns of left hands of 1056 males were recorded. L- Loop, RL- Radial Loop, UL- Ulnar Loop, A- Arch, PA- Plain Arch, TA- Tented Arch, SW- Single Whorl, DW- Double Whorl. N- Total number of finger print patterns of left hands of males (1056 males x 5 left hand fingers = 5280).

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Finger Print Patterns</th>
<th>Number(n) (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loop (RL +UL)</td>
<td>4764 (95.2)</td>
</tr>
<tr>
<td>2</td>
<td>Arch (PA + TA)</td>
<td>385 (3.1)</td>
</tr>
<tr>
<td>3</td>
<td>Whorl (SW + DW)</td>
<td>131 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5280 (100)</td>
</tr>
</tbody>
</table>
Table 5: Finger print patterns of right hands of 1056 females were recorded. L- Loop, RL- Radial Loop, UL- Ulnar Loop, A- Arch, PA- Plain Arch, TA- Tented Arch, SW- Single Whorl, DW- Double Whorl. N- Total number of finger print patterns of right hands of females (1056 females x 5 right hand fingers = 5280).

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Finger Print Patterns</th>
<th>Number(N) (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loop (RL +UL)</td>
<td>322 (6.1)</td>
</tr>
<tr>
<td>2</td>
<td>Arch (PA + TA)</td>
<td>540 (10.2)</td>
</tr>
<tr>
<td>3</td>
<td>Whorl (SW + DW)</td>
<td>4418 (83.7)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5280 (100)</td>
</tr>
</tbody>
</table>

Table 6: Finger print patterns of left hands of 1056 females were recorded. L- Loop, RL- Radial Loop, UL- Ulnar Loop, A- Arch, PA- Plain Arch, TA- Tented Arch, SW- Single Whorl, DW- Double Whorl. N- Total number of finger print patterns of left hands of females (1056 females x 5 left hand fingers = 5280).

<table>
<thead>
<tr>
<th>No</th>
<th>Finger Print Patterns</th>
<th>Number (N) (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loop (RL +UL)</td>
<td>459 (8.7)</td>
</tr>
<tr>
<td>2</td>
<td>Arch (PA + TA)</td>
<td>497 (9.4)</td>
</tr>
<tr>
<td>3</td>
<td>Whorl (SW + DW)</td>
<td>4324 (81.9)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5280 (100)</td>
</tr>
</tbody>
</table>

Table 7: Gender wise comparison of correlation between predominant finger pattern and lip pattern among males and females. Correlation done by Karl Pearson’s correlation coefficient p value <0.010: HS

<table>
<thead>
<tr>
<th>Gender</th>
<th>Correlation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>r = +0.8</td>
<td>0.003 HS</td>
</tr>
<tr>
<td>Female</td>
<td>R = +0.75</td>
<td>0.005 HS</td>
</tr>
</tbody>
</table>

Discussion

Forensic science plays a definite role in criminal investigation. The victimizers of the infamous Nirbhaya gang rape case (2012) were identified by forensic records. The woman who was part of the hit squad during the Paris attack (2017) was identified by finger prints. Many more such cases were proved by forensic records.

The current research revealed the predominance of Type IV ,Type V patterns among females and Type I’, Type I patterns among males. Costa and Caldas and Kumar et al stated that lip groove patterns can be used as a potential aid in gender determination which is in accordance with the results of current research.[5] Lip groove patterns varies in different parts of lips and it reiterates the fact of uniqueness of lip groove patterns. [8, 9,10, 11]

In our study the Loop finger print patterns were found to be predominant among males and Whorl finger print patterns were found to be predominant among females. The study results were in accordance with tendon et al. [12]
The predominant pattern of finger print and lip print also show regional variations. In Iranian sample population, (96 individuals) Type V lip groove patterns was found predominant pattern. [11] In saudi population (150 individuals), males showed Type III pattern and females showed Type I pattern as the predominant type. [8] In our study, Loop finger print patterns with type 1 lip print were found to be predominant among males. Whorl finger print patterns with type 4 lip print were found to be predominant among females. Similarly Gaba et al found that lip prints were unique to an individual and the patterns varied among males and females and concluded that lip print analysis can be used for forensic analysis and gender determination. [15] Our results are in association with Metgad et al results, who found that correlation between lip prints and finger prints for gender identification was statistically significant. [16] From our study it can be observed that there is various in lip print and finger print form one person to other and among gender hence it can be used for identification of gender in forensic medicine. Further long term studies are required on larger sample size with other variables for gender determination.

**Conclusion**

Most studies on lip prints and thumb prints have limited samples. [13,14] The current study was done on a larger sample of population, however more studies in different geographic areas with larger populations are needed to establish cheiloscopy and dactyloscopy as distinct branches with wider scope in forensic science.

**Conflict of Interest:** Nil

**Source of Funding:** Nil

**References**


A Study to Compare the Stress Patterns in the Edentulous Mandibular Bone Around the Two Implant Retained Over Denture and the Prosthesis Restored with All-On-Four Concept Using the Three Dimensional Finite Element Analysis

Puneeth Hegde¹, Satish Shenoy², Shobha Rodrigues³, Thilak Shetty⁴, Umesh Pai¹, Sharon Saldhana¹, Mahesh M¹
¹Associate Professor, Department of Prosthodontics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, ²Professor & Head, Department of Aeronautical Engineering, Manipal Institute of Technology, Manipal, Manipal Academy of Higher Education, Karnataka, India, ³Professor & Head, ⁴Professor, Department of Prosthodontics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal

Abstract

When we consider the rehabilitation of patients with the mandibular implant supported over denture, the influence of the implant number and the cantilever design on the stress distribution on the bone needs to be assessed precisely.

Purpose: The purpose of the simulation study was to compare the biomechanical behavior of the two implant supported over denture design with the All-On-Four Concept using the three dimensional finite element method thereby evaluating the Von Misses stresses induced on the implant under different loading simulations.

Materials & Method: Three dimensional models representing mandible restored with two implant supported prosthesis was compared with the models that were restored based on the ‘All-On-Four’ concept. The models were then subjected to four different loading simulations (full mouth biting, canine disclusion, load on cantilever, and load in the absence of cantilever). The maximum von Mises stresses were localized and quantified for comparison.

Results: Among the models, under all loading simulations, the maximum stress concentrations were along the neck of the implant. The stress levels for full mouth loading simulation was highest for two implant supported overdenture design and the least for All-On-Four overdenture design. In all the designs, the least stress was when the implants were loaded in a lateral direction. The stress levels for cantilever and non-cantilevered designs were nearly the same for all the simulated designs.

Conclusion: When tested under different loading simulations, both models showed similar location and distribution of stress patterns. Thus from the study it can be concluded that the All-On-Four Concept is a clinically applicable treatment option for the atrophic edentulous ridges and induces least amount of stresses on the edentulous ridges. Therefore the overall longevity of the prosthesis is greatly enhanced.

Key words: atrophic mandible, biomechanics, finite element analysis, implants supported prosthesis, tilted implants.

Introduction

Recent advancements in the field of oral implantology has greatly improved the options available for rehabilitation of edentulous patients with highly resorbed ridges. Other than enhanced retention and stability the implant supported over denture design demonstrates lot of advantages like preservation of the
alveolar ridges, increased comfort, chewing efficiency and overall improved quality of life when compared to the conventional complete dentures. A new concept named All-On-Four is now widely being applied for rehabilitation of completely edentulous arches with implant supported prosthesis \(^1,^2,^3,^6\).

According to this technique, the use of tilted implant permits cantilever reduction while warding off important anatomical structures. Poor planning and designing of the implant supported prosthesis can result in stress concentration and peri-implant bone loss. This eventually would lead to complete loss of implant. Hence an indepth understanding regarding the regions of stress concentration encountered by the implant and the supporting bone is essential for the long term success of the implant and the prosthesis \(^3,^5,^6\).

**Aim**

To compare the stress patterns in the edentulous mandibular bone around two implant retained overdenture and the prosthesis restored with All-On-Four Concept using finite element analysis.

**Objectives**

To compare the biomechanical behaviour of the prosthesis restored with All-On-Four Concept with that of two implant supported mandibular overdenture using three dimensional finite element analysis.

To localize and quantify the Von Mises stresses induced on the implants under different loading simulations.

**Material & Method**

**Armamentarium used for the study**

- CT Scan of edentulous mandible
- Replace Select Tapered TiU NP 3.5 x 13mm (Nobel Biocare)
- The Profile Projector (METZ-801)
- Cylindrical Retainer of 4mm diameter.
- ANSYS - 11 Workbench Software.

Preparation of FEM model of the Edentulous Mandible.

The computerized tomographic (CT) image of the human edentulous mandible was introduced into the Computer Aided Design (CAD) software. The ANSYS software was then used to simplify the CT image of the mandible into an arch shaped bone block with dimensions of 7.5mm thick and 15mm high. To simulate the type III bone which is commonly found in the mandibular posterior region, 1mm cortical bone layer was established overlying the entire mandible whereas the trabecular bone was used in the internal structure. After obtaining the computerized 3-Dimensional model, incorporation of the implant design into the model was planned.

**Preparation of the FEM implant model**

For a precise and more accurate analysis, the contour and design of the threaded implant is very important. However the shape and depth of the threads could not be evaluated and reproduced in the 3 dimensional model with the help of computerized tomography. Hence an instrument called ‘Profile Projector Optical System’ was used in the study. The values thus obtained were then used to design an accurate 3-D model of the threaded implant along with the retainer.

**Profile Projector (METZ-801) Optical System**

The profile projectors have the ability to display a two dimensional projection of a part rather than just simple linear dimension as in case of other gauging devices. The images are displayed in a viewing screen as an aid to precise determination of the dimension, form and physical characteristics.

This instrument creates work piece image on the projection screen at desired magnifications (10x, 20x, 50x) to provide accurate dimensional measurement as well as inspection of the contour and surface condition of the work piece. The accuracy of this instrument is known to be 0.001mm.

**Preparation of the working model**

Three dimensional working models were constructed using 3D computer aided design software (ANSYS). The models represented the mandible restored with 2 - implant supported prosthetic design and the design restored with the All On Four Concept. A rigid type III gold prosthetic bar, 6mm thick and 4mm high and in the shape of an arc was then designed and joined to the abutments.\(^1\)
For the 3-Dimensional two implant supported prosthesis model, the threaded implants were strategically placed vertically in the region of lateral incisors bilaterally.

For the 3-dimensional ‘All-On-Four’ model, two anterior implants were placed vertically in the position of the lateral incisors and two implants were placed bilaterally in the position of second premolars and tilted distally to 30º angle.

To evaluate and compare the distribution of stresses on the implant on the three models, four loading situations were simulated in each model using load values similar to those of functional bite movements from patients with implant supported prostheses.

- **Loading 1**: Full mouth biting – bilateral and simultaneous vertical static loads of
  - 200 N was applied on the occlusal surface of the first molars (Cantilevers)
  - 150 N on the occlusal surface of second premolars
  - 150 N on the occlusal surface of first premolars
  - 100 N on the distal of canines
- **Loading 2**: Lateral Load – Unilateral static load of 50 N applied in the region of left canine.
- **Loading 3**: Cantilever Load – Unilateral vertical static load of 200 N was applied in the region adjacent to the left second premolar, simulating absence of cantilever.
- **Loading 4**: Load without the cantilever - Unilateral vertical static load of 200 N was applied in the region adjacent to the left second premolar, simulating absence of cantilever.

The results of the mathematical solutions were later converted into visual results and expressed in colour gradients, ranging from shades of red, orange, yellow, green and blue, with red representing highest stress values. The stress values in the two models were collected and compared, with the points of greatest magnitude identified by the Von Mises equivalent stress levels.

**Results**

The results of the numerical analysis are shown in Table - 2 for Von Mises stresses occurring for the FEM models.
Discussion

Bone remodeling is a constant process and continues throughout the life of an individual. However, despite having progressive medical and technological advancements, as age advances, the bone resorption pattern is faster and aggressive. This leads to a severely resorbed ridge thus compromising the function and esthetics when a conventional denture is delivered. Implant supported denture could be a good option but in patients with resorbed ridges, the amount of bone support might be compromised to assist in osseointegration and withstand masticatory load.

Among several techniques being used in rehabilitation of completely edentulous patients with implant supported prosthesis, the All-On-Four technique is considered to be the most successful and rewarding technique. A proper understanding of the load transfer and stress concentration under implant supported prosthesis is very essential before planning an implant supported prosthesis.  

The finite element analysis is a very effective technique to obtain a detailed stress strain analysis at any location. The three dimensional finite element analysis has been used to evaluate the stresses around the various implant systems.

Keeping in mind the consequences of unwanted stresses, this study was an attempt to compare the Von Mises Stresses around the implant by different loading conditions, on two different finite element models. The models were simulated on the basis of implant number, position, angulation and the type of prosthesis which is a Type III gold bar.

References

6. Dorothy E Eger et al, Comparison of angled and standard abutments and their effect on clinical outcome,

Assessment of Needle Stick Injury in an Institutional Set up: A Retrospective Analysis

Shobha Rodrigues¹, Thilak Shetty², Umesh Pai³, Sharon Saldanha³, Mahesh M³, Puneeth Hegde³, Mandakini Mohan⁴

¹Professor & Head, ²Professor, ³Associate Professor, Department of Prosthodontics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, ⁴Senior Lecturer, Division of Clinical Dentistry, International Medical University, Kuala Lumpur, Malaysia

Abstract

Purpose: Occupational exposure to blood borne pathogens with needle stick or other sharp tool injuries is a serious but preventable problem. this study will be conducted to investigate the incidence of Needle stick injury (NSI) among the Dental health care providers (DHCP) of Manipal College of Dental Sciences, Mangalore.

Method: The NSI incidence, demographics, contributing factors, and follow up following NSI report were reviewed.

Results: Six, eleven, seventeen and nineteen needle stick injuries were reported in 2010, 2011, 2012 and 2013 respectively. Most injuries were experienced by students and least by the Faculty.

Conclusion: Based on the results and within the limitations of this surveillance study it is concluded that occupational exposures can be reduced and reporting of all exposures is vital. The institution of appropriate PEP, psychological support and counselling of affected HCWs and stringent follow-up are all needed to reduce the burden of occupationally acquired infections in HCWs.

Keywords: Needle stick injury (NSI), Health care worker(HCW), dental students

Introduction

Occupational exposure to blood borne pathogens with needle stick or other sharp tool injuries is a serious but preventable problem. Health care workers (HCWs) are at a risk of occupational acquisition of human immunodeficiency virus (HIV) infection and other viral infections (HCV and HBV) due to accidental exposure to infected blood and body fluids.¹ The reporting of such injuries is a critical step in initiating early prophylaxis.²

Needle stick injury (NSI) is a significant problem in general dental practice and exposes personnel to a serious risk of infection from blood-borne transmissible agents. Dental students are exposed to various oral infections or lesions, which may be due to manifestations of AIDS. As dental students lack knowledge, experience and skill, they are vulnerable to accidental exposure to blood and other body fluids when performing clinical activities. Even if there are timely and effective post exposure prophylaxis, such as for HBV and HIV, there can be serious psychological and economic consequences following NSIs. Also, unsafe practices such as not wearing gloves and the resheathing of needles predispose dental students to NSI. There should be a well-formulated coordinated approach for the provision of information support, and referral from healthcare workers who sustain occupationally related management of occupational exposures varies between institutions and often reflects the level of staff education.

Corresponding Author:
Dr. Sharon Saldanha
Email ID- shobha.j@manipal.edu Contact No- +91-9448100464, Address: Department No. 7, Manipal College of Dental Sciences, Lighthouse Hill road, Mangalore 575001
and previous experience in areas of infection control and transmission of blood-borne diseases. Despite published guidelines and training programs, needle stick injuries remain an ongoing problem. All patients should be considered to pose a potentially high risk of infection; also, recommended precautionary measures should be followed at all times. An effective and multifaceted management plan must be prepared for prevention and management of needle stick injuries in healthcare workers. After an occupational exposure, the healthcare worker should be counseled about the degree of risk associated with the type of exposure: needlestick injuries pose a greater risk than splashes, and those from a hollow-bore needle are a greater risk than from a solid needle.

As dental students lack knowledge, experience and skill, they are vulnerable to accidental exposure to blood and other body fluids when performing clinical activities. This places them at the potential risk of blood-borne diseases such as hepatitis B virus (HBV), hepatitis C virus and human immunodeficiency virus (HIV). Even if there are timely and effective post exposure prophylaxis, such as for HBV and HIV, there can be serious psychological and economic consequences following NSIs. Also, unsafe practices such as not wearing gloves and the resheathing of needles predispose dental students to NSIs.

There are various approaches and differences in the program curricula of various dental schools to prevent NSIs among medical students. One preventive strategy is to enhance training programmes on infection control, including the prevention of NSIs, while another is to improve students’ knowledge of the management and reporting procedures following an NSI. An effective and multi focused training programme is essential, as its implementation can decrease the overall rate of NSIs in hospitals. Dental students are oriented about infection control during the course as part of the measures taken to reduce the incidence of NSIs among medical students. There are also standard operating protocols on measures to be taken and injury notification available at this Institution.

While there is a declining trend for the incidence of NSIs, there has been so such study performed in our Institution. In light of the above findings, this study will be conducted to investigate the incidence of NSI among the Dental health care providers (DHCP) of Manipal College of Dental Sciences, Mangalore.

Materials and Method:

Reports of NSI occurrences of DHCP originating from MCODS, Mangalore that were entered in Adverse Event Reporting System, Kasturba Medical College, Attavar during the period from May 2010-November 2013 were the source of information. The NSI incidence, demographics, contributing factors, and follow up owing NSI report were reviewed. A standard operating protocol was introduced in January 2013 to help reduce the incidence of NSI. The incidence of NSI before and after introduction of this protocol was also reviewed. The results was then subjected to a descriptive analysis and areas of improvement in the current training system will be suggested.

Results

Are presented in Table 1 & 2

Table 1: Number of Dental injuries reported (2010-2013) as against the total injuries occurring in the Medical College

<table>
<thead>
<tr>
<th>Year</th>
<th>Faculty</th>
<th>Post-graduates</th>
<th>Students</th>
<th>DSA</th>
<th>Housekeeping Staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>6</td>
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<td></td>
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<tr>
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<td>2012</td>
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<td>52</td>
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<td></td>
</tr>
<tr>
<td>2013</td>
<td>19</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Number of Needle stick injuries sustained by different Dental Health care workers Yearwise

<table>
<thead>
<tr>
<th>Year</th>
<th>Faculty</th>
<th>Post-graduates</th>
<th>Students</th>
<th>DSA</th>
<th>Housekeeping Staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>1</td>
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<tr>
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<td>1</td>
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<td>2012</td>
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<tr>
<td>2013</td>
<td>2</td>
<td>3</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Prevention and management of occupational exposures has been a thrust area of the hospital infection control committee since the last several years. Continuing education and training for HCWs has been very successfully carried out at our institute for many years. There has been a decline in sharps injuries from garbage bags, needle recapping and during injection administration, over the years due to constant awareness programs conducted at our hospital on infection control.

Regular education is also very important to discourage practices like needle recapping and encourage responsible storage, use and disposal of sharps. Only constant surveillance can ascertain exactly what interventions are wise to make and whether they will result in a positive change. Most of the NSI injuries were experienced by students. This may be because of the inexperience of the students.

In the study by Yang et al, 8 trainee nurses were exposed to NSIs about 2-4 times more frequently than other nurses.

Similarly, data presented in this study corroborates with other publications.9

The fact that NSIs were detected at lower rates among doctors may have been owing to less reporting of the injury in this group.

It is a known fact that not all NSI cases are reported. It is also known that the awareness of the identity of the source and the status of bloodborne infections causes a false sense of security after exposure and decreases the report frequency of reports.10 The percentage of unreported NSIs in the literature ranges from 22%-82%.11-13 Voide et al11 conducted a questionnaire survey about NSIs among health care professionals. They found that although the most frequent injuries occurred among doctors (49.2%), the group that least reported NSIs was also doctors.

The results emphasize the risk of needle stick injuries in HCWs. The reporting date of needle stick injury is varied by job categories. In a study from United States, nurses accounted for 68%, interns for 35%, and resident doctors for 31% of BBF exposures.14 Another study conducted in Mumbai, India, observed that the incidence of occupational exposure due to infected blood and body fluids were highest among resident doctors (76%), followed by nurses (11%), and interns (5%).15

Since students in our hospital have more patient contact there are more frequent uses of needles by them than other HCW’s, and therefore they report a higher incidence of needle stick injury. These findings suggest the need for improved continuing education programs which verify competency of HCWs, especially students about standard precautions and risk of needle stick injuries.

The first aid is a crucial factor to decrease the risk of infection from NSI. Washing the wound immediately and thoroughly with soap and water is the most important management. It is ensured that unsafe work practices such as recapping needles are avoided, used needles are disposed of promptly in an appropriate sharp instrument disposal container. Finally, efforts are being made to improve the surveillance systems for needle stick injuries and collected data are analyzed to develop and assess methods to decrease the risk to HCW exposure.

Safe use of dental equipment, provision of education, Implementation of institutional protocol, and their reflection on outcomes and behaviors have seen a decreasing trend in NSI.

Conclusion

Based on the results and within the limitations of this surveillance study it is concluded that occupational exposures can be reduced and reporting of all exposures is vital. The institution of appropriate PEP, psychological support and counselling of affected HCWs and stringent follow-up are all needed to reduce the burden of occupationally acquired infections in HCWs.

Conflict of Interest : Nil

Source of Funding : Self

References

3. Kinlin LM, Mittleman MA, Harris AD, Rubin MA, Fisman DN. Use of gloves and reduction of risk of injury caused by needles or sharp medical


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<td></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>11</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>17</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>19</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Number of Needle stick injuries sustained by different Dental Health care workers Yearwise

<table>
<thead>
<tr>
<th>Year</th>
<th>Faculty</th>
<th>Postgraduates</th>
<th>Students</th>
<th>DSA</th>
<th>Housekeeping Staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2011</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>2</td>
<td>3</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2013</td>
<td>2</td>
<td>3</td>
<td>14</td>
<td>--</td>
<td>-</td>
</tr>
</tbody>
</table>

Discussion

Prevention and management of occupational exposures has been a thrust area of the hospital infection control committee since the last several years. Continuing education and training for HCWs has been very successfully carried out at our institute for many years. There has been a decline in sharps injuries from garbage bags, needle recapping and during injection administration, over the years due to constant awareness programs conducted at our hospital on infection control.

Regular education is also very important to discourage practices like needle recapping and encourage responsible storage, use and disposal of sharps. Only constant surveillance can ascertain exactly what interventions are wise to make and whether they
will result in a positive change. Most of the NSI injuries were experienced by students. This may be because of the inexperience of the students.

In the study by Yang et al., trainee nurses were exposed to NSIs about 2-4 times more frequently than other nurses.

Similarly, data presented in this study corroborates with other publications. The fact that NSIs were detected at lower rates among doctors may have been owing to less reporting of the injury in this group.

It is a known fact that not all NSI cases are reported. It is also known that the awareness of the identity of the source and the status of bloodborne infections causes a false sense of security after exposure and decreases the report frequency of reports. The percentage of unreported NSIs in the literature ranges from 22%-82%. Voide et al conducted a questionnaire survey about NSIs among health care professionals. They found that although the most frequent injuries occurred among doctors (49.2%), the group that least reported NSIs was also doctors.

The results emphasize the risk of needle stick injuries in HCWs. The reporting date of needle stick injury is varied by job categories. In a study from United States, nurses accounted for 68%, interns for 35%, and resident doctors for 31% of BBF exposures. Another study conducted in Mumbai, India, observed that the incidence of occupational exposure due to infected blood and body fluids were highest among resident doctors (76%), followed by nurses (11%), and interns (5%).

Since students in our hospital have more patient contact there are more frequent uses of needles by them than other HCW’s, and therefore they report a higher incidence of needle stick injury. These findings suggest the need for improved continuing education programs which verify competency of HCWs, especially students about standard precautions and risk of needle stick injuries.

The first aid is a crucial factor to decrease the risk of infection from NSI. Washing the wound immediately and thoroughly with soap and water is the most important management. It is ensured that unsafe work practices such as recapping needles are avoided, used needles are disposed of promptly in an appropriate sharp instrument disposal container. Finally, efforts are being made to improve the surveillance systems for needle stick injuries and collected data are analyzed to develop and assess methods to decrease the risk to HCW exposure.

Safe use of dental equipment, provision of education, Implementation of institutional protocol, and their reflection on outcomes and behaviors have seen a decreasing trend in NSI.

**Conclusion**

Based on the results and within the limitations of this surveillance study it is concluded that occupational exposures can be reduced and reporting of all exposures is vital. The institution of appropriate PEP, psychological support and counselling of affected HCWs and stringent follow-up are all needed to reduce the burden of occupationally acquired infections in HCWs.

**Conflict of Interest :** Nil

**Source of Funding :** Self

**References**


Root Canal Morphology of Mandibular Second Molars Using CBCT

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Abstract

Background: A thorough knowledge of root canal system, anatomy and variation in morphological plays an important role in all scopes of endodontic treatment. The practitioner should have a great understanding of the detailed morphological anatomy of root canal system in order to utilize the proper technique and method during the endodontic treatment especially in mandibular second molar.

Aim: To evaluate root canal morphology of mandibular second molars using Cone Beam Computed Tomography (CBCT) in Chennai Population based on Vertucci’s classification.

Materials and Method: The CBCT scans of patient age between 20 years to 40 years were retrieved from Department of Oral Medicine and Radiology, Saveetha Dental College, Chennai, India from September 2018 to January 2019. The teeth were observed for the root canals system using Vertucci classification, and other parameters. The comparison between males and female were made.

Results: Generally the parameter values are more in females when compared to males and the most prevalence root canal configuration are Type 4, followed by Type 2 in mesial and distal roots for both gender.

Conclusion: The study indicates the significant values to certain extend and can serve as aids to utilize the proper technique and method during the endodontic treatment especially in permanent mandibular second molar.

Key words: Root canal; Morphology; Second Mandibular Molar; CBCT; Chennai population

Introduction

A thorough knowledge of root canal system, anatomy and variation in morphological plays an important role in all scopes of endodontic treatment. The practitioner should have a great understanding of the detailed morphological anatomy of root canal system in order to utilize the proper technique and method during the endodontic treatment. The proper technique of the treatment will increase the success rate of the treatment.¹

The anatomical variations of the root canal morphology are the most important aspect in the endodontic treatment.² The untreated missing root canal may lead to the abnormal pain caused by the contamination of microorganism colonies and production of necrotic tissue inside the canal. These condition will lead to the apical pathosis development.³

The study of the root canal anatomy and their variations has been reported in various research articles from the past and more recent update. Hence, it is show the importance of the morphology of root canals which dictates the final results of the root canal procedures.⁵
Few types of root canal system morphology has been proposed by various authors. The different root canal systems classification of human permanent including the Weine’s classification, Vertucci’s classification, and Gulabivala classifications. Vertucci’s classification is considered the most widely used and includes eight categories: Type I (1), Type II (2-1), Type III (1-2-1), Type IV (2), Type V (1-2), Type VI (2-1-2), Type VII (1-2-12), and Type VIII (3).  

The methods that have been utilized to investigate the root canal system morphology can be divided into in vitro and in vivo methods. Methods in the in-vivo consist of clinical evaluation during root canal treatment, retrospective examination of patient records, conventional assessment of radiographic imaging, and advanced radiographic techniques such as CBCT. The methods in the in-vitro are canal staining and tooth clearing, root sectioning, microscopic examination, examination of conventional radiographs, and using three-dimensional modalities such as microcomputed tomography.  

The CBCT methods has the ability to examined and precise detection of root canal morphology same as the root canal staining and the clearing techniques, which in the past were considered as the advanced to conventional methods of studying root canal system because its ability to shows 3-dimensional views and details on complete morphologic.  

The CT application in the endodontic treatment had been reported at earliest by Tachibana and Matsumoto in 1990. The advantage of CT is can shows the 3-dimensional views and facilitates the root canal system reconstruction. The recent introduction of CBCT has provide dentistry with advanced practical. The CBCT is a practical radiographic tools which is less invasive and provide 3D reconstruction imaging that can be used in endodontic and morphologic study.  

The radiographic images generated by CBCT were processed and analysed for these parameters of current research. The pattern of the root canals was evaluated and classified according to Vertucci’s classification shown in Figure 1. There are 6 types of root canals system based on Vertucci’s classification. The types of root canals system was observed in mesial and distal roots of the mandibular second molars. The mesial and distal root length, the distance between occlusal pit and pulp chamber, the distance between pulp floor to furcation, were observed by using the digital system. Later, the the descriptive analysis of all parameters were analysed in term of mean values, standard deviation and their significance of result. The comparison between descriptive values were done between males and females.
Result

A total of 83 teeth were examined by using the CBCT scans in the current study. Out of the total, 28 are males and 22 are females. The result for both genders can be seen as mean value and standard deviation, p-values and its significance of result. The p-value less than 0.5 shown the significant value.

The descriptive analysis of permanent mandibular second molar root canal types in males can be seen in Table 1. Out of 50 teeth examined and assessed, the most common root canal morphology in males are Type 1 and Type 4 morphology, which are 46% and 20% respectively. Both Type 1 and Type 4 are dominant in the mesial root and distal root of mandibular second molar. There are no finding of Type 7 and Type 8 during the examination in mesial roots and distal roots.

In females, out of 33 mandibular second molar teeth examined and assessed, the descriptive analysis of root canal types shown that Type 1 and Type 4 are the most dominant root morphology in distal roots and mesial roots with total percentage of 53% and 19.6% respectively [Table 2].

The result of the descriptive analysis of mesial root length for permanent mandibular second molar in males and females are shown in Table 3. The mean value of mesial length for males is 11.9468mm meanwhile for females is 12.6313mm. This shown that females having longer length of mesial roots compared to males. Both the values collected from males and females sample having significant value of p-value which are less than 0.5.

The result of the descriptive analysis of distal root length for permanent mandibular second molar in males and females are shown in Table 4. The mean value of distal root length for males is 11.1636mm meanwhile for females is 11.8147mm. This shown that females having longer length of distal roots compared to males. Both the values collected from males and females sample having significant value of p-value which are less than 0.5.

The result of the descriptive analysis of distance between occlusal pit and pulp chamber for permanent mandibular second molar in males and females are shown in Table 5. The mean value of distance between occlusal pit and pulp chamber for males is 4.165mm meanwhile for females is 4.262mm. This shown that females having more distance between occlusal pit and pulp compared to males. Both the values collected from males and females sample having significant value of p-value which are less than 0.5.

The result of the descriptive analysis of distance between pulp floor to furcation for permanent mandibular second molar in males and females are shown in Table 6. The mean value of distance between pulp floor to furcation for males is 2.5977mm meanwhile for females is 2.5993mm. This shown that there is no significant differences between males and females for distance between occlusal pit and pulp. The standard deviation between both genders also shown slight differences. Both the values collected from males and females sample having significant value of p-value which are less than 0.5.
Table 1: The result of the descriptive analysis of permanent mandibular second molar root canal types in males

<table>
<thead>
<tr>
<th>Type</th>
<th>Mesial Root, n (%)</th>
<th>Distal root, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>21 (21%)</td>
<td>25 (25%)</td>
<td>46 (46%)</td>
</tr>
<tr>
<td>Type 2</td>
<td>8 (8%)</td>
<td>5 (5%)</td>
<td>13 (13%)</td>
</tr>
<tr>
<td>Type 3</td>
<td>5 (5%)</td>
<td>5 (5%)</td>
<td>10 (10%)</td>
</tr>
<tr>
<td>Type 4</td>
<td>10 (10%)</td>
<td>10 (10%)</td>
<td>20 (20%)</td>
</tr>
<tr>
<td>Type 5</td>
<td>4 (4%)</td>
<td>4 (4%)</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Type 6</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Type 7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Type 8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: The result of the descriptive analysis of permanent mandibular second molar root canal types in females

<table>
<thead>
<tr>
<th>Type</th>
<th>Mesial Root, n (%)</th>
<th>Distal root, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>13 (19.6%)</td>
<td>22 (33.3%)</td>
<td>35 (53%)</td>
</tr>
<tr>
<td>Type 2</td>
<td>5 (7.6%)</td>
<td>1 (1.5%)</td>
<td>6 (9.1%)</td>
</tr>
<tr>
<td>Type 3</td>
<td>3 (4.5%)</td>
<td>2 (3%)</td>
<td>5 (7.6%)</td>
</tr>
<tr>
<td>Type 4</td>
<td>7 (10.6%)</td>
<td>6 (9%)</td>
<td>13 (19.6%)</td>
</tr>
<tr>
<td>Type 5</td>
<td>2 (3%)</td>
<td>2 (3%)</td>
<td>4 (6.1%)</td>
</tr>
<tr>
<td>Type 6</td>
<td>3 (4.5%)</td>
<td>0</td>
<td>3 (4.5%)</td>
</tr>
<tr>
<td>Type 7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Type 8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3: The result of the descriptive analysis of mesial root length for permanent mandibular second molar in males and females

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean value (mm)</th>
<th>Standard deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>50</td>
<td>11.9468</td>
<td>1.1046</td>
<td>4.020331</td>
<td>95</td>
<td>0.000619</td>
<td>Significant</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>12.6313</td>
<td>1.3506</td>
<td>4.831118</td>
<td>95</td>
<td>0.00022</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table 4: The result of the descriptive analysis of distal root length for permanent mandibular second molar in males and females

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean value (mm)</th>
<th>Standard deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>50</td>
<td>11.1636</td>
<td>1.679</td>
<td>-2.336465</td>
<td>95</td>
<td>0.029457</td>
<td>Significant</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>11.8147</td>
<td>2.4361</td>
<td>2.885045</td>
<td>95</td>
<td>0.011991</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table 5: The result of the descriptive analysis of distance between occlusal pit and pulp chamber for permanent mandibular second molar in males and females

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean value (mm)</th>
<th>Standard deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>50</td>
<td>4.165</td>
<td>0.834</td>
<td>4.02137</td>
<td>95</td>
<td>0.000617</td>
<td>Significant</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>4.262</td>
<td>1.0154</td>
<td>2.905381</td>
<td>95</td>
<td>0.011496</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table 6: The result of the descriptive analysis of distance between pulp floor to furcation for permanent mandibular second molar in males and females

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean value (mm)</th>
<th>Standard deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>50</td>
<td>2.5977</td>
<td>0.5795</td>
<td>4.838027</td>
<td>95</td>
<td>0.000088</td>
<td>Significant</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>2.5993</td>
<td>0.5756</td>
<td>4.032468</td>
<td>95</td>
<td>0.001235</td>
<td>Significant</td>
</tr>
</tbody>
</table>
**Discussion**

The study of the root canal morphology of mandibular second molars using CBCT had been studied in various research papers previously. The CBCT is a practical radiographic tools which is less invasive and provide 3D reconstruction imaging that can be used in endodontic and morphologic study.\(^\text{14}\)

In the current study in Chennai population, 83 permanent mandibular second molars were examined and assessed by using CBCT. In this study, Type 4 and Type 2 root canals configuration was the most common in mesial and distal roots, both males and females. The finding of this current study is consistent with other research result in few previous studies.

The study done in 850 south-eastern Turkish population by Bil Gulsum Nur et al. shown that Type 4 configuration was the most prevalent which were 89% in females and 93% in males for mesial roots. Type 4 canal configuration was the most common in the mesial roots, whereas type 1 canal configuration was the most common in the distal roots. Moreover, type 2, type 3, and type 5 canal configurations were also observed in mesial and distal roots, and the incidences of varying root canal configurations did not statistically differ between females and males with p-valued more than 0.05 (\(P>0.05\)), with the exception being the mesial canal of the left mandibular second molars with its p-values less than 0.05 (\(P<0.05\)).\(^\text{15}\)

The study done by Zaatar et al. and al-Nazhan et al. shown that type 2 were the most common root canals configuration followed by Type 4 configuration. In their studies, only one female patient had a Type 8 root canal configuration in the mesial root.\(^\text{3, 16}\)

The present study shown the contrast with the previous study done by Vertucci and Williams in a Caucasian population. In their studies, they observed Type 1 as the most common root canal configuration in both males and females for mandibular permanent second molars.\(^\text{17}\)

In the previous study by Marwan et al. between 2005 and 2007 where he investigated four hundred and thirty-six mandibular molars in an Egyptian Ismailia population shown that most common root canals configuration of mandibular second molars was Type 2 (57.71%) in the mesial roots and Type 1 (95.02%) in distal roots. He also concluded that there are not gender roles in determination of any root canals configuration.\(^\text{18}\)

The study by Gulabivana et al. in 2002 in Mongoloid traits shown the same results as in the Korean population. The author had concluded that the root and canal morphology of mandibular second molars also can be used in tracing the racial origins of populations.\(^\text{20}\)

In the current study, there are few limitations that may affect the precision of result in an evaluation of root canal morphology of mandibular second molars by using CBCT. The specific technique and methods need to be used during the handling of CBCT software. Technical defect may contribute in wrong evaluation of canal configuration.

**Conclusion**

The study concluded that the root canal morphology of mandibular second molars using CBCT in Chennai Population based on Vertucci’s classification shows significant result in certain extend. . There is a significant difference which can be differentiated between the genders. Generally the parameter values are more in females when compared to males and the most prevalence root canal configuration are Type 4, followed by Type 2 in both gender. Therefore the study indicates that the study can serve as aids to utilize the proper technique and method during the endodontic treatment especially in permanent mandibular second molar.

**Ethical Clearance**- All datas are taken from examination of outpatients of Saveetha Dental College and Hospital

**Source of Funding**- Saveetha Institute of Medical and Technical Sciences

**Conflict of Interest** - Nil

**References**


Comparative Evaluation of Effect of Auxillary Grooves on Retention of Complete Cast Crowns in Molar Teeth – An In-Vitro Study

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Abstract

Aim: The aim of this study is to compare and evaluate the effects of auxillary grooves on retention of complete cast crown on molars. Method: Thirty mounted teeth were divided randomly into three groups of 10 each and grooves are placed using NOF104R diamond point. In group A, no groove is placed – control, group B, one groove is placed proximally in the centre of mesial surface – Test group. In group C, two groove is placed proximally, each in centre of mesial surface and distal surface - Test group. Castings were prepared luted and retention was checked using a Universal Testing machine Results were subjected to one way ANOVA and Student T Test. Results: Auxiliary retentive features produces a significant increase in retention of complete cast crowns. Incorporation of two proximal grooves on the mesial and distal side produces the most retention as compared to one groove or conventional tooth preparation Conclusion: Retentive features produces a significant increase in retention of complete cast crowns

Key words: auxillary grooves, complete cast crowns, tooth preparations, mandibular molar

Introduction

A full complement of natural dentition plays an important role in mastication, speech and aesthetics. Conditions like dental caries, periodontal problems, congenital abnormalities, neoplasm, trauma affects the dental health causing loss of teeth, which results in loss of function and aesthetics. Restorative materials are used to re-establish form and function of teeth.

Different modalities of tooth replacement are removable dental prosthesis, fixed dental prosthesis, & Implant supported prosthesis, and among them the most frequently done is fixed dental prosthesis. Complete coverage restoration are the commonly fabricated restoration to regain the morphology, function and contour of the damaged tooth structure caused by caries, trauma and for protection of root canal treated teeth as well as retainers for fixed partial dentures. They are also indicated for establishing occlusal rest and guide planes in removable partial dentures.

Teeth preparation is defined as the mechanical treatment of dental diseases that restores tooth to original form. Tooth preparation must follow certain mechanical, biological and esthetic principles. Retention prevents removal of restoration along the path of insertion or along the tooth preparation. Retention depends on taper, height, surface area and texture of tooth preparation.

Sometimes we come across tooth with inadequate crown height which results in reduced crown retention causing dislodgment of the crown by masticatory or para-functional forces.
Clinically a short crown requires crown lengthening, which is a periodontal surgical procedure before crown preparation which adds to the cost and complexity of treatment. Other modalities for inadequate crown height to improve retention are incorporation of grooves, boxes, pins & ledges. The aim of this study is to compare and evaluate the effects of auxiliary grooves on retention of complete cast crown on molars.

**Materials and Method**

Thirty extracted mandibular first molars, of equal dimensions mesiodistally, buccolingually and cervico-incisally were selected for the study. The roots of teeth were notched for anchorage and mounted vertically in auto polymerizing acrylic resin block 2mm below the cementoenamel junction. A dental surveyor is used to position the long axis of the tooth. These mounted teeth were divided randomly into three groups of 10 each and grooves are placed using NOF104R diamond point.

In group A, no groove is placed – control group

In group B, one groove is placed proximally in the centre of mesial surface – Test group

In group C, two grooves are placed proximally, each in centre of mesial surface and distal surface - Test group.

The groove were placed parallel to the path of insertion and minimum of 3mm long. Groove should be terminated 0.5mm above the chamfer finish line and are parallel to each other with the axial walls converging towards the occlusal surface. Size of groove should be half the diameter of the diamond point with buccal and lingual walls diverging from each other.

**Tooth Preparation:**

Tooth preparation was done with a high speed airotor handpiece attached to vertical arm of surveyor using a lockable joint. A custom made paralleling device is used to attain parallelism of axial walls and constant taper for each crown preparation. All teeth were then photographed (at an image size of 4:1) for facial and/or lingual aspect as well as mesial and/or distal aspect. A protractor was used to measure the degree of taper. A constant taper of 10 + /- 1 degree was obtained for all the preparations. A uniform chamfer finish line of 0.5mm width is established at the cemento-enamel junction. All preparations are refined using SF-102R diamond point. Teeth are prepared to constant height of 3.5mm as measured at the mid-buccal surface.

**IMPRESSION TECHNIQUE:**

Impressions of the prepared teeth were made with multiple mix single step technique with polyvinyl siloxane impression material. and cast is poured in type IV die stone following manufacturer’s instructions taking care to see mix is free of any defects or air bubbles by mixing with a vacuum mixer.

**PREPARATION OF WAX PATTERNS:** The dies are painted with three coats of die spacer 1mm short of finish lines. Die lubricant (Isolit) is painted on the master die and excess of lubricant is removed with a gentle stream of air. Wax copings of 1mm is prepared by using blue inlay wax(BEGO), carved with PKT wax instrument simulating a cylinder axially and flat occlusally. A 2mm sprue wax is made as loop and attached to the centre of occlusal surfaces of wax pattern for testing in a universal testing machine. Wax patterns are designated with a code number for convenience of identifying it for corresponding prepared tooth. These wax patterns are sprued and invested with phosphate bonded investment. Burn out is done by lost wax process.

**Casting And Finishing:**

Test copings are cast using nickel chromium alloys (Wirolloy Bego, Germany) in electronic centrifugal induction casting machine (Gallioni). These castings are retrieved from investments and sprues are cut using high speed grinder (Speed Master). Minor adjustments to seat the casting are done with a small round bur mounted on a laboratory hand piece. Internal surface of the castings are air abraded with 100-150 um Aluminium oxide particles at 40 psi and fit of casting are verified on the preparation. All the casting are cleaned with ultrasonic cleaning unit (USG 4000 Ultrashall, Dentarum) for 15 minutes.

**Cementation:**

Teeth and crowns are thoroughly cleansed and dried with filtered compressed air before cementation. All these teeth are cemented with Type I glass ionomer cement according to manufacturers recommendation. Each casting is filled with sufficient luting cement to evenly cover the inner surface, seated with digital pressure on the tooth taking care to see that excess cement is flown all around. An explorer is used to carefully remove the excess cement.
To verify the complete seating of casting, micrometer measurements are made before and after cementation. Each casting is seated on tooth without cement and the distance from the bottom margin of the acrylic resin base is measured. Measurement is repeated after cementation and two readings are compared. Tests specimens are stored for 24 hours at 37 degree at 100% relative humidity.

**Measurement Of Retention:**

Crowns are subjected to vertical dislodgement force until failure on a universal testing machine at a cross head speed of 5mm/ min. Castings are pulled along the apicoronal axis of each tooth using a “J” hook attached to the upper member of the testing machine. Force of dislodgement and debonding are recorded in Megapascals and tabulated. Intergroup comparison was done using ANOVA. Level of significance was set at $p \leq 0.05$.

**Results**

Results are presented in Table 1 and 2.

**Table 1: Mean and Standard Deviations of the forces required to dislodge each casting from its preparations**

<table>
<thead>
<tr>
<th>Samples</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>10</td>
<td>256.8</td>
<td>4.135</td>
</tr>
<tr>
<td>Group B</td>
<td>10</td>
<td>34.553</td>
<td>7.173</td>
</tr>
<tr>
<td>Group C</td>
<td>10</td>
<td>57.67</td>
<td>10.58</td>
</tr>
</tbody>
</table>

**Table 2: Comparison of different groups using least significant difference test**

<table>
<thead>
<tr>
<th>Group (X)</th>
<th>Group (Y)</th>
<th>Mean difference (X-Y)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Group B</td>
<td>-8.693</td>
<td>0.047</td>
</tr>
<tr>
<td>Group A</td>
<td>Group C</td>
<td>-31.814</td>
<td>.001</td>
</tr>
<tr>
<td>Group B</td>
<td>Group C</td>
<td>-23.121</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Discussion**

Full cover restorations are required to restore teeth with minimal remaining coronal tooth structure or when fixed partial denture is fabricated. A steadily increasing demand for restorations of edentulous regions with fixed partial dentures has directed attention to the effectiveness of retention of fixed partial denture on abutment teeth. Several factors affect the retention of the crowns. They are geometry of preparation, type of luting agent, occlusal forces acting, position of crown and tensio-frictional resistance offered by the dentin. Loss of retention of a full coverage restoration may be due to poor geometrical preparation of the crown, dissolution of the luting agent and improper occlusal contacts.

This study demonstrated that clinically compromised complete coverage tooth preparation including reduced occluso cervical dimension, increased TOC, and a reduced occluso cervical –to buccolingual dimension ratio offered greater retention with grooves. Thirty freshly extracted mandibular molars are used. These teeth were divided into three groups. Group A had teeth prepared in a conventional manner, group B had teeth prepared with one proximal groove & group C had teeth prepared with two proximal grooves. Tooth occlusal convergence is one of aspect of preparation of complete cast crowns. Retention of crown is determined at various TOC angles by applying a tensile force to cemented crown. Maximum tensile retentive values are recorded at 5 degree TOC. Wilson and Chan in 1994 reported maximal retention occurred between 6 to 12 degrees. Occlusal views were used to clinically assess TOC but of limited value, hence during tooth preparation mouth mirror has been recommended so that facial and lingual view of prepared tooth is established as effective means of assessing TOC.

Mandibular teeth are generally prepared with greater TOC compared to maxillary teeth, hence axial grooves are routinely incorporated in preparation of mandibular molars for fixed partial dentures. Also FPD abutments are prepared with greater TOC compared to individual crowns.

Woosley and Matisch determined that proximal grooves provided complete resistance to faciolingual forces, whereas facial or lingual grooves provided only partial resistance to faciolingual dislodgment. Proximal grooves increase the resistance and retention of restoration. Ayad et al in a study found a marked difference between the degree of taper of full crown
preparation of 18-22 degree and that of boxes and grooves in the axial surface of preparations as shorter distance between walls allows dentist to prepare more precisely. Also Woosley and Matich reported that proximal grooves on short 15 degree dies provide complete resistance to faciolingual horizontal displacement.

The results showed highly significant amount of separation force required to dislodge casting between one groove (Group B) and no grooves (Group A)  \( p \leq 0.047 \) and very highly significant forces required to dislodge castings with two grooves \( p \leq 0.001 \). This suggests incorporation of proximal grooves improves the retention of cast crowns. The increased retention may be attributed to the fact that the lateral walls of the grooves will help in the tensiofrictional resistance and will limit the freedom of displacement. This results are in confirmation with previous studies done by Kishimoto et al and, Omar et al 2,3

**Limitations :**

Unidirectional forces were applied for dislodgement of crowns in this study. Masticatory forces are multidirectional. In addition only one luting agent that is Type I glass ionomer was under consideration. This study was an invitro study and did not consider the role of saliva which has an influence of the physical properties of the luting agent.

**Conclusion**

1. Auxiliary retentive features produces a significant increase in retention of complete cast crowns

2. Incorporation of two proximal grooves on the mesial and distal side produces the most retention as compared to one groove or conventional tooth preparation

**Conflict of Interest :** Nil

**Source of Funding :** Self

**Ethical Clearance** for this study is taken from our college institutional ethical committee. Enclosed certificate of ethical clearance.

**References**

Risk Assessment, Risk Management, and Risk Communication of Welding Work at PT Dok and Perkapalan Surabaya (Persero)

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Abstract

PT Dok dan Perkapalan Surabaya Is one of the manufacturing industry sectors in which operational activities can cause accidents and work-related illnesses such as the activities of joining the ship’s hull parts, namely the welding process. The risk of welding work needs to be done risk management to determine the prevention of possible work accidents through Risk Assessment activities which are then communicated to all parties. The purpose of this study is to identify hazards, conduct risk assessments and study risk control on welding work. This research is a descriptive study with an observational approach. From the results of the risk assessment of the 3 stages with 7 job descriptions, the percentage of risk categories was obtained, namely high risk 43% and medium risk 57%, and there was no low risk category Control carried out as part of risk management went well but residual risk remained so need a strong commitment from human resources or management and awareness of the workers on the use of personal protective equipment in every work. Risk communication is carried out through coaching or training, conducting safety induction, safety talk, Safety campaigns such as the activity of installing banners, posters, conducting meetings of the Health and Safety Guidance Committee at the end of each month to discuss the problems that occur and make improvements.

Keywords: Risk Assessment, Risk Management, Risk Communication, Job Safety Analysis (JSA), Welding

Introduction

In the current era of globalization, it is necessary for advanced technology to meet human needs as a whole, sometimes without us realizing the absence of good control causes harm to humans themselves. In the use of technology it is unavoidable that in the current era of industrialization the transformation process in technology cannot be prevented from developing. With the use of advanced technology, it will affect the increased use of materials that have potential hazards according to current industrial needs. In the use of advanced technology, it will provide convenience for users, but it will also have an unavoidable impact, namely the increase in the number of hazard risks and the variety of sources of accidents for technology users themselves¹.

The East Java Manpower and Transmigration Office claims that there were 21,631 cases of work-related accidents in 2017. That number rose around 200 cases compared to the previous year. While at PT Dok dan Perkapalan Surabaya every year there are still work accidents. In 2010 to 2015 there were a total of 36 occupational accidents with a risk rating of occupational deaths to minor risks².

PT. Dok and Perkapalan Surabaya (Persero) is one of the processing industries (manufacturing) of BUMN with international standards, and has main activities including: ship building, steel structure fabrication,
design & engineering, offshore construction and ship repair & conversion which in each production uses equipment or machinery that has the potential to cause accidents and there are also other potential dangerous and toxic accidents that can cause occupational diseases. As for its operational activities, it covers: welding, cutting steel plates or ship material, lifting iron or heavy equipment ships, assembling ships, installing pipes in ships and other operational activities.

The activity of uniting ship blocks is inseparable from welding activities. Welding is the connection of two or more materials based on the principles of the diffusion process, so that a part of the material is joined together. This welding process is very important because to join the hull parts of the ship. Where in the welding process there should not be the slightest mistake whether it is a crack, over heat, and so on. This welding process is a very vital part of the ship building process. The types of hazards that occur due to welding are exposed to ultraviolet and infrared radiation, inhaled fumes that exist in welding, fires, electrocuted. Therefore, to find out what hazards there are in the welding activity and control.

The problem faced by companies in this modern era is that there is no comprehensive analysis of all hazard factors in the company so that the control of each hazard factor is often done partially. Thus, to look at the problems in the company in a comprehensive manner, the company’s problems must be analyzed in the risk risk analysis. Risk analysis is an effort to analyze all hazard factors in the company based on risk assessment, risk management and risk communication. Risk Communication is an interactive process that involves exchanging information and opinions about risk, both Risk assessment and risk management. Risk Management is an effort to manage occupational health and safety risks with its implementation component including hazard identification, risk assessment and risk control. Risk assessment is an attempt to calculate the magnitude of a risk and place whether the risk is recognized or not. Before conducting a risk assessment, first identify the hazard. One of the hazard identification techniques is Job Safety Analysis (JSA).

One way to prevent accidents in the workplace is to establish and arrange work procedures and train all workers to implement efficient and safe work methods. Compiling work procedures that are correct and safe is one of the advantages of applying Job Safety Analysis. Job Safety Analysis is one method for identifying hazards which in its implementation emphasizes the identification of risks from hazards that arise at each stage of the work or task. This is in line with the approach to the cause of work accidents that starts from the condition or action that is not safe when carrying out an activity. Therefore, by identifying hazards for each type of work, appropriate and effective preventive measures can be taken. Positive things that can be obtained from the implementation of JSA, are:

1. As an effort to prevent accidents
2. As a safety contact tool for new workers
3. Review the job procedures after an accident
4. Provide pre job instruction on a new job
5. Providing personal training to employees

The Law of the Republic of Indonesia Number 1 of 1970 is the legal basis for Occupational Safety in Indonesia. This law discusses labor rights and obligations, as well as work safety requirements that must be applied in each company. Another related law is Law No. 13 of 2003 concerning Labor, article 86 in this Law states that every organization must implement occupational safety and health efforts to protect the safety of workers, while article 87 in this Law states that each company is required to have a Occupational Health and Safety Management System that is integrated with the management of other companies. To comply with the law in Indonesia and to minimize workplace accidents in the company, efforts are needed to identify potential hazards in the company. Identification of potential hazards and control can also use the Job Safety Analysis method.

The purpose of this study is to identify hazards, conduct risk assessments and study risk control on welding work at PT. Dock dan Perkapalan Surabaya with Job Safety Analysis method, so that later it will be communicated to workers.

**Material and Method**

This type of research is a descriptive study with an observational approach. This research was conducted by interview and direct observation. This research was conducted at PT Dock and Shipping Surabaya, Surabaya, East Java. The type of welding process that
will be identified is SMAW. SMAW (Shield Metal Arch Welding) is an arc welding electric flame protected by using an electric arc as a heat source for melting metal. The risk assessment method uses the JSA with the following risk assessment steps:

1. Select the place to be analyzed
2. Explain the stages of work
3. Identify the various hazards and risks that occur at each step of the work, and identify the various possibilities that have the potential to become accidents;
4. Risk assessment (likelihood and severity)
5. Categorize risk (risk assessment matrix)
6. Provide control recommendations

In its implementation, risk assessment looks at the workplace to identify equipment, situations, and processes, which can cause damage especially to humans. After identification is made, the risk of the possibility and severity of the risk must be evaluated, then determining the effective steps to prevent or control the loss.

### Table 1 Scale of Severity

<table>
<thead>
<tr>
<th>Level</th>
<th>Descriptor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Insignificant</td>
<td>There was no injury, low financial loss</td>
</tr>
<tr>
<td>2</td>
<td>Minor</td>
<td>Minor injuries, moderate financial losses</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Moderate injuries, need medical treatment, High financial losses</td>
</tr>
<tr>
<td>4</td>
<td>Major</td>
<td>More than one serious injury, large loss, production disruption</td>
</tr>
<tr>
<td>5</td>
<td>Catastrophic</td>
<td>Fatal is more than one person, a huge loss of extensive impact that has a long impact, stops all activities</td>
</tr>
</tbody>
</table>

Source: 5

### Table 2 Scale of Probability

<table>
<thead>
<tr>
<th>Value</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Never happened or ever happened once a year</td>
</tr>
<tr>
<td>2</td>
<td>Usually it doesn’t happen but the possibility of it happening still exists (every month)</td>
</tr>
<tr>
<td>3</td>
<td>There may be a small danger or a coincidence (once or once a week)</td>
</tr>
<tr>
<td>4</td>
<td>Possibility of occurring in certain circumstances (more than once per week)</td>
</tr>
<tr>
<td>5</td>
<td>Very likely to happen (every day)</td>
</tr>
</tbody>
</table>

Source: 9

### Table 3. Risk Assessment Matrix

<table>
<thead>
<tr>
<th>Probability</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>5</td>
<td>5 10 15 20 25</td>
</tr>
<tr>
<td>4</td>
<td>4 8 12 16 20</td>
</tr>
<tr>
<td>3</td>
<td>3 6 9 12 15</td>
</tr>
<tr>
<td>2</td>
<td>2 4 6 6 10</td>
</tr>
<tr>
<td>1</td>
<td>1 2 3 4 5</td>
</tr>
</tbody>
</table>

Source: 5

Risk Assessment shows the level of risk acceptable or unacceptable. Low risk categories range from grades 1-4, moderate risk 5-14, while 15-25 are high risk categories. Risk assessment matrix is used as the basis for carrying out further actions, so that the level of risk received can be reduced to a level that can be tolerated. Determining risk control measures is an effort to reduce or eliminate hazardous exposure in the work environment. Activities in the control are: elimination, substitution, technical engineering, administration, and finally personal protective equipment.

**Findings**

### A. Risk Assessment

In the welding process Using the type of welding SMAW (Shield Metal Arch Welding) there are 3 stages with 7 job descriptions. The first stage begins with preparation, where there are steps to assemble a welding
device, adjust the amount of electric current as needed, and install the filler on the holder. At the preparation stage the potential hazards are electrical hazards such as electric shock, burning wires, and short circuit. There are 2 risk levels for the High Risk category and 3 Medium Risk categories.

The Second Stage is Implementation. At this stage is the welding process or the stage of the workpiece merge. The dominant danger present at this stage is the physical chemical hazard of toxins such as metal fume fever. Fume is a solid particle that is toxic, collects, and forms bonds with larger molecules. Fume particle size ranges from 0.001-0.1 microns which can endanger health problems\(^{11}\). Other hazards at this stage are cataract eye irritation, visible light and sparks from UV-B radiation and infrared. All risks at this stage fall into the High category.

The third stage is Finishing. At this stage is the activity of cleaning workpieces after welding, using both grinding machines and hammers. At this stage there is the risk of noise, burns, injury to the eyes due to grinding of work materials and sparks of welding material. At this stage all risks fall into the medium category.

B. Risk Management

From the results of the risk assessment, the percentage of risk categories is high risk 43% and medium risk 57%, and there is no low risk category, meaning that the welding work at PT Dok and Perkapalan Surabaya is included in the work that requires control measures to prevent accidents from occurring.

PT Dok dan Perkapalan Surabaya implemented one of the global occupational health and safety management systems that is in effect OHSAS 18001.2007. In the OHSAS 18001.2007 concept, risk management is a core element mentioned in clause 4.3.1\(^{10}\).

Based on the results of Hazard identification and Risk Assessment in the welding section, there are several forms of control applied by PT Dok dan Perkapalan Surabaya, such as using a respiratory protective device type cartridge respirator to prevent the risk of inhalation of fume particles through inhalation and using a face shield to prevent risk of eye irritation and eye fatigue for exposure to UV-B radiation and infrared at the high-risk stage of the welding process.

Other controls applied by PT Dok dan Perkapalan Surabaya in the welding process are based on the control hierarchy, including engineering control. This is to create a footing board for road access when the work area is wet, installing a safety line in a hazardous work area flammable work equipment, tidy up scattered cables so as not to interfere with road access. Control which includes administrative control on welding is a warning sign installed in the work area, carried out safety talk before doing work, communication with fellow workers, coordination with the Health Safety and Environmental Officer, work permit for welding in hazardous places, standard operating standards (SOP) on each job, selection of employees to do certain jobs, conducting patrol safety and field inspection training on certain jobs, maintenance of work equipment, calibrating work tools.

Control by using personal protective equipment. The forms of personal protective equipment provided by the company are: safety helmet, safety belt, safety shoes, gloves, safety harness, ear plug / ear muff, mask and respiro mask, safety spectacles, goggles, face shields.

However, all of these control methods do not necessarily reduce danger and risk at zero, meaning that workers are still more likely to be exposed to hazards at work. Research conducted by Wahyu, P.D and Tualeka, A.R., 2013 in one of the welding industries in East Java, Indonesia explained that there is still residual risk despite risk control. So that residual risk assessment needs to be done so that it can further determine additional risk control recommendations\(^{12}\). For this reason, we need a strong commitment from human resources or management and the awareness of the workers on the use of personal protective equipment in every work.

C. Risk Communication

The results of risk assessment and risk management in the form of Job Safety Analysis Sheet must be communicated so that it can be known by all parties including workers. Communication must be easily understood by all parties so that it needs to be designed in accordance with the desired goals\(^{5}\).

Risk Communication carried out at PT Dok dan Perkapalan Surabaya is through occupational health and safety development aimed at socializing and raising awareness of all workers about safety culture in the work environment for example: conducting safety induction is intended for new workers and transfer workers to enter
the welding activity area talk is held every Monday and Friday at the time before the start of an activity that contains informs of potential hazards at work. Safety campaign is an activity to install banners, posters that contain appeals for work safety, it is hoped that with these posters the workers are interested in reading and implementing their work, and conducting meetings of the Occupational Health and Safety Management Committee at the end of each month to discuss issues that occur improvement. Through the findings of patrol safety and inspection.

**Conclusion**

From the results of the risk assessment of the 3 stages with 7 job descriptions, the percentage of risk categories was obtained, namely high risk 43% and medium risk 57%, and there was no low risk category, meaning that welding work at PT Dok dan Perkapalan Surabaya included work requiring control to prevent accidents. Control carried out as part of risk management is going well but residual risk still exists so that it needs a strong commitment from human resources or management and the awareness of the workers on the use of personal protective equipment in every work. Risk communication is carried out through coaching or training, conducting safety induction, safety talk, Safety campaigns such as the activity of banner installation, posters, conducting meetings of the Worker Health and Safety Advisory Committee at the end of each month to discuss the problems that occur and make improvements.

**Conflicts of Interest:** All authors have no conflicts of interest to declare.

**Source of Funding:** The source of this research costs from self.

**Ethical Clearance:** The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

**References**

Prediction of The Needs for Benzene Detox with Foods Intake Containing CYP2E1 Enzyme, Sulfation, and Glutathione at Gas Stations Pancoranmas Depok, Indonesia

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Abstract

Background: Benzene was a dangerous chemical compound which was one of the products of gas stations and one of the chemicals contained in gasoline and it was carcinogenic. To reduce and eliminate toxin of benzene from human body, could be used the detoxification process. One of the detoxification process approach was using foods. The aim of this research was to calculate the foods intake containing CYP2E1 enzyme, sulfation, and glutathione to improve benzene detox.

Method: The type of research was descriptive study. The subjects was 15 workers. Location of this research was in gas station Pancoranmas Depok. Variables were body weight, duration of work, working time perweek, working time perday, and benzene concentration. After getting all variables above, breathing rate and intake non-carcinogen per respondent can be calculated. Then, effective doses of foods containing CYP2E1 enzyme, sulfation, and glutathione would be obtained.

Results: All respondents were at workplace shows benzene concentration below the TLV. The highest effective dose of foods containing CYP2E1 enzyme was cow brain, sulfation was tuna, and glutathione was carrot.

Conclusion: The level of adequacy of enzyme of each respondent was different. Effective dose of each respondent depending on body weight, duration of work, and benzene concentration at workplace. Every respondent could choose foods depending on their needs and taste.

Keyword: Benzene, CYP2E1, Detoxification, Glutathione, Sulfation

Introduction

According to the Central Bureau of Statistics, the number of vehicle users such as private cars has reached almost 15 million, for private motorbike users reaching 105 million in 2016. The use of the vehicle certainly requires fuel. One of the efforts to obtain fuel is through a Gas Station (SPBU). Every day, gas stations will refuel. The odor can cause a smell that is quite stinging so that it can be inhaled into the workers’ body. The smell of this fuel contains organic compounds, namely benzene.

Benzene is a colorless and sweet-smelling liquid, evaporates very quickly in the air but difficult to dissolve in water. Based on the results of the NCI (National Cancer Institute) and CAPM (Chinese Academy of
Preventive Medicine) studies, it was stated that fopoiotic malignancies lymphohema and hematological disorders in 74,828 benzene-exposed workers in 672 factories in 12 cities in China, increased the risk of all types of ANLL leukemia (Acute Nonlymphocytic Leukemia) and a combination of ANLL and precursor MDS (Myelodisplastic Syndrome)\[^{1}\] (Travis et.al, 1994). If workers are exposed to benzene it will cause a variety of negative things such as dizziness, nausea, kidney and liver problems, even if in long-term exposure and in a long duration it will cause cancer to cause death. Body receptors that will interact with toxins namely cells, enzymes, DNA/RNA, oxygen transport. The interaction between benzene and the receptors above causes work-related diseases.

Regulation of Indonesian Ministry of Manpower and Transmigration Number PER13/MEN/X/2011 and American Conference of Government Industrial Hygienists (ACGIH) in 2007, set the threshold limit value (TLV) for benzene exposure at workplace is 0.5 ppm (1.6 mg/m\(^3\))\[^{2-3}\]. Benzene included as a carcinogenic group in humans. National Institute for Occupational Health and Safety (NIOSH) in 2005, set the recommended exposure limit for 8 hours of work at 0.1 ppm (0.32 mg/m\(^3\))\[^{4}\]. While according to Agency for Toxic Substance and Disease Registry (ATSDR) in 2007, set the minimal risk levels (MRLs) for benzene exposure is 0.009 ppm (0.02 mg/m\(^3\)) per day which for acute effect and 0.003 ppm (0.009 mg/m\(^3\)) per day for chronic effect\[^{5}\]. The most vulnerable population to the accumulation of benzene exposure is gas stations workers. This is because SPBU workers work every day at the place so that continuous exposure can occur which over time accumulates the concentration of benzene can become quite high in the workers’ body.

Previous research, states that the benzene concentration at gas station Pancoranmas Depok is 0.02 ppm (0.06 mg/m\(^3\)) at both administration and operational units\[^{6}\]. That means it is still below the threshold limit value (TLV) according to Regulation of Ministry of Manpower and Transmigration Number PER13/MEN/X/2011 and American Conference of Government Industrial Hygienists (ACGIH) in 2007. This concentration value also below the TLV based on manual calculation of safe concentration at gas station Pancoranmas Depok is 0.03 ppm (0.09 mg/m\(^3\)). But this concentration value is above the TLV of minimum risk level (MRL) according to ATSDR\[^{5}\].

Although the benzene concentration in the gas station Pancoranmas Depok is below TLV, solution is needed to reduce negative impact of benzene exposure for gas station workers. One way is detoxify toxin from the body. One detoxification method that can be used is detoxification using food. Before toxins can be detoxified from the body, a biotransformation process is needed in the body. The purpose of biotransformation is to convert non-polar to polar, then to become hydrophilic so that it can be excreted out of the body. Biotransformation occurs in two phases. The first phase is the functional phase where the functional group matches the oxidation, reduction and hydrolosis reactions. Then the second phase is the conjugate reaction phase involving several types of endogenous metabolites in the body in the endoplasmic reticulum\[^{7}\] (Tualeka, 2013).

Research using foods approach as benzene detoxification is still very limited. Foods containing CYP2E1 enzyme such as cow liver, cow brains, and salmon \[^{8-9}\]. Foods containing sulfation such as egg, chicken, and tuna, and foods containing glutathione such as broccoli (Forman et al, 2010), carrot, and tomato \[^{10-11}\] (Dhivya, 2012). But there has never been a research that explains how much intake of these foods is needed to improve benzene detoxification, especially in populations that exposed to benzene in the long time. Based on the background above, the aim of this research is to calculate the intake of foods (effective doses) containing CYP2E1 enzyme, sulfation, and glutathione to detoxify benzene at gas station workers in Pancoranmas Depok.

**Method and Materials**

The type of research was descriptive study. Subjects were workers in gas station Pancoranmas Depok. The inclusion criteria were workers who had worked in this industry for 10 years or more and willing to be used as research respondents. The sample of was 15 respondents.

Variables calculated were body weight, duration of working (years), working time per week (days), average of working everyday (hours) of respondents, benzene concentration at 2 locations in this gas station. Measurement of respondents weight using manual measurement method with body scales. Measurement of duration of work, working time per week, and average of working everyday were obtained with indepth interview with respondents. Then, measurement of benzene concentration at environment using
measurement method of NIOSH 1501 (2003) with aromatic hydrocarbon sampling method [4]. Air samples were taken using a calibrated personal sampler pump. The filter used to absorb toluene vapour was a charcoal tube SKC 226-01. Air samples were analyzed using Gas Chromatography-Flame Ionization Detector (GC-FID). Willingness to participate in research was made in writing through informed consent and this study had received prior ethical approval by the Ethics Committee of the Faculty of Public Health, Airlangga University with ethical number 516 KEP-K.

After getting all variables above, can be found breathing rate and intake non-carcinogen of benzene per respondent. Then, effective dose of foods containing CYP2E1 enzyme, sulfation, and glutathione would be obtained by manual calculating, use the formula below:

\[ \text{Intake nc (non-carcinogen)} = \frac{C}{R} \times Dt \times fE \times tE \times Wb \times C_{\text{enzyme}} \]

**Explanation:**

- \( C \): Benzene concentration (mg/m^3)
- \( R \): Breathing rate (m^3/hour)
- \( Dt \): Duration of working (years)
- \( fE \): Working time per week (days)
- \( tE \): Average of working time per day (hours)
- \( Wb \): Weight (kg)
- \( C_{\text{enzyme}} \): Concentration of enzyme in 100 grams of food

**CYP2E1 enzyme**

\[ C_{\text{CYP2E1}} = 0.0000088 \text{ mmol/ml} \]

**Sulfation**

\[ C_{\text{Sulfation}} = 0.0001 \text{ mmol/ml} \]

**Glutathione**

\[ C_{\text{Glutathione}} = 0.00000099 \text{ mmol/ml} \]

**A** = Content of enzyme in 100 grams of food

- **CYP2E1 enzyme**
  - Cow liver: 5.6 mg
  - Cow brain: 1.8 mg
  - Salmon: 6.6 mg

- **Sulfation**
  - Egg: 1.477 g
  - Chicken: 0.801 g
  - Tuna: 0.755 g

**Results**

**Distribution of Benzene Concentration at Workplace**

In Figure 1 shows that all respondents are at workplace with benzene concentration below the threshold limit value (TLV). The TLV of benzene concentration in the air is 0.5 ppm (1.6 mg/m^3). While, there is no respondent who is at workplace with benzene concentration above TLV. Measurements of benzene concentrations are carried out at administration and operational units. The benzene concentration in both units are 0.02 ppm (0.06 mg/m^3).

**Effective Dose of Foods Containing CYP2E1 Enzyme to Benzene Detox**

- Egg: 1.477 g
- Chicken: 0.801 g
- Tuna: 0.755 g
- Broccoli: 7.8 mg
- Carrot: 5.9 mg
- Tomato: 10.9 mg

![Figure 1. Distribution of Benzene Concentration at Workplace](image)

![Figure 2. Effective Dose of Foods Containing CYP2E1 Enzyme to Benzene Detox](image)
In Figure 2 shows that the effective dose of cow brain has the highest dose, while salmon has the lowest dose from foods containing CYP2E1 enzyme. The highest effective dose of cow liver, cow brain, and salmon are 25.08 mg, 78.05 mg, and 21.28 mg (respondent 11). While the lowest effective dose of cow liver, cow brain, and salmon are 15.36 mg, 47.8 mg, and 13.03 mg (respondent 12).

Effective Dose of Foods Containing Sulfation to Benzene Detox

In Figure 3 shows that the effective dose of tuna has the highest dose, while egg has the lowest dose from foods containing sulfation. The highest effective dose of egg, chicken, and tuna are 53,093 mg, 97,901 mg, and 103,866 mg (respondent 11). While the lowest effective dose of egg, chicken, and tuna are 32,346 mg, 59,644 mg, and 63,278 mg (respondent 12).

Effective Dose of Foods Containing Glutathione to Benzene Detox

In Figure 4 shows that the effective dose of carrot has the highest dose, while tomato has the lowest dose from foods containing glutathione. The highest effective dose of broccoli, carrot, and tomato are 0.09 mg, 0.12 mg, and 0.06 mg (respondent 11). While the lowest effective dose of broccoli, carrot, and tomato are 0.05 mg, 0.07 mg, and 0.04 mg (respondent 12).

Discussion

Distribution diagram analysis between benzene concentration and the threshold limit value (TLV) shows that all respondents are at workplace with benzene concentration below TLV according to Regulation of Ministry of Manpower and Transmigration Number PER13/MEN/X/2011 about The Threshold Limit Value of Physical and Chemical Factors at Workplace [2]. Detoxification is very important to be done in order to remove harmful chemicals in the body. Many toxins are spread on this earth such as in the sea, rivers, food, drinks, and objects around us. Detoxification can be done uses food approach. Food-based nutrition be involved in the detoxification process. Several publications that had used cells, animals and clinical studies show that food-based components and nutrients could modulate the process of conversion and excretion of toxins from the body [13].

CYP2E1 enzyme catalyzes the oxidation reaction of benzene to benzene oxide which is in balance with benzene oxepin. Then metabolized to phenol (the main metabolite product of benzene). Phenol is then oxidized with CYP2E1 catalyst to catechol or hydroquinone, which is then oxidized by the enzyme myeloperoxidase (MPO) to be a reactive metabolite 1,2- and 1,4-benziunon. Catechol and hydroquinone can be converted to metabolites 1,2,4-benzenetriol with CYP2E1 catalysts [14-15]. Food sources of CYP2E1 enzyme like cow liver, cow brain, and salmon [8-9]. Specific foods and nutrients can induce metabolic enzymes, such as sulfation. Sulfation can be catalyzed by enzyme sulfotransferase. This enzyme can be found in the liver, intestines, adrenal glands, brain, and skin. The sulfating process is a detoxification process that can reduce the toxicology of a chemical. The decline in the function of this enzyme can be caused by genetic or certain chemicals. The activity of the sulfotransferase enzyme depends on inorganic sulfate reserves. Food sources of sulfation-containing compounds such as egg, chicken, and tuna [9]. Enzyme that responsible for metabolizing glutathione is the glutathione s-transferase enzyme. Involving antioxidant responsive elements and xenobiotic responsive elements. Many foods to be
upregulation of this enzyme, including garlic, fish oil, black soybean, broccoli, carrot, tomato, curcumin, etc. Genetic variances, gender, and maybe body weight can play a role in the effects of dietary factors on GST enzymes [9].

Based on the results of data analysis, the effective dose of cow liver, cow brain, and salmon that the body needs to remove benzene out of the body as shown in Figure 2-4. The effective dose of each food is different depending on the physical of individual. The higher concentration of benzene in the body, the higher detox mass of cow liver, cow brain, and salmon needed. Also with detox mass of egg, chicken, tuna, broccoli, carrot, and tomato. This is consistent with the formulation that has been made in previous studies which states that it has a synergistic relationship with substance concentration [16]. Maximum consumption mass of foods containing CYP2E1 enzyme like cow liver is 26.08 mg, cow brain is 78.05 mg, and salmon is 21.28 mg. Maximum consumption of foods containing sulfation and glutathione like egg is 53.09 g, chicken is 97.9 g, tuna is 103.86 g, and broccoli is 0.09 mg, carrot is 0.12 mg, and tomato is 0.06 mg. Foods in the diagram can be chosen by each respondent needs and tastes. If the respondent does not like the cow liver and cow brain, he/she can consume salmon according to the effective dose of salmon needed, and vice versa. Also with foods containing sulfation and glutathione. The consumption of each food can be regulated by each respondent, can be divided into several days.

**Conclusion**

All of respondents were at workplace with benzene concentrations below the threshold limit value (TLV). Intake of foods that contain CYP2E1 enzyme (cow liver, cow brain, and salmon), sulfation (egg, chicken, and tuna) and glutathione (broccoli, carrot, and tomato) were expected to increase detoxification of benzene. The effective dose was required by the respondents depending on body weight, duration of work, and benzene concentration at workplace. The higher the benzene concentration, the higher the needs for foods containing CYP2E1 enzyme, sulfation, and glutathione that the body needs. Body weight and duration of work could also be another factors in differences of individual intake. Every respondent could choose foods depending on their needs and tastes.

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**References**


Determination of Safe Benzene Concentration at Ciputat Gas Station

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Abstract

Benzene is a simple cyclic organic compounds whose concentrations are found usually low dilikungan. Benzene is often used in the industrial world, both home industry and the oil and gas industry. Benzene can enter into the body through the lungs, it can be through inhalation, gastrointestinal tract, and through the skin. If someone exposed to benzene at high concentrations, the levels of benzene into the lungs roughly half of the levels of benzene is absorbed, so that the incoming kealiran blood. This research is descriptive. The population in this study is a gas station worker Ciputat region totaling 10 people. The results of the study then analyzed quantitatively to determine the concentration secure benzene for workers obtained from the data concentration of benzene in the workplace, height workers, the weight of workers, heavy mice, respiration rate workers, time spent working, the surface area of the worker’s body surface area the body of laboratory mice, the highest dose of the toxin no effects in animal experiments (NOAEL), Km factors in animals (animal Km), Km factors in workers (Human Km), and the safe limit for workers toxin dose (SHD). The results of measurement of the concentration of benzene in petrol stations in the region Ciputat is 0.58mg/m³ (0.18 ppm), which means it is still below the Threshold Limit Value (TLV) according Permenakertrans 13 / MEN / X / 2011, the year 2011 is equal to 0.5 ppm. In contrast to the NAB which have been set at 0.18 ppm, based on manual calculations for safe concentration limit of benzene obtained value of 0.02 ppm.

Keyword: Benzene, Safe Concentration, Gas Stations, Workers

Introduction

In the era of the fastest growing technology, transportation is a very necessary thing humans in performing daily life. Means of transport is a tool that has a main component of a machine that needs fuel to run, usually referred to by fuel oil (BBM). In Indonesia alone type of fuel oil used on a variety of vehicles, such as gasoline and diesel.

There are various kinds of chemical substances contained in the fuel dinataranya benzene, toluene, xylene, ethylene, etc. which is certainly harmful to human health. The nature of the chemicals benzene, namely colorless liquid with a sweet odor. Benzene evaporates into the air very quickly, slightly soluble in water but soluble in fats, and flammable. Benzene can be found in air, water, and soil. Benzene is a chemical that is harmful and carcinogenic to humans and is a colorless liquid and volatile. Benzene is widely used in the rubber industry, oil refining, shoe factories, chemical plants and other industries related to oil including age refueling stations (gas stations).

Indonesia itself has created rules limit benzene exposure in the work environment specified in the Threshold Limit Value (TLV) of benzene of 0.5 ppm in accordance with the Regulation of the Minister of Manpower and Transmigration Republic of Indonesia number PER / 13 / MEN / X / 2011 on the Threshold Limit Value Physical and chemical factors in the Workplace.

Acute benzene exposure would have an impact like that cause disturbances in the nervous system, lack of oxygen supply to the brain, dizziness, rapid heart
rate, headaches, tremors, confusion and fainting. While chronic benzene exposure can cause decreased production of red blood cells causes anemia. Benzene can also get to the bone marrow and impair the production of blood cells so that persons exposed to benzene can suffer diseases related to decreased production of blood cells in the bone marrow. Some studies indicate a relationship between the quantity of benzene exposure by inhalation to the trans, trans-Muconic Acid (ttMA) as a biomarker of exposure to benzene. trans, trans-Muconic Acid (ttMA) is a minor metabolite of benzene that can be used as biological indicators of exposure to benzene. The occurrence of abnormalities in the hematopoietic system caused by exposure to benzene is a major concern. Examination and laboratory testing conducted on workers at risk of benzene exposure include a Complete Blood Count (CBC) with leukocyte count, hematocrit, hemoglobin (Hb), red cell count, erythrocyte indices (MCV, MCH, MCHC), and platelet counts.

Based on previous studies of benzene in the workplace, there is very little research on the safe concentration (C safe) in the work environment that have exposure to benzene. Area gas stations as a working environment that has a high exposure to benzene, should the concentration of benzene in the safe limits so as not to cause health problems for workers. Therefore, based on the explanation above, the writer will measure the safe concentration limit of benzene in the area of the pump.

This study aims to determine safe concentrations of benzene in petrol stations in the region namely Ciputat. The results of this study are expected to be on filling stations in protecting workers from exposure to benzene.

Material and Method

This research is descriptive. The population in this study were gas station workers who worked at number 1 and point 2, totaling 10 people. The collection of primary data that the air concentration of benzene in the workplace, the duration of working time, and the weight of workers. It also conducted a secondary data collection on laboratory animals, namely the weight of mice.

The variable in this study is the concentration of benzene in the workplace, height workers, the weight of workers, heavy mice, respiration rate workers, time spent working, the surface area of the worker’s body, body surface area of mice, the highest dose of the toxin no effects in animal experiments (NOAEL), Km factors in animals (animal Km), Km factors in workers (Human Km), the safe limit for workers toxic dose (SHD), and the concentration of benzene in the air that is safe for workers (C safe).

Findings

A. Characteristics of Animals Try and Try

Animal Body Surface Area (Rats)

The toxicity of compounds in general can be interpreted by the potential chemicals that cause damage when it entered into the human body. In the implementation of toxicity tests using experimental animals are mice. In general, the human response to toxicant is qualitatively similar to the response of the animals, so that this fact is the basis of extrapolation from animal data to humans.

Table 1. Characteristics of Animal Experiments (Rats)

<table>
<thead>
<tr>
<th>Animals Try (Rats)</th>
<th>W (kg)</th>
<th>BSA (m2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1405</td>
<td>0.024165</td>
</tr>
<tr>
<td>2</td>
<td>0.1405</td>
<td>0.024165</td>
</tr>
<tr>
<td>3</td>
<td>0.1410</td>
<td>0.024223</td>
</tr>
<tr>
<td>4</td>
<td>0.1410</td>
<td>0.024223</td>
</tr>
<tr>
<td>5</td>
<td>0.1395</td>
<td>0.024050</td>
</tr>
<tr>
<td>6</td>
<td>0.1405</td>
<td>0.024165</td>
</tr>
</tbody>
</table>

Based on data from white rat body weight, it can be calculated body surface area white mice using the following formula:

\[ BSA \text{ hewan} = 0.09 W^{0.67} \]

Information:

- BSA : Body Surface Area / body surface area (m2)
- W : Weight / Weight (kg)

B. Characteristics of Workers, Body Surface Area Workers and Workers Respiratory rate

Characteristics of workers in this study include the weight and working time of 10 respondents working
in the area of gas stations in Region Ciputat. Based on Table 2, the highest known weight 67 Kg, 44 Kg lowest weight, and average weight of 51.4 kg. Long time working day is 8 hours. As for the height using the average value of height of an adult male Indonesia is 159 cm. Based on data from trade body weight and height workers, can be calculated body surface area workers and workers breathing rate using the following formula.

1. Full Body Surface Area

\[ BSA = \sqrt{\frac{W \times h}{3600}} \]

Information :

BSA : Body Surface Area / body surface area (m²)

W : Weight / Weight (kg)

h : Height / Height (cm)

2. Respiratory rate of Workers

\[ BR = \frac{5.3 \times \ln W - 6.9}{24} \]

Information :

BR : Breathing rate / respiratory rate (m³ / h)

W : Weight / Weight (kg)

<table>
<thead>
<tr>
<th>worker</th>
<th>W (kg)</th>
<th>h (cm)</th>
<th>BSA (m²)</th>
<th>BR (m³ / hr)</th>
<th>t (hours / day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>159</td>
<td>1.49</td>
<td>0.58</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>159</td>
<td>1.49</td>
<td>0.58</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>159</td>
<td>1.60</td>
<td>0.60</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>159</td>
<td>1.62</td>
<td>0.61</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>159</td>
<td>1.47</td>
<td>0.57</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>159</td>
<td>1.40</td>
<td>0.56</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>159</td>
<td>1.39</td>
<td>0.54</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>46</td>
<td>159</td>
<td>1.42</td>
<td>0.55</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>67</td>
<td>159</td>
<td>1.72</td>
<td>0.64</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>159</td>
<td>1.40</td>
<td>0.56</td>
<td>8</td>
</tr>
<tr>
<td>Average</td>
<td>51.4</td>
<td>159</td>
<td>1.50</td>
<td>0.57</td>
<td>8</td>
</tr>
</tbody>
</table>

Based on Table 2, the highest known weight of workers at gas stations Ciputat region that is 67 kg, while the lowest body weight 44 kg. The length of time worked in a day is all of 8 hours and height using the average value of height of an adult male Indonesia is 159 cm.

The results of the analysis of body surface area calculation and respiratory rate workers according to table 2 shows that the average worker’s body surface area is 1.59 m² and the average worker respiratory rate is 0.57 m³ / h.

C. Benzene concentration

The results of measurement of the concentration of benzene in the workplace stations in Ciputat area is either at point 1 and point 2 equals that is 0.58 mg / m³ (0.18 ppm)
Table 3. Distribution of Benzene concentration at the pump stations in Region Ciputat

<table>
<thead>
<tr>
<th>location Measurement</th>
<th>Benzene concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
</tr>
<tr>
<td>Point 1</td>
<td>0.18</td>
</tr>
<tr>
<td>Point 2</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Based on the results of measurements taken, the concentration of benzene in the working environment Ciputat Regional gas station is 0.18 ppm. The benzene concentration is below the Threshold Limit Value (TLV) of 0.5 ppm in accordance with the Regulation of the Minister of Manpower and Transmigration No. PER.13 / MEN / X / 2011 on the Threshold Value Factor of Physical and Chemical Factors in the Workplace. However, the benzene concentration is above the Minimum Risk Level (MRL), inhalation of benzene exposure levels set by ATSDR, namely for acute exposure (≤14 days) = 0.009 ppm, exposure to moderate (15-364 days) = 0.006 ppm, and chronic exposure (≥365 days) = 0.003 ppm.

D. Animal and Human Km Km

The determination of safe dose limit for workers toxin begins prior to the calculation of Animal and Human Km Km.

1. Animal Km

\[ Animal \ Km = \]

Information:

Animal Km: Km factors in animals

\( W \) : Weight of experimental animals (rats)

BSA : Body Surface Area of experimental animals (rat White)

Animal Km ditunjukka calculation results in Table 4, with an average of Animal Km on white rats is 5.81.

Table 4. Calculation Results Km Animal Animal Try (Rats)

<table>
<thead>
<tr>
<th>Animals Try (Rats)</th>
<th>animal Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.81420952</td>
</tr>
<tr>
<td>2</td>
<td>5.81420952</td>
</tr>
<tr>
<td>3</td>
<td>5.82102947</td>
</tr>
<tr>
<td>4</td>
<td>5.82102947</td>
</tr>
<tr>
<td>5</td>
<td>5.80052067</td>
</tr>
<tr>
<td>6</td>
<td>5.81420952</td>
</tr>
<tr>
<td>Average</td>
<td>5.81</td>
</tr>
</tbody>
</table>

2. Human Km

\[ Human \ Km = \]

Information:

Human Km: Km factors in human / workers

\( W \) : Weight workers

BSA : Body Surface Area workers

Table 5, with an average of Human Km at gas station worker is 34.09.

<table>
<thead>
<tr>
<th>worker</th>
<th>Human Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.55</td>
</tr>
<tr>
<td>2</td>
<td>33.55</td>
</tr>
<tr>
<td>3</td>
<td>36.25</td>
</tr>
<tr>
<td>4</td>
<td>37.03</td>
</tr>
<tr>
<td>5</td>
<td>33.33</td>
</tr>
<tr>
<td>6</td>
<td>32.14</td>
</tr>
<tr>
<td>7</td>
<td>31.65</td>
</tr>
<tr>
<td>8</td>
<td>32.39</td>
</tr>
<tr>
<td>9</td>
<td>38.95</td>
</tr>
<tr>
<td>10</td>
<td>32.14</td>
</tr>
<tr>
<td>Average</td>
<td>34.09</td>
</tr>
</tbody>
</table>

E. NOAEL

One objective of the research activities in the field of Tiksiologi is to be able to evaluate the safety of a substance. To determine the safety limit concentration of a chemical substance toxicity test begins with the
determination of the highest dose without effect on animal or No Observed Adverse Effect Level (NOAEL). The research results Swaen et al. (2010) states that benzene NOAEL was 3.0 mg / m³, equivalent to 0.022 mg / kg obtained from the following calculation formula.

\[
\text{Benzene NOAEL (mg / m³)} = 0.022 \text{ mg / kg}
\]

**F. Safe Human Dose**

Safe Human Dose safe dose limits for workers or Safe toxin Human Dose (SHD) found begin using the formula from Shaw et al. (2007) below.

\[
\text{SHD} = \text{NOAEL}
\]

Information:

- **SHD**: Safe Human Dose (mg / kg)
- **animal Km**: Km factors in animals
- **Human Km**: Km factors in human / workers

Based on this equation, the calculation results obtained from the SHD NOAEL value, average animal Km, and the average human Km are:

\[
\text{SHD} = 0.022 \times
\]

\[
= 0.003 \text{ mg / kg}
\]

**G. Safe limit concentration of Benzene**

Determining the safety limit concentration of benzene in the workplace (filling station) using the formula (William, 1985; Soemirat, 2003; Davis, 1991) the following:

\[
\text{C aman} = \frac{\text{mg}}{\text{m}^3}
\]

To convert the units of mg / m³ to ppm use the following formula.

\[
\text{C aman} = \times 24.5 \text{ ppm}
\]

Information:

- **C safe**: The concentration of toxins in the air that is safe for workers (mg / m³)
- **SHD**: Safe Human Dose (mg / kg)
- **W**: Weight (kg)
- **\(\delta\)**: % Of the absorbed substances lungs

Based on the formula above, the result of calculation of a safe concentration of benzene in petrol stations Ciputat region derived from SHD value, the average weight of workers, the percentage of absorption of the average worker respiratory rate and the average length of working time is:

\[
\text{C safe (mg / m³)} =
\]

\[
= 0.067 \text{ mg / m³}
\]

\[
\text{C aman (Ppm)} = \times 24.5 \text{ ppm}
\]

\[
= 0.02 \text{ ppm}
\]

The result of the calculation of the safety limit in the air that is safe for workers above can be used for (William, 1985 in Tualeka, 2013): predict the concentration of toxins in the air a safe working environment for the workers when there has been no determination of the Threshold Limit Value (TLV), and for comparison with NAB which has been established by various agencies both by Manpower, the National Standardization Agency, ACGIH, NIOSH and OSHA.

**Conclusion**

The results of measurement of the concentration of benzene in petrol stations in the region Ciputat is 0.58 mg / m³ (0.18 ppm), which means it is still below the Threshold Limit Value (TLV) according Permenakertrans 13 / MEN / X / 2011, the year 2011 is equal to 0, 5 ppm. In contrast to the NAB which have been set at 0.18 ppm, based on manual calculations for safe concentration limit of benzene obtained value of 0.02 ppm. While based on the Minimum Risk Level (MRL) set ATSDR 2007, the concentration of 0.009 ppm benzene every day can give the effect of acute and 0.003 ppm daily for chronic effects, so as the concentration of benzene in petrol stations work environment has the potential to provide health effects for workers.

Thus the necessary control measures so that workers are protected from the adverse effects of benzene on health. Control recommendations are to consume the
enzyme CYP2E1 contained in beef liver and salmon which serves to reduce the levels of benzene in the body, use of personal protective equipment appropriate in the form of half-mask respirator with organic vapor cartridge for meminimalisir exposure to benzene, and planted a number of plants that can absorb and lowering the concentration of benzene such as Boston and Golden Phothos 14.

Conflicts of Interest: All authors have no conflict interest to declare.

Source of Funding: The source of the research cost from self.

Ethical Clearance: This study was approved by the institutional Ethical Bord Airlangga University, Faculty of Public Health.

All subjects were fully informed about the procedures and objectives of this study each subject prior to the study signed an informed consent form.

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4. Republic of Indonesia’s Minister of Manpower and Transmigration. Regulation of the Minister of Manpower and Transmigration Republic of Indonesia Number Per.13 / Men / X / 2011 Concerning ValueThreshold Factor Physical And Chemical Factors in the Workplace. 2011; 1-54.
Forensic Implications of Saliva: An Overview

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Abstract

Over past years, investigators and researchers have been repeatedly attracted by possible applications of saliva related evidence in forensics. It is specifically looked for in cases of sexual assault, drug abuse, driving while intoxicated, and in cases involving animal bites. Oral fluid has a role in linking suspects or victims to a crime scene, as well as in profiling of the unknown individual. Collection of salivary evidence should thus be given deserved importance and should be carried out using scientific methods, considering the progression of time, type of case, sequence and type of analytical methods to be performed, nature of surface etc. This review emphasizes the comprehensive use of saliva in forensics.

Key words: Identification, Investigation, Forensics, Analysis, Oral Fluid, Saliva

Introduction

Mandel in 1990 quoted that “saliva lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears”. [1] An exocrine secretion from the salivary glands, saliva is the predominant secretion in the oral cavity. With an average secretion of about a litre per day, and a slightly alkaline pH, it has a multifunctional role. [2] Having a range of molecules in content, saliva has an indispensable role in forensic odontology. [3]

Although with few solutes, saliva is has a varied scope in drug detection, identification, DNA fingerprinting, cases of abuse, analysis of psyche, and so on. Saliva can be collected from bite-marks on skin and edible items, from stains in surfaces, and from various items like utensils, straws, phones, cigarette butts, stamps etc. [4]

In the past, forensic investigations have revealed saliva in lip-prints, bite-marks, and other objects, helping in identification to a greater extent. [5] As saliva enters the oral cavity, it coalesces with blood cells, oral microflora, food, and upper airway secretions. This increases the chance of saliva containing information about a person. [6]

The current article will summarise on the use of saliva in various context of forensics.

Presence of Saliva at the Crime Scene

In numerous crimes, saliva is stored in ‘nibble marks’. [7] The advantages of using saliva its ease of access, ease of handling, its non-invasive collection, and economical analysis. [3] On the other hand, oral fluid deposited on substrate can’t be specifically submitted to extraction procedures. [8]

Recovery and Detection:

• Visual Examination: As dry colourless stains, saliva is difficult to identify in a crime scene. However with the use of alternate light sources and ultraviolet lights, discovery becomes easier. Under ALS, ranging 415–490nm (using orange/red goggles) [9], quartz arch tube or argon ion laser, saliva presents soft edged white spots, less intense than other stains. [4] Saliva stains will appear bluish-white under an ultraviolet light. However, it degrades the DNA in the sample. [10]

• Presumptive tests: Due to lack of solutes, there are no confirmatory tests for saliva. [8] The presence of saliva stains can be detected through test for amylase, present in high concentrations in saliva, pancreatic

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fluid, and faeces. Detection of amylase is particularly important in cases involving fellatio.\textsuperscript{[11]}

Test for saliva involves starch and Florence iodine solution (~1:100), with the sample. Starch and iodine form a deep blue complex. Amylase hydrolyses starch and the colour fades (time: 15 mins, temperature: 37°C). However, albumin, gamma-globulin, semen, etc. may give a false positive result.\textsuperscript{[10]} Elevated amylase levels may also be from oral sex/fellatio or from vaginal cavity.\textsuperscript{[9]}

The method has limitations and variable sensitivity depending upon the age of the stain and quantity of deposit.\textsuperscript{[9]}

- **Collection:** Collection involves three aspects: the crime scene, the victim and from suspect(s). The classical technique is using a single wet cotton swab/filter paper laid passively on the surfaces.\textsuperscript{[4]}

  Sweet et al developed the double swab technique, to provide a better yield through rehydration of the saliva traces by swabbing with a sterile swab, wetted in nuclease free water with moderate pressure in a rolling motion for about ten seconds, followed by a second dry swab.\textsuperscript{[4, 12]}

  Stimulated saliva can be collected from the suspect using paraffin or citric acid crystals, and non-stimulated saliva by simple rinse with mouthwash. While these samplings are considered as diluted saliva, whole saliva is directly milked from the opening of Stenson’s duct. Commercial kits like Oragene are also available, when salivary DNA is required.

  FTA cards (Fritz Technology Associates) are useful for the collection and storage of DNA from body fluids, including saliva.

  **Laboratory analysis:** Amylase mapping is performed for saliva stains on samples of larger area [Figure-1].\textsuperscript{[9]}

  \begin{itemize}
  \item Amylase substrate is sprayed on a sheet of filter paper
  \item Substrate-containing paper is placed over the area to be tested
  \item The orientation of the filter paper is marked to aid in locating the stain
  \item The filter paper is dampened by spraying with water and plastic wrap is placed on top to prevent the paper from drying.
  \item Brown colour indicates a positive reaction
  \end{itemize}

**Figure 1: Procedure for amylase mapping**

A radial diffusion assay has been used to distinguish sources of AMY1 (amylase found in saliva, breast milk, perspiration) and AMY2 (amylase in the pancreas, semen, and vaginal secretions) [Figure-2]
**Immunological methods:** ELISA is not widely used. α-amylase is detected using a horseradish peroxidase conjugate combined with monoclonal antibodies. The Phadebas1 test reagent, used in tube and press tests, with sensitivity up to 1:12810, incorporates procion red amylopectin.

**Detection of Chemicals:** Dried saliva stains can be identified with soluble phosphatase, starch and amylase resulting in a red precipitate. Nitrites and thiocyanates can also be detected. Tryptophan also helps recognize dried oral fluid, giving a trademark emanation range on fluorescent spectroscopy.\[13\]

Near-infrared (NIR) Raman spectroscopy has been used to measure spectra of pure dried human saliva samples, showing its heterogeneity.\[14\]

The RSID (Rapid Stain Identification) test, specific for human salivary α-amylase, has been very useful and quick to identify saliva. It is similar to a pregnancy test (parallel stream immuno-chromatography) and is the principal high sensitivity specific non-enzymatic test for amylase.\[12\]

Investigation of Drugs of Abuse in Saliva

Oral fluid is second only to urine for checking medications.\[15\] Medications enter the salivation by the passive exchange.\[16\] Most illicit and addictive drugs of abuse can be distinguished in saliva.\[17\] Radioimmunoassay (RIA) has also proved useful.\[1, 18\] Recently, Drug wipe, an immunochemical based test strip has been introduced to detect medications of mishandle in the fluid.\[19\]

Peel et al. discovered quantifiable amounts of medications in salivation removed with methanol and broke down by immunoassay and gas chromatography/mass spectrophotometry, which can be adjunct to serological testing.\[20\]

Deoxyribonucleic Acid Profiling/Fingerprinting

DNA analysis is of great dependency when traditional identification methods fail.\[21\] In spite of affecting the security of human DNA dirt and microscopic organisms don’t pose much threat as the tests utilized for profiling are particular to person only.\[22\]

Saliva contains leukocytes and exfoliated epithelial cells as a useful DNA source.\[23\] Since secretory genes are present, saliva can also be used for blood grouping.

Saliva yields a very small amount of DNA. However, DNA tests are intensified by polymerase chain response for DNA writing utilizing short tandem repeats (STRs), unique, lifelong and durable markers consisting of repeated DNA sequences. STRs are suitable for maternity/paternity assurance and scientific examination, requiring only 0.5ng of DNA template.\[26\]

Mitochondrial DNA (mtDNA) is present in cells found in saliva, which has a high copy number proves useful when nuclear DNA testing fails, due to degradation.\[27\] mtDNA is maternally inherited\[28\], thus, distant maternal relatives can be used as a reference source for identification.\[29\]

Methylation and telomere shortening, has been observed in salivary DNA to estimate age.\[32\]

Salivary DNA lasts even after about fifteen days.

**Sex Determination from Saliva**

The peeled epithelial cells present in salivation have expanded the likelihood of sex assurance of the culprit. Even in degraded samples, sex chromatin like Barr bodies (in females) and F bodies (in males) can be observed; and sex hormones detected using fluorescent dyes or radioimmunoassay.\[22\]

**Salivary Biomarkers**

Salivary nucleic acids and proteins contain vital information. Saliva biomarkers have been used in diagnosis of various diseases but they have wider role in forensics. Markers for any particular disease can be matched and on tracking that disease to hospital visits, positive identification can be achieved.\[6\]

**Determining Psychology**

Different hormones in saliva helps in deciding the mind of a person.\[11\] Salivary level of steroid hormones reflects the free, unbound circulating fraction,\[30\] helps in determining mind-set of a person. High testosterone in spit has demonstrated people as rough, less consistent and related with individual mischief.\[11\] Low salivary cortisol could likewise be related with fierce criminal conduct.\[31\]

**Conclusion**

Rarely, people think of saliva, as a convenience,
Practicing dentists find it’s a nuisance, to be sponged, evacuated or dammed. However, over the years, the need and importance of saliva as an investigative body fluid is increasing rapidly in forensics. With constant examination, and a communitarian work in serological and odontological fields, salivation is developing as a fundamental device for scientific examination. A careful learning of what at a dentist faces each time he/she works in a mouth, may increase the significance of an odontologist in a criminal examination group by manifolds. One can only ponder what more is behind each drop of spit in the mouth.

**Conflict of Interest:** Nil

**Source of Funding:** Nil

**Ethical Clearance:** Nil

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Risk Management by Implementing Hazard Identification, Risk Assessment and Determinant Control (HIRADC) Method in the Research Center of the University in Surabaya, Indonesia

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Abstract

The Research Center of the Faculty of Dentistry, Universitas Airlangga is one of workplaces that have various risks to cause occupational accidents. The use of various chemicals, specimens, and special instruments at the Research Center has potential to cause various risks that can threaten workers, assets, and environment. To prevent and control every hazard with potential negative risks, a risk management using HIRADC method must be implemented. This study aims to implement Hazard Identification, Risk Assessment and Determinant Control (HIRADC) method in the Research Center of the Faculty of Dentistry, Universitas Airlangga. This study is descriptive research with a cross-sectional design. Data collection was done through direct observation in the workplace and interview with a laboratory worker at the Research Center. The Research Center of the Faculty of Dentistry, Universitas Airlangga has a moderate risk. The research results showed that of the 5 work activities, there were 20 potential hazards with 20 risks identified. Based on the risk assessment, there are 1 extreme risk, 1 high risk, 11 moderate risks, and 7 low risks. The extreme risks identified are explosion, fire, and death caused by LPG. The high risks identified are explosion, poisoning, and death caused by CO2 gas. Based on the determinant control, there are 15 controls implemented including substitution control, engineering control, administration control, and wearing personal protective equipment. However, there are still 5 controls that have not been implemented. Further recommendations are needed for 5 controls that have not been implemented in every activity conducted in the Research Center of the Faculty of Dentistry, Universitas Airlangga.

Keywords: HIRADC, Hazard Identification, Risk Assessment, Determinant Control, Research Center, Laboratory

Introduction

Every job has its own potential hazards depending on the production type, machines and instruments used, materials used, environment, and management and worker quality. Hazards are everything including situations or actions that have the potential to cause occupational accidents or injuries to humans, damage or other disturbances(1). If a hazard is linked to the likelihood of occurrence of an accident and the severity it causes, it becomes a risk. Uncontrolled risks that are not managed effectively have potential to cause occupational accidents to workers.

According to data from the International Labour Organization (ILO) (2017), as many as 2,780,000 workers die each year due to occupational accidents and occupational diseases. About more than 380,000 (13.7%) of work deaths were caused by occupational accidents. In addition to causing high number of deaths of workers, occupational accidents also cause losses. Estimates of
losses due to occupational accidents reach 3.94% of global Gross Domestic Product (GDP) each year\(^{(2)}\). In Indonesia, the number of occupational accidents is also quite high. According to data from the Ministry of Health of the Republic of Indonesia (2015), from the number of occupational accident cases in Indonesia during 2011-2014, the highest number occurred in 2013 with 35,917 cases. Therefore, it is known that every hour as many as 4 workers die due to occupational accidents\(^{(3)}\).

The Research Center of the Faculty of Dentistry, Universitas Airlangga is one of workplaces that have various risks to cause occupational accidents. The Research Center of the Faculty of Dentistry, Universitas Airlangga is a research center that aims to facilitate basic research, applied and innovative research for prevention of various diseases in oral cavity based on science and technology. The Research Center has five laboratories in it, which include immunology laboratory, molecular biology laboratory, karyotype laboratory, microbiology laboratory, dental biomaterial laboratory and stem cells.

The large number of work activities carried out at the Research Center has potential to cause various risks that can threaten workers, assets, and environment. The use of various chemicals, specimens, and special equipment at the Research Center also has potential to cause explosions, fires, occupational accidents such as being cut off, slashed, poisoning, exposed to chemicals, and also occupational diseases such as contact dermatitis, infertility and cancer. To prevent and control every hazard with potential negative risks, a risk management using HIRADC method must be implemented.

Hazard Identification, Risk Assessment and Determinant Control (HIRADC) method is a system for conducting risk analysis consisting of 3 stages, including hazard identification, risk assessment and determinant control. The output of risk management using the HIRADC method is to minimize any risk that can cause occupational accidents in the workplace\(^{(4)}\). Therefore, the objective of this study is to implement Hazard Identification, Risk Assessment and Determinant Control (HIRADC) method at the Research Center of the Faculty of Dentistry, Universitas Airlangga.

**Material and Method**

This study is a descriptive research, which is a study conducted to describe or explain a phenomenon that occurs\(^{(5)}\). This study is an observational study with a cross-sectional design. The population in this study was a laboratory worker at the Research Center of the Faculty of Dentistry, Airlangga University. This study uses the total population as respondents. This study was done during October 2018. The data collected in this study is primary data through direct observation in the workplace and interview with a laboratory worker. The variables that will be used in this study are hazard identification, risk assessment and determinant control.

Hazard identification completed through direct observation in the workplace using the HIRADC form and interview with a laboratory worker at the Research Center using an interview sheet. Based on the results of hazard identification, the potential hazards and risks in each work activity carried out are known. Then, the level of likelihood and severity at each risk are determined using risk assessment matrix of Australian Standard / New Zealand Standard 4360:2004 (AS/NZS 4360:2004) \(^{(6)}\). The results of multiplication between likelihood and severity level scores will result in total risk. Based on the total risk, the analysis is carried out using a risk assessment matrix to determine the level of risk. After knowing the level of risk, then determinant controls were given in accordance with the level of risk.
Table 1. Categories of Likelihood Level Based on AS/NZS 4360:2004

<table>
<thead>
<tr>
<th>Levels</th>
<th>Description</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rare</td>
<td>The likelihood of hazards is very small, almost never happens</td>
</tr>
<tr>
<td>2</td>
<td>Unlikely</td>
<td>It usually not happens, but the likelihood is unlikely, the frequency is annual</td>
</tr>
<tr>
<td>3</td>
<td>Possible</td>
<td>The likelihood of hazard is small hazard or a coincidence, the frequency is monthly</td>
</tr>
<tr>
<td>4</td>
<td>Likely</td>
<td>The likelihood of hazard in a particular circumstances, the frequency is almost 100%</td>
</tr>
<tr>
<td>5</td>
<td>Almost Certain</td>
<td>Very likely to happen, the frequency is certain</td>
</tr>
</tbody>
</table>

Table 2. Categories of Severity Levels Based on AS/NZS 4360:2004

<table>
<thead>
<tr>
<th>Levels</th>
<th>Description</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Insignificant</td>
<td>No injury</td>
</tr>
<tr>
<td>2</td>
<td>Minor</td>
<td>First aid kit, employees continue to work</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Injuries that need medical treatment, employees do not go to work</td>
</tr>
<tr>
<td>4</td>
<td>Major</td>
<td>Severe injuries (limb or partial disability), loss of production capacity</td>
</tr>
<tr>
<td>5</td>
<td>Catastrophic</td>
<td>Death</td>
</tr>
</tbody>
</table>

Table 3. Risk Assessment Matrix Based on AS/NZS 4360:2004

<table>
<thead>
<tr>
<th>Likelihood</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insignificant 1</td>
<td>Minor</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Almost Certain</td>
<td>Moderate</td>
</tr>
<tr>
<td>Likely</td>
<td>4</td>
</tr>
<tr>
<td>Possible</td>
<td>3</td>
</tr>
<tr>
<td>Unlikely</td>
<td>2</td>
</tr>
<tr>
<td>Rare</td>
<td>1</td>
</tr>
</tbody>
</table>

Findings

Hazard Identification

Hazard identification is a systematic process carried out to identify all circumstances and events that have potential to cause occupational accidents and occupational diseases that may arise in the workplace\(^1\). Hazard identification method in this study is a proactive method using Job Safety Analysis (JSA). There are 5 work activities identified at the Research Center, including research in immunology and histology laboratory, research in microbiology laboratory, research in molecular biology laboratory, research in stem cell laboratory, and cleaning of research medium used.

When carrying out work activities in each
laboratory at the Research Center, there are various potential hazards that can threaten workers health and safety. Based on the results of the hazard identification, it is known that from 5 work activities carried out at the Research Center of the Faculty of Dentistry, Universitas Airlangga there are 20 potential hazards with 20 risks that can occur. Potential hazards identified at the Research Center include physical hazards, chemical hazards, biological hazards, electrical hazards, and ergonomic hazards. Physical hazards identified at the Research Center are illumination, syringe, and fire. Chemical hazards identified at the Research Center are formalin, acids, dental paint, CO\(_2\) gas, and LPG. Biological hazard identified at the Research Center is bacteria. Electrical hazard identified at the Research Center is electricity. Ergonomic hazard identified at the Research Center is long sitting positions while conducting research.

In addition, the risks that identified at the Research Center of the Faculty of Dentistry, Universitas Airlangga are eye fatigue caused by inadequate illumination; finger puncture wound caused by using syringe; fire caused by using fire; suffering from cancer and gas poisoning caused by formalin exposure; skin and eye irritation caused by exposure to acids; stains marks that are hard to remove on the hands caused by dental paint exposure; explosion, poisoning, and death caused by CO\(_2\) gas; explosion, fire, and death caused by LPG; disease from residual organisms in research activity caused by bacteria; mild electric shock in workers caused by electricity; Musculoskeletal Disorders (MSDs) caused by long sitting positions while researching.

Risk Assessment

After all potential hazards and risks were identified, a risk assessment was then carried out. Risk assessment method used in this study is a qualitative method. Risk assessment includes 2 stages, namely risk analysis and risk evaluation. Risk analysis is carried out to determine the total risk that is the result of multiplication between the level of likelihood and the severity at each risk. Meanwhile, a risk evaluation is carried out to assess whether the risk is acceptable or not, compared to the applicable standards or the ability of the organization to face a risk\(^{(1)}\). Risk assessment is done by finding the value of relative risk using risk assessment matrix of AS/NZS 4360:2004\(^{(6)}\).

According to AS/NZS 4360:2004, the risks included in the low risk category is risks with a relative risk score of 1-4, the moderate risk category is risks with a relative risk score of 5-9, the high risk category is risks with a relative risk score of 10-19, and the extreme risk category is risks with a relative risk value of 20-25\(^{(6)}\). Based on the results of the risk assessment, in the risk analysis it is known from 5 work activities carried out at the Research Center of the Faculty of Dentistry of Universitas Airlangga, there are 20 risks consisting of 1 extreme risk, 1 high risk, 11 moderate risks, and 7 low risks.

In the risk evaluation, it is determined whether the risk is acceptable or not which determines the prioritisation of risk. Based on ALARP concept (As Low as Reasonably Practicable), the risks included in the category of high risk and extreme risk are unacceptable risks. Because these risks cannot be accepted or tolerated, risk controls must be taken so that the risk level can decrease to an acceptable level\(^{(1)}\). In work activities carried out at the Research Center, the risk that included in the extreme risk is explosions, fires, and deaths caused by LPG, and the risk that included in the high risk category is explosion, poisoning, and death caused by CO\(_2\) gas.

On the results of the risk assessment of LPG hazard potential, the likelihood level is 4 and the severity level is 5, so the total risk is 20. Based on the results of observations and interviews, it is known that the likelihood of LPG exposure when conducting cleaning activities is likely. The likelihood of hazard in a particular circumstances and the frequency of the occurrence is almost 100%. The severity it causes included to the catastrophic category, which can result in human death. Therefore, the risk of explosion, fire, and death due to LPG included to the extreme risk category.

On the results of the risk assessment of CO\(_2\) gas hazard potential, the likelihood level score is 2 and the severity level is 5, so the total risk is 10. Based on the results of observations and interviews, it is known that the likelihood of CO\(_2\) gas exposure when conducting research is unlikely. The likelihood usually not happens, is unlikely and the frequency of the occurrence is annual. For the severity it causes included to the catastrophic category, which can result in human death. Therefore, the risk of explosion, poisoning, and death due to CO\(_2\) gas included to the high risk category.

Based on the results of the risk assessment, it is known that all hazards at the Research Center must be given determinant controls to minimize the occurrence
of work accidents, especially for extreme risks and high risks. Determinant control is also needed to reduce the impact on worker health and safety.

Determinant Control

After the assessment of all existing risks, the next step is to determinant control. Determinant control aims to minimize or reduce the level of risk to the lowest or tolerable level\(^{1}\). Based on the results of the risk assessment, then control is determined according to the level of risk that has been analyzed. The determined control is adjusted to the risk control that has been implemented at the Research Center of the Faculty of Dentistry, Universitas Airlangga.

Based on the results of determinant control, there are 15 controls that have been implemented including substitution control, engineering control, administration control, and wearing personal protective equipment. The controls that have been implemented at the Research Center for potential hazards of bacteria are assistance by laboratory workers when conducting research, use of disposable gloves, hand washing with alcohol before and after conducting research, collecting in jerry cans, and washing autoclave 2 times with detergent. For potential hazards of illumination is use of bright colors on the ceiling and wall paints in laboratory, and adjusting use of lighting.

For potential hazards of formaldehyde, risk controls have also been implemented are storing formalin in tight specific places and controlled use of formalin when conducting research. For potential hazards of a long sitting position while conducting research is use of movable seats that can be used for stretching the body. Then, for potential fire hazards are the provision of small fire extinguishers in laboratory and use of fire as sterilization method only carried out by laboratory workers. For potential hazards of dental paint, risk controls have been implemented is washing the stains of dental paint on the hands slowly for several days. For \(\text{CO}_2\) gas hazard potential are the provision of backup electricity in the form of generators and UVS in the laboratory, and assistance by laboratory workers when conducting research.

Most of controls have been implemented to every activity carried out at the Research Center of the Faculty of Dentistry, Universitas Airlangga. However, there are still 5 controls that have not been implemented, including potential hazards of syringes, potential electrical hazards, potential hazards of acids, and potential hazards of LPG.

Conclusion

Based on the results of this study, it can be concluded that the Research Center of the Faculty of Dentistry, Universitas Airlangga has a moderate risk. The research results showed that of the 5 work activities, there were 20 potential hazards with 20 risks identified. Based on the risk assessment, there are 1 extreme risk, 1 high risk, 11 moderate risks, and 7 low risks. The extreme risks identified are explosion, fire, and death caused by LPG. The high risks identified are explosion, poisoning, and death caused by \(\text{CO}_2\) gas. Based on the determinant control, there are 15 controls implemented including substitution control, engineering control, administration control, and wearing personal protective equipment. However, there are still 5 controls that have not been implemented to some activities carried out at the Research Center of the Faculty of Dentistry, Universitas Airlangga.

Further recommendations are needed for 5 controls that have not been implemented in every activity conducted in the Research Center. For research activities with potential hazards of syringes, it is recommended to provide temporary waste shelters around the Research Center area. For potential electrical hazards, it is recommended to provide without a small electric current voltage and the installation of protective equipment for laboratory equipment on the outside that is made of metal. For potential hazards of acids, it is recommended in the form of making Material Safety Data Sheets. For potential hazards of LPG, it is recommended to store and put LPG cylinders in a well-ventilated place.

Conflict of Interest: None.

Source of Funding: This study was sponsored by Universitas Airlangga.

Ethical Clearance: Ethical clearance was obtained from the Ethics Committee of the Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia.

References


Evaluation to Identify Benzene Safe Concentration in Oil and Gas processing Facility in East Java Area Due to Process Fugitive Emission

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Abstract

Benzene is an organic chemical compound with the chemical formula C6H6. The benzene molecule is composed of six carbon atoms joined in a ring with one hydrogen atom attached to each. Acute benzene exposure can cause central nervous system depression. Longterm exposure can result in depression of the blood-forming system and may increase risks associated with anemia and leukemia. The purpose of this research is to identify benzene safe concentration in crude oil processing plant in east java area due to process fugitive emission. Sample is taken from 20 workers include work duration and weight of the worker also crude oil plant air quality monitoring is measured using direct-measure benzene detectors.

In the benzene measurement on the crude oil plant in east java area, 2 spot sample is taken with resulting data 0.96 mg/m3 or 0.30 ppm and 0.86 mg/m3 or 0.27 ppm, and according to final manual calculation for safe benzene concentration with the result 1.12 mg/m3 or 0.35 ppm, all of those number are still below safe concentration limit by refer to minister of man power No.13 / MEN / X / 2011 regulation and The Occupational Exposure Limits (OELs) which have been adopted globally as a company standard which is 0.5 ppm as an eight hour time-weighted average (TWA8) and 1.0 ppm as a Short Term Exposure Limit (STEL) (averaged over fifteen minutes). But according to Minimum Risk Level (MRL) ATSDR 2007, those value already above threshold which is 0,009 ppm daily exposure for acute effect and 0,003 ppm daily exposure for chronic effect, Recommendation to control and reduce fugitive emission which resulting in number of benzene is by reviewing engineering design for equipment causing fugitive emission, and since this company regulation for respirator usage in benzene case are 0.5–5.0 ppm must use Half-face mask with organic vapor cartridge, 5.0–25.0 ppm use Full-face mask with organic vapor cartridge, and Greater than 25 ppm use Self-Contained Breathing Air (SCBA) then need to re asses all area which has an obligation to wear personal protective equipment (Half-full / full mask with organic vapor cartridge or Self contain breathing apparatus) by not only based on benzene level but also considering the exposure duration.

Keywords: Benzene, safe concentration, Crude Oil Plant, Fugitive emission, engineering design.

Introduction

Oil and gas processing facility is place to separate raw oil from water, sediment, and unwanted gas. According Agency for Toxic Substances and Disease Register (ATSDR), dangerous chemical substances in the crude oil proprietary is benzene, toluene, xylene, ethylene, TPH (Total Petroleum Hydrocarbon), and Polycyclic Aromatic Hydrocarbon (PAHs). From six of them, benzene has the highest carcinogen effect to which can cause high severity harmful effect to the body.

Airborne benzene is the most common route of exposure. Even at low doses of airborne benzene (<1 parts per million (ppm)), As a result of inhalation
exposure, lungs are the major site of benzene metabolism (Arnold et al., 2013)\(^3\). Worker in here continually exposed by benzene from process fugitive emission which come from process fugitive emission on the oil and gas processing facility\(^4\).

The results of the measurement of benzene at two points in the Oil and Gas processing plant Industry in East Java are 0.96 mg/m\(^3\) or 0.30 ppm and 0.86 mg/m\(^3\) or 0.27 ppm. These results are still below safe concentration limit according to minister of man power No.13 / MEN / X / 2011 and The Occupational Exposure Limits (OELs) which have been adopted globally as a company standard which is 0.5 ppm as an eight hour time-weighted average (TWA8) and 1.0 ppm as a Short Term Exposure Limit (STEL) (averaged over fifteen minutes)\(^5\), but workers who are exposed to benzene will still have a carcinogenic effect. A similar study at the Pancoranmas Depok gas station by Abdul rohim (2012), even though benzene concentrations at the site had yields below the safe concentration limit, but workers at the exposed area had a risk of carcinogenic effects at the duration of the lifetime exposure\(^6\).

The authors will measure the limits of the safe concentration of benzene in different areas, namely in the Oil and Gas Industry processing plant in east java region.

**Material and Method**

This type of research is an observational, cross sectional and descriptive study. The population in this study are all production operators in oil and gas company workers in the East Java region. The sampling technique is the total population, so the sample is 20 workers.

The research variables is benzene concentration in the workplace, worker body weight, worker height, respiration rate of ayworkers, length of day working, body surface area, weight of white mice, body surface of white mice, highest dose of toxin without effect on experimental animals NOAEL, Km factor in animals (Animal Km), factor Km in workers (Human Km), safe dose limit for toxins for workers (SHD), and safe benzene concentration in the air (safe C).

Data analysis in this study was carried out by using quantitative data analysis manually to determine the safe concentration of benzene for oil and gas company workers in the East Java region.

**Findings**

A. **Characteristics of Try Animals and the Surface Area of Try Animals (White Mice).**

According to Saridewi and Tualeka, the implementation of a toxicity test using experimental animals which is white rats\(^6\). In general, the human response to toxicity is qualitatively similar to the response of animals, so this fact forms the basis of extrapolation from animal to human data.

In table 1, the characteristics of experimental animals in the form of white rat body weight are displayed on below table.

**Table 1. Distribution of Characteristics of Animals**

<table>
<thead>
<tr>
<th>Animal research (White Mice)</th>
<th>W (kg)</th>
<th>BSA (m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0,1405</td>
<td>0,024165</td>
</tr>
<tr>
<td>2</td>
<td>0,1405</td>
<td>0,024165</td>
</tr>
<tr>
<td>3</td>
<td>0,1410</td>
<td>0,024223</td>
</tr>
<tr>
<td>4</td>
<td>0,1410</td>
<td>0,024223</td>
</tr>
<tr>
<td>5</td>
<td>0,1395</td>
<td>0,024050</td>
</tr>
<tr>
<td>6</td>
<td>0,1415</td>
<td>0,024165</td>
</tr>
</tbody>
</table>

Based on the data of white rat body weight, the body surface of the white mouse can be calculated using the following formula\(^7\).

\[
\text{BSA Animal} = 0,09 \cdot W^{0,67}
\]

Explanation:

- BSA : Body Surface Area (m\(^2\))
- W : Weight (kg)

B. **Worker Characteristics, Worker’s Body Surface Area and Worker’s Respiratory Rate.**
Table 2. Workers characteristics distribution, Worker's Respiratory Rate and Length of Working Time in oil processing companies in East Java region.

<table>
<thead>
<tr>
<th>Workers</th>
<th>W (kg)</th>
<th>H (cm)</th>
<th>BSA (m²)</th>
<th>BR (m³/h)</th>
<th>t (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>159</td>
<td>1.69</td>
<td>0.63</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>159</td>
<td>1.63</td>
<td>0.62</td>
<td>12</td>
</tr>
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<td>3</td>
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<tr>
<td>4</td>
<td>97</td>
<td>159</td>
<td>2.07</td>
<td>0.72</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>159</td>
<td>1.99</td>
<td>0.71</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>159</td>
<td>1.80</td>
<td>0.66</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>159</td>
<td>1.72</td>
<td>0.64</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>159</td>
<td>1.63</td>
<td>0.62</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>70</td>
<td>159</td>
<td>1.76</td>
<td>0.65</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>79</td>
<td>159</td>
<td>1.87</td>
<td>0.68</td>
<td>12</td>
</tr>
<tr>
<td>11</td>
<td>77</td>
<td>159</td>
<td>1.84</td>
<td>0.67</td>
<td>12</td>
</tr>
<tr>
<td>12</td>
<td>69</td>
<td>159</td>
<td>1.75</td>
<td>0.65</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>65</td>
<td>159</td>
<td>1.69</td>
<td>0.63</td>
<td>12</td>
</tr>
<tr>
<td>14</td>
<td>66</td>
<td>159</td>
<td>1.71</td>
<td>0.64</td>
<td>12</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
<td>159</td>
<td>1.76</td>
<td>0.65</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>90</td>
<td>159</td>
<td>1.99</td>
<td>0.71</td>
<td>12</td>
</tr>
<tr>
<td>17</td>
<td>85</td>
<td>159</td>
<td>1.94</td>
<td>0.69</td>
<td>12</td>
</tr>
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<td>18</td>
<td>76</td>
<td>159</td>
<td>1.83</td>
<td>0.67</td>
<td>12</td>
</tr>
<tr>
<td>19</td>
<td>66</td>
<td>159</td>
<td>1.71</td>
<td>0.64</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>93</td>
<td>159</td>
<td>2.03</td>
<td>0.71</td>
<td>12</td>
</tr>
<tr>
<td>Average</td>
<td>74.05</td>
<td>159</td>
<td>1.80</td>
<td>0.66</td>
<td>12</td>
</tr>
</tbody>
</table>

Based on data on worker weight and height of workers, the body surface area and the rate of respiration of workers can be calculated using the following formula.

1. Worker Body Surface Area

\[ BSA = \sqrt{W \cdot h/3600} \]

Explanation:

BSA : Body surface area (m²)

W : Weight (kg)

h : Height (cm)

2. Workers Breathing Rate.

\[ = 5.3 \ln - 6.9/24 \]

Explanation:

Table 2 shows that the average body surface area of workers is 1.80 m² and the average respiration rate of workers is 0.66 m³ / hour.

B. Benzene Concentration.

The measurement results of the concentration of benzene at two points in the Oil and Gas processing plant Industry in East Java are 1.44 mg / m³ or 0.45 ppm and 1.18 mg / m³.

Table 3. Distribution of Benzene Concentration in oil and gas processing plant in East Java region.

<table>
<thead>
<tr>
<th>Measuring Location</th>
<th>Benzene Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point 1</td>
<td>0.45</td>
</tr>
<tr>
<td>Point 2</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Those benzene concentration is still below the threshold limit value of 0.5 ppm in accordance with the provisions of the Minister of Manpower Regulation No.13 / MEN / X / 2011 concerning Occupational Safety and Health at the Work Environment. However, the concentration of benzene is above the Minimum Risk Level (MRL), the level of benzene inhalation exposure determined by ATSDR which is for acute exposure (≤14 days) = 0.009 ppm, moderate exposure (15-364 days) = 0.006 ppm, and chronic exposure (≥365 days) 8

C. Animal Km dan Human Km

The first step to determine safe dosage of toxin for workers is by calculating Animal Km and Human Km.

1. Animal Km

\[ \text{Animal Km} = \frac{W_{\text{animal}}}{BSA_{\text{animal}}} \]

Keterangan :

Animal Km : Animal Km Factor.

W : Weight of research animal (White Mice).

BSA : Body Surface Area (White Mice).

The results of the Animal Km calculation are shown in table 4. The average Animal Km in the experimental animal is white rats which are 5.81.
Table 4. Result of Animal Km for Research animal (white mice).

<table>
<thead>
<tr>
<th>Animal research (White Mice)</th>
<th>Animal Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5,81420952</td>
</tr>
<tr>
<td>2</td>
<td>5,81420952</td>
</tr>
<tr>
<td>3</td>
<td>5,82102947</td>
</tr>
<tr>
<td>4</td>
<td>5,82102947</td>
</tr>
<tr>
<td>5</td>
<td>5,80052067</td>
</tr>
<tr>
<td>6</td>
<td>5,81420952</td>
</tr>
<tr>
<td>Average</td>
<td>5,81</td>
</tr>
</tbody>
</table>

Source: 6

The results of the Human Km calculation are shown in Table 5. Based on Table 5, the average Human Km

\[
\text{Human Km} = \frac{W \text{ human}}{BSA \text{ human}} \times \frac{1}{24,45} \times 0.66 \times 12 \times 0.1405 \times 0.022 \text{ mg/kg} \times 0.94 \text{ ppm} \times 0.1405 \times 0.022 \text{ mg/kg} \times 0.94 \text{ ppm} \times 0.1405
\]


in the oil and gas company environment in East Java is 36.70.

Table 5. Calculation result of Human Km in oil and gas operators in east java region.

<table>
<thead>
<tr>
<th>Workers</th>
<th>Human KM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38,36271775</td>
</tr>
<tr>
<td>2</td>
<td>36,85770701</td>
</tr>
<tr>
<td>3</td>
<td>37,76791091</td>
</tr>
<tr>
<td>4</td>
<td>46,86391378</td>
</tr>
<tr>
<td>5</td>
<td>45,14128763</td>
</tr>
<tr>
<td>6</td>
<td>40,65501431</td>
</tr>
<tr>
<td>7</td>
<td>38,94844197</td>
</tr>
<tr>
<td>8</td>
<td>36,85770701</td>
</tr>
<tr>
<td>9</td>
<td>39,81087364</td>
</tr>
<tr>
<td>10</td>
<td>42,29278006</td>
</tr>
<tr>
<td>11</td>
<td>41,75399653</td>
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<tr>
<td>12</td>
<td>39,52548736</td>
</tr>
<tr>
<td>13</td>
<td>38,36271775</td>
</tr>
<tr>
<td>14</td>
<td>38,65668924</td>
</tr>
<tr>
<td>15</td>
<td>39,81087364</td>
</tr>
<tr>
<td>16</td>
<td>45,14128763</td>
</tr>
<tr>
<td>17</td>
<td>43,86944611</td>
</tr>
<tr>
<td>18</td>
<td>41,48198063</td>
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<tr>
<td>19</td>
<td>38,65668924</td>
</tr>
<tr>
<td>20</td>
<td>45,88747517</td>
</tr>
<tr>
<td>Average</td>
<td>40,84</td>
</tr>
</tbody>
</table>

D. NOAEL

One of the objectives of toxicology research is to be able to evaluate the safety limit of a substances in order to determine the safe concentration limit of a chemical begins with a toxicity test to determine the highest dose without causing effects on research animal or No Observed Adverse Effect Level (NOAEL).

There was a research from Swaen et al. (2010) revealed that benzene NOAEL is 3.0 mg/m³ (0.94 ppm) or equivalent with 0.022 mg/kg which come from below calculation.9

\[
\text{NOAEL benzene} = \frac{3 \times 0.00013 \times 12}{0.1405} = 0.022 \text{ mg/kg}
\]

F. Safe Human Dose

\[
\text{SHD} = \frac{\text{NOAEL}}{\text{Human Km}} \times \frac{W \text{ human}}{BSA \text{ human}} \times 0.1405
\]

According to above formula then from the SHD calculation which come from NOAEL value, average from animal KM and human KM are:

\[
\text{SHD} = \frac{0.022 \text{ mg/kg}}{0.1405} = 0.0398 \text{ ppm}
\]

G. Benzene safe concentration limit.

Below formula (William, 1985)9 is used to determine benzene safe concentration limit in process area of oil and gas processing plant in east java region.

\[
C \text{ aman} = \frac{(\text{SHD})(W)}{(\delta)(BR)(t)} \text{ mg/m}^3
\]

And to convert unit of mg/m³ to ppm can use below formula.

\[
C \text{ aman} = \frac{\# \text{ mg/m}^3}{(MW)} \times 24.5 \text{ ppm}
\]

Explanation:

C Safe: safe toxin concentration in the air for worker (mg/m³). SHD: Safe Human Dose (mg/kg). W: Weight (kg). \(\delta\): % absorbed substances in lungs. BR: Human breathing rate (m³/jam). t: Working time duration (jam). MW: Molecular Weight.

The results of calculating the benzene safe concentration in the oil and gas process area in east java region are obtained from the SHD value, the average body weight, the percentage of absorbed substances, the average workers breathing rate and the average working time duration are:

\[
C \text{ aman} = \frac{(0.0398)(74.05)}{0.1405} = 0.729 \text{ mg/m}^3
\]

\[
C \text{ aman} = \frac{0.729 \times 24.45}{78.11} = 0.35 \text{ ppm}
\]
Discussion

The risks of benzene are caused by the increasing accumulation of benzene exposure in the body through inhalation. It is also in line with the research of Maryiantari et al (2016), enter the duration exposure workers with a high level of exposure has adverse effects against the risk of developing health problems\(^\text{11}\).

In this study involving 20 outside operators who work on the detected benzene area. Under normal condition, outside operators will stay on the benzene exposed area for doing routine job, but there will be potential higher benzene exposure where the activity in abnormal state (equipment failure, equipment under preparation for maintenance, pigging pipeline, or other breaking containment job).

OHSAS 18001 (2007) provides specific control guidelines for Occupational Health and Safety Hazards through elimination, substitution, technical, administrative and personal protective equipment approaches. The approach of elimination and substitution at oil and gas processing plant in east java will require big project means will demand high cost. For the technical approach can be done by making sure the preventive maintenance to minimize equipment failure, and continue re assessing area which producing harm benzene value and providing personal benzene detector to increase awareness when time to use benzene respirator protection as a personal protective equipment approach.\(^\text{12}\)

Conclusion

In the benzene measurement on the crude oil plant in east java area, 2 spot sample is taken with resulting data 0.96 mg/m\(^3\) or 0.30 ppm and 0.86 mg/m\(^3\) or 0.27 ppm, those number are still below safe concentration limit according to minister of man power number No.13 / MEN / X / 2011 and The Occupational Exposure Limits (OELs) which have been adopted globally as a company standard which is 0.5 ppm as an eight hour time-weighted average (TWA8) and 1.0 ppm as a Short Term Exposure Limit (STEL) (averaged over fifteen minutes), and also according to final manual calculation for safe benzene concentration with the result 1.12 mg/m\(^3\) or 0.35 ppm, but according to Minimum Risk Level (MRL) ATSDR 2007, all of those value already above threshold which is 0,009 ppm daily exposure for acute effect and 0,003 ppm daily exposure for chronic effect, then control action needed to protect workers from benzene harm effect to worker occupational health. Recommendation is reviewing engineering design for equipment causing fugitive emission (review type of gasket on flanges, change to double seal on the pumps or compressors, and re-routing hazardous gas vent), and since this company regulation for respirator usage in benzene case are 0.5-5.0 ppm must use Half-face mask with organic vapor cartridge, 5.0-25.0 ppm use Full-face mask with organic vapor cartridge, and Greater than 25 ppm use Self-Contained Breathing Air (SCBA) then need to re assess all area which has an obligation to wear personal protective equipment (Half-full / full mask with organic vapor cartridge or Self contain breathing apparatus) by not only based on benzene level but also considering the exposure duration\(^\text{4}\).

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

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Common Types and Methods of Drug Usage In Iran: A Systematic Review and Meta-Analysis

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Abstract

Introduction: Drug usage is a prevalent social and health problem in most countries. The highest prevalence of drug abuse has been reported among people aged 30-40 years old. The purpose of this study was to determination of the types of drugs and the methods of drug usage in Iran in a systematic and meta-analysis study.

Methods: This study was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Databases of SID, Iran Doc, ISI, Cochrane library, Science direct, PubMed/Medline, and Scopus were independently searched by two researchers using MeSH keywords. Articles published between 2004 and 2018 were recruited. Data was analyzed using random effects model using STATA-SE12 software. Heterogeneity among studies was investigated using, T² and I²statistical indices.

Results: Initial search retrieved 90 articles from which 7 articles with desirable quality were finally analyzed. Opium was the most common in used narcotic50 %(95% CI: 35-66%) followed by opium extract44%(95% CI: 5-83%), and heroin 19%(95% CI: 12-26%). Using more than one narcotic was observed in 9% (95% CI: 4-15%). The inhalation was the most common method of usage72 %(95% CI: 61-83%) followed by oral consumption 32%(95% CI: 9-54%) and injection 14%(95% CI: 05-24%).

Conclusion: Drug abuseis a common phenomenon in Iran. It is amenable to reduce the tendency to using drugs by providing appropriate training, informing the risks of drug abuse, and identifying and educating high-risk groups.

Keywords: Drug usage, Meta-analysis, Iran

Introduction

Iranian scientists, have been aware of the beneficial and detrimental impacts of opium and had used it as an antidote (¹, ²) Besides other major crises including nuclear threats, environmental pollution and poverty, drug addiction has turned into an acute problem of the today’s world. Addiction to drugs has also boosted in Iran in recent years. The primary official statistics have estimated that around 2 million drug addicts and abusers are present in the country (³, ⁴). In addition to the direct economic burden, serious health threats such as respiratory illnesses, AIDS, hepatitis, tuberculosis etc., as well as numerous socio-economic complications

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and problems such as increased drug-related crimes shrinkage the country’s large material capital (5-6).

This is while most professionals specify that familial support, as well as social support can be key contributors to increase the effectiveness of pharmaceutical treatment. In fact, social support can boost the therapeutic efficiency of pharmaceutical treatment through him parting information related to addicts’ health, encouraging healthy behaviors and preventing risky health attitudes, as well as providing access to psychological facilities to augment social capital and social support (7, 8).

Previous studies have reported the rates of drug usage recurrence as 50% to 80% in the first year after quitting (10, 9). Therefore, it is very important to identify the factors responsible for reusing drugs to prevent this phenomenon by implementing appropriate controlling policies and measures, (11-12). The chemicals in the drug have different effects (35). Therefore, the purpose of this study was to determine the types of drugs and the methods of drug usage in Iran.

Method

This was a systematic review and meta-analysis. The data of this review was acquired from studies conducted around the world. Articles published in SID, Iran doc, Science direct, PubMed/Medline, Scopus, ISI, and Cochrane library within 2004-2018 were recruited. MeSH keywords encompassing the type of drugs and the methods of drug usage in Iran were applied.

Selection of studies and data extraction: All the articles related to drug usage in Iran were collected. A checklist of abstracts of the found articles was initially prepared. Then all articles that mentioned the type of drug and the method of usage in their titles were recorded in the checklist. Studies mentioning merely suggestions or recommendations on drug usage were excluded.

Next, the required information from each study including author’s name, article title, year and location of study, sample size, number of women and men, the type of substance, and age groups were recorded into a secondary checklist.

Statistical analysis: Given that the types of narcotics and sample sizes had been extracted from each study, binomial distribution was used to calculate the variance of each study. Weighted mean was used to combine the frequencies reported indifferent studies. Each study was inversely weighted according to its variance. Due to the great heterogeneity in frequencies reported by different studies and statistically significant heterogeneity index (I²), the random effects model was used to conduct meta-analysis. The rate of heterogeneity in this study was 94.5% falling into the category of studies with high heterogeneity (i.e. I² indices < 25%, 25-75%, and >75% represent low, moderate, and high heterogeneities respectively) (13-14). Meta-regression was used to evaluate the trend of drug abuse in Iran in terms of the year of study and sample size. Meta-regression was also used to investigate the cause of heterogeneity between results. Subgroup analysis was performed to investigate the rate of drug abuse in Iran based on contributing factors and age groups. The data was analyzed using STATA software (version 12).

Results

The initial search yielded 90 articles related to the topic. Two independent researchers reviewed the titles and abstracts. If the title or abstract was relevant, the related full text was prepared for further reviewing. During screening, 25 duplicated and 25 irrelevant articles were excluded. Also, 33 studies were omitted because of providing insufficient information. Finally, 7 articles with adequate quality entered the systematic review process.

The rate of heterogeneity in this study was 94.5% representing a high heterogeneity (I² indices of < 25%, 25-75%, and >75% indicate low, moderate, and high heterogeneities respectively) (13-14). The total sample size was 6375 rendering the average sample size of 911 per study. Table 1 shows the characteristics of each included study.

The Forest plot of opium consumption frequency showing the rate of 50% (95% CI: 35-66%). The Forest plot of the frequency of opium extract consumption representing the prevalence of 44% (95% CI: 05-83). The Forest plot of the frequency of heroin usage representing the prevalence of 19% (95% CI: 12-26%). The Forest plot of the frequency of using more than one narcotic. The overall frequency was obtained as 9% (95% CI: 4-15%). The Forest plot of inhalation method of drug consumption representing the frequency of 72% (95% CI: 61-83%). The Forest plot of oral consumption of narcotics representing the prevalence of 32% (95% CI: 9-54%). The Forest plot of the rate of drug injection showing the overall prevalence of 14% (95% CI: 05-24).
Table 1: The characteristics of the article

<table>
<thead>
<tr>
<th>References</th>
<th>Author</th>
<th>Year</th>
<th>Place</th>
<th>Total(n)</th>
<th>Age</th>
</tr>
</thead>
<tbody>
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<td>Sadegheh</td>
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<tr>
<td>16</td>
<td>Feyzi</td>
<td>2015</td>
<td>Kermansh</td>
<td>768</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>Aghakhan</td>
<td>2017</td>
<td>Uromieh</td>
<td>400</td>
<td>-</td>
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<tr>
<td>18</td>
<td>Naghizad</td>
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<td>Isfahan</td>
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<td>20</td>
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<td>2018</td>
<td>Uromieh</td>
<td>812</td>
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<tr>
<td>21</td>
<td>Moshki</td>
<td>2015</td>
<td>Ghochan</td>
<td>753</td>
<td>36</td>
</tr>
</tbody>
</table>

The meta-regression analysis of drug usage based on sample size and the year of study publication indicated that the rate of drug consumption decreased in more recent studies and increased in studies with larger sample sizes (Figure 3, P<0.001). The mean age of drug users was 34.5 (95% CI: -25-44) years old.

Table 2. Frequency of types of drugs and their use

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Articles(N)</th>
<th>%</th>
<th>CI/95</th>
<th>I²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>opium</td>
<td>7</td>
<td>50</td>
<td>35-66</td>
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<td>0.000</td>
</tr>
<tr>
<td>opium extract</td>
<td>4</td>
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<td>05-83</td>
<td>99</td>
<td>0.000</td>
</tr>
<tr>
<td>heroin</td>
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<td>19</td>
<td>12-26</td>
<td>98.4</td>
<td>0.000</td>
</tr>
<tr>
<td>more than one narcotic</td>
<td>3</td>
<td>9</td>
<td>4-15</td>
<td>97.9</td>
<td>0.000</td>
</tr>
<tr>
<td>inhalation method of drug</td>
<td>3</td>
<td>72</td>
<td>61-83</td>
<td>43.5</td>
<td>0.170</td>
</tr>
<tr>
<td>oral consumption</td>
<td>3</td>
<td>32</td>
<td>9-54</td>
<td>96.1</td>
<td>0.001</td>
</tr>
<tr>
<td>drug injection</td>
<td>3</td>
<td>14</td>
<td>05-24</td>
<td>95.5</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure 1a. Meta-regression analysis of drug abuse based on the year of study conduction
Discussion

The review studies provide various evidence to the researchers\(^{(32-34)}\). Also considering the impact of drugs on mental health\(^{(3,4)}\) and the importance of paying attention to mental health\(^{(35,34)}\), For this reason, this review study was conducted. The tendency for using narcotics has increased especially in adults of 30-40 years old. The aim of this systematic review and meta-analysis was to determine the types of drugs and the methods of drug usage by Iranians. The highest drug consumption was observed in >35-year-old age group which was in line with the report of the Iranian Drug Control Headquarter that reported the highest prevalence of drug abuse in the 20-29-year-old age group\(^{(22)}\). However, in the study of Sadeghiyeh Aberiet al, the highest rate of drug abuse was observed in individuals around 40 years old\(^{(15)}\). As people usually raise a family in this age group\(^{(20-29)}\), providing occupation opportunities to provide their family’s expenses may help them to avoid drug abuse.

The present study showed that the most consumed substance was opium with 50% frequency (95% CI: 35-66) followed by opium extract and heroin with frequencies of 44% (95% CI: 5-83) and 19% (95% CI: 12-26) respectively. In addition, consuming more than one type of drug was observed in 9% (95% CI: 4-15). It seems that higher tendency to consume opium and opium extract roots in their faster and easier availability, as well as their cheaper prices than other narcotics. Over the past two decades, policies such as intense monitoring of Eastern borders to prevent drug trafficking, arresting drug dealers, establishing rehabilitation centers, and producing and distributing advertising videos and posters to illustrate the negative consequences of drug abuse have been followed in Iran. Nevertheless, drug abuse and its subsequent health problems continue to be serious challenges in the country\(^{(16, 23)}\). Measures such as training life skills through mass media, increasing public awareness of the adverse effects of drug abuse, emotional support for susceptible individuals, and educating on drug resistance of narcotics have been effective in reducing drug consumption\(^{(16, 24)}\). In addition to the aforementioned actions, focusing of families on educating their children and controlling their relationships, providing appropriate environment for routine activities and healthy entertainments, and informing community and families about the consequences of narcotics can be effective to prevent drug abuse\(^{(16, 23, 24)}\).
A study by Bagheri et al in Qazvin found that the most common used substances were opium and heroin (25). Also, Abbasi et al. reported that opium constituted the most common in used narcotic in Gorgan (26). In the study of Ahmadipour et al in Kermanshah, heroin was the most popular drug among addicts, while the lowest frequency was related to hashish (27).

Nevertheless, Di et al. stated that drug injection was on the rise (28) in people working in various occupations including health care professions (29). This is critical as if drug abused disseminates among health care personnel, their activities and functioning are disrupted endangering patients’ lives and increasing occupational violence (30-31).

**Conclusion**

Drug abuse is a common phenomenon in Iran. It is amenable to reduce the tendency to using drugs by providing appropriate training, informing the risks of drug abuse, and identifying and educating high-risk groups.

**Funding:** This study was approved by Ilam University of Medical Sciences. We thank the Deputy of Research and Technology of the University for Financial Support.

**Ethical Clearance:** Ilam University of Medical Sciences committee (A-10-2612-1)

**Conflict of Interest:** There is no conflict of interest between authors.

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Factors Related to the Incidence of Pesticide Toxicity in Pesticide Traders in Paser District in 2018

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Abstract

Pesticide sale circumstances in Paser District through measuring cholinesterase enzymes by the laboratory of the Paser District on the blood of pesticide traders found 37.5% experienced organophosphat group pesticide toxicity. This research aim to analyze risk factors related to the level of pesticide toxicity in pesticide traders namely age, nutritional status, educational level, length of work, work period and use of personal protective equipment. The study design used was observational with a cross sectional approach. The number of samples is 42 people taken by simple random sampling. Measurement data using observation and examination sheet cholinesterase levels in the blood of respondents using Tintometer tool brands in ScienPro Pest 100. The data were analyzed using Chi-square and multiple logistic regression. Pesticide traders who experienced pesticide toxicity were 42.9%. Result of Chi-Square test shows three variables that have a significant relationship with the incidence of pesticide toxicity. Risk factors for pesticide toxicity were age (p=0.021; OR=6.250; 95% CI=1.511-25.860), length of work (p=0.038; OR=6.769; 95% CI=1.268-36.139) and work period (p=0.043; OR=4.900; 95% CI=1.238-19.399). Multiple logistic regression showed that the most dominant factor associated with the incidence of pesticide toxicity namely the length of work and work period. Risk factors associated with the incidence of pesticide toxicity are age, length of work and work period where is the last two are the dominant factors.

Keywords: pesticide, toxicity, age, length of work, work period

Introduction

Pesticides are toxic and dangerous materials, if not managed properly it will have a negative impact on health. One of the places that manages pesticides is the pesticide management sites. Scope of supervision of pesticide management sites, namely pesticide factories, plantations, shops, or Village Unit Cooperatives. The place to pesticides selling that are not properly managed will cause pollution to the surrounding environment and can cause health problems for the surrounding community or its management whether acute or chronic.¹

Based on data from the Agriculture Office East Kalimantan Province in 2016, the number of pesticide sellers in East Kalimantan was 228, spread across 10 municipal Districts. Use of chemical pesticides for the delivery of plant pests and disease vectors tends to increase constantly. High use of pesticides increase to the health risks faced by both pesticide operators and the community at large. Health risks experienced by users of pesticides (industrial workers, formulators, applicators) usually related to the security method, storage, and use of pesticides. While the health risks experienced by the wider community are generally due to contamination of pesticides that enter the food chain, and poisoning of pesticides due to ingestion, inhalation and direct contact with the skin.²,³,⁴
Previous studies in Subang concluded that, 66.1% of workers working at pesticide sales sites experienced organophosphate group pesticide toxicity. Likewise, 78.8% of pesticide sales people in the city of Bengkulu experienced pesticide toxicity. The same was shown in the Pasamanan sub-District where nearly 50% of pesticide management workers experienced pesticide toxicity.5,6

Data from the Paser District Health Laboratory (2018), which states that 37.5% of pesticide traders were identified as organophosphate group pesticide poisoning with an average age of 39 years. The data also states that the people who are poisoned are male, with an average work period of 8 years and a long working average of 8 hours per day. In addition, 25% of traders have poor nutritional status, with 75% of traders having high school education, and 100% of pesticide traders not using Personal Protective Equipment (PPE) when managing pesticides.

This research was conducted to find out the relationship between pesticide toxicity in pesticide traders in Paser District, especially the relationship between age, nutritional status, education level, length of work, length of service, and use of PPE.

**Materials and Method**

This study was an analytic observational study with a cross sectional design. The population in this study are all traders or workers who work in pesticide shops as many as 97 people. Sampling technic by simple random sampling, the sample size is determined by the formula of Lemeshow is 42. Sources of data from the primary data were collected using observation and interview sheets and blood sampling of respondents. Data taken includes data on pesticide toxicity, age, nutritional status, education level, length of work, work period, and use of PPE, as well as reference books related to research. Analysis using univariate, bivariate analysis using Chi-square test and multivariate analysis using the Logistic Regression Test.

**Findings and Discussion**

**Table 1. Frequency Distribution of Variables**

<table>
<thead>
<tr>
<th>No.</th>
<th>Variable</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Poisoning</td>
<td>18</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>24</td>
<td>42.9</td>
</tr>
<tr>
<td>2</td>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;18 years or &gt; 45 years</td>
<td>14</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>18 - 45 years old</td>
<td>28</td>
<td>66.7</td>
</tr>
<tr>
<td>3</td>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>basic education</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Middle education</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>higher education</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Nutritional status</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>29</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>Length of working</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 5 hours / day</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 hours / day</td>
<td>29</td>
<td>69</td>
</tr>
<tr>
<td>6</td>
<td>Years of service</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 5 years</td>
<td>18</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 years</td>
<td>24</td>
<td>57.1</td>
</tr>
<tr>
<td>7</td>
<td>Use of PPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 4 PPE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&lt;4 PPE</td>
<td>42</td>
<td>100</td>
</tr>
</tbody>
</table>
Age

The results of Chi-square test with p-value 0.021 (p<0.05) with OR 6.25 which means that there is a significant relationship between the age of the trader and the incidence of pesticide toxicity. The farmers aged 40 years are more likely to suffer from pesticide poisoning. The age of pesticide traders over 35 years is more likely to experience pesticide toxicity compared to under 35 years of age. People who are older, they get more experience and exposure. Their metabolic function will decrease and result in a cholinesterase activity so that it will facilitate pesticide toxicity. Age is also related to immunity the body in overcoming the level of toxicity of a substance, the older the age of a person, the effectiveness of the immune system in the body will decrease.

Nutritional status

The results of Chi-square test obtained p-value 0.162 (p<0.05), which means that there is no significant relationship between the nutritional status of traders and the incidence of pesticide toxicity on traders. The number of pesticide traders who have good/normal nutrition is 58.1% compared to pesticide traders who have poor nutritional status. There are no relationship between nutritional status and the incidence of pesticide toxicity in the pesticide trader in Subang and West Lombok District, Wonosobo and Magelang District farmers.

Respondent with a poor category of nutritional status 69.23% is a pesticide trader who experiences overweight, this can result in toxic substances that can be stored in body fat tissue, so that the effects of poisoning cannot be detected. There is a positive relationship between the percentage of body fat and respondent’s cholinesterase levels, meaning that the higher the body fat percentage, the more the number/dose of pesticides needed for the occurrence of low cholinesterase levels.

Education Level

The results of statistical analysis using the Chi-square test with p-value 0.119 (p <0.05) which means that there is no significant relationship between the level of education with the incidence of pesticide toxicity in pesticide traders. There is no relationship between education level and pesticide toxicity on traders.

The formal education received by respondents does not necessarily indicate the actual level of knowledge about pesticides, a lot of knowledge about pesticides is actually obtained through education and training activities specifically for this matter, and especially for traders of pesticides that this can be done by the agency agriculture, health services and agents of pesticide brands.

Length of Work

The results of statistical analysis using the Chi-square test obtained p-value 0.119 (p<0.05) with OR 6.769 which means that there is a significant relationship between the length of work of traders and the incidence of pesticide toxicity at pesticide traders. There was a relationship between the length of work and the incidence of pesticide toxicity in farmers in the Jambi and Brebes Muaro.

Respondent who has more than five hours of work per day with a working period of more than 5 years as much as 40.5% where the respondent who works more than five hours with a larger work period is 8.85 years compared with respondents with working time of less than 5 hours which has an average working period of 6.76 years. This shows that the longer people work, the more likely they are to be exposed to dangerous substances. In addition, respondents who work more than 5 hours per day and also work long periods of time will cause continuous and repeated exposure to pesticides which causes a very high risk of pesticide toxicity. The main factors causing toxicity are the length of exposure, concentration/dose of exposure and thickness in individuals affected by age, nutritional status, sex and others.

Work Period

The results of statistical analysis using the Chi-square test obtained p-value 0.019 (p<0.05) with OR 4.900 which means that there is a significant relationship between the length of work of traders and the incidence of pesticide toxicity at pesticide traders. There was a relationship between the length of work and the incidence of pesticide toxicity in farmers in Tomohon and Brebes. There are significant differences in toxicity due to pesticides among farmers with employment <5 years and ≥ 5 years.

Work period is one of the factors that influence the level of cholinesterase in the blood of the respondent, where the longer the period of work, the lower the level of cholinesterase in the blood of the respondent so cause the risk of pesticide toxicity. The length of work as a pesticide trader influences the incidence of poisoning.
because exposure to pesticides in a short time can lead to toxicity in traders. Chronic organophosphate poisoning symptoms arise due to inhibition of cholinesterase and will mene tap for 2-6 weeks, resembling mild acute poisoning. But, when exposed again in a small number of severe symptoms may arise.

**Use of PPE**

Influence personal protective equipment (PPE) at the pesticides sale sites has no relationship. The results of this study indicate that the proportion of pesticide traders does not use complete personal protective equipment (PPE), so statistical analysis cannot be done. Although the results of the study showed no association between the use of PPE and the incidence of poisoning in traders but it is necessary to recommend the use of PPE when mixing/repacking pesticides because of the potential for the most contact to occur at that time.

**Dominant factors**

Based on the results of logistic regression analysis, the variables have p-value <0.05 is the length of work p-value 0.028, OR 9.587 and work period p-value 0.044, OR 6.005. The calculation of probability for the incidence of pesticide toxicity in pesticide traders can be predicted based on influential variables, P = 0.725 or 72.5%. So, pesticide trader with a service period of> 5 years; length of work per day> 5 hours have probability for pesticide poisoning of 72.5%.

The length of work and working period is a strong determinant of the incidence of pesticide toxicity in farmers.13 Both of these risk factors (length of work and working period) reinforce each other based on multivariate analysis. Results of research shows that the longer the life of the farmer, the lower the enzyme activity of blood kolinesterase explain that vegetable farmers who have been exposed for a long time or are persistent are at risk of getting poisoned at the next level. This is made worse by all pesticide traders in Paser regencies who make contact with pesticides, which are not used to wearing personal protective equipment (PPE), even if wearing does not meet the requirements. Length of work and work period with pesticides must be wary, because the longer the contact pesticide, the greater the exposure to pesticides in the sale.16

**Conclusion**

As many as 18 respondents (42.9%) experienced pesticide toxicity and 24 others (57.1%) did not experienced. There is a relationship between age, length of work and working period with the incidence of pesticide toxicity in pesticide traders in Paser Regency, while the results of the study show that all respondents did not use personal protective equipment in a complete manner so that they were more at risk of experiencing pesticide toxicity. The need to conduct counseling or training for managers and workers at the pesticide sale sites on how to properly manage pesticides and mitigate the negative effects of hazards caused by pesticides which include the management of appropriate pesticides, the use of personal protective equipment, the practice of handling pesticides, health labor aspects, how to repack pesticides, work environment sanitation as well as ways of dealing with poisoning. As well as for traders to conduct medical check-up every 6 months and provide PPE for workers and themselves.

**Ethical Clearance:** This study approved ethical clearance from the Committee of Public Health Research Ethics of Medical Faculty, Lambung Mangkurat University, Indonesia. We followed guideline from Committee of Public Health Research Ethics of Medical Faculty, Lambung Mangkurat University, Indonesia for ethical clearance and informed consent. The informed consent included the research title, purpose, participants’ right, confidentiality, and signature.

**Source Funding:** This study was done by self-funding from the authors.

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

**References**


Determination of Safe Benzene Concentration in Tank Car Crew at PT Pertamina Patria Niaga

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Abstract

Benzene is a compound that can cause carcinogenic effects in humans. The purpose of this study was to determine the level of health risk due to exposure to benzene through inhalation on tank car crews at Pertamina Patra Niaga. This type of research is a descriptive, observational and cross sectional study. The population in this study were all the Tank Car Crew (AMT) who worked in Pertamina Patra Niaga, amounting to 8 people. The sampling technique is the total population, so the sample is 8 workers. Data analysis used quantitative data to determine the safe concentration of (C) benzene in workers from animal body weight of white rats (W animals), body surface of experimental animals (BSA animals), body weight (W), height of workers (h), worker body surface area (BSA), worker respiratory rate (BR), working time (t), benzene (C) concentration, animal km. Human km, NOAEL and safe dose toxin limit (SHD).

The results showed measurements of benzene concentration at PT. Pertamina Patra Niaga is 0.26 mg / m³ (0.08 ppm), which means the concentration of benzene is still below the Threshold Value (NAB) according to Minister of Manpower Regulation No. 13 / MEN / X / 2011 at 0.5 ppm. Based on the calculations that have been done, the safe limit value is 0.02 ppm. This value if according to the Minimum Risk Level (MRL) of 2007 ATSDR exceeds that which is set at 0.009 ppm daily for acute effects and 0.003 ppm daily for chronic effects, so that control efforts are needed to be protected from the adverse effects of benzene on the health of workers. Control recommendations are to consume CYP2E1 enzyme contained in beef liver and salmon which serves to reduce benzene levels in the body, use Personal Protective Equipment (PPE) in the form of mask respirators and plant a number of ornamental plants that can absorb and reduce benzene concentrations such as Boston and Golden Photos¹

Keywords : Benzene, safe concentration, workers of PT Pertamina Petra Niaga

Introduction

Industrial progress and development requires the availability of adequate transportation so that it can meet economic needs and development. According to data from the Central Statistics Agency (BPS) since 2014-2016 there has been an increase in the number of vehicles in Indonesia by 5-6% per year.²

Increasing the number of vehicles will support the economy of the community, but on the other hand it can cause air pollution and energy needs which continue to increase every year.³

PT Pertamina Patra Niaga is one of the motorbike fuel terminals which is in charge of distributing fuel in Central Java. In fuel distribution activities, workers called the Tank Car Crew (AMT) must come in contact with benzene.

Benzene is a compound that can cause carcinogenic effects in humans. Benzene can enter the human body through 3 pathways, namely breathing, inhalation and skin. Prolonged exposure to benzene in the work environment such as fuel terminals can cause an increased risk of nervous system disorders, blood pressure, headaches and loss of consciousness.⁴
Based on observations made at Pertamina Patra Niaga, it was found that tank crews did not use personal protective equipment (PPE) such as masks and gloves to protect themselves from exposure to gasoline vapors containing benzene and many workers who smoke also added to the risk of benzene exposure.

Based on the background described, the researchers wanted to conduct a study that aimed to determine the level of health risk due to exposure to benzene through inhalation on tank car crews at Pertamina Patra Niaga.

**Research Method**

This study was a descriptive, observational and cross sectional study. The population in this study were all the Tank Car Crew (AMT) who worked in Pertamina Patra Niaga, amounting to 8 people.

The research design begins with the collection of secondary data related to work processes which include chemicals in the work area and the number of workers involved. Furthermore, primary data collection was carried out related to the concentration of benzene in the workplace air, length of work time, and worker body weight. In addition, primary data collection was also carried out on experimental animals, namely the weight of white mice. Data on the concentration of benzene in the air was obtained by direct measurement using Coconut shell charcoal and analyzed by Gas Chromatography (GC), referring to the NIOSH 1501 method.

The research variables were benzene concentration in the workplace, worker body weight, worker height, respiration rate of workers, length of day working, body surface area, weight of white mice, body surface of white mice, highest dose of toxin without effect on experimental animals (NOAEL), Km factor in animals (Animal Km), factor Km in workers (Human Km), safe dose limit for toxins for workers (SHD), and benzene concentration in safe air for workers (C is safe).

Data analysis in this study was carried out by using quantitative data analysis manually to determine the safe concentration of benzene for tank car crews at PT Pertamina Patra Niaga.

**Findings**

A. Characteristics of Try Animals and the Surface Area of Try Animals (White Mice)

In general, the toxicity of compounds is interpreted by the potential of chemicals that can cause damage when it enters the human body. In general, the response of the human body qualitatively to toxic substances is similar to the response of animals, so this becomes the basis of extrapolation from animal to human data.

Table 1 shows the characteristics of experimental animals in the form of white rat body weight.

<table>
<thead>
<tr>
<th>Try Animals (White and White)</th>
<th>W (kg)</th>
<th>BSA (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0,1405</td>
<td>0,024165</td>
</tr>
<tr>
<td>2</td>
<td>0,1405</td>
<td>0,024165</td>
</tr>
<tr>
<td>3</td>
<td>0,1410</td>
<td>0,024223</td>
</tr>
<tr>
<td>4</td>
<td>0,1410</td>
<td>0,024223</td>
</tr>
<tr>
<td>5</td>
<td>0,1395</td>
<td>0,024050</td>
</tr>
<tr>
<td>6</td>
<td>0,1415</td>
<td>0,024165</td>
</tr>
</tbody>
</table>

Based on the data of white rat body weight, the body surface of the white mouse can be calculated using the following formula:

\[ BSA_{\text{animal}} = 0,09 \ W^{0,67} \]

Description :

BSA: Body Surface Area / body surface area (m²)

W: Weight / weight (kg)

B. Worker Characteristics, Worker’s Body Surface Area and Worker’s Respiratory Rate

The surface area of the worker body

\[ BSA = \sqrt{W.h/3600} \]

Description : 

BSA: Body surface area (m2)

W: Weight / weight (kg)

h: Height / Height (cm)

C. Occupational respiratory rate

\[ BR = 5,3\ln W - 6,9 / 24 \]
Description:

BR: Breathing Rate (m³/hr)

W: Weight (kg)

**Table 2. Worker’s Body Surface Area, Worker’s Respiratory Rate and Characteristics of Workers**

<table>
<thead>
<tr>
<th>Workers</th>
<th>Wb (kg)</th>
<th>h (cm)</th>
<th>BSA (m²)</th>
<th>t (hour/day)</th>
<th>BR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>171</td>
<td>1.63</td>
<td>8</td>
<td>0.60</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>160</td>
<td>1.75</td>
<td>8</td>
<td>0.64</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>169</td>
<td>1.82</td>
<td>8</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>159</td>
<td>1.82</td>
<td>8</td>
<td>0.65</td>
</tr>
<tr>
<td>5</td>
<td>79</td>
<td>161</td>
<td>1.88</td>
<td>8</td>
<td>0.67</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>155</td>
<td>1.72</td>
<td>8</td>
<td>0.64</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>166</td>
<td>1.52</td>
<td>8</td>
<td>0.57</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>167</td>
<td>1.61</td>
<td>8</td>
<td>0.60</td>
</tr>
<tr>
<td>Total</td>
<td>525</td>
<td>1308</td>
<td>13.75</td>
<td>64</td>
<td>5.02</td>
</tr>
<tr>
<td>Average</td>
<td>65.62</td>
<td>163.5</td>
<td>1.72</td>
<td>8</td>
<td>0.63</td>
</tr>
</tbody>
</table>

The characteristics of the workers in this study included the body weight and working time of 6 workers in the Tank Car Crew area at PT Pertamina Patra Niaga. Based on Table 2, it is known that the highest body weight is 79 Kg, the lowest weight is 50 Kg, and the average weight is 65.62 Kg. The duration of work in a day is 8 hours. Whereas the highest height is 171 cm, the shortest is 155 cm with an average height of workers that is 163.5 cm. The results of the analysis of the calculation of body surface area and worker respiratory rate according to table 2 show that the average body surface area of workers is 1.72 m² and the average respiration rate of workers is 0.63 m³/hour.

**D. Benzene Concentration**

**Tabel 3. Benzene Concentration**

<table>
<thead>
<tr>
<th>Measurement Location</th>
<th>Results of Benzene Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/m³)</td>
</tr>
<tr>
<td>Tanks In PT Pertamina Patra Niaga</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Based on the results of air measurements of the workplace environment at PT Pertamina Patra Niaga where the results of these measurements showed a concentration of Benzene of 0.08 ppm or 0.26 mg/m³. Based on the Minister of Manpower and Transmigration Regulation No. Per.13 / MEN / X / 2011 concerning the threshold values of physical factors and chemical factors in the workplace for 0.5 ppm Benzene concentration so that benzene concentrations in the Tank Car Crew at PT Pertamina Patra Niaga Benzene concentration values below NAB.

**E. Animal Km dan Human Km**

Determination of safe dosage of toxin for workers begins with the calculation of Animal Km and Human Km.
Animal Km and Human Km.

1. Animal Km

\[ \text{Animal Km} = \frac{W_{\text{animal}}}{\text{BSA}_{\text{animal}}} \]

Description:
Animal Km = Km factor in animals
W = experimental animal body weight (white mouse)
BSA = Body surface area can be tried

<table>
<thead>
<tr>
<th>Workers</th>
<th>Animal Km = W/BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.81</td>
</tr>
<tr>
<td>2</td>
<td>5.81</td>
</tr>
<tr>
<td>3</td>
<td>5.82</td>
</tr>
<tr>
<td>4</td>
<td>5.82</td>
</tr>
<tr>
<td>5</td>
<td>5.80</td>
</tr>
<tr>
<td>6</td>
<td>5.86</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>34.93</strong></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>5.82</strong></td>
</tr>
</tbody>
</table>

The results of the Animal Km calculation are shown in table 4, with the average Animal Km in the experimental white rat being 5.82

2. Human Km

\[ \text{Human Km} = \frac{W_{\text{human}}}{\text{BSA}_{\text{human}}} \]

Description:
Human Km = Km factor on workers
W = worker weight
BSA = Worker's body surface area

<table>
<thead>
<tr>
<th>Workers</th>
<th>Human Km = W/BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.36</td>
</tr>
<tr>
<td>2</td>
<td>39.43</td>
</tr>
<tr>
<td>3</td>
<td>39.01</td>
</tr>
<tr>
<td>4</td>
<td>41.21</td>
</tr>
<tr>
<td>5</td>
<td>42.02</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>303.82</strong></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>37.98</strong></td>
</tr>
</tbody>
</table>

The results of the Human Km calculation are shown in table 5, with an average Human Km in labor force of 37.98.

F. NOAEL

One of the objectives of research activities in the field of toxicology is to be able to evaluate the safety of a substance. To determine the safe limit of the concentration of a chemical begins with a toxicity test determining the highest dose without causing effects on experimental animals or No Observed Adverse Effect Level (NOAEL).

NOAEL Benzena (mg/m³) = \( \frac{3 \times 0.00013 \times 8}{0.1405} \)
= 0.022 mg/kg

Swaen et al. (2010) research results state that benzene NOAEL is 3.0 mg / m³ or equivalent to 0.022 mg / kg obtained from the calculation of the formula above.

G. Safe Human Dose

Safe dosage of toxin for workers or Safe Human Dose (SHD) is found to be initiated using the following formula from Shaw et al. (2007):

\[ \text{SHD} = \frac{\text{NOAEL} \times \text{Animal Km}}{\text{Human Km}} \]
\[ = \frac{0.022 \times 5.82}{37.98} \]
\[ = 0.003 \text{ mg/kg} \]
H. Safe Limit for Benzene Concentration

Determination of safe limits of Benzene concentration in the working environment of the Tank Car Crew uses the following formula:

\[
C_{\text{safe}} = \frac{(SHD)(W)}{(\delta)(BR)(t)} \text{mg/m}^3
\]

Description:
- C safe: concentration of toxin in the air that is safe for workers (mg / m³)
- SHD: Safe Human Dose (mg / kg)
- W: Weight (kg)
- δ: % of substances absorbed by the lungs
- BR: Human respiratory rate (m³ / hour)
- t: Working time (hours)
- MW: Molecular Weight / Molecular Weight

Based on the above formula, the results of calculating the safe concentration of benzene at PT Pertamina Patra Niaga are obtained from the SHD value, average worker weight, percentage of substance absorption, average worker respiratory rate and average length of work time is:

\[
C_{\text{safe}} = \frac{(0.003 \times 65.62 \times 0.50 \times 0.6 \times 8)}{2.4} = 0.07 \text{mg/m}^3
\]

\[
C_{\text{safe (ppm)}} = \frac{0.07 \times 24.5}{78.11} = 0.02 \text{ppm}
\]

The results of the calculation of safe limits in the air for workers above can be used to predict the concentration of toxins in the air of a safe work environment for workers if there is no determination of the Threshold Limit Value (NAV), and to be compared with the NAV determined by various institutions either by Ministry of Manpower and Transmigration, National Standardization Agency, ACGIH, NIOSH and OSHA.

CONCLUSION

The results of the measurement of benzene concentration in PT Pertamina Patra Niaga were 0.26 mg / m³ (0.08 ppm). Based on Permenakertrans No. 13 / MEN / X / 2011 Threshold Value The benzene limit is 0.5 ppm. From the measurement results when compared to the NAB, it is still below the set threshold value.

While based on the 2007 ATSDR Minimum Risk Level (MRL), daily benzene concentration of 0.009 ppm can have an acute effect and 0.003 ppm every day for chronic effects, so that the concentration of benzene in the work environment of PT Pertamina Patra Niaga
has the potential to provide health effects for workers. Thus control efforts are needed so that workers are protected from the adverse effects of benzene on the health of workers.

Control recommendations are to consume CYP2E1 enzyme contained in beef liver and salmon which serves to reduce benzene levels in the body, use the appropriate Personal Protective Equipment in the form of half mask respirator with organic vapor cartridge to minimize benzene exposure, and plant a number of ornamental plants that can absorb and reduce the concentration of benzene such as Boston and Golden Phothos.

Conflicts of Interest: all authors have no conflict interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

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Polymorphic Relationship of rs7526700, rs2278651, and rs611386 Of SLC30A1 Mothers’ Gene with Mothers’ High Levels of Zinc

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Abstract

The most common facial malformation in all populations and ethnic groups is Cleft lip and palate (CB/L). Various factors cause nonsyndromic cleft lip and palate abnormalities (CB/L NS) such as interactions between environmental and genetic factors, so that CB/L embryopathy is not clear. Deficiency of Zn with certain severity in pregnant women can be a risk factor for CB/L. The purpose of this study was to analyze the relationship of polymorphism of rs7526700, rs2278651, and rs611386 of the gene SLC30A1 mother with a group of deficiency events of Zinc maternal. The type of this research is an observational study through cross sectional approach with the research subjects are mothers and newborns diagnosed with CB/L NS in the Perinatology Department of Dr. Hasan Sadikin Hospital Bandung and other Network Hospitals. The research was conducted in September 2016 to June 2017 with 34 groups samples. Data were analyzed using fisher’s exact test and correlation test. The results showed that the majority of mothers who became the research respondents aged 25-29 years were 34% and the majority of infants who became the respondents were female as much as 60%, and most types of lip and palate disorders experienced by infants who became the respondents was Unilateral CB/L which is as much as 66%. Furthermore, the results of statistical tests indicated that there was no significant relationship between polymorphism of rs7526700, rs2278651, and rs611386SLC30A1 maternal gene with levels of Zinc maternal.

Keywords: Polymorphism, rs7526700, rs2278651, rs611386, SLC30A1, Zinc, Mother

Introduction

Most common facial malformations in all populations and ethnic groups are the cleft lip and palate (CB/L) reported from all anomalies in the head and neck around 65%. CB/L prevalence is quite high in Asian populations and American-Indian, of which there is around 1 in every 500 births. In Indonesia, the number of CB/L incidence is not yet known certainly. According to Kembaren L (2012), people with cleft lip disorders in Indonesia have increased by an average of 7,500 people per year.1,2

Failed unification of central facial skeleton part in the fourth to the tenth week of pregnancy is the cause of cleft lip and palate since the embryo experiences development and growth of facial skeleton at the end of the fourth week and the end of the eighth week of pregnancy. The process of fusion of the palate ends at the end of the tenth week of pregnancy.3,4

Various factors cause nonsyndromic cleft lip and palate abnormalities (CB/L NS) such as interactions between environmental and genetic factors so that CB/L of the embryopathy is not clear.4 Both have complex

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genetic traits, environmental factors have a role in the pathogenesis of CB/L NS and genetic factors are still difficult to understand. Some genes become candidate genes related to CB/L NS events such as transforming growth factor beta 3 (TGFβ3) gene, orofacial cleft (OFC1) gene, the transforming growth factor alpha (TGFα) gene, the methylene tetrahydrofolate reductase (MTHFR) gene, drosophilamsh homebox homolog-1 (MSX1) gene, alpha retinoic acid receptor (RARα) gene, orofacial cleft 2 (OFC2) gene, and orofacial cleft 3 (OFC3) gene. 

Micronutrient Deficiencies are found in developing countries, especially in Indonesia, especially deficiency of Zinc (Zn) and folic acid. Women in modern times like today consume a lot of food but experience less nutrient. The cause of micronutrient deficiency is due to the lack of food intake and consumption of Zn and iron which usually present together in food sources. Oysters, beef, turkey, chicken, cereals, and processed nuts are examples of foods containing Zn and iron.

Zinc is a micronutrient belongs to the category of essential minerals, in which about 3 - 10% of genes encode proteins in humans having a domain of binding Zinc. Zinc ions cannot pass through the cell membranes, thus the Zinc transportation requires a Zinc Transporter. The biological functions of Zn in cells are divided into 3 categories, those are catalytic, structural and regulatory systems. The catalytic system of more than 300 human body enzymes requires Zn to function properly. In structural system, Zn plays an important role in protein synthesis, both at the cellular and molecular stages. In the molecular level, Zn acts as a catalyst for DNA replication enzymes, gene transcription, RNA synthesis and protein. In cellular level, Zn is important to maintain cell survival, affecting the signal transduction, transcription and replication. The regulatory system of Zn’s role is very broad, among others, as a counterweight to the levels of a number of hormones, bone metabolism, neuropsychiatry, immune system, taste and olfactory sensation systems. 

Zn deficiency with certain severity in pregnant women can be a risk factor for CB/ L. Research in mice shows the role of Zinc transporters (ZnT) in fetal development. Zn transporters are classified as family solute-linked carriers 30A (SLC30A / ZnTs) and SLC39A (ZiPs). The SLC30A1 gene is one of the genes in which ZnT is known to be actively expressed in developing mouse embryos and this expression depends on the mothers’ intake of Zinc. SLC30A1 and SLC30A5 proteins have an essential function in the process of transporting zinc from mother to fetus.

The purpose of this study was to analyze the relationship of polymorphism of rs7526700, rs2278651, and rs611386 of SLC30A1 maternal gene with the incidence of Zinc maternal deficiency.

Research Method

This research was an observational study with a cross sectional approach in which the research subjects are mothers and newborns diagnosed with CB/L NS in the Perinatology Department of Dr. Hasan Sadikin Hospital Bandung and the Network Hospital, among others: RSUD of Bandung City of Ujung Berung, Cibabat City Hospital of Cimahi, Special Hospital for Women and Children in Bandung City, and those who came to the Foundation for Lip and Palate Disease Foundation (YPPCBL) Bandung conducted in September 2016 to June 2017. The research subjects were selected based on inclusion criteria including new mothers who gave birth to babies diagnosed with CB/L NS, newborns diagnosed with CB/L NS, mothers and babies diagnosed with CB/L NS from the Deutero Melayu tribe, as well as mothers and babies diagnosed with CB/L NS who were in good health and good general condition, selected through consecutively Sampling. The sample size in this study was 34 people per group.

The variables in this study were level of zinc maternal and infant suffered from CB/L NS as the dependent variable and polymorphism of rs7526700, rs2278651, and rs611386 of the SLC30A1 gene for mothers as the independent variables. Clinical examination was carried out to determine the diagnosis of cleft lip and palate (CB/L NS) and maternal blood taking from CB/L NS infants and CB/L NS infants whose Zn plasma level was examined and continued by genetic analysis (DNA isolation, PCR and RFLP and Sanger Sequencing). Data were analyzed through statistical test which was fisher’s exact test. If the result did not meet the requirements but normally distributed, Pearson correlation test was performed. Meanwhile, if the data were not normally distributed, the Spearman correlation test rank was used.

Results

1. Characteristics of Research Subjects
Table 1 Characteristics of Respondents (n = 35)

<table>
<thead>
<tr>
<th>No</th>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mother</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>15 – 19</td>
<td>3 (8.57%)</td>
<td></td>
</tr>
<tr>
<td>20 – 24</td>
<td>4 (11.43%)</td>
<td></td>
</tr>
<tr>
<td>25 – 29</td>
<td>12 (34%)</td>
<td></td>
</tr>
<tr>
<td>30 – 34</td>
<td>10 (28%)</td>
<td></td>
</tr>
<tr>
<td>35 – 40</td>
<td>4 (11.43%)</td>
<td></td>
</tr>
<tr>
<td>≥40</td>
<td>2 (5.71%)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Infants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>14 (40%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21 (60%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 shows that the majority of mothers who became respondents and aged 25-29 years old were 34% and the majority of infants who became the respondents were female, which was 60%.

Figure 1 Distribution of Respondents by type of cleft lip and palate

Figure 1 shows that most types of lip and palate disruption experienced by infants who were respondents were Unilateral CB/L which is as much as 66% of the respondents.

2. PCR and Sequencing of rs7526700 polymorphisms

PCR products produced polymorphisms of rs7526700 for SLC30A1 gene by 352 bp. A single band of 352 bp indicated that the PCR technique has been optimized. The PCR results were then sequenced to analyze the polymorphism of rs7526700. Taq1 was primarily used in this study. From 70 samples, it was found in the mother and baby.

3. PCR - RFLPs / MboI Digestion polymorphism rs2278651 Digestion

Process along with enzymes MboI carried out for 4 hours at 37°C produces a PCR product of 566 base pairs, rs2278651 truncated produces a PCR product of 566 base pairs, rs2278651 truncated homozygou homozygot produces base pieces 345 base pairs and 277 base pairs, while those that do not produces base pieces 345 base pairs and 277 base pairs, while those that do not the result was 566 base pairs. RFLP results were then confirmed by using the sequencing method.

4. PCR - RFLPs / MboI Digestion polymorphism rs611386

The digestion process along with the enzyme MboI which was carried out for 4 hours at 37°C produced a PCR product of 679 base pairs. rs611386 truncated homozygou produces base pieces of 572 base pairs and 121 base pairs, while uncollected results are 679 base pairs. Only normal (variants wild type) and heterozygous were found in this fragment.

5. Relationship of polymorphism rs7526700, rs2278651, and rs611386 gene SCL30A1 mother with group events deficiency Zinc mother

Relationships polymorphism rs7526700, rs2278651, and rs611386 gene SCL30A1 mother with group events deficiency Zinc mother can be seen in Table 2.
Table 2: Relationship of polymorphisms of rs7526700, rs2278651, and rs611386 of SCL30A1 maternal gene with zinc maternal deficiency incidence

<table>
<thead>
<tr>
<th>Mother’s Gene SLC30A1</th>
<th>Zink maternal level</th>
<th>Total</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;7 μg/dl</td>
<td>&gt;7μg/dl</td>
<td></td>
</tr>
<tr>
<td>rs7526700</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphism (GC)</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Non polymorphism (GG)</td>
<td>24</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>rs2278651</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphism (CT, TT)</td>
<td>15</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>Non polymorphism (CC)</td>
<td>13</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>rs611386</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphism (GA)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non polymorphic (GG)</td>
<td>28</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>7</td>
<td>35</td>
</tr>
</tbody>
</table>

*Fisher’s Exact

Table 2 shows statistical analysis results of the rs7526700 gene polymorphism in mothers which is not significantly related to Zinc maternal level (p = 1.00 or p > 0.05), which means that there was no differences in levels found Zinc maternal between the polymorphism and non-polymorphism of rs7526700. In addition, gene polymorphisms of rs2278651 also did not have any significant relationship with Zinc maternal level (p = 0.69 or p > 0.05) or there were no differences in Zinc maternal level between the polymorphisms and non-polymorphisms of rs2278651.

Discussion

Based on the respondents’ characteristic with lower zinc level in infants compared to mothers in this study, the results of experimental studies and human epidemiological case reports indicate that severe Zn deficiency can cause fetal malformations including cleft lip and palate. In this study, the clinical manifestations of CB/L NS are mostly unilateral CB/L. This is consistent with previous research, in which the incidence of cleft lip and palate is the largest, amounting to 45%, ceiling slit was 30%, and lip slit was only 25%. However, the results of this study is different from bilateral CB/L which has more severe clinical manifestations compared to unilateral CB/L and appear less frequently. Cases of CB/L and CL are considered to be two different effects and each has a different genetic etiology.

The results showed that polymorphism can disturb the balance of transporting Zn from mother to baby. Polymorphism in the mother directly influences the levels of Zn in the mother’s body or in the baby’s body. This assumption raises the hypothesis that gene polymorphism of SLC30A1 in mothers can affect the levels of Zn in mothers and infants, but the results of research and statistical analysis showed that the three polymorphisms of SLC30A1 genes in mothers do not affect levels of Zn in mothers. This happens because polymorphism in the mother influences levels of Zn from the beginning of embryogenesis to the second trimester, thereby increasing the occurrence of CB/L during fusion between the maxillary process and the medial process. This will not be observed in this study because the measured level is the level of Zn in the serum of babies who have been born (~ 9 months).

The results of genetic studies in humans showed a significant relationship between the gene expression profile for Zn Transporter in chronic diseases (diabetes, asthma) and carcinogenesis. Since craniofacial malformations arises from tissue misregulation which is usually coordinated during early embryogenesis. Another interesting aspect is the aspect of Zn Transporter’s function with the possible involvement of this protein in
Genetic and environmental factors, including maternal nutritional status, are known to take part in the process of orofacial gaps including CB, CB/L and CL. Some previous studies have shown that nutritional deficiencies, such as folic acid, cholesterol, multivitamins and micronutrients such as Zinc in mothers, can increase the risk of developing orofacial gaps in infants. In contrast, fortification of food by adding folic acid to pregnant women has been shown in several studies to reduce the risk of developing orofacial gaps in infants.

**Conclusion**

Most of the mothers who were respondents were aged 25-29 years and most of the babies who were respondents were female. Most types of cleft lip and palate disorders experienced by infants who were respondents were Unilateral CB/L, and there was no correlation between polymorphism of rs7526700, rs2278651, and rs611386SLC30A1 mother gene with Zinc maternal levels.

**Conflict of Interest:** Nil

**Ethical Clearance:** This research has been proved by Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran Bandung Indonesia Number 837/UN6.C1.3.2/KEPK/PN/2016 approved in 26th August 2016

**Source of Funding:** Self

**Reference**


Conventional Detection of Resistance of *Aedes aegypti* Larvae as DHF Vector in Kediri District Against Temephos

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¹Health Polytechnic of Surabaya, Indonesia

**Abstract**

Dengue Haemorrhagic Fever (DHF) becomes health problem in the world. The most number of DHF sufferers in Kediri District in 2016 were in Pare Subdistrict, Ngasem Subdistrict, and Kunjang. However, controlling technique of DHF vector is such as fogging by using active substance of melathion for adult stadium of mosquito and larvasidation by using active substance of temephos for larvae stadium of mosquito. Moreover, resistance of vector against insecticide is global phenomenon, particularly for program management of infectious disease controlling vector and as a singular barrier in the success of vector control chemically. The vector resistance detection can be conducted by using Detection Conventionally through WHO standard method of Susceptibility test in impregnated paper. This research aimed at analyzing detection conventionally the resistance of *Aedes aegypti* as DHF vector in Kediri District against Malathion and Themepos. This research was True Experiment research and temephos was scattered to the larvae with concentration of 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, and 0.04 mg/l in contact time of 15, 30, 45, and 60 minutes. Data analysis included determination of resistance status by referring to category standard from WHO and analyzing the difference of biota test mortality by using statistical different test of Anova. The result of this research was *Aedes aegypti* larvae in Kediri District was resistant against temephos with concentration in 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, 0.04 mg/l and there was a significant influence of contact time against the death of *Aedes aegypti* larvae. Furthermore, suggestion for Health Office in Kediri District was the use of temephos as larvacide was needed in concentration of more than 0.04ml/l. Besides, it was also needed resistance test of *Aedes aegypti* larvae with concentration of temephos in more than 0.04 mg/l.

**Keywords:** resistance, *Aedes aegypti*, temephos

**Introduction**

DHF is infectious disease that is caused by dengue virus from *Falvivirus* genus. Until nowadays, DHF still becomes main health problem in the world. Geographical distribution of DHF case is found to be scattered in either tropical area or subtropical area. Based on¹, since 1968 until 2009, World Health Organisation (WHO) noted that Indonesia was the highest country with DHF case in Southeast Asia. DHF case in Surabaya in 1968 was 58 people who were infected and the 24 people died. Afterwards, DHF disease scattered to all areas in Indonesia and attacked all people, particularly children, as well as for DHF case in Kediri District.

In 2016, Kediri District had been occurred DHF case with 993 people (IR = 64.19/ 100,000 population) with total of mortality in 18 people (CFR = 1.8%). If it was compared with the case total in 2015, it had been occurred a tremendous increase in which total of DHF case was 702 people with total of mortality in 7 people. Meanwhile, the case total in 2014 was 221 people, but there was no mortality case. Case total in 2013 was 832 people with total of mortality in 11 people and in 2012 was 492 people with total of mortality in 7 people. Among 38 Districts/Cities in East Java, Kediri District was one of the districts that was categorized as Outbreak area of DHF because in 2015, it was occurred more rather than in 2014. The most number of distribution of DHF sufferers’s domicile in Kediri District in 2016 was in Pare Subdistrict with sufferer total in 107 sufferers. Then, it was in Ngasem Subdistrict with 90 sufferers.
and it was followed by Kunjang Subdistrict with 74 sufferers.\(^{(2)}\)

Efforts that had been done in preventing and controlling DHF in Kediri District in 2015 were such as: socializing how to prevent and control DHF; monitoring and training the group of control worker (Pokjanal) in eradication of Mosquito Nest (PSN-DBD) at Subdistrict level, countryside level, and school; training for the mosquito larva monitor for conducting periodic check; fogging focus if it was known that there was transmission of DHF case around sufferer’s house, PSN 3M plus, fishization and larvasidation.\(^{(2)}\)

Technique to control DHF vector was such as: fogging by using active substance of malathion for adult stadium of mosquito and larvasidation by using active substance of temephos for larva stadium of mosquito. Both of the active substances were organophosphate insecticide. Furthermore, this insecticide was used in Indonesia since 1970, but until nowadays, DHF case was still more reported. The condition made question whether it had been occurred the resistance of \textit{Aedes aegypti} as DHF vector against Malathion and Temephos or not, particularly in Kediri District which was an endemic area of DHF.

Temephos is one of larvasidations from organophosphate class that is more used by Health Office or non-governmental organization for controlling population of \textit{Aedes aegypti} larvae. The use of temephos was began since the government proclaimed abatitation in 1980 for determining DHF transmission chain by distributing abate powder to the society. This program was conducted continuously throughout the year without any larvicidal rotation by proposing to avoid the epidemic. Resistance of insect against an insecticide would be occurred if it was used intensively for 2 until 20 years and continuously throughout the year.\(^{(3)}\)

The resistance of temephos and malathion in 0.8\% had been occurred to \textit{Aedes aegypti} in West Jakarta, East Jakarta, and South Jakarta.\(^{(4)}\) \textit{Aedes aegypti} larvae was resistant against temephos in 0.02 mg/l at Plosokerep Village, Sumobito Subdistrict, Jombang District, East Java-Indonesia.\(^{(5)}\)

Concerning with the use of temephos had been used in Kediri City for more than 20 years as an effort of preventative and chain breake of DHF transmission when it was occurred the outbreak or infectious disease epidemic of dengue. Hence, it was needed to be conducted a determination of the susceptibility status of \textit{Aedes aegypti} larvae against temephos at three Subdistricts of endemic DHF.

This research aimed at detecting conventionally the resistance of \textit{Aedes aegypti} larvae as DHF vector in Kediri District against temephos.

**Methods**

This research was true experiment research and the sample was 3\textsuperscript{rd} descent (F3) of \textit{Aedes aegypti} larvae stadium that was bred in laboratory of Department of Environmental Health, Health Polytechnic of Surabaya, from parental that came from trapped egg in ovitrap in DHF endemic area in Pare, Ngasem, Kandat, and Kunjang, Kediri District, East Java Province, Indonesia. Temephos was scattered to larvae with concentration in 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, 0.04 mg/l with contact time in 15, 30, 45, and 60 minutes. Data analysis included determination of resistance status in Kediri District by referring to category standard from WHO and analyzing the difference of biota test mortality by using statistical different test of Anova.

**Findings**

Result of Conventional Resistance test which scattering 20 \textit{Aedes aegypti} larvae that came from Kediri District for 60 minutes by using temephos with either concentration in 0.01; 0.02; 0.03; or 0.04 mg/l, for control group, it used etanol 1 cc. Every 15 minutes, it was conducted an observation. Result that was obtained in either first 15 minutes, second 15 minutes, third 15 minutes, or fourth 15 minutes was there was no dead \textit{Aedes aegypti} larva, except for concentration in 0.03 mg/l, the percentage average of \textit{Aedes aegypti} larva death was in 0.83%. However, \textit{Aedes aegypti} larva was dead that was caused by residual temephos with concentration in 0.01; 0.02; 0.03; and 0.04 mg/l and also etanol 1 cc for 24 hours (1440 minutes) was in 1.25%; 0.42%; 1.25%; 0.42% ; and 2.08%.

\textit{Aedes aegypti} larva in Kediri District was resistant against temephos with concentration in 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, and 0.04 mg/l (Table 1).
### Table 1. Resistance Status Against Temephos in Kediri District 2018

<table>
<thead>
<tr>
<th>Contact Time (minute)</th>
<th>Concentration of Temephos</th>
<th>%</th>
<th>Status</th>
<th>%</th>
<th>Status</th>
<th>%</th>
<th>Status</th>
<th>%</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 mg/l</td>
<td>0.02 mg/l</td>
<td>0.03 mg/l</td>
<td>0.04 mg/l</td>
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<tr>
<td>15</td>
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<td>0</td>
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<td>0</td>
<td>Resistant</td>
<td>0</td>
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<tr>
<td>30</td>
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<tr>
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<td>60</td>
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<tr>
<td>1440 (24 hours)</td>
<td>1.25</td>
<td>0.42</td>
<td>Resistant</td>
<td>1.25</td>
<td>Resistant</td>
<td>0.33</td>
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</table>

### Discussion

*Aedes aegypti* larva from Kediri District that was scattered by using temephos with concentration in 0.01; 0.02; 0.03; and 0.04 mg/l until 60th minutes, there was no dead *Aedes aegypti* larvae. In certain concentration, *Aedes aegypti* larvae died because as the effect of residue from temephos with concentration 0.03 mg/l. As we know that temephos is the substance of organophosphate insecticide which is effective to be used in water and to be able to kill mosquito larvae. This substance is substance from *abate* that is often used by the society. How to use this temephos insecticide is by hindering enzyme in the insect nervous system, thus, it is not able to have normal function. Larvae of *Aedes aegypti* mosquito in Kediri District which was almost all in varied concentration did not undergo death and this was caused by the concentration that was used was perhaps too small. Similar research was conducted by Merty, et al.\(^6\) in Proceedings of Medical Education who researched resistance of Malathion in 0.8% and Temephos in 1% to adult *Aedes aegypti* mosquito and larvae in Bandung City. Observation was conducted during these two weeks for *Aedes aegypti* larvae that was still sensitive with insecticide of temephos 1% with percentage average of death in 100%. However, the difference with recent conducted research was the concentration that was used in this research was in mg/l, meanwhile, in conducted research by Merty et al.\(^6\) used percent. Concentration and similar units which were conducted by Ridha, et al.\(^7\) who researched by using insecticide of temephos 0.02 mg/l to *Aedes aegypti* larvae in Generation F1 in Banjarbaru City, that the average percentage of death for larvae in the area was in 95%.

Insecticide that was used by society commonly was *abate*, which another name of *abate* was temephos. Dosage of *abate* use in society was 10 gram/100 liter, thus, dosage of *abate* that was used by society was 10%. In this research, the substance of *abate* that was used was temephos with concentration in 0.02 mg/l. Result of the research was all larva of *Aedes aegypti* mosquito in Kediri District was resistant in concentration 0.01; 0.02; 0.03; and 0.04 mg/l. This was caused by toxicity that was given to larva was too small.

Similar research was conducted by Handayanji\(^8\) who used temephos with concentration in mg/l. Her research by using concentration 0.625; 0.31; 0.15; 0.078; and 0.039 mg/l concluded that larvae in perimetry area was in tolerant category with mortality rate in 96%, meanwhile, in buffer area was in resistant category with mortality rate in 68%. Another conducted research by Sinaga, et al.\(^9\) used concentration 0.0025 mg/l; 0.005 mg/l; 0.02 mg/l; 0.01 mg/l; 0.02 mg/l; 0.04 mg/l; and 0.08 mg/l. Mortality percentage of *Aedes aegypti* larvae reached 100% in concentration 0.08 mg/l.

All in all, the use of *abate* continuously will make larvae to be able to adapt with the environment around, hence, it causes larvae will be resistant with chemical substance. This condition is proved from several areas where have endemic for DHF and always use *abate* as one of mosquito controls. Giving *abate* with concentration...
0.02 mg/l that is conducted continuosly causes larvae is resistant, thus, it needs higher concentration. Controlling by using abate must be noticed more. Furthermore, abate cannot be given continuosly. It can be conducted periodiically in order to avoid resistant mosquito larvae against chemical substance. However, the main mosquito control is through controlling mosquito population in the environment. One of the most effective ways in controlling the mosquito is through draining, closing, and burying, which in Indonesian.

Analysis result showed that there was a significant influence between contact time and larva death, but concentration of temephos and interaction of contact time with temephos did not have any significant influence against death of Aedes aegypti larvae. It meant that larvae will die with longer contact time. The influence of several dosages of bacillus thuringiensis var israelensis Serotype H14 against Aedes aegypti larvae in West Kalimantan was a conducted research by Perwitasari. In her research, she stated that the use of insecticide in Bacillus thuringiensis type with dosage based on WHO that was 0.02 ml/l could kill Aedes aegypti larva of LT50 with contact time in 5,046 hours, meanwhile, in contact time of 24.68 hours, larva population was dead (95%). Furthermore, temephos is insecticide in organophosphate which is insecticide category from organic substance that is added by phosphate substance. Phosphate is non-toxic acid type. Characteristic of this acid will impede growth process from mosquito larvae. The larvae can be alive in pH 5.8-8.8. If pH of the water under 5.8, larva will die. However, controlling larvae through insecticide must be kept and controlled well. Safe use for killing larvae is by using insecticide concentration from organophosphate, which is in small concentration in quite long exposure time. Giving insecticide is not allowed continuosly, but it can be given periodically and insecticide substance, is always evaluated.

Conclusion

Aedes aegypti Larvae in Kediri District was resistant against temephos with concentration in 0.01 mg/l, 0.02 mg/l, 0.03 mg/l, 0.04 mg/l and there was a significant influence between contact time and larva death.

The use of temephos as larvacide was needed with concentration in more than 0.04 mg/l. Giving abate was needed to be done periodically and also evaluated every time. Moreover, it needed resistance test for larvae by using other insecticide substance besides from organophosphate type. In addition, it needed to be conducted monitoring of vector resistance periodically in different area.

Conflict of Interest- No

Source of Funding- Authors

Ethical Clearance- Yes

References

8. Handayani N. Resistance Status of Larvae Aedes aegypti Against Temephos in the Region of the Perimeter and Buffer Port of Tanjung Emas Semarang. 2015.

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¹Department of Economics, Faculty of Economics and Business, Universitas Airlangga, ²Faculty of Public Health, Universitas Jember, ³Health Polytechnic of Surabaya

Abstract

Indonesia is known as an agrarian country, should rely on the agricultural sector as an economic source as well as supporting development. In addition to the economic aspects, the progress of agriculture is also very important for the provision of nutrients for the maintenance and improvement of public health. This study uses the Location Quotient method to obtain a base subsector in the agricultural sector, so that the results can be used as material for consideration of export specialties. This study uses East Java GRDP data and as a comparison using Indonesia’s GRDP in 2010-2016. The results in this study indicate that the food crop and livestock sub-sector has an advantage compared to other sub-sectors and becomes a subsector of the base, so that these two sub-sectors can be used as export products for East Java.

Keywords: Location Quotient, Agriculture, East Java

Introduction

Background: The economic growth of a region can be seen from the increase in Gross Regional Domestic Products (GRDP).¹ Sector in GRDP is agriculture, mining and excavation, processing industry, electricity, gas and water sector, building sector, trade, hotel and restaurant sector, transportation and communication sector, financial sector, leasing and business services, and services sector.² A region is said to be agrarian if the role of the agricultural sector is very dominant in its GDP, and vice versa is said to be an industrial area if the dominant sector is the industry.

The existence of agricultural potential in an area does not have meaning for the agricultural growth of the region if there is no effort to utilize and develop the potential of agriculture optimally. Therefore, the utilization and development of all potential agricultural potentials must be a top priority to be explored and developed in implementing regional agricultural development as a whole.³

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Indonesia is known as an agrarian country, should rely on the agricultural sector as an economic source as well as supporting development. In addition to the economic aspects, the progress of agriculture is also very important for the provision of nutrients for the maintenance and improvement of public health. The role of the agricultural sector in economic development is very important, because most members of Indonesian society depend their lives on the sector. If the planners really pay attention to the welfare of their people, then the only way is to improve the welfare of most members of their community who live in the agricultural sector.⁴ The agricultural sector is still a part of potential development resources to be used as a strategic sector of current and future development planning, both at the national and regional levels.⁵

The percentage of East Java’s agricultural sector to East Java GRDP during 2010-2017 averaged 14% with an increase every year. The Agriculture, Animal Husbandry, Hunting and Agricultural Services sub-sector has the largest contribution compared to the lack and logging and fisheries. Food crops have the largest contribution to the subsector of Agriculture, Livestock, Hunting and Agricultural Services and always increase every year.⁶ The potential of East Java province with
agricultural and non-agricultural land resources reached 4,598,266 Ha at the end of 2016, with donations of 1,176,650 hectares of rice fields, 2,477,669 hectares of non-rice fields, and 943,946 hectares of non-agricultural land.\(^{(7)}\)

The Agricultural sector has a forward and backward relationship that is relatively strong with the manufacturing industry sector; construction; trade, hotel and restaurant; transportation and communication; and services. This relatively strong forward link means that output from the agricultural sector is widely used as input by these sectors, the relatively strong backward link means that these sectors need a lot of raw materials from the agricultural sector.\(^{(2)}\)

Economic growth is an important part that is inseparable from achieving economic development. Therefore, an analysis of the region that is the mainstay of the leading sector is needed to encourage economic growth in the East Java province. So that economic development planning can be realized in a structured manner based on its sectoral potential. If viewed from the regional GRDP sector value, the agricultural sector has a large role in increasing East Java GRDP. Nevertheless, it cannot be ascertained that the agricultural sector is the basis of the East Java Province, because comparisons with the wider area are needed. In this case, East Java Province is a comparison of the value of the East Java Regency GRDP sector. Calculation of Location Quotient (LQ) value is needed to find out the base and non-base sectors owned by East Java Province. LQ calculation can be used to find a Leading sector so that with industrial development it will spur and lift the development of other sectors, such as the agricultural sector. Rapid industrial growth will stimulate the growth of the agricultural sector to provide raw materials for industry.\(^{(8)}\)

**Purpose**

Identification of the agricultural subsector which is the base sub-sector of East Java Province as the A Reference for Law and Policy on Economics, Nutrition and Public Health.

**Method**

**Location Quotient (LQ)**

LQ techniques are widely used to discuss economic conditions, leading to the identification of specialization of economic activities or measuring the relative concentration of economic activities to get a picture in determining the leading sectors as a leading sector of an industrial economic activity.

Commodities that produce LQ> 1 are normative standards to be determined as superior commodities. However, when there are many commodities in an area that produce LQ> 1, while only one is sought, then the one that must be selected is the commodity that gets the highest LQ. Because the higher the value of LQ in an area shows the higher the potential for superiority of the commodity. Thus, the LQ analysis method or location quotient can be used easily and efficiently if you want to calculate a leading sector somewhere. Deficiencies encountered in the field can be reduced carefully and diligently in collecting data. The results obtained later are accuracy and leading sectors that can be empowered and developed by all communities in a particular region.

Based on GRDP data, sectoral contributions and economic growth rates in East Java and Indonesia, calculations can be made using the Location Quotient (LQ) method to find the comparative advantage possessed by East Java Province towards Indonesia by identifying the base and non-base sectors. The data used is data on the basis of constant prices.

With the aim to see economic developments in real terms whose growth is not affected by changes in prices or inflation / deflation. Sectors included in the base sector indicate that the sector has a comparative advantage. With the increase in the number of base activities in a region, the income inflows will increase due to export activities. On the other hand, non-base activities cause the outflow of income from the region to the monetary outflow caused by the region importing shortages in demand in the sector to meet needs within the region.

Base and Non-Base Sectors According to Arsyad\(^{(8)}\) economic base theory states that the main determinant of economic growth in a region is directly related to the demand for goods and services from outside the region. Economic base theory essentially differentiates base sector activities and non-base sector activities. Base sector activity is the growth of the sector that determines the overall development of the area, while the activities of the non-base sector constitute the city polowing sector, which means that it depends on developments that occur from comprehensive development.
Economic base theory seeks to find and recognize the activity of the basis of a region, then predict that activity and analyze the additional impact of the export activity. The key concept of economic base theory is that export activities are the engine of growth. Whether or not a region grows is determined by how the region’s performance is against demand for goods and services from outside.

Furthermore, in economic base theory analysis, the theory can be used to determine potential sectors and sub-sectors in East Java Province based on Gross Regional Domestic Product (GRDP). If the potential sector can be developed properly it certainly has a significant influence on the economic growth of a region, which in turn can increase regional income optimally. According to this theory an area can be differentiated into a mainstay area and not a mainstay, which is subsequently modified into a potential economic sector / subsector and not a potential economic sector / sub-sector.

### Discussion

**Table 1. GRDP at Constant Price Based on Business Fields in East Java and Indonesia in 2010 up to 2017 (Billion Rupiahs)**

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<tr>
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<td>150463.70</td>
<td>155784.00</td>
<td>160889.40</td>
<td>164760.40</td>
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<td>49679.10</td>
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Table 1 is the data on GRDP of the Agriculture, Forestry and Fisheries sector of East Java Province and Indonesia’s GRDP in billion Rupiah as comparative material for the analysis of the agricultural sector location quotient. In the agricultural sector including the subsector of agriculture, livestock, hunting and agricultural services (food crops, agricultural crops, plantation crops, livestock and agricultural and plantation services), forestry and logging and fisheries. Based on GRDP, the eastern subsector of agriculture, animal husbandry, hunting and agricultural services has a greater contribution to East Java’s GRDP compared to the forestry subsector and logging and fisheries.

The following are the results of East Java Agricultural sector LQ Calculation for the 2010-2017 period.

Table 2. LQ Processing Results

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<td>0.67</td>
</tr>
<tr>
<td>3 Fisheries</td>
<td>0.99</td>
<td>0.98</td>
<td>1.01</td>
<td>1.04</td>
<td>1.03</td>
<td>1.01</td>
<td>1.00</td>
<td>0.99</td>
</tr>
</tbody>
</table>

LQ >1 and consistent from 2010-2017, it is the food crop subsector and livestock, with the highest LQ value being the livestock subsector. The two sectors can be interpreted as the provisional sector base of East Java while the non-base sector is the subsector of Horticulture Plants, Plantation Plants and Agricultural Services and Hunting.

In the Food Crop sub-sector, the following information about the results of the calculation of Food Crop LQ value in East Java Province in 2010-2017: In 2010 the Food Crop sector has a LQ value of 1.26 >1 with a GRDP amounting to 46010.30 (Billion Rupiah) for one year. In 2011 the food crop sector had an LQ value of 1.28 >1 with a GDP value of 46435.30 (Billion Rupiah) for one year. In 2011, the value of LQ did not experience a significant change from 2010 to 2011 with an increase in the value of GDP that was also not too high. There is no significant change to the LQ value because this occurs when the economic growth rate in the Indonesian sector is greater than that of the East Java Province.

In 2012 the food crop sector had a LQ value of 1.24 >1, the LQ value in 2012 decreased compared to the LQ value in 2011 with a GDP value of 47585.50 in billion rupiah. In 2013 the food crop sector value of LQ dropped back to become the number 1.23 >1, with a GRDP value of 48241.20. In 2014 the LQ value rose again to 1.26 >1 with a GDP value of 49679.10. However, in 2015-2017 the value of LQ has decreased continuously, namely 1.23 1.22 and 1.16 with a GDP value of 51233.80 in 2015, 52136.80 in 2016 and 51001.70 in 2017 in billion rupiah.

In the subsector of livestock, the value of LQ has continued to decline from 2010 to 2017. In 2010 the value of livestock subsector LQ was 1.73 with a GRDP value of 26996.40 in the rupiah bill. In 2011 the LQ value decreased by 1.71 with a GRDP value of 28126.70 in billion rupiah. And in 2012-2017 livestock subsector LQ values were 1.67 1.60 1.52 1.49 1.47 1.47 with a GDP value of 29055.80 in 2012, 29365.60 in 2013 29704.60 in 2014, 30302.80 in 2015, 31357.80 in 2016 and 32579.10 in 2017 in billion rupiah.

East Java Province has great potential in the food and livestock sector, including Malang and its surroundings. The food crop sector is the sector that has the largest increase in output as a result of changes in the demand for the end of East Java Province. Capital support is considered very much needed in both sectors. The
shift in the structure of the economy from agriculture to the industrial sector is feared to have an impact on the state of economic stability, where the agricultural sector also contributes to the food sector. Food security and increased regional income can be achieved through maximization and optimization in the agricultural sector. Land use and use of machinery are expected to increase agricultural productivity in East Java, so that sustainable development in the agricultural sector can work.\(^{(10)}\)

The contribution of the food and livestock sector in East Java to GDP is the second largest after trade. Malang Regency is the largest producer of beef cattle in Indonesia.\(^{(11)}\) Livestock subsector has a very important role in supporting the achievement of national food security. This is because livestock is a provider of animal food from livestock through increased production of various commodities, as well as providers of raw materials for industry. In addition, livestock indirectly plays a role in alleviating poverty, because livestock is a process that uses production factors from the community so as to provide remuneration to the community that will affect their income, as well as an alternative energy source for environmental sustainability life (Zulkifli et al., 2015).

**Conclusion**

Based on the analysis of the agricultural sector location quotient in East Java, the food crop and livestock sector can be regarded as a base sector, both sectors have LQ >1. So that both sectors can be used as leading sectors to specialize in exports. Livestock has a greater LQ value compared to the food crop sector, this is because East Java is more precisely in Malang Regency, the largest producer of beef cattle in Indonesia. Food security and increased regional income can be achieved through maximization and optimization in the agricultural sector. As it is understood that food security in turn is a source for nutritional needs and improving public health.

**Ethical Clearance:** Yes

**Conflict of Interest:** None

**Source of Funding:** Authors

**References**

Association between Soluble ST2 Basal and Global Longitudinal Strain 2D-Speckle on Tracking Echocardiography with Left Ventricle Remodeling after an Acute Myocardial Infarction

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Abstract

Background: Left ventricle remodeling (LVR) is an important prognosis post acute myocardial infarction (AMI). Soluble ST2 is a novel biomarker for myocardial fibrosis and left ventricular remodeling. Global Longitudinal Strain (GLS) which is a reflection of the longitudinal layer of cardiac muscle can be detected in the early ischemia phase, and has been proven to predict the occurrence of left ventricular remodeling post AMI. Objective: To identify the association between soluble ST2 basal and GLS with LVR post AMI. Method: This research was conducted from August to December 2015. This is an analytic observational study with one group pretest and post test design. Soluble ST2 and GLS examinations were performed twice (2-5 days after AMI and 12 weeks after therapy). The results were analyzed using Spearman’s correlation test. Result: The sample size was 45 respondents (82.2% males, average age of 55.47 ± 10.13 years, 84.4% STEMI). There was a strong correlation between high ST2 basal levels with LVR (p = 0.0001 r = +0.723) and ΔST2 with LVR (p = 0.0001 r = 0.639). The association of low GLS with LVR was p = 0.015 and r = + 0.362. Conclusion: A significant LVR was found post-AMI, the high soluble ST2 basal and low GLS basal may be a factor for predicting LVR.

Keywords: Left Ventricle Remodeling, Soluble ST2 basal, GLS, Acute myocardial infarction

Introduction

Heart disease is the main death leading cause worldwide. In the United States, acute coronary syndromes occur every 34 seconds resulting death in every minute¹. Indonesia’s Health Profile 2009 released by the Health Ministry of the Republic Indonesia reported that in 2008, vascular system disease took the highest place (11.06%) of all death causes in hospitals².

Acute myocardial infarction (AMI) causes myocardial regional damage leading to systolic dysfunction, after an infarction then left ventricular remodeling (LVR) occurs. LVR is associated with high cardiovascular cases including heart failure. The characteristics are progressive left ventricular dilatation (LVESV) >15% or >20% (LVEDV). Several epidemiological studies and clinical studies suggested that heart failure was a 30-40% complication of AMI. The incidence of left ventricular systolic dysfunction (LVSD) after myocardial infarction was still poorly documented, but LVSD appeared in 25-60% of AMI, and 50% of patients with LVSD would have heart failure³,⁴.

Echocardiography is an affordable screening tool and can be used to study the regional and global functions of the left ventricle on AMI. Left ventricular ejection fraction (LVEF) measured by Simpson biplane method at the time of hospitalization is a well-known marker for left ventricular global function and predicting short-term or long-term morbidity and mortality of AMI patients. 2D Speckle Tracking Echocardiography (2D-STE) is a new non-invasive method of ultrasonic imaging capable...
of quantitatively and objectively assessing the global and regional function of myocardium in both systolic and diastolic function. This modality is considered more sensitive and accurate in predicting or detecting the presence of coronary heart disease. The change of regional function occurs earlier than the global damage, and can be detected by 2D STE. Examination with 2D STE is done by the per segmental strain and strain rate.

Longitudinal strains disturbed earlier at the time of ischemia. Studies in AMI patients found that longitudinal strains were associated with peak level cTt and extensive infarction. Longitudinal strains measurement after reperfusion is a very good predictor of LV remodeling, heart failure and mortality. Another study showed a Global Longitudinal Strain (GLS) examination before reperfusion could also predict LV remodeling and complications in STEMI patients.

Biomarkers become an important thing in improving the accuracy of diagnosis and providing disease information therefore it can determine the appropriate treatment options. The soluble ST2 (Interleukin 1 like receptor) is part of IL-1 receptor (IL-1R) consisting of ST2 transmembrane (ST2L) and dissolved (sST2). The soluble ST2 has no transmembrane or intracellular domains and is thought to have a function as a bait receptor neutralizing IL-33, known as the ligand of ST2 transmembrane. IL-33 has a function of inhibiting hypertrophy effect on cardiomyocytes and otherwise on the soluble ST2 giving excessive intracardiac pressure on marmots. The data showed that cardiac protection role was from IL-33/ST2 signal pathway.

SST2 biomarkers for myocardial fibrosis had been included in the ACC/AHA guideline for monitoring heart failure patients. Increased soluble ST2 in AMI patients suggested that a good myocardium acquired additional hemodynamic pressure as compensation for the necrotic area. An increase of baseline sST2 >35 μg/L was an independent predictor of mortality, sudden death and heart failure within 30 days and demonstrated sST2 biomarker’s role in providing prognostic information of acute coronary syndromes patients. SST2 role studies in ST Elevation Myocardial Infarction (STEMI) patients showed an increase of initial SST2 level indicating mortality risk and heart failure in patients treated with both fibrinolysis and percutaneous coronary intervention (PCI).
Table 1. Descriptive Data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
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<td></td>
</tr>
<tr>
<td>31-40</td>
<td>3 (6.7)</td>
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</tr>
<tr>
<td>41-50</td>
<td>12 (26.7)</td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>14 (31.1)</td>
<td></td>
</tr>
<tr>
<td>61-70</td>
<td>13 (28.8)</td>
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</tr>
<tr>
<td>71-80</td>
<td>3 (6.7)</td>
<td></td>
</tr>
<tr>
<td>55.47 ± 10.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>37 (82.2)</td>
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</tr>
<tr>
<td>Female</td>
<td>8 (17.8)</td>
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<tr>
<td>Risk Factors</td>
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<tr>
<td>Hypertension</td>
<td>28 (62.2)</td>
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<tr>
<td>Smoking</td>
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<tr>
<td>Dyslipidemia</td>
<td>19 (42.2)</td>
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<tr>
<td>Diabetes</td>
<td>42 (93.3)</td>
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<tr>
<td>CHD</td>
<td>9 (20.0)</td>
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<tr>
<td>55.47 ± 10.13</td>
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<tr>
<td>Diagnosis</td>
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<tr>
<td>STEMI</td>
<td>38 (84.4)</td>
<td></td>
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<tr>
<td>NSTEMI</td>
<td>7 (15.6)</td>
<td></td>
</tr>
<tr>
<td>Therapy</td>
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</tr>
<tr>
<td>PPCI</td>
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</tr>
<tr>
<td>Thrombolytic</td>
<td>10 (22.3)</td>
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</tr>
<tr>
<td>PCI</td>
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<tr>
<td>Conservative</td>
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<td>ASA</td>
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<td></td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>45 (100)</td>
<td></td>
</tr>
<tr>
<td>ACEI/ARB</td>
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<td></td>
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<tr>
<td>Beta Blocker</td>
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<td></td>
</tr>
<tr>
<td>Statin</td>
<td>45 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of echocardiographic characteristic difference obtained from not normally data distribution was used by Mann Whitney U Test. There was an increase of LVEDV from $76 \pm 29.69 \text{ ml/m}^2$ to $98.11 \pm 36.84 \text{ ml/m}^2$ of subjects undergoing LVR compared to non LVR ($p = 0.003$), LVESV was increased from $40.89 \pm 23.89 \text{ ml/m}^2$ to $51.96 \pm 26.43 \text{ ml/m}^2$ ($p = 0.006$) The basal value of soluble ST2 was much higher in LVR 34 (12-71) ng/mL ($p = 0.0001$). The basal value of GLS was lower in those with LVR -10.0 (-20-3) compared to non LVR ($p = 0.017$; Table 2).
Based on the type of AMI the most subjects who experienced LVR were patients with NSTEMI (6 people), then inferior STEMI (6 people), anteroseptal STEMI (4 people), broad anterior STEMI, anterior, inferior VR posterior (2 people), and the rest was STEMI inferior and anteroseptal inferior (1 person).

**Table 3. Correlation between basal levels of LVEF biplane and LVR**

<table>
<thead>
<tr>
<th>Variable</th>
<th>p</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal LVEF</td>
<td>0.44</td>
<td>0.117</td>
<td>45</td>
</tr>
</tbody>
</table>

**Table 4. Correlation between basal soluble ST2 and LVR**

<table>
<thead>
<tr>
<th>Variable</th>
<th>p</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal ST2</td>
<td>0.0001</td>
<td>0.723</td>
<td>45</td>
</tr>
</tbody>
</table>

**Table 5. Correlation between Δ soluble ST 12 weeks and LVR**

<table>
<thead>
<tr>
<th>Variable</th>
<th>p</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal ST2</td>
<td>0.001</td>
<td>-0.639</td>
<td>45</td>
</tr>
</tbody>
</table>

**Table 6. Correlation between basal GLS levels and LVR**

<table>
<thead>
<tr>
<th>Variable</th>
<th>p</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal ST2</td>
<td>0.015</td>
<td>0.362</td>
<td>45</td>
</tr>
</tbody>
</table>
Statistical analysis of not normally distributed data using Spearman correlation showed no correlation between LVEF and LVR \((p = 0.44; r = 0.117; \text{Table 3})\). Statistical analysis using Spearman correlation obtained significant correlation between soluble ST2 and LVR \((p = 0.0001; r = +0.723; \text{Table 4})\). It also obtained significant correlation between \(\Delta\) soluble ST2 in 12 weeks with LVR \((p = 0.001; r = 64\%; \text{Table 5})\). The analysis showed correlation between basal level of GLS with LVR \((p = 0.015; r = +0.362; \text{Table 6})\).

**Discussion**

In this study, the incidence of LVR was 27 people (60%), more than in the previous study, probably it caused by the subjects used were all primary PCI and PCI, and aggressive antiremodelling drugs, whereas in this study most of them received conservative therapy because most of the AMI patients came more than 24 hours, thus passing the onset of doing reperfusion either mechanically or pharmacologically \(^{14}\). In addition, not all patients wanted to do reperfusion for cost reasons. In the study found 29/93 patients who experienced remodeling (31\%) \(^{15}\).

An increase of LVEDV >20% from basal or an increase in LVESV >15% from the basal were the criteria used in LVR such as in the previous study \(^{14,16}\). LVEDV values after 12 weeks were increased, it was higher in LVR compared to non LVR. This was consistent with previous studies either associated with GLS or soluble ST2 \((p = 0.003)\), as well as LVESV after 12 weeks \((p = 0.006)\). This was similar to the previous study, there was an increase in LVEDV and LVESV after 90 days on AMI patients treated with PPCI. Basal level of soluble ST2 and GLS were also significant in LVR \((p = 0.0001\) and \(p = 0.017\) respectively) \(^{14}\). In the study there was an increase in LVEDV after 8 weeks from \(36 \pm 6\) to \(56 \pm 22\) with \(p <0.05\), and LVESV from \(20 \pm 5\) to \(33 \pm 18\) with \(p <0.05\) \(^{17}\).

NSTEMI and inferior STEMI were the most infarct type of LVR in the study, It was similar with this study, most of them were NSTEMI and inferior STEMI patients \(^{14}\). Literature states that infrak areas often experience remodeling if the infarction is widespread or spreading to LAD, but it is also said that diabetes, smoking, and previous infarction also often lead to LVR after AMI \(^{18}\).

This study found a significant correlation between basal level soluble ST2 and LVR \((p = 0.0001; r = +0.723)\). The previous study found an increase in basal soluble ST2 was associated with a change in LVEDV after 24 weeks that was measured by Cardiac Magnetic Resonance (CMR) proving that the increase in basal ST2 soluble was associated LVR. In the study basal soluble ST2 was also associated with lower basal LVEF after AMI. Changes in ST2 after 12 weeks (\(\Delta\)ST2) were also associated with \(\Delta\)LVEDV after 24 weeks \(^{15}\).

Study of ST2 roles in STEMI patients showed an increased of initial ST2 level indicating mortality and heart failure risk in patients treated with both fibrinolysis and PCI \(^{19}\). GLS basal had weak correlation \((p = 0.015 r = +0.362)\). This was similar to previous research that obtained a correlation between GLS and LVR \(^{14}\). Many studies using STE to predict LVR, either with GLS, torque or circumferential \(^{14,16,19}\). STEMI patients treated with PPCI found that the GLS examination at the time before the patient was repatriated was a novel examination to predict the occurrence of LVR. In patients with decreased GLS, LVR was obtained after 3 months and 6 months \(^{20}\). A study obtained a low basal GLS \(-11.05 \pm 4.1\) in LVR compared to non LVR \(-15.2 \pm 3.2\) with \(p <0.001\) \(^{21}\). This study only used GLS because of measurement limitation of echocardiography in Dr. Soetomo General Hospital Surabaya.

Generally, longitudinal LV mechanics, which are influenced largely by subendocard region, are the most vulnerable components of global LV mechanics because they are very sensitive to the presence of myocardial disease. Single longitudinal mechanical assessment is more adequate in early myocardial disease or has not shown significant abnormalities. The subendocardium region is the most remote area of perfusion therefore it is susceptible to hypoperfusion and ischaemia \(^{4,5}\).

**Conclusion**

There was a positive correlation between basal soluble ST2 and LVR after AMI and there was a negative correlation between basal GLS and LVR after AMI.

**Ethical Clearance:** This study protocol was approved by ethical clearance Dr. Soetomo Surabaya, Indonesia teaching hospital research.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.
References


Gynecomastia Treatment by Liposuction with Gland Excision and H.H. Method

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Abstract

Background: Gynecomastia (GM) is benign enlargement of male breast (glandular enlargement with fat accumulation). We classify in 6 grades depend on size and shape of breast. All cases were treated surgically as combine of gland excision and liposuction and added new method HH (Haider and Husam) method which is specific for grade 5 and 6 GM.

Method: From 2013 to 2018, a chart review was performed for 300 patients. Preoperative examination included endocrine and urological examination and exclusion of other pathological conditions. The surgical technique consisted of liposuction through small incision in lateral side of breast, combine with gland excision by sub areolar incision and sometime HH method depend on grade of gynecomastia.

Results: Total number of patient 300 cases all of them under want combination of liposuction and gland excision with drain keep for about 8 hours. Recurrent rate very rare and complication like infection or areolar necrosis also rare and 9 case only suffering from early postoperative bleeding and 22 cases develop seroma within 2 weeks.

Conclusions: This analysis data that include treatment of GM grade 1-6 is performed by liposuction combined with subcutaneous resection of the glandular tissue by sub areolar incision the procedure had low rate of complications and excellent patients’ satisfaction about the results. Preoperative fellow up is important to rule out and prevent specific complication of procedure and to exclude diseases or malignancy causing the GM as all gland sent to histopathology.

Keywords: Gynecomastia, HH method, liposuction, gland excision, surgery, combine treatment.

Introduction

Gynecomastia (GM) is a common condition but with variable severity that occurs in men at all ages and refers to a benign condition of breast enlargement with benign glandular proliferation of various causes. It can be found at autopsy in 40-55% of all men [1 - 5]. GM most often presents bilaterally, although it can occur asymmetrically. Patients with GM most frequently present suffering from aesthetic or psychological symptoms [6].

Three peaks in the prevalence of palpable GM are described: The first one occurs in infants, caused by high materno-placental estrogen levels, and regresses in the first weeks after birth [7]. The second peak appears during puberty between the ages of 13 and 14 years, with a high rate of regression before the 17th year of life [8 - 10]. This transient form is related to the hormonal changes during puberty, which shows an earlier peak of estrogen before testosterone reaches its peak levels. The third peak occurs in men between 50 and 80 years of age related to diverse conditions [4]. Besides physiological categories, GM can be categorized by the triggers (25%) persistent pubertal GM, 10-25% drug induced, 8% cirrhosis or malnutrition, 10% male hypogonadism, 3% testicular tumors, 1.5% hyperthyroidism, 1% chronic renal insufficiency, 25% idiopathic [11]. GM results from an increased volume of glandular tissue or adipose tissue (pseudogynecomastia) or a mixture of both. All causes share an imbalance of androgens and estrogens with a decreased testosterone-to-estradiol ratio, which directly affects the breast tissue [12]. Altered ratios of androgens and estrogens or increased sensitivity of breast tissue to normal circulating estrogen levels gives rise to ductal...
hyperplasia, elongation and branching of the ducts correlated with proliferation of fibroblasts and neovascularization\textsuperscript{[13 & 14]} which altogether form the clinical appearance of GM.

Figure 1: Male patient with enlargement beast (A). patient after gland excision and liposuction (B)

Gland of breast

Figure 2: Same Male Patient, before bilateral gland excision (A). After bilateral gland excision and a bilateral liposuction were performed (B). Glandular tissue and adipose tissue from breast (appearance of GM) after removal from the patient(C)

All pictures show the same patient before and after in all cases, a bilateral gland excision and a bilateral liposuction were performed.

The classification of GM

- Type 1: puffy look of the nipple coin like.
- Type 2: more extreme amount of glandular tissue and fat. As, fatty feeling in the outer zones.
- Type 3: includes breast sagging.
- Type 4: excess breast tissue sags below the chest fold, and the breasts look like a woman’s breasts.
- Type 5: A more extreme degree of sagging, under armpits, and may extend toward the back.
- Type 6: the nipple appearing at the bottom of a large amount of saggy breast tissue.

Most often used\textsuperscript{[15]}. Its groups patients into categories according to the size of the GM. Group (I) is characterized by minor but visible breast enlargement without skin redundancy. Group IIA features moderate breast enlargement without skin redundancy. Group IIB shows moderate breast enlargement with minor skin redundancy. Group III is characterized by gross breast enlargement with skin redundancy that simulates a pendulous female breast. Patients in groups I and II do not require skin excision during potential surgery, but the breast development associated with group III is so marked that excess skin must be removed Figure (2). Therapy of GM depends on several factors including its duration, grade, etiology, and the presence or absence of clinical symptoms. Treatment options include drug therapy and surgical removal of breast glandular tissue\textsuperscript{[16 & 17]}. After a persistence of 12 months or longer, GM is likely to have reached the fibrotic status, in which conservative treatment is not effective.

The aim of this study was to analyze the surgical strategies in GM grade I-II, their clinical outcome and, based on this data, the development of an algorithm for surgical treatment.
**Patients and Method**

Between 2013 and 2018, we surgically treated 300 patients with GM.

All patients underwent presurgical workup like a physical examination, and investigation and sometime ultrasound to breast. If any abnormalities, we found so send the patient to MRI to breast and some time to abdomen or fine needle aspiration from the testis and other laboratory workup about hormonal assessment of the patient.

**Surgical Technique**

Subcutaneous fatty tissue area of the breast was infiltrated with normal saline 0.9% solution 500 cc with one ampule of adrenaline[18]. Under guide monitoring of anastasis for any arrhythmia of change in cardiac condition[19]. One stab incision in lateral side near anterior axillary line for infiltration then for liposuction the breast by vesper or traditional method, complete clear the area and also below the gland to be dissected easy without use cattery unipolar figure (1).

This procedure for all grade of gynecomastia.

After finishing the liposuction then sub areolar incision to excision the gland very gentle release of the gland with secure homeostasis without need for bipolar and routinely. we put corrugate drain from the side of liposuction cannula for about 6-8 hours.

The size of liposuction cannula it’s about 4 -6 mm.

For grade 4 GH we do small skin excision below the areola look like <> on both side of areola.

For grade 5 and 6 we do my method name HH method (Haider, Husam) by which we do liposuction as usual then supra areolar incision look like inverted - U shape to elevate the areola up to 4 intercostal area; slightly lateral to mid clavicular line and also we put corrugate drain from side of liposuction cannula then the wound closed by 3/0 PDS and drain was keep for about 8 hours to avoid any hamartoma.

In all procedure we advice the patient to wear chest corsea for about 4 to 8 weeks to avoid redundant skin appear and also to avoid any seroma.

**Results**

Clinical data from 300 patients all cases bilateral gynecomastia mean age was 34 years (rang from 14 to 55) and mean body mass index about 25 – 35% all of the patients under want liposuction and gland excision under general anaestasia for about one to one and half hour and discharge to home in the same day on in the next day after drain remove and sure no any collection of haematoma.

Postoperatively no pain only parastesia remain for about 3 to 8 weeks.

9 cases suffering from immediate haematoma after 2-4 hours and immediate evacuation was done.

33 cases were suffered from seroma after 10 days and aspiration was done for one time only with covering antibiotic and compression of chest by coarsa.

Only one case develop areolar necrosis on one side only treated conservatively.

All glands after excision sent to histopathology no any case of malignant report

And no any case of wound infection.

**Discussion**

Surgery is the best way to treatment of gynecomastia in man start from grad one to grad six the procedure include combination of liposuction and gland excision or subcutaneous mastectomy[20] the duration of operation about one hour and drain routinely was keep for a few hours follow by compression of breast to avoid any collection of fluid like blood or serous[21] the postoperative complication are rare as infected wound or bleeding or areolar necrosis and recurrent[22] some time asymmetrical of right and left breast occure postoperatively in 3-4 weeks so need to simple liposuction under local anesthiasia or mesotherapy lipolysis injection it benefit[23].

The conservative treatment may be trail in early stage of GM combine with psychological support[24]. Some time we do operation to early stage of GM duo to aesthetic cousce[25].

The operation cost it’s not expensive it slightly depend on stage of GM.

Again we strongly recommended to do sub areolar gland excision to all stage of GM combine with liposuction.
Patient with drags that cause GM should be stop these drags or change to other type of medication [26]. Nowadays with elevated the body awareness will raise the discussion and curve toward surgical treatment of GM and will be raise more in future.

Source of Funding: Self

Ethical Clearance – Not required

Conflict of Interest: None

References

A View on Patterns of Weight Loss after Intragastric Balloon Insertion in Iraqi Patients

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Abstract

Obesity is a major metabolic illness that results from increased body fat and leads to negative health consequences. Obesity increases the prevalence of various diseases, including diabetes mellitus, hypertension, coronary heart disease, sleep apnea, CVA, GERD disease, gall bladder disease, certain types of malignancy, and non-alcoholic fatty liver disease. Moreover, it is also a major avoidable health detriment. Current therapeutic approaches to obesity are lifestyle changes, pharmacologic treatment, and bariatric surgery. Materials and Method: Twenty-seven patients visited our obesity clinic in Al-Dewaniyah Teaching Hospital from September 2016 to September 2017 and selected for intra-gastric balloon insertion after discussion with the patients, all current bariatric operative options beside the discussion to choose different balloon types. Air filled balloon was chosen. Results: female to male ratio 3.1:1, with mean age 34±6.1 and mean body mass index 40.48 ± 5.16 had excess body weight 38.93 ± 8.44 Kg, all patients had been received IGB heliosphere 720 ml as treatment of their obesity. Each patient has been followed up 6 months and weight loss patterns observed. Mean weight loss after six month 9.6 ± 4.8 Kg. Conclusion: In regards of patient selection to the procedure, Patients should be selected according to their commitment to the recommended diet and life style modification, this would largely affect the outcome.

Key words: Intragastric , Balons, AL-Diwaniyah general hospital

Introduction

Obesity is a major metabolic illness that results from increased body fat and leads to negative health consequences. Obesity increases the prevalence of various diseases, including diabetes mellitus, hypertension, coronary heart disease, sleep apnea, CVA, GERD disease, gall bladder disease, certain types of malignancy, and non-alcoholic fatty liver disease. Moreover, it is also a major avoidable health detriment. Current therapeutic approaches to obesity are lifestyle changes, pharmacologic treatment, and bariatric surgery. Although intensive lifestyle modification was reportedly associated with only limited weight reduction when it is combined with weight-loss drugs approved for long-term use, an additional weight reduction of 3%-9% can occur within 1 year. Such drugs are said to improve several cardio-metabolic risk factors, but they are also related to harmful adverse effects. Although new obesity medications have recently been approved and introduced, they are associated with issues of safety and high costs. Weight-loss surgery provides the most sustained and effective therapeutic choice for obesity. Accessible methods include the adjustable gastric band, Roux-en-Y gastric bypass, or sleeve gastrectomy. Regardless of its proven effectiveness, only 1% of obese patients eligible for the surgical procedure choose to undergo it. The major issues with surgery are difficult accessibility, high costs, patient non-preference, and significant morbidity and mortality. Although its associated mortality has decreased considerably, the complication rate in the early and late stages of the bariatric procedure persist at 17% (95%CI: 11%-23%)9. However, several adverse events were associated with its use, including small bowel obstruction associated with spontaneous deflation and gastric mucosal injury. Although the GEBG is no longer used, considerable advancements to its design have led to the development of a more effective and safer intra-gastric balloon. It is now being used in numerous countries. The increased prevalence of obesity has motivated experts...
in bariatric medicine to advance minimally invasive endoscopic treatment for obesity management as well as innovative techniques that address important features of treatments, such as their efficiency and safety. A new meta-analysis showed that endoscopic obesity treatment could be effective and of substantial value if combined with a multidisciplinary and comprehensive treatment plan. Intra-gastric balloon is placed in the stomach using endoscopic procedures under mild sedation in an outpatient setting. Intra-gastric balloons allow patients to sense fullness and ultimately reduce their food intake. It is hypothesized that the intra-gastric balloon facilitates satiety peripherally by being an obstacle to food consumption, decreasing intragastric volume, and delaying gastric emptying. Additionally, signals transmitted centrally through the vagal nerves by activated gastric stretching receptors could affect satiety. The intragastric balloon permits an early feeling of satiety, which is thought to be a consequence of gastric distention. The mechanical intragastric distention to a meaningful volume during mealtime significantly decreases the amount of food eaten. This study aimed to evaluate the role of Intragastric Balloon insertion to decrease body weight in obese patients.

**Materials and Method**

Twenty-seven patients visited our obesity clinic in Al Dewania Teaching Hospital from September 2016 to September 2017 and selected for intra-gastric balloon insertion after discussion with the patients, all current bariatric operative options beside the discussion to choose different balloon types. Air filled balloon was chosen. Heliosphere IGB 720 cc positioning was performed, the manufacturer’s instructions were followed in positioning the device, after diagnostic endoscopy, under unconscious sedation. After placement, the balloon was slowly inflated with room air to the final volume of 720 ml. All patients were discharged on the same day of insertion with omeprazole (po 20 mg/d), ondansetron (po 8 mg/d) and butyl scopolamine bromide (po 20 mg t.i.d.).

All patients from day 3 after placement began liquid diet and were on a 1000 kcal diet (carbohydrate 146 g, lipid 68 g, protein 1 g/kg ideal weight). The patients were followed monthly, and complications and their treatment, post-placement symptoms, BMI and %EWL were recorded. After 6 months, the Heliosphere Bag was removed. Data were analyzed by SPSS 18.

**Results**

Twenty-seven patients enrolled in these study, female to male ratio 3.1:1, with mean age 34±6.1 and mean body mass index 40.48 ± 5.16 had excess body weight 38.93 ± 8.44, all patients had been received IGB heliosphere 720 ml as treatment of their obesity. Each patient has been followed up 6 months and weight loss patterns observed.

The table (1) shows the difference in excess body weight and body mass index with gender. In male the excess body weight were 44.45±10.58 while in female 36.38±15.2 the difference not statistically significant. The BMI in male were 43.52±4.89 and in female were 39.2±5.914 which is not significantly difference between them.

<table>
<thead>
<tr>
<th>Table 1: Distribution of weight according to gender.</th>
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<tr>
<td>variable</td>
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<tr>
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<tr>
<td>Excess WT(kg)</td>
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<td></td>
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<tr>
<td>BMI</td>
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</table>

P-value for Male and female: Excess WT(kg)= 0.83 , BMI=0.25

After six months mean BMI of all samples were 33.28±6.21, mean loss of BMI 4.75 ± 1.87, mean of body weight loss 9.6 ± 4.8. More weight loss occurred in two months after insertion of balloon in mean loss 9.4±5.35.

Other result reveal male had more weight loss than female, in male mean 13.45±5.41and in female 8.47±3.52, on other hand percent of weight loss according to body weight in female 27.12±11.01and in male 26.05±10.71 as in table 2.

<table>
<thead>
<tr>
<th>Table 2: shows difference in weight loss between gender.</th>
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<tbody>
<tr>
<td>Variables</td>
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<tr>
<td>WT loss within six month(kg)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Percent weight loss</td>
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</tbody>
</table>
P-value for Male and female: WT loss within six month(kg)0.02, Percent weight loss =0.25

During the follow up period many patients had developed complication after insertion of intragastric balloon, 49% had vomiting, 42% had only nausea, 56% feel epigastric discomfort and 11% patient need admission, 3.75 develop gastric perforation as show in figure 1

![Figure 1: shows percent of each complication](image)

Intra-gastric balloon placement can be performed through a simple endoscopic method and is easily reversible. This simplicity offers an expansive role in obesity treatment based on the degree of obesity.

Intra-gastric balloon treatment might produce only short-lasting effects in obesity treatment. Thus, it is important to maintain weight loss following intra-gastric balloon removal. Long-term management for weight reduction after intra-gastric balloon removal can also comprise intensive lifestyle modification, alone or with pharmacotherapy, and could be suggested to protect against weight regain

According to our results, maximum weight loss achieved, was in the first two weeks of IGB insertion. In this period, nausea and epigastric discomfort were maximum, soon later when patients started to tolerate these symptoms, the weight loss achievement decreases and oral feeding becomes more tolerable.

The cornerstone of obesity management is patient compliance. It is crucial and vital. One of our patient who lost only 4 kgs after 6 months, said he was dividing sweet food into pieces so can ingest them without difficulty and without inducing nausea and epigastric cramps.

Three patients developed severe abdominal cramps and they asked to retrieve the IGB, we responded by readmission and prescribing more analgesia and IV fluid without improvement in their symptoms and final decision of retrieval of the IGB was taken, based on patients request.

All our patients received IGB insertion as their definitive obesity treatment but with possible second line bariatric surgery based on patients' weight loss achieved, all our patients have been informed that IGB could be their definitive therapy if the commit to the diet and life style modification. This was our goal to achieve maximal patient compliance. To encourage the patients to commit to the diet and life style modifications, we informed the patient that the IGB could be their final bariatric intervention.

This point is against most of studies that suggest IGB as bridge to bariatric surgery. One patient developed acute abdominal pain and tenderness 12 hours after
insertion with air under diaphragm. At Laparotomy, we found tiny pin point fundal gastric perforation and was repaired with primary closure and drainage of sub phrenic space with nasogastric suction tube patient recovered well and discharged home on the sixth postop day without complication. Such complication was not reported till the date of writing this paper as we did extensive online researches. This was life threatening complication and need urgent intervention. Thus, close clinical follow up is indicated in the first 24 hours after insertion. We recommended adding such complication to the informed consent.

The results of our study support most of the studies that recommend intra-gastric endoscopic bariatric interventions and concluded that it is promising procedure. the average of our patient BMI decreases to below 35 % where surgery is not indicated at such level, a part from the higher BMI in those studies, all other variables included, were the same. However no one of these studies BMI decreases below 35 %, due to the higher Baseline BMI 16,17,18.

**Conclusion**

In regards of patient selection to the procedure, Patients should be selected according to their commitment to the recommended diet and life style modification; this would largely affect the outcome. Larger scales and volume studies are needed particularly those studies that divide patients into groups according to the compliance and commitment to the diet and behavioral and life style changes they need to achieve the ideal body weight beside IGB insertion, those patients who understand that any bariatric procedure is not the only needed to lose weight. Incidence of life threatening complication was 3.7 %, Close follow up is indicated in the first 24 hours to detect gastric perforation .Material and safety issues regarding the intra-gastric balloon should be further investigated. More research should be performed to investigate a pathophysiologic pattern of obesity, the uncertain role of gut hormones, potential predictive factors for the efficacy of the intra-gastric balloon in obesity treatment, and individualized treatment-induced changes.

**Conflict of Interests:** Authors have declared that no competing interest.

**Source of Funding:** Self-funding

**No Ethical Issues Involved**

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Comparison between AMH level in Multipara and Nullipara in Women More than 40 Years Old

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1University of Babylon, College of Medicine, Iraq, 2Teeba IVF Center, Babyl, Iraq, 3Jabir Ibn Hayyan Medical University, 4Karbala Alzahraa Hospital for Maternity and Children

Abstract
Decreased ovarian reserve is considered as one of the main causes of infertility. It is about the availability of preovulatory oocytes in the ovaries. With increase age, ovarian reserve decreases. Nowadays, “ovarian reserve” assessment became a strategy to assess female infertility.

Objective: The current study aims to find the relationship between age and infertility type with measures of ovarian reserve (FSH, AMH and AFC) in infertile patients.

Material and Method: The cross-sectional study design was used for this study and a survey was conducted among 100 infertile women.

Result: Results revealed that Relationships between age and ovarian reserve indicators show a highly significant negative correlation with AFC (p= 0.001) and AMH (p= 0.007) level while positive correlation is found with FSH level (p= 0.001). The relation between age and FSH was moderate (rs=0.38, p<0.0001) and revealed that AMH and AFC level decreases while FSH level increases with age.

Conclusion Study of AMH level is the most reliable source to measure age-specific changes.

Key words: Female infertility, ovarian reserve, anti-mullerian hormone.

Introduction
In the modern world, the determination of the strategy to manage female infertility by assessing ovarian reserve has become necessary. Traditionally, ovarian reserve used to be evaluated by age and ultrasound investigation of Follicle stimulating hormone, Antral follicle count, and estradiol levels at the early follicular phase [1]. AMH (Anti-Mullerian Hormone) or Mullerian inhibiting substance is promising marker for ovarian reserves. It is a dimeric glycoprotein and involved in growth and differentiation. It is gonadal factor causes Mullerian duct regression and precursor of female reproductive tract. Plasma level of AMH is detectable at birth, gradually increases till puberty and then decreases until undetectable at menopause1. For evaluation of low ovarian reserve, FSH and E2 levels were considered to be determining biochemical markers for several years. However, it has been recently found that high level of FSH is associated with decreased functioning of ovary. Moreover, evaluation of AFC at later stages are considered to be more reliable in the assessment of ovarian reserve. High-resolution sonographic systems help obtain follicle count quickly, but there are certain difficulties in assessing correct AFC such as anatomical variations and high inter-observer differences 2 . AFC is considered the first choice to determine ovarian reserve by several health care professionals because it predicts poor response effectively when compared to basal FSH. Recently, it has been suggested that Anti-mullerian hormone are essential in the evaluation of ovarian response. It is a Mullerian inhibiting hormone which is a dimeric glycoprotein. This hormone belongs to the family of transforming growth factor – β. In women of reproductive age, AMH is produced in the ovary by granulosa cells and is secreted by small antral follicles 3 . The primary function of AMH in the ovary is resisting the accumulation of primordial follicle and reducing pre-antral and small antral follicles sensitivity to FSH in the ovary. There are certain advantages of AMH when compared with other tests in the assessment of ovarian reserve.
reserve. One of the significant advantages is AMH levels can be evaluated at any day of the menstrual cycle as they are stable throughout the cycle. Moreover, they are not affected by variations in any other hormone as well as the use of oral contraceptives. Bentzen et al. have suggested in a recent study that makers of ovarian reserve are lower in women who use contraceptive methods involving steroids.

On the other hand, AMH cannot be detected in women until they reach puberty and reaches its highest level at the age of 24-25 years, while it cannot be detected after menopause. With increasing age, AMH level, as well as the quality of oocytes, reduces. According to recent studies, depletion of follicles doubles when the amount of primordial follicle reaches approximately to the value of 25,000. Women reach at this critical stage at about 37-38 years, after which ovarian reserves sharply reduced. The normal AMH level value with female age is 5.4, 3.5, 2.3, 1.3 and 0.7ng/ml in 25, 30, 35, 40 and >43 years respectively (fertility, 2019). These changes in ovarian reserves are not only associated with age; hence, a woman’s age alone is not sufficient to evaluate ovarian reproductive potential. This enables the need for implementation of individual biological age-specific ovarian reserve determining tests. These tests can be highly reliable in determining ovarian reserves and reproductive potential of a woman at the early stages of infertility. According to recent studies, AMH can be a good predictor of ovarian reserves and the success rates of in vitro fertilization.

On the other hand, some other studies have shown that pregnancy can also be achieved even at low levels of AMH. Evaluation of ovarian reserves by identifying AMH levels is a modern method, and the data obtained are contrasting, implementation of further studies and collecting more data in the field are deemed to be reasonable. Hence, this study aimed to explore the relationship between different age groups and type of infertility with measures of ovarian reserve (FSH, AMH, and AFC) in infertile women.

## Material and Method

This was a cross-sectional study which consisted of the study population of 100 infertile women. Patients were recruited from outpatient’s private clinics during the period from June 2017- March 2019. These women were divided into two groups by age above and below 45-years old and by type of infertility into primary and secondary infertile groups. All patients underwent detailed fertility assessment including history, physical examination, laboratory and radiological (transvaginal ultrasound) investigations. AFC and serum FSH and AMH were measures and compared between groups. The study was approved by the Ethics Committee of University of Babylon. All patients consented to participate in the study. Statistical analysis was performed using SPSS v.24 (IBM, USA).

## Results

The study population was distributed based on age as follows: group I comprises of female of age less than 45 years (83%) and group II comprises of female of age more than 45 years (17%). There were 48% (n=48) primary infertile patients and 52% (n=52) secondary infertile patients. Table 1 and 2 show the values for AMH, FSH and AFC, respectively, according to the study groups. All of the three indicators of ovarian reserves in both age groups differed significantly from each other (AMH: p=0.007; FSH: p=0.001; AFC: $\chi^2=15.45, p=0.001$). These indicators varied according to age. Results are expressed as mean± SD.

### Table 1. AMH and FSH levels in women with age below and above 45 years

<table>
<thead>
<tr>
<th></th>
<th>Age (&lt;45 years) (n=83)</th>
<th>Age (&gt;45 years) (n=17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMH (ng/ml)</strong></td>
<td>1.94±2.65</td>
<td>0.17±0.34</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>FSH (IU/ml)</strong></td>
<td>10.48±6.06</td>
<td>26.3±23.84</td>
<td>0.001</td>
</tr>
</tbody>
</table>

AMH (ng/ml) level was higher in women <45 years (1.94±2.65, p value: 0.007) while the level of FSH (IU/ml) was higher in women >45 years (26.3±23.84, p value: 0.001).

### Table 2. Antral follicles count in women with age below and above 45 years

<table>
<thead>
<tr>
<th></th>
<th>Age (&lt;45 years) (n=83)</th>
<th>Age (&gt;45 years) (n=17)</th>
<th>X2=15.45,1 P=0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal AFC</strong></td>
<td>43 (51.8%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Decreased AFC</strong></td>
<td>40 (48.2%)</td>
<td>17 (100%)</td>
<td></td>
</tr>
</tbody>
</table>


Antral follicle number was normal and decreased in women of >45 years age 0% and 100% respectively, with p-value 0.001.

**Table 3. Parity in women with age below and above 45 years**

<table>
<thead>
<tr>
<th></th>
<th>Secondary infertility (n=52)</th>
<th>Primary infertility (n=48)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH (ng/ml)</td>
<td>2.16±3.2</td>
<td>1.07±1.20</td>
<td>0.02</td>
</tr>
<tr>
<td>FSH (IU/ml)</td>
<td>13.12±13.7</td>
<td>13.84±11.19</td>
<td>0.77</td>
</tr>
</tbody>
</table>

AMH (ng/ml) has high parity effect on secondary infertility (2.16±3.2, p value: 0.02) while FSH (IU/ml) has high effect on primary infertility (13.84±11.19, p value: 0.77)

**Table 4. AMH and FSH levels in nulliparous and multiparous women**

<table>
<thead>
<tr>
<th></th>
<th>Age (&lt;45 years)</th>
<th>Age (&gt;45 years)</th>
<th>X²=</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary infertility</td>
<td>45 (54.2%)</td>
<td>7 (41.2%)</td>
<td>0.96,1</td>
<td>0.23</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>38 (45.8%)</td>
<td>10 (58.8%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Secondary and primary infertility in nulliparous and multiparous women associated with AMH and FSH level was higher in women of <45 years as 54.2% and 45.8% respectively with p-value: 0.23

**Table 5. Antral follicles count in nulliparous and multiparous women**

<table>
<thead>
<tr>
<th></th>
<th>Secondary infertility (n=52)</th>
<th>Primary infertility (n=48)</th>
<th>X²= 3.51,1</th>
<th>P=0.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal AFC</td>
<td>27 (51.9%)</td>
<td>16 (33.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased AFC</td>
<td>25 (48.1%)</td>
<td>32 (66.7%)</td>
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</tbody>
</table>

Normal AFC count was observed in secondary infertility in nulliparous and multiparous women (51.9%) while decreased AFC count was observed in primary infertility in nulliparous and multiparous women (66.7%) with p-value: 0.07

**Discussion**

Results from the current study revealed that assessment tests of ovarian reserve reflected age-specific changes in both age groups. As an ovarian reserve marker, AMH is useful to estimate the reproductive lifespan of healthy young women and to predict the ovarian response to stimulation for in vitro fertilization (IVF), namely poor and hyperresponses. Reduction in AMH and increased in FSH level is associated with age increase while there is no significant association among age and AFC level. No clear link between infertile women age and AMH, FSH values with infertility type and there is no association between primary and secondary infertilities and AFC values is evident from current study. AFC and AMH serum level decreases with age. MH levels vary in both age groups, AFC levels significantly higher in women below 45 years, while FSH levels were higher in women above 45 years. AMH value is more reliable in assessing age-specific changes when compared to other indicators. Relationships between age and ovarian reserve indicators show difference between indicator levels. The relation between age and FSH was moderate and revealed that AMH and AFC level decreases while FSH level increases with age. Above mentioned trends are also confirmed by other researchers. Similar results were found by different studies. We found a significant difference existed in the mean FSH of fertile and infertile women. There is a significant difference in AMH between fertile and infertile women. There was a negative correlation between FSH and AMH in both fertile and infertile, stated that the plasma AMH levels were significantly higher in women with the polycystic ovarian syndrome. The significant association was seen between FSH and AFC with AMH. However, no significant association was observed between AMH levels with age, BMI, ovarian volume and type of treatment protocols.

It is cleared from current and previous studies that observed AMH value was significant in all age groups, while AFC value was significant in women above 45 years. Hence, we conclude that age-specific changes are better reflected by considering AMH values. Study of AMH level is the most reliable source to measure age-specific changes. This correlation is also confirmed by other researchers.

**Conclusion**

Among ovarian reserve evaluation tests that are used in modern practice, the serum levels of AMH
should be considered more authentic. Measuring serum AMH levels along with AFC levels may enhance the evaluation of ovarian reserve for assessing fertility potential and examining infertility treatment. Study of AMH level is the most reliable source to measure age-specific changes.

**Ethical Clearance** - Taken from University of Babylon committee

**Source of Funding** - Self

**Conflict of Interest** - None

**References**


Evaluation of Risk Factors and Outcome of Incarcerated Inguinal Hernia in Pediatric Age Group

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Abstract

Background: Incarcerated inguinal hernia is one of emergency conditions in children. It results from entrapment of bowel or other viscera within the hernia sac. High incidence of incarcerated inguinal hernia occurs in young age and preterm. The incidence of incarcerated hernia is variable and ranges from 12–17%. The aim is to evaluate the risk factor / or factors and outcomes for patients with incarcerated inguinal hernia.

Patients and method: This prospective study directed in department of pediatric Surgery, Central Child’s Teaching Hospital, Baghdad, Iraq. Fifty-five patients with incarcerated inguinal hernia were managed for 10 months. They were evaluated for history and sent for investigations. All patients initially treated by manual reduction. Later on they treated by elective operation. Overall outcomes were evaluated in all patients.

Results: The patient’s age range from 17 days to 5.5 years. There was male predominance with male: female ratio was 10:1. The frequency of right side incarcerated inguinal hernia was more than the left. Fourty (72.7%) patients were under first year. Thirty-two (58.2%) patients were preterm. Fourty-four (80%) patients have successful manual reduction and eleven (20%) patients were failed and underwent emergency surgery. Intestinal resection done to 4 patients and rate was 7.3%, and orchiectomy done to 3 patients and rate was 5.4%.

Conclusion: Premature and younger age patients are risk factors for incarcerated inguinal hernia. Incarceration increases the rate of overall complications. Any patient, especially young patient and preterm, with reducible hernia should be treated as soon as possible.

Key words: Incarcerated inguinal hernia; Risk factors; Outcomes.

Introduction

Indirect inguinal hernias are fundamentally the result of failure of closure of the processus vaginalis (¹, ²). Incarcerated inguinal hernia can result from entrapment of bowel or other viscera within the hernia sac (¹). Approximately 1–5% of all children will develop an inguinal hernia and a positive family history is found in about 10%, while the male-to-female ratio was 5:1, and right-sided hernias were twice as common as those on the left. The mean age at diagnosis was 3.3 years. The overall incidence of inguinal hernia in premature infants is estimated to be 10–30%, whereas term newborns have a rate of 3–5 % (³, ⁴). Younger age and prematurity are risk factors for incarceration (⁵).

Incarcerated hernia

Inguinal hernias that are difficult to reduce are “incarcerated. A strangulated hernia happens once there’s vascular compromise of the entrapped viscera. This results from constriction by a tight internal or external ring. Most children can progress speedily to strangulation if the hernia isn’t reduced. This method will take as very little as a pair of hours. If this process is unchecked, this will lead to gangrene and perforation of the bowel or other viscera. Incarceration and strangulation can also damage the testicle by compromising the blood supply to the testis (¹).
Clinical presentation

Incarceration symptoms are often manifested as a fussy or inconsolable child with intermittent abdominal pain and vomiting. A tender and at times erythematous irreducible mass is noted within the groin. Abdominal distention may be a late sign; as are bloody stools. Peritoneal signs indicate strangulation. Incarceration may be the presenting sign of the hernia, especially in an infant (3). The diagnosis of incarcerated inguinal hernia is usually made on clinical grounds. Abdominal x-rays may occasionally show bowel gas within the lump in the groin and confirm the diagnosis (6). In uncertain cases ultrasound can be useful to distinguish bowel from hydrocele fluid or a testicular torsion (1). Differential diagnosis for incarcerated hernia is the primary distinction to be made is between an inguinal hernia and a hydrocele. Femoral and direct inguinal hernia are rare but should be kept in mind; a retractile or undescended testis may mislead the unwary, and in young children, an inguinal lymph node may be situated close to the external inguinal ring (7).

Treatment

If the patients without obvious signs of shock or peritonitis, non-operative management is first tried (1). The incarcerated hernia should be reduced by taxis. Taxis is a manipulative trick, not a matter of force, and if necessary may be attempted several times. As long as the necessary monitoring is available, a distressed child may be sedated with midazolam or opiate analgesia. Taxis is successful in over 90% of cases, with virtually no chance of complication by en masse reduction (7). If positive, the hernia contents can steadily disappear into the internal ring. To be certain it is reduced; compare it with the contralateral side (1). Once an incarcerated hernia is reduced, a delay of 24 to 48 hours to allow resolution of edema is reasonable (3, 8).

Complications

The risk of recurrence after an elective inguinal hernia repair is less than 1% in several large series. Injury to the spermatic cord or testis is a rare occurrence in elective hernia repairs. Also the infection and hematoma can occurs in 1–3% of cases. Moreover loss of domain due to a huge hernia is another problem more frequently seen in adults, but it can occur in infants or children and may require staged repair or other measures. The iatrogenic cryptorchidism from failure to replace the testis in normal anatomic position is a rare occurrence (<1%). Bladder injury in infants in whom the medial wall of the sac contains urinary bladder is also a rare complication. While Mortality directly related to inguinal hernia or its repair is exceedingly rare (<1%) (3).

Patients and methods:

Fifty five patients with incarcerated inguinal hernia were evaluated in term of risk factors and outcome at surgical department of Central Child’s Teaching Hospital in Baghdad from 1st October 2015 to 2nd August 2016. The information’s been collected from the patients according to special data sheet and collected data involved:

1- History included: name, age, gender, maturity, presenting symptoms and sings with duration, side, and history of reducible hernia.

2- Investigations involved: plain erect abdominal x-ray, U/S (abdomen and inguinal), and preoperative routine investigations.

3- Treatment by taxis: reduction by taxis followed by elective operation and failed reduction followed by emergency operation.

4- Post-operative complications for elective and emergency operation.

5- Operative time and hospital stay.

The diagnosis depended mainly on clinical examination. Erect abdominal X-ray was taken to all patients. U/S of abdomen and inguinal was taken for some patients. Blood sample was taken and sent for preoperative routine investigations. Manual reduction by taxis was tried to all patients as they didn’t have contraindication. For elective patients who succeeded by manual reduction, formal herniotomies through inguinal approach were done later on with high ligation of sac. Patients were followed in term of wound infection, hematoma, recurrence, and testicular atrophy.

Statistical Analysis

Statistical package for social sciences version 22 (SPSS v22) was used for data input and analysis. Discrete variables presented as numbers and percentages and continuous data presented as means. Chi-square test for independence was used to test the significance of association between discrete variables. Findings with P values less than 0.05 were considered significant.
Results

The study includes fifty five patients with incarcerated inguinal hernia. Age range from 17 days to 5.5 years and mean age was 11 months. It consists of 50 males (90.9%) and 5 (9.1%) females with ratio male: female ratio is 10:1. In term of age distribution; 28 (50.9%) patients were less than 6 months, 12(21.8%) patients were between 6-12 months, 10 (18.2%) patients were between 12-24 months, and 5(9.1%) patients were more than 24 months. About 2/3 (72.7%) of patients were less than one year old which showed in table1.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Gender mean (percentage %)</th>
<th>Total mean (percentage %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>&lt;6</td>
<td>24(43.6%)</td>
<td>4(7.3%)</td>
</tr>
<tr>
<td>6-12</td>
<td>12(21.8%)</td>
<td>0</td>
</tr>
<tr>
<td>12-24</td>
<td>10(18.2%)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;24</td>
<td>4(7.3%)</td>
<td>1(1.8%)</td>
</tr>
<tr>
<td>Total mean (percentage %)</td>
<td>50(90.9%)</td>
<td>5(9.1%)</td>
</tr>
</tbody>
</table>

The most common presenting symptom was irritability(pain) and/or excessive crying that found in 51(92.7%) patients. vomiting (bilious & non-bilious) occur in 37(67.2%) patients, poor feeding in 44(80%) patients, constipation in 17(30.9%), and abdominal distension in 12(21.8%), bleeding per rectum in 1(1.8%) patient. Tenderness of inguinal or inguinoscrotal swelling occurred in 44(80%), and redness occurred in 8 (14.5%) patients. No signs of peritonitis (Tender abdomen, high fever, guarding) were reported, as shown in table2.

<table>
<thead>
<tr>
<th>No.</th>
<th>Signs &amp; symptoms</th>
<th>Total No. (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Irritability(pain) and/or excessive crying</td>
<td>51(92.7%)</td>
</tr>
<tr>
<td>2.</td>
<td>Vomiting (bilious &amp; non-bilious)</td>
<td>37(67.2%)</td>
</tr>
<tr>
<td>3.</td>
<td>Poor feeding</td>
<td>44(80%)</td>
</tr>
<tr>
<td>4.</td>
<td>Constipation</td>
<td>17(30.9%)</td>
</tr>
<tr>
<td>5.</td>
<td>Abdominal distension</td>
<td>12(21.8%)</td>
</tr>
<tr>
<td>6.</td>
<td>Bleeding per rectum</td>
<td>1(1.8%)</td>
</tr>
<tr>
<td>7.</td>
<td>(peritonitis)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>8.</td>
<td>Swelling</td>
<td>Tenderness 44(80%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Redness 8(14.5%)</td>
</tr>
</tbody>
</table>

Inguinal swelling less than 24 hrs. duration, 31(56.4%) of 32(58.2%) patients treated by taxis, 12(21.8%) of 17(30.9%) patients who presented between 24 – 48 hours treated by taxis , and only 1(1.8%) patient of 6 (10.9%) patients presented more than 48 hours duration and treated by taxis. Overall mean duration was 21.6±18.4 hrs. (R= 4-72 hrs.) as showed in table3.
Table 3: Duration of presentation and treatment of incarcerated hernia.

<table>
<thead>
<tr>
<th>Duration (hrs)</th>
<th>Total</th>
<th>reduction by taxis &amp; elective operation</th>
<th>Emergency operation after taxis failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24</td>
<td>32(58.2%)</td>
<td>31(56.4%)</td>
<td>1(1.8%)</td>
</tr>
<tr>
<td>24-48</td>
<td>17(30.9%)</td>
<td>12(21.8%)</td>
<td>5(9.1%)</td>
</tr>
<tr>
<td>&gt;48</td>
<td>6 (10.9%)</td>
<td>1(1.8)</td>
<td>5(9.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>55(100%)</td>
<td>44(80%)</td>
<td>11(20%)</td>
</tr>
</tbody>
</table>

* P value < 0.001

* There is a significant association between duration and manual reduction (p value<0.05)

No intestinal necrosis found in patients with history of incarcerated inguinal hernia less than 24 hours of duration, while it found in 4 patients delayed more than 24 hours as showed in table 4. For these 4 patients, mean duration was 45 hrs. and mean age was 6 months.

Table 4: Duration of symptoms and intestinal necrosis.

<table>
<thead>
<tr>
<th>Duration (hrs)</th>
<th>Frequency of incarceration</th>
<th>Intestinal necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24</td>
<td>32(58.2%)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;= 24</td>
<td>23(41.8%)</td>
<td>4(7.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>55(100%)</td>
<td></td>
</tr>
</tbody>
</table>

* P value = 0.014

* There is a significant association between duration & intestinal necrosis (P<0.05)

The overall post-operative complications of these patients with incarcerated hernia were 7 (12.7%) patients. For elective operation, only 2 (4.5%) of 44 patients had post-operative complications including: 1 (2.25%) patient had hematoma and 1(2.25%) had wound infection. For emergency operation, 5(45.4%) of 11 cases had complications including: 1(9.1%) hematoma, 1(9.1%) recurrence, 2(18.2%) wound infection, and 1(9.1%) testicular atrophy as showed in table 5.

Table 5: Frequency of post-operative complications in patients subjected for emergency and elective operations.

<table>
<thead>
<tr>
<th>Type of complication</th>
<th>Emergency N=11</th>
<th>Elective N=44</th>
<th>Total N=55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoma</td>
<td>1(9.1%)</td>
<td>1(2.25%)</td>
<td>2(3.6%)</td>
</tr>
<tr>
<td>Wound infection</td>
<td>2(18.2%)</td>
<td>1(2.25%)</td>
<td>3(5.4%)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>1(9.1%)</td>
<td>0(0%)</td>
<td>1(1.8%)</td>
</tr>
<tr>
<td>Testicular atrophy</td>
<td>1(9.1%)</td>
<td>0(0%)</td>
<td>1(1.8%)</td>
</tr>
<tr>
<td>total</td>
<td>5(45.5%)</td>
<td>2(4.5%)</td>
<td>7(12.6%)</td>
</tr>
</tbody>
</table>

* P value < 0.001

* There is significance for overall complications (P value < 0.05)
Regarding emergency patients, the mean duration of surgery was 55min (range 35-100 min), and mean post-operative hospital stay 63.1 hours (range 30 – 140 hrs.). For elective cases, the mean duration of surgery was 22.7 min (range 15-30) and mean post-operative hospital stay 2.7 hours (range 2-4 hours) as showed in table 6.

**Table 6: Mean operative time and hospital stay for emergency and elective patients.**

<table>
<thead>
<tr>
<th>Mean operative time and hospital stay</th>
<th>Emergency N=11</th>
<th>Elective N=44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Operative time</td>
<td>55min (R 35-100 min)</td>
<td>22.7 min (R 15-30)</td>
</tr>
<tr>
<td>Mean Hospital stay</td>
<td>63.1hrs (R 30 – 140 hrs)</td>
<td>2.7 hrs (R 2-4 hrs)</td>
</tr>
</tbody>
</table>

**Discussion**

The incidence of hernia incarceration is variable and ranges from 12–17 % (3, 4). In this study, 28 (50.9%) patients were less than 6 months, 12(21.8%) patients were between 6-12 months, 10 (18.2%) patients were between 12-24 months, and 5(9.1%) patients were more than 24 months. About two third of patients (72.7%) were infants as in Kayode T. Bamigbola etal (9) study and Mohammed Zamakhshary etal (10) study which assume that two third of incarcerated hernia were infants. With higher incidence among infants, this is due to narrow rings which easily trap the herniating loops of bowel or other viscera. Mean age of occurrence was 11 months with age range from 17 days to 5.5 years, common in male 50(90.9%), and in right side.

In this study preterm infants were 32(58.2%), and 23(41.8%) were terms. prematurity considered as risk factor for incarceration and this is mentioned by Rowe MI etal (4) study, EinSH etal (8) study, and Hughes K etal (11) study.

Regarding clinical presentation, 51(92.7%) of patients presented with irritability (pain) and/or excessive crying. Other presentations were poor feeding in 44(80%), vomiting (bilious or non-bilious) in 37(67.2%), constipation in 17(30.9%), abdominal distension in 12(21.8%), and bleeding per rectum in 1(1.8%). No signs of peritonitis were presented. Examination of swelling demonstrates tenderness in 44(80%), and redness in 8(14.5%). The most common symptom was irritability (pain) and/or excessive crying and the most common sign of swelling was tenderness which is comparable with Wael Mohammed etal (12) study.

Thirty two (58.2%) patients presented less than 24 hrs. duration and twenty three (41.8%) patients were more than 24 hrs. duration. Total manual reduction rate of incarcerated inguinal hernia by taxis succeeded in 80% of patients followed by elective herniotomy later on. Less 24hrs duration, 96.8% (31 of 32 cases) had succeeded by manual reduction, this rate decreased to 56.5% (13 of 23 cases) between 24-48 hrs. duration and only 16.6%(1 of 6) above 48 hrs. The total successful rate of manual reduction is less than Puri P. etal (32) study, and Ein SH etal(8) study which range from 90-95%. This is a result of the high rate of delayed presentation with 41.8% of cases presented after 24 hrs.

Eleven patients had emergency operations because of failed manual reduction. During operation, the sac must be opened and content examined. Bowel necrosis occur in 4 patients and give rate of 7.3% and the rate equal to Kayode T. Bamigbola etal (9) study which is 7.3%. Frankly gangrenous testis was 3 and give rate of 5.4%. This rate is less than Kayode T. Bamigbola etal (9) study which is 14 % and this may be due to high operation rate in neonate due to high failure of manual reduction in his study but near to 2.6-5% as mentioned in Arnold G. Coran etal (1). The overall complications rate was 12.7% and this is comparable with reports in studies of Niedzielski etal (13), Meier AH etal (14), Chen LE etal (15), and Ameh EA (16) with rate range between 11 and 31%. Complications rate of emergency operations was 45.4% and it is comparable to 51.7% in Sadik H Kadhem etal (17) study.

Mean operative time for patient with incarcerated hernia treated emergently was 55 minutes range from 35 – 100 minutes while mean time was 22.7 minutes with range of 15-30 minutes for patients who were treated electively. Mean post-operative hospital stay was 2.7 hours range from 2-4 hours for elective patients and 63.1 hours (2.7 days) range from 30-140 hours for emergency operations. So, there is prolonged operative time for emergency treated patients that will increase risk of anesthesia and complications. And there is prolong hospital stay which means more cost on hospital and more cost on patients.
Conclusion

Younger age and prematurity are risk factors for developing incarcerated inguinal hernia. Longer duration and younger age will decrease rate of manual reduction and increase rate of testicular and intestinal necrosis. Overall complications in incarcerated hernia will be higher than those with reducible hernia and especially high in emergently operated patients. Incarcerated inguinal hernia is more costly for patient and hospital than reducible hernia, as there is prolonged operative time and hospital stay.

Conflict of Interest: Authors have no conflict of interest.

Source of Funding: The current study which was directed at Central Child Teaching Hospital, Department of Pediatric surgery in Baghdad, where personal finance was adopted by the authors.

Ethical Clearance: The information’s been collected from the patients according to special data sheet and collected data. All patients were joining up in the study after signing a written informed consent, and their selection follows the above criteria’s.

Reference


Harmonic Scalpel Vs Conventional Cautery Use In Hemorrhoidectomy

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Abstract

Introduction: The evidence-based literature emphasizes the requirement of prioritizing harmonic scalpel haemorrhoidectomy over the conventional surgical interventions in the context of minimizing the intra-operative and post-operative complications in the treated patients.

Aim: The presented research study evaluated the significance of the harmonic scalpel (HS) as compared to the conventional electrocautery in the context of facilitating safe and effective haemorrhoidectomy in the selected patients. The study question focusses on analysing the potential of HS haemorrhoidectomy in terms of minimizing the post-operative complications in the patients affected with grade III and IV hemorrhoids. The study hypothesized beneficial treatment outcomes of HS haemorrhoidectomy as compared to the conventional electrocautery-based haemorrhoidectomy.

Methodology: The prospective cohort study shortlisted 20 candidates for HS and electrocautery-based haemorrhoidectomies. Initial 10 patients underwent the conventional haemorrhoidectomy; however, the other 10 received HS haemorrhoidectomy.

Result: The research findings categorically revealed limited post-operative pain and bleeding in the harmonic scalpel group as compared to the conventional electrocautery group. HS haemorrhoidectomy substantially reduced the extent of collateral thermal damage in the treated patients.

Conclusion: The study findings affirmed the effectiveness of HS haemorrhoidectomy over traditional intervention in terms of minimizing postoperative complications including bleeding, pain, urinary incontinence, fecal incontinence, anal stricture, and anal abscess.

Keywords: Harmonic scalpel, Electrocautery, Haemorrhoids, Bleeding, Collateral thermal damage

Introduction

Harmonic scalpels (HS) include the ultrasonic scalpel instruments that effectively incorporate automatic vessel-sealing systems for undertaking the surgical intervention including hemorrhoidectomy. These scalpels include the hemostatic, electrothermal, and bipolar devices that systematically utilize pressure and radio frequencies in the context of coagulating and dissecting the vessels of 0.7cm diameter. Utilization of harmonic scalpels leads to minimal tissue charring and limited thermal spread that reduce the risk of intraoperative hemorrhage. Harmonic scalpels increase surgical field visibility during hemorrhoidectomy. The concomitant undertaking of hemostasis and resection by the harmonic scalpels results in the reduction of mucosal tissue damage and post-surgical infection. Furthermore, harmonic scalpels’ use minimizes the risk of surgical site pain and post-hemorrhoidectomy bleeding in the treated patients. Contrarily, the hemorrhoidectomy through conventional cautery is performed in two distinct stages. The first stage facilitates the damage of blood vessels and mucosal tissues during hemorrhoidal resection.
The second stage is based on hemostasis and suturing at the resection site. These facts affirm the significance of harmonic scalps in terms of reducing the intra-operative hemorrhage as well as operation duration.

The analysis by Bulus et al. advocates the significance of the harmonic scalpel in terms of effectively treating the Grade III/IV hemorrhoids and associated complications. Various research studies affirm the safety of HS-based hemorrhoidectomy in comparison to the conventional operative interventions. Patients who undergo harmonic scalpel hemorrhoidectomy rapidly resume their work as compared to other patients who receive conventional hemorrhoid excision interventions in the operative settings. However, evidence-based findings regarding the effectiveness of harmonic scalpel hemorrhoidectomy over conventional techniques, lack scalability. Some of the significant studies do not report any reduction in postoperative pain intensity, bleeding and other postsurgical complications through the prioritized use of an ultrasonic scalpel over conventional hemorrhoidectomy techniques. The analysis by Homayounfar et al. reveals similar thermal injury and greater extent of necrosis under the impact of the ultrasonic scalpel as compared to the conventional monopolar electrocautery. Similarly, the findings by Khan et al. do not reveal the claimed benefits of HS hemorrhoidectomy over traditional electrocautery-based hemorrhoidectomy. These findings radically reveal the limited generalizability of the harmonic scalpel (safety/efficacy-related) findings in the scientific community. This necessitates the requirement of prospective interventions to comparatively analyze the safety and efficacy of HS-based hemorrhoidectomy in comparison to the conventional cautery method.

The analysis by Bilgin et al. affirms the potential of a harmonic scalpel (HS) hemorrhoidectomy in terms of reducing the recurrence of hemorrhoids in the treated patients. The assessment affirms the capacity of the harmonic scalpel in facilitating fast and safe hemorrhoidectomy. The study findings advocated the use of HS hemorrhoidectomy over the conventional staple hemorrhoidopexy. However, harmonic scalpel-based reduction in the initial postoperative complications, including the post-surgical pain and intra-operative bleeding substantiates the prioritization of this technique in comparison to conventional hemorrhoidectomy approaches. Postoperative pain is the major complication experienced by the treated patients following the administration of hemorrhoidectomy. Harmonic scalpel-based technique is more advantageous as compared to other conventional interventions, including cryotherapy, photocoagulation, sclerotherapy, electrocautery, and rubber band ligation. This is majorly because of limited postoperative pain and decreased lateral thermal trauma (0.5 – 1.5mm) under the impact of harmonic scalpel utilization. The assessment by reveals no rational evidence of the systematic utilization of the harmonic scalpel in hemorrhoidectomy. The study findings also relate the post-operative pain with the extent of thermal injury in the treated patients. Monopolar cautery leads to the thermal injury (up-to 15mm) to the porcine small bowel mesentery during hemorrhoidectomy. This injury substantially elevates the intensity of post-operative pain in the treated patients. Contrarily, the analgesic effect of the harmonic scalpel reduces these outcomes to a considerable extent.

Harmonic scalpel utilizes sound waves that effectively induce vibrations at 55, 000/second in the context of coagulating the medium/small size blood vessels during hemorrhoidectomy. Utilization of conventional electrocautery increases the risk of perianal pain-related post-operative complications including constipation and urinary retention. This eventually elevates patients’ treatment burden and length of their hospital stay during the post-operative period. Contrarily, no such clinical complications are reported after utilizing harmonic scalpel during hemorrhoidectomy. This resultantly reduces the operative time and risk of blood loss-related clinical complications in the treated patients. In summary, the HS hemorrhoidectomy is a closed and suture-less intervention that not only saves the operative time, but also increases patient safety to a considerable extent. The reduction in intra-operative and post-operative complications through the utilization of HS technique justifies its benefits over the conventional electrocautery technique in the hemorrhoidectomy-based surgical settings. We hypothesized that the Harmonic scalpel use in hemorrhoidectomy reduces the intra-operative and postoperative complications in comparison to the conventional electrocautery intervention. The presented research study accordingly evaluated the safety and outcomes of HS hemorrhoidectomy while comparing them with the conventional hemorrhoidectomy results.

**Methodology**

The prospective study selected 20 subjects affected with grade III/IV internal hemorrhoids. These candidates underwent hemorrhoidectomy in the year 2017 in
a private hospital in Tikrit city. Indeed, 10 patients received the conventional monopolar electrocautery-based hemorrhoidectomy. However, the other 10 of them underwent HS hemorrhoidectomy in the operative setting. The medical records of the selected patients were effectively retrieved from the hospital database. The study participants included 9-females and 11-male patients. The entire subjects had to undertake pre-operative lab interventions prior to hemorrhoidectomy. Saline enema was administered to each patient one night before surgery. Administration of prophylactic antibiotics was effectively undertaken before the initiation of surgical hemorrhoidectomy. 15 patients received general anesthesia for hemorrhoidectomy. However, 5 patients (including 2 female subjects) received spinal anesthesia prior to surgery. The surgical field acquisition was performed through anoscope. Forceps were utilized for lifting the hemorrhoidal stems from the anal sphincter.

The conventional hemorrhoidectomy was performed in the selected group through monopolar electrocautery in accordance with the Ferguson’s closed hemorrhoidectomy method. The anal sphincter and hemorrhoidal tissue were sequentially excised through the monopolar electrocautery device. The hemorrhoid mucosal resection was followed by hemostasis and closure of the surgical wound by Vicryl 3-0 surgical sutures. Contrarily, the ultrasonic scalpel was utilized for excising the hemorrhoidal pedicle and tissue up-to-the apex region. Vascular forceps were used in the context of minimizing the internal sphincter injury during the intra-operative period. Coagulated blood vessels and hemorrhoidal mucosa were subsequently excised through harmonic scalpel. 3-0 vicryl was used for placing the mucosal sutures.

Acetaminophen (2-tables TDS) were prescribed to each patient for post-operative pain management. Patients underwent ‘sitz bath’ three times a day during the post-operative tenure. VAS (visual analog scale, 0-10) was utilized for recording the post-defecation/resting pain, urinary retention, and hemorrhage. The occurrence of fecal incontinence, anal structure, and anal abscess was effectively monitored during the post-operative period. Indeed, 0-10-points on a VAS scale indicated no-pain to severe pain. Major bleeding was identified by the requirement of intensive medical interventions (including close-monitoring, reoperation, blood transfusion). Contrarily, minor bleeding was indicated by the minimal bleeding pattern during defecation that did not require consistent monitoring.

**Results**

The findings of the prospective study revealed limited post-defecation pain in the patients who received the harmonic scalpel intervention. The pain pattern was marked between the range of 1-2 on VAS scale. However, subjects of conventional electrocautery reported post-defecation pain on the VAS scale of 5-6. Major bleeding was not reported in both study groups. However, electrocautery-based subjects reportedly experienced elevated bleeding as compared to patients who underwent HS hemorrhoidectomy in the surgical setting. The subjects who received electrocautery-based hemorrhoidectomy encountered greater tissue injury that caused increased bleeding and anal pain as compared to the other treatment group. The elevated pain and tissue injury at the surgical site resulted in the clinical complications including fecal incontinence, anal abscess, and urinary retention in the initial treatment group. These postoperative complications variably impacted the study subjects in accordance with the extent of their tissue injury and immunity level. However, the other group did not experience these complications (except minimal anal pain/post-defecation bleeding) to any level during the post-operative period. Therefore, the study findings revealed the safety and efficacy of HS hemorrhoidectomy over the conventional electrocautery-based hemorrhoidectomy in the selected patients.

**Discussion**

The study findings effectively concord with the evidence-based outcomes that emphasize the effectiveness of HS hemorrhoidectomy over conventional electrocautery intervention in terms of minimizing the hospital stay duration, post-operative pain, blood loss and surgery time. Similarly, the systematic review by Mushaya et al. affirms the efficacy and safety of harmonic scalpel hemorrhoidectomy in terms of enhancing the quality of life and minimizing post-operative pain in the surgically intervened patients.

Numerous research interventions advocate the single-handed utilization of the harmonic scalpel method in comparison to the conventional electrocautery technique. Some studies also emphasize the requirement of the harmonic scalpel with other devices and techniques including the cushion suspension clamp, electric knife, and Milligan–Morgan hemorrhoidectomy for treating stage III/IV hemorrhoids. These mixed
method interventions also generate better outcomes as compared to the conventional electrocautery-based Ferguson’s closed hemorrhoidectomy. The analysis by Lohsiriwat\textsuperscript{14} emphasizes the effectiveness of non-conventional hemorrhoidectomies over the traditional methods in terms of limited convalescence duration, reduced postoperative pain, and shorter operative time. However, the findings do not indicate the greater effectiveness of non-conventional techniques (related to the long-term outcomes) as compared to the conventional hemorrhoidectomy methods.

The findings of the presented study revealed the reduction in post-hemorrhoidectomy pain under the sustained impact of pain-relieving medication and as well as minimally invasive harmonic scalpel-based intervention. These findings lack generalizability because of the small sample size. However, the findings still prove to be credible in the context of the systematic methodological approaches that the presented study utilized effectively for retrieving the desired outcomes. The findings of the presented study revealed minimum post-defecation pain in patients who underwent harmonic scalpel hemorrhoidectomy in comparison to the conventional technique. These findings effectively concord with the evidence-based outcomes that advocate the effectiveness of Harmonic Scalpel Hemorrhoidectomy in terms of minimizing the risk of sphincter lesions and associated fecal incontinence\textsuperscript{15}. Eventually, the absence of post-hemorrhoidectomy continence disorders minimizes the risk of post-defecation pain in the treated patients.

Undoubtedly, the harmonic scalpel-based hemorrhoidectomy offers the scope of the concomitant utilization of other non-conventional methods with the core objective of reducing the extent of the lateral thermal injury and resultant postoperative complications. The study by Abo-hashem, et al.\textsuperscript{16} revealed similar findings that indicated the reduced risk of excessive lateral thermal injury through the utilization of the harmonic scalpel while undertaking non-conventional hemorrhoidectomy. Similarly, the findings by Ababaikere, et al.\textsuperscript{17} reveal the limited risk of thermal injury to the subjacent tissues and resultant postoperative bleeding through the administration of harmonic scalpel-based hemorrhoidectomy. The findings also reveal the effectiveness of harmonic scalpel technique for minimizing the risk of postoperative mucous discharge.

The findings of the presented study revealed the absence of postoperative urinary retention and anal abscess in patient groups who received harmonic scalpel-based treatment. This occurred under the impact of minimal collateral thermal damage during the operative process. Evidence-based research literature substantially affirms these findings while indicating the minimal lacerating impact of harmonic scalpel-based ultrasound waves on the adjacent healthy tissues\textsuperscript{18}. The harmonic scalpel-based technique precisely dissects and coagulates the targeted hemorrhoidal tissues, while minimizing the surgical site complications. This eventually reduces the risk of fecal incontinence, urinary retention, and anal abscess during the post-operative period. The conventional electrocautery-based hemorrhoidectomy fails to minimize this post-operative complications that eventually lead to the extended hospital stay and reduced quality of life of the treated patients. These findings categorically support the authenticity of the outcomes of our prospective cohort study that advocates the use of HS hemorrhoidectomy over conventional monopolar electric cauterity in the surgical units.

Conclusions

The presented research study effectively authenticated the potential of HS hemorrhoidectomy in terms of minimizing the post-operative complications in the treated patients. The findings revealed a substantial reduction in postoperative bleeding, anal pain, urinary retention, and fecal incontinence through the utilization of the harmonic scalpel as compared to the conventional electrocautery during hemorrhoidectomy. The findings advocate the scalable utilization of HS hemorrhoidectomy for minimizing the post-surgical complications. The findings prove to be a milestone for healthcare professionals and the scientific community in terms of improving the safety and efficacy of hemorrhoidectomy methods for the treated patients. However, the study did not perform a cost-benefit analysis to evaluate the economic implications associated with HS hemorrhoidectomy as compared to the conventional treatments in the operative settings. This substantiates the need for organizing prospective studies to understand the economic implications of HS hemorrhoidectomy in the healthcare sector.

Ethical Clearance: The blood was collected from the a private hospital in Tikrit city after their investigation. Oral consent was taken before enrolled the patients in the study.
Source of Funding: Self
Conflict of Interest: Nil

References
The Implementation of Hiradc Method in Computer Laboratory

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Abstract

The computer laboratory of the Faculty of Public Health, Universitas Airlangga is used as a research site, measurement or scientific training for about 15,000 students. There are various types of activities which are at high risk, especially in the use of electricity, which can cause short-circuit and leads to fire. The objective of this study was to apply the Hazard Identification, Risk Assessment and Determining Control (HIRADC) methods in the computer laboratory as an effort to prevent occupational accidents and illness of the user. This study was an observational research using cross sectional design. The observation was conducted on the source of hazard and control efforts on the computer laboratory. Interview was conducted on the lecturers and administrative staff who are in charge of the computer laboratory. The results of the hazard identification indicated that there were 17 hazard sources that can cause 18 risks. Based on the risk assessment conducted on a activities practicum, there are 18 risks classified into 2 risk levels, those are 3 risks with moderate risk level and 15 risks with low risk level. The three level of moderate risks include taking off the shoes in standing position which can cause sprains, there is no first aid box and using electric current which can cause short circuit, electric shock and fire risk. The control that can be carried out by management laboratory to reduce the three moderate risk are by giving advice to taking off the shoes in sitting position, covering the socket which has risky placement by using duct tape to avoid the risk of electric shock as well as the provision of a light fire extinguisher for fire risk and the last providing first aid kits to treat the accidents that occur in the laboratory as soon as possible.

Keywords: HIRADC, risk, computer laboratory

Introduction

The management of Occupational Health and Safety (OHS) risk is the effort to manage hazard which has a potential risk to the workers’ safety and health. The aim is to prevent accidents and illnesses caused by the occupation in the workplace comprehensively, planned and structurally in a good system. The magnitude of the potential risk is determined by the accident and its severity which is possibly caused (¹). According to OHSAS 18001, organization must establish procedures and make efforts in terms of Hazard Identification, Risk Assessment and Determining Control or known as HIRADC (²).

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HIRADC is an essential element in the occupational safety and health management system because it is directly related to the prevention and control of hazards used to determine OHS purposes and plans (¹). The OHS Management System in Indonesia refers to the Government Regulation Number 50 of 2012 on the Implementation of Occupational Health and Safety Systems. HIRADC is a part of the implementation of OHSS. The regulation in appendix II point 2.1.2 states that as the OHS strategy, the identification of potential hazards, as well as the assessment and control of OHS risks plan needs to be implemented by competent officers (³).

The computer library of the Faculty of Public Health is one of the largest laboratories at Universitas Airlangga. This laboratory is used as a research site,
and scientific measurement or training for about 15,000 students. The hazards which can occur in such place can be in the form of electric shock, tripping of cables, low back pain interference and fire or short circuit. The computer laboratory practicum are usually done 5 days a week. This laboratory is also supervised and protected by the Department of Biostatistics, Faculty of Public Health. This laboratory has not implemented the OHS Management System in spite of the number of thousands students participated in the laboratory which is potentially can cause hazards. Therefore, this place has bigger risk of accidents.

Based on the preliminary observation, hazard of electric shock or short circuit is a major hazard which has a high risk of causing fire. Power sources come from various types of cables connected to the computer and electric socket next to the computer desk. In addition, this laboratory has some rules which prohibited the students to wear any shoes or footwear and bring food or drink into the laboratory.

The objective of this study was to apply the Hazard Identification, Risk Assessment and Determining Control (HIRADC) method in the computer laboratory of the Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia.

**Material and Method**

Based on the analysis characteristics and system, this research is a descriptive research. Based on the length of time this research was conducted, this research belongs to a cross sectional study. In addition, based on the research site, this study is an observational study since the data is obtained through observation and interviews. The population of this research is the computer laboratory staff which consists of two people. The sample was taken using total sampling method. The research was conducted for one month in July 2018.

This study was completed by implementing HIRADC method. The first step was doing hazard identification which include analysis of work activities, sources of hazards and risks. The second step was doing risk assessment by considering the likelihood and severity of risk. The assessment process is done by multiplying the likelihood value and severity value. Furthermore, multiplication results are used to determine the level of risk with Low (L), Moderate (M), High (H), or Extreme (E) risk categories. And the last step is determining the controls that have been carried out by management and giving the recomendation.

The category level of Likelihood, Severity, and Risk Matriks accordance with AS/NZS 4360 standard (1,4).

**Table 1: Level of Likelihood**

<table>
<thead>
<tr>
<th>Levels</th>
<th>Description</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rare</td>
<td>The possibility of hazards is very small, almost never happens</td>
</tr>
<tr>
<td>2</td>
<td>Unlikely</td>
<td>It usually do not occur, but the likelihood is rare, the frequency is annual</td>
</tr>
<tr>
<td>3</td>
<td>Possible</td>
<td>The possibility of hazard is small or coincidental, the frequency is monthly</td>
</tr>
<tr>
<td>4</td>
<td>Likely</td>
<td>The likelihood of hazard in particular circumstances, the frequency is almost 100%</td>
</tr>
<tr>
<td>5</td>
<td>Almost Certain</td>
<td>Very likely to happen, the frequency is certain</td>
</tr>
</tbody>
</table>

**Table 2: Level of Severity**

<table>
<thead>
<tr>
<th>Levels</th>
<th>Description</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Insignificant</td>
<td>No injuries</td>
</tr>
<tr>
<td>2</td>
<td>Minor</td>
<td>First aid kit, employees continue to work</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Injuries that need medical treatment, employees do not go to work</td>
</tr>
<tr>
<td>4</td>
<td>Major</td>
<td>Severe injuries, loss of production capacity</td>
</tr>
<tr>
<td>5</td>
<td>Catastrophic</td>
<td>Death</td>
</tr>
</tbody>
</table>
### Findings

The activities conducted in student practicum in the computer laboratory of the Faculty of Public Health consist of seven activities, including taking off shoes before entering the laboratory, putting the bag into the laboratory’s locker, sitting on the seat, the lecture gives lectures using wired microphone, practicum preparation which include checking the computer equipment, the implementation of the computer practicum and the closing of computer practicum. According to the identification results, there are 17 potential hazards from all of the activities which can lead to 18 risks. After the hazards were identified in the practicum activities, the next step is to carry out the risk assessment. Based on the risk assessment conducted on all work activities, there are 18 risks classified into 2 risk levels, those are 3 risks with moderate risk level and 15 risks with low risk level. The three level of moderate risks include taking off the shoes in standing position which can cause sprains, there is no first aid box and using electric current which can cause short circuit, electric shock and fire risk. Then, the management’s existing control were giving advice to taking off the shoes in sitting position, covering the socket which has risky placement by using duct tape to avoid the risk of electric shock as well as the provision of a light fire extinguisher for fire risk and the last providing first aid kits to treat the accidents that occur in the laboratory as soon as possible.

### Hazard Identification

Hazard identification is a process that can be done to recognize the whole situation which potentially can cause occupational accidents and illness in the workplace. This research used the proactive method. Proactive methods are the best method for identifying hazards.

Laboratory regulations prohibit footwear from entering the computer laboratory, therefore students must take off their shoes and put them in the locker. The students usually take off their shoes in a standing position, which makes their body unbalanced and can cause their legs to dislocate. In a certain time, the floor in the front of the laboratory is sometimes slippery which can cause risks to the students.

The activity sitting on the chair was found a source of hazard, in which the sitting position which was less ergonomic for a long time can cause a low back pain disorder. According to Sumekar’s research (2010), a good sitting position caused 27 out of 65 respondents (41.5%) experienced low back pain, while in a bad sitting position, 11 of 12 respondents (91.7%) also experienced low back pain with a risk of 15,481 times.

The source of hazard in the computer practicum preparation activity was turning on the computer that can cause electric shock. The electric shock hazard during plugging in or removing an electronic cable can cause electric shock and weakness. Then, the second source of hazard is the use of electric current sources in the form of a socket which can cause a short circuit leading to fire and electric shock. This is due to the type and the placement of the socket which is appropriate. The third source of hazard is in the form of the sparks of the electrical cables installation which is unknown by the people since it is covered by the plywood, so that it can cause a fire. The fire extinguishers must be near the workplace to help extinguish the fire. The fourth source of hazard is the noise of a damaged computer that can cause hearing disorder. This has been
experienced by the students when they are operating a damaged computer. The fifth source of hazard is the unavailability of first aid kits in the laboratory. Based on the Law Number 1 of 1970 article 3 paragraph 1, one of the requirements for occupational safety is to provide first aid kits \(^9\). The absence of this equipment can make the injury occurred becomes worse.

The computer practicum implementation activity have two sources of hazard, those are ultraviolet (UV) radiation and cold temperatures in the laboratory. A long exposure to UV radiation from a monitor screen can cause eye disorders, which can lead to a disorder of Computer Vision Syndrome (CVS). When Computer Vision Syndrome is not treated well, it can decrease one’s daily work productivity, increase the level of error in work, and decrease the job satisfaction \(^10\).

The source of hazard in the form of cold temperatures in the laboratory can cause unbalance fluid or water on body (mild dehydration) with the symptoms of dry and cracked skin, sleepy, dry mouth as well as reduced saliva.

**Risk Assessment**

Risk assessment is the process of evaluating risks caused by hazards, taking into account the adequacy of controls owned, and determining whether the risks are acceptable or not \(^2\). This study used a qualitative risk assessment method according to AS/NZS 4360:2004 standard which consists of likelihood and severity criteria \(^4\). This method uses a risk matrix that describes the level of likelihood and severity of an event that is expressed in the form of a range from the lowest to the highest risk. The danger of taking off the shoes in standing position, there is no first aid and using electric current gets likelihood 2 value and severity value 3. If multiplied then gets a total value of 6. Value 6 is included in the moderate risk category in the risk matrix.

The danger of sitting less ergonomic for a long time get likelihood value of 3 and a severity value of 1. If multiplied then gets a total value of 3. The danger of turning on the computer get likelihood value of 2 and a severity value of 2. If multiplied then gets a total value of 4. This means that risk is included in the low risk category. The danger of sparks on electric cables under plywood get likelihood value of 1 and a severity value of 4. If multiplied then gets a total value of 4. The danger of damaged computer causes noise get likelihood value of 1 and a severity value of 3. If multiplied then gets a total value of 3. And the last, the danger of laboratory temperature is quite cold and ultra violet radiation from monitor screen gets likelihood value of 3 and a severity value of 1. If multiplied then gets a total value of 3. This means, all of the total values above are included in the low risk category in the risk matrix.

Work activities with *moderate risk* requires action to reduce risks including prevention costs that are needed to be carefully calculated and limited and measurement of risk reduction needs to be implemented properly and correctly \(^4\). Work activities with *low risk* does not require additional control measures, but requires monitoring actions to ensure that controls are maintained and implemented properly and correctly \(^4\).

**Determining Control**

Risk control plays a role in minimizing or reducing the level of risk to the lowest level. There are five hierarchies of risk control including elimination, substitution, engineering, administration and the use of personal protective equipment \(^2\). The determining controls carried out by the laboratory management were adjusted to the existing hazards, including:

For moderate risk, the determining controls were the student should taking off the shoes in sitting position to avoid sprains, covering the socket which has risky placement by using duct tape to avoid the risk of electric shock as well as regularly checking the socket to avoid the hazard risk of circuit and fire and the last providing first aid kits to treat the accidents that occur in the laboratory as soon as possible.

For low risk, the determining controls were chair design is adjusted to the computer desk to avoid musculoskeletal disorders on the student, giving a warning to carefully contact with the electrical appliance and attaching warning stickers to prohibit the students to bring food and drinks into the laboratory room to avoid the risk of electric shock and short circuit, checking the condition of the cable installation under the plywood and the provision of a light fire extinguisher for fire risk, do not operate the damage computer to avoid hearing disorders, and giving attention to rest their eyes for a moment after long facing the computer screen to avoid computer vision syndrome.

**Conclusions**

The computer laboratory of the Faculty of Public
Health, Airlangga University is used as a research site, measurement or scientific training for about 15,000 students. There are various types of activities which are at high risk, especially in the use of electricity, which can cause short-circuit and leads to fire. Based on HIRADC analysis, there are three level of moderate risks include taking off the shoes in standing position which can cause sprains, there is no first aid box and using electric current which can cause short circuit, electric shock and fire risk. Then, the management’s existing control were giving advice to taking off the shoes in sitting position, covering the socket which has risky placement by using duct tape to avoid the risk of electric shock as well as the provision of a light fire extinguisher for fire risk and the last providing first aid kits to treat the accidents that occur in the laboratory.

Acknowledgement: The authors acknowledge Mr. Andri and Miss Rahmah as the laboratory workers of the Laboratory of Computer, Universitas Airlangga, Surabaya, for providing information regarding the research of risk analysis of Occupational Safety and Health.

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Evaluation of Factors Affecting Quality of Nursing Cares for Dying Patients in ICUs

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Abstract

Nursing managers have paid special attention to quality assessment as an important management tool for improving nursing performance and the quality of nursing care. This study aimed to evaluate the aspects and factors involved in the quality of nursing cares for dying patients in ICUs in 2016-7. This descriptive and analytical study was conducted in ICUs of Imam Khomeini Hospital Complex and Shahid Rajaei Cardiovascular Medical & Research Center, Tehran. In total, 191 working nurses were selected via convenience sampling. Data collection tool was quality patient care scale (QUALPACS). In addition, data analysis was performed in PSSS version 20 using descriptive and inferential statistics. The mean score for end-of-life care was 83.62±8.62 in the psychosocial, 85.36±7.74 in the communication and 92.23±10.88 in the physical aspects. According to the results, a significant relationship was observed between passing educational courses related to end-of-life care and quality of end-of-life care in the physical (P=0.002), psychosocial (P=0.02) and communication (P=0.01) aspects. According to the results of the study, the quality of care for dying patients was higher in the physical aspect, compared to other aspects. However, the quality of nursing care was poor in the psychosocial aspect.

Keywords: Quality, End-of-Life Care, Nursing Care, Intensive Care Unit

Introduction

Today, with the advancements in technology and new therapies in recent decades, we are faced with a high incidence and long-term hospitalization of critically ill patients in intensive care units (ICUs), which is accompanied with the suffering of the patient and the family without hope for improvement and, on the other hand, imposing heavy financial and mental burdens on the patient, the family and the health system. Helping patients in the final stages of life involves a period of the imminent death of patients. In this regard, the great number of demands and needs of patients and companions and stressful situations prevent participation in care decisions, caregivers' dissatisfaction, disregard for care details, and poor quality of care for patients.

Similar to other countries, with regard to the advances made in the field of medical and paramedical sciences, we are faced with a higher number of people in need of special care due to chronic diseases or problems caused by life-threatening and incurable diseases in Iran. Recently, national attention and focus have been on the provision of end-of-life care, where palliative care is the center of attention and includes a range of management of physical, mental, and social sufferings associated with health status for which the treatable cure is not a remedy.

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In the field of modern medical ethics, special attention has been paid to the rights of patients (6). All over the world, numerous differences are observed in end-of-life treatment methods for dying patients considering the ethical principles (7). While the goal of end-of-life care is death with dignity and respect, its concept is vague in articles and has no comprehensive definition (8).

Nurses and physicians in intensive care units (ICU) are a group of professional healthcare providers, who deal with problems related to end-of-life care on a daily basis (1). While nurses all over the world share many commonalities in terms of different physical, psychological, and social needs of patients, there are differences in patterns of end-of-life care that are rooted primarily in the culture and beliefs of the community (9).

Since nurses are the largest group of medical staff in the hospital to provide care services (10, 11), Quality is a multi-dimensional concept with mental, social, and spiritual aspects (12). The quality of care provided is an important factor in the satisfaction of patients and their families and even the job satisfaction of nurses in the health system and is recognized as a priority of the health care system, especially in the field of nursing. In most countries, including Iran, a hospital’s grading and accreditation are influenced by the quality of nursing care provided and is assessed along with other validation measures (13, 14). In Iran, Haghighi and Khoshkhoo (2004) confirmed the validity and reliability of this scale in a research conducted to assess the quality of nursing cares from the perspective of nurses and patients hospitalized in training healthcare centers affiliated to Tabriz University of Medical Sciences, Tabriz, Iran (16).

In addition, the scale was used in studies by Akbari Kaji and Farahmini (2009) and Neyshabouri et al. (2010) (17, 18). In the present study, the reliability of this tool was confirmed at 0.82 using the split-half method. Data analysis was performed in SPSS version 20 using Chi-square, correlation bivariate, and Pearson’s correlation to assess descriptive and inferential statistics. Moreover, P-value of less than 0.05 was considered statistically significant.

Results

In this study, the majority of nurses (54.5%) were within the age range of 30-40 years. In addition, 74.3% of the participants were female, whereas 51.3% of the subjects were married. In terms of work experience, 58.1% had less than 10 years of work experience. Moreover, 59.7% of the nurses had permanent contracts, and 136 subjects were working in special surgical sections, including heart surgery, transplantation, neurology, cancer, and gynecology. Furthermore, 87.4% of nurses had a BSc degree, and 66.5% of the subjects were highly interested in working in ICUs.

Statistical tests demonstrated a significant relationship between the physical aspect and type of employment (P=0.01), the experience of the death of a family member or close friend (P=0.005), passing critical care courses (P=0.03), and gender (P=0.02). (Table 1).
Table 1: Relationship between Demographic Characteristics and Aspects of End-of-life Care in Dying Patients in ICUs

<table>
<thead>
<tr>
<th>Various aspects of end-of-life care</th>
<th>Psychosocial aspect</th>
<th>Physical aspect</th>
<th>Communication aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>P-Value</td>
<td>P-Value</td>
<td>P-Value</td>
</tr>
<tr>
<td>Type of employment</td>
<td>0.3</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Type of ICU (internal and surgical)</td>
<td>0.2</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Number of ICU beds</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Level of education</td>
<td>0.7</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Interest in working in the current ward</td>
<td>0.1</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Average working hours per week</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Type of work shift</td>
<td>0.9</td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Caring for dying patients</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Caring for how many dying patients</td>
<td>0.3</td>
<td>0.08</td>
<td>0.2</td>
</tr>
<tr>
<td>experience of the death of a family member or close friend</td>
<td>0.06</td>
<td>*0.005</td>
<td>0.9</td>
</tr>
<tr>
<td>Passing intensive care courses</td>
<td>0.6</td>
<td>*0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>Passing educational courses related to end-of-life cares</td>
<td>*0.02</td>
<td>*0.002</td>
<td>*0.01</td>
</tr>
<tr>
<td>History of working in ICU</td>
<td>0.07</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Marital status</td>
<td>0.8</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Gender</td>
<td>0.4</td>
<td>*0.02</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The quality of end-of-life care for dying patients showed an undesirable level (46.1%=low) considering the overall score of end-of-life care areas, which was favorable (53.9%=moderate). In addition, the mean score of end-of-life care in the psychosocial aspect was 83.62±8.63 (%37.7 favorable and 62.3% lower), whereas the same mean in the communication aspect was 85.36±7.74. In addition, the mean score of end-of-life care in physical aspect was 92.23±10.88 (Table 2). Moreover, the score of psychosocial aspect of end-of-life care in ICUs of Imam Khomeini and Shahid Rajaei Cardiovascular Medical Center of Tehran was reported to be 82.61±8.52 and 85.42±8.59, respectively.
Table 2: Quality of End-of-life Care in Dying Patients in Various Aspects in ICUs

<table>
<thead>
<tr>
<th>Quality of end-of-life care Aspects</th>
<th>Unfavorable</th>
<th>Favorable</th>
<th>Mean±standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Psychosocial</td>
<td>119 (62.3)</td>
<td>72 (37.7)</td>
<td>83.62±8.63</td>
</tr>
<tr>
<td>Physical</td>
<td>35 (18.3)</td>
<td>156 (81.7)</td>
<td>92.23±10.88</td>
</tr>
<tr>
<td>Communication</td>
<td>95 (49.7)</td>
<td>96 (50.3)</td>
<td>85.36±7.74</td>
</tr>
<tr>
<td>Total score</td>
<td>53.9 (103)</td>
<td>46.1 (88)</td>
<td>173.31±2.1</td>
</tr>
</tbody>
</table>

According to correlation bivariate analysis, the level of correlation between psychosocial aspect with the physical aspect (R=0.7, P=0.001) and between psychosocial aspect with the physical aspect (R=0.6, P=0.001) was significant and direct. On the other hand, a reverse association was found between the physical aspect and psychosocial aspect (R=-0.7, P=0.001) (Table 3).

Table 3: Relationship between Quality of End-of-life Care for Dying Patients in ICUs of Hospitals

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Psychosocial</th>
<th>Physical</th>
<th>Communications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychosocial</td>
<td>Pearson’s correlation coefficient</td>
<td>1</td>
<td>0.652</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>Physical</td>
<td>Pearson’s correlation coefficient</td>
<td>0.652</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>Communications</td>
<td>Pearson’s correlation coefficient</td>
<td>0.715</td>
<td>0.605</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td></td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Discussion

The results showed that the total mean quality of end-of-life care provided by nurses in ICU was desirable while the quality of nursing care was undesirable in the psychosocial aspect and desirable in the communication and physical aspects. Similar results showed that the quality of care in the psychosocial aspect was desirable from the perspective of 31.6% patients and 92.6% nurses. In the communication aspects, quality of care was favorable from the viewpoint of 24.7% of patients and 56.8% of nurses. In the psychosocial aspect, 73.5% of patients stated that nurses would rarely introduce themselves to patients. Moreover, 77.9% claimed that new patients were rarely introduced to patients. In the communication aspect, 42.5% of patients stated that they were rarely assured of the confidentiality terms (19).

Although various studies conducted in Iran have demonstrated attention to functional and care aspects, studies have shown that lack of time and fatigue, and any factor that leads to a negative attitude and emotional stress in nurses, can result in emotional and physical withdrawal of nurses from patients, and in many cases, neglecting the emotional needs of patients and ignoring the aspects of care quality (20,15). Meanwhile, nurses are legally and morally responsible for the quality of care they provide and must be aware that both psychosocial aspects, as well as their expertise and skills in providing care, are effective in understanding the quality of care by patients.

Since nurses primarily aim to meet the basic needs through communication, intervention and assisting in treatment, these communications must be increased and
appropriate and accurate care must be provided so that patient satisfaction could be ultimately achieved (21). By better identification of the quality of nursing care for dying patients (in the face of death), we can provide evidence-based care and reduce the severity of illness in the final stages of life. Convincing evidence has shown that the inadequate association between the ICU group and the family members of patients is common, which can have serious consequences. When ICU staff are not well-trained in this aspect, families often regard communication with ICU personnel as a major concern. Accurate, sensitive, and inclusive communication is the key to the success of the discussion and the problems that arise in end-of-life care (16).

Statistical tests showed that the correlation between psychosocial aspect and the physical aspect was direct and significant since the increase in social psychosocial score was associated with an increased score of the physical aspect. In this respect, Izadji et al. (2013) marked that the highest priority of caring behaviors by nurses was related to a relationship based on trust and emotional and physical well-being of patients, whereas the availability and anticipation of the needs of patients was the last care priority, which could reduce the quality of care (22). According to the results of the present research, end-of-life care had a higher quality in the physical aspect, compared to communication and psychosocial aspects. In addition, the professional attitude of nurses toward the subject of death affected caring for dying patients. Therefore, discussion of attitudes toward death, including the inevitability of death and fear of death, must be included in the education of these individuals.

**Ethical Clearance:** This article is the result of a research project (a master’s thesis critical care nursing) approved by the in Shahid Rajaei Cardiovascular Research and Academic Center. After the necessary coordination and receiving the relevant licenses, the researcher met with the subjects at their working hour in the hospital to introduce himself, explain about the objectives of the research, and obtain oral and written consents prior to the research. Moreover, the participants were assured of the confidentiality terms regarding their personal information since the questionnaires would be completed anonymously.

**Conflicts of Interest:** None declared

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Effect of Crude Bacteriocin Isolated from Locally *Lactococcus lactis* on Cancer Cell Lines

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Abstract

*Lactococcus lactis* is a Gram-positive bacterium used widely in the production of cheese and buttermilk, but has moreover become well-known as the first genetically modified organism to be used alive for the treatment of human diseases. This study was designed to display the efficacy of lactococcus lactis, which was isolated from raw cow’s milk isolated by using the liquid MRS agar, culture, and microscopically was tested moreover, chemically was also tested to check the presence of (catalase, oxidase, protease production, gelatinease, clot formation). The isolates were confirmed using molecular diagnosis. The Bacterial isolates were developed on the M17 liquid medium at 30 °C for 24 hrs to obtain the raw bacteriocin after separation by centrifuge. The toxicity and bacterial susceptibility was tested as well the effect of Bacteriocin on *E.coli*, *P.aerugionsa*, *staph.areus*. The results showed a clear inhibitory effect of bacteriocin on pathogenic bacteria. The toxic effect of crude bacteriocin was investigated and its effect on cancer cell lines of breast cancer cells (MCF-7) lymphoid cell line (CCL-119) using different concentrations of the extract. The toxic effect was also examined on normal liver cell lines (WRL) with a 72 hrs incubation period. The results show a clear and high-significant effect of crude bacteriocin on cancer cells. It was also observed that the severity of toxicity increased with the increases of concentration with no effect on normal cell lines.

Keywords: lactococcus lactis, cow milk, bacteriocin, cancer cell lines.

Introduction

Lactic Acid Bacteria (LAB) are beneficial microorganisms and contribute to develop people fitness. These beneficial bacteria are alive microorganism which is necessary for mammalian healthiness. Probiotic bacterial strain comprise of bacteria belongs to *Bifidobacterium*, *Lactobacillus* groups, these group are varied include numerous kinds of bacterial strain, the probiotic term refers to nutritious matters. That benefit bacteria can grow above benefit strain and have ability to produce bacteriocin that found in raw caw milk, heterogenous group of bacteriocins with anti-microbial activity. Bacteriocins are protein that have different spectrum of activity mode of action, biochemical properties, genetic origin, and molecular weight. Probiotic bacterial strain can be isolated and identified from this diet product, LAB is one of the greatest groups of probiotic organisms (¹, ²).

*lactococcus* are helpful bacteria that inhibit the growth of pathogen, stimulate digestion, improve immune function and rise resistance to infectious diseases, in addition to its physiological aids in eliminating carcinogens. In spite of development in care, medication, recognition and the presence chemotherapeutic treatment, the disadvantage of cancer treatment remains non-specific in damaging the normal body cells, beside that cancer cell during the course of treatment has ability to improve resistance to chemotherapy, as a result, it’s difficult to treat cancer cell by using usual anticancer drugs, therefore, the request for different anticancer therapy is very necessary to develop a unique biological control methodology (³-⁵).

Bacteriocin is a complex protein produced from LAB and used therapeutically; it is rapidly digested by the protease enzyme and have an inhibitory effect on Gram-positive bacteria by directing the (cell envelope-associated mechanisms), several types of bacteriocins such as antibiotics and some class II bacteriocins target Lipid II found in the middle in the biosynthesis of peptidoglycan in the envelope of the bacterial cell in this way they are capable to inhibit peptidoglycan synthesis, other bacteriocins have the potential to destroy the target
cell by inhibiting gene expression (6).

The bacteria products that contain toxins, several proteins, bacterial enzymes, diverse peptides with low molecular weight products also have anticancer properties though, merely a rare of these peptides considered as anticancer, these peptides are modified by bioengineering technique (7, 8).

In this study the researchers display the efficacy of lactococcus lactis, which was isolated from raw cow’s milk isolated and was tested against microbes and breast cancer cell lines (MCF-7).

**Material and Method**

**Lactic acid bacteria Isolation and Identification**

Thirty sample of goat and cow milk were collected from different barn in Baghdad. The lactation period was under sterile conditions in hygienic cap and was transferred to Graduate Laboratory, treated within 1 hr. Milk sample was diluted with distilled water, after that a serial of dilution and culture on M17 and MRS media. All colonies cultures were purified by subculture on the same media and incubated at 30 °C for 24 hrs. Cultures were tested its morphology, physiological, biochemical features and molecular characteristics up to the genetic level.

**Identification of lactococcus bacteria by sequencing of the 16S rRNA gene**

The genomic DNA was isolated from the isolate. Amplification of the 16s rRNA gene was performed using the universal primers. Sequence analysis was carried out using NCBI online tools. DNA extraction from bacteria using G- Spin DNA extraction kit, intron biotechnology, Cat. No. 17045.

**Sequencing and Sequence Alignment**

The Polymerase Chain Reaction (PCR) products were alienated on a (2%) agarose gel electrophoresis and U.V spectrum at 302 nm after staining, gene sequencing was done by National Instrumentation Center for Environmental Management (NICEM) online at, biotechnology lab, machine used for DNA sequence was 3730XL, similar search was directed using Basic Local Alignment Search Technique (BLAST) which is available at the National Center Biotechnology Information online at (http:// www.ncbi.nlm.nih.gov).

**Screening and identification of bacteriocin producing strain**

The first step to detect the bacteriocin production was to assess the inhibitory effect of the isolated bacteria through microbiological tests by using disc diffusion test.

**Indicator bacteria used in the study**

Indicator pathogenic bacteria and biofilm formation belong to the different genera were used in capping plate method, and agar well diffusion method for selection bacteriocin activity, Gram positive bacteria *Staphylococcus aureus* and two strains of Gram negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* were collect from Medical City Hospital-Baghdad.

**Bacteriocin production media**

MRS and M17 modified media was prepared to increase bacteriocin production composed from:

<table>
<thead>
<tr>
<th>Percentage %</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>4%</td>
<td>Glucose</td>
</tr>
<tr>
<td>1%</td>
<td>Tween 80</td>
</tr>
<tr>
<td>12%</td>
<td>Skim milk</td>
</tr>
</tbody>
</table>

Effect of bacteriocin on CCL-119 and MCF-7 cancer cell line used in this study

*(MCF-7) cancer cell line*

Brest cancer cell was derivative from the pleural effusion from female 69 year old Michigan—cancer-foundation, (MCF-7) regret from a breast adenocarcinoma.

*(CCL-119) cancers cell line*

(ATCC No. CCL-119™) cell line collected from tissue peripheral blood type of T lymphoblast cancer occurred in patient with acute lymphoblastic leukemia.

*(WRL-68) normal cell line*

Hepatic cell line was gathered from human body (WRL 68) that exhibit morphology like to hepatic primary cultures and hepatocytes and, cells have been revealed to secrete alpha-feto protein ,albumin , and precise liver enzymes such as (alanine amino transferase) (9). Cytotoxicity influence crude bacteriocin that was
isolated from L. lactis and was carry out by via (MTT) protocol by using ready kit:

Contents of Kit:

a. Solution MTT (1 ml x 10) vials.

b. Solubilize solution (50 ml x 2) bottle.

Detection Caspase (9) Assay in CCL-119 and MCF-7 Cell Line (10).

This assay is a standardized glowing assay that measures activity of caspase-(9) that play a key role in the (intrinsic apoptotic pathway) of animal and human cells.

Contents of kit:

a- CaspaseGlo9 (Buffer1 × 10mL).

b- CaspaseGlo9 Substrate lyophilized.

c- (MG 132) Inhibitor 30 μL.

Result and Discussion

L. lactis Cultural Characteristics

Cultural for bacterial isolates carried on when isolated bacteria grown on MRS agar and M17 agar plates. On M17 agar, L. lactis colonies were seemed with distinctive features as a pure white colony, the size of the colony was small (2-3 mm). After that, identification of the isolates was carried out by measuring its ability to form clot on both skim milk, and lysis skim milk.

Molecular identification of L. lactis subsp.lactis

After genomic extraction of the DNA from L. lactis which was carried out via (PCR) and gel electrophoresis and recognize the L. lactis using 16S RNA, PCR was accomplish on an unpolluted DNA template to every bacterial isolates particularly for intensification of (16S RNA) by using (oligonucleotide primer), which increase the existence of DNA.

Thermo stable polymerase with (Mg ions) in a buffer solution was added to PCR, denaturation was used to separate both strand, and then temperature was lowered to start the primer annealing. The initial temperature was around (72 °C) which is ideal for the polymerase to extend the primer with the incorporation of (dNtp), the results indicate the bacterial isolates were L. lactis subsp. lactis . The primer that was use produced (1250 bp) and was appear in electrophoresis gel and was measured using a (100 bp) of DNA ladder.

Lactococcus lactis strain CAU9592 16S ribosomal RNA gene, partial sequence Sequence ID: MF098001.1Length: 1362

<table>
<thead>
<tr>
<th>Score</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
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<tbody>
<tr>
<td>1294 bits(1434)</td>
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<td>737/750(98%)</td>
<td>0/750(0%)</td>
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</table>

Query

GCTGACGAGTGGCGGAGGGTGAGTAATGTCTGGGAAAACTGCCTGATGGAGGGG
GATAAC 60

Sbjct

GCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAAACTGCCTGATGGAGGGG
GATAAC 78

Query

TACTGGAAACCGGTAGCTAATACGCATAACGTCGCAAGACCAAAGAGGGGGACCT
TCGGG 120

Sbjct
Sbjct
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TCGGG 138

Query
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Sbjct
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Sbjct
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Query
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Sbjct
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Query
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GGAAG 360//

Sbjct
GCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGA
GGAAG 378

Query
GGAGTAAAGTTAATACCTTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCAGGCTA
ACTC 420

Sbjct
GGAGTAAAGTTAATACCTTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCAGGCTA
ACTC 438
It was found that the greatest number of the isolates can prevent the growth of *S. aureus*, *p. aeruginosa* and *E. coli* (11). Consuming raw cow milk and fermented products can assist human health, fitness and defend the body against probiotic infection (12).

Raw camel milk analysis usually shows the presence of *L. pentosus*, *L. plantarum*, and *L. lactis* as probiotic lactic acid bacteria (13) and it could be a source to isolate probiotic (LAB) strains, furthermore it can be considered as an antimicrobial against pathogenic bacteria due to the presence of bacteriocin production (13).

**Bacteriocin cytotoxicity on normal and cancer cell lines using MTT assay**

MTT assay was achieved to determine the (IC$_{50}$) for crude bacteriocin on human breast cancer cell line (MCF7), lymphoblast leukemia (CCL 119), and normal embryonic liver cell line (WRL-6).

Data analysis carried out in ($\mu$g/ml) and the log value ($\mu$g/ml) and plotted in graph cushion prism using log Inhibitor against regularized response curve. Greatest values were selected for the maximum significant (IC$_{50}$) values. Cell viability at each time-point was firm by MTT colorimetric assay.

Figure 1 shows crude bacteriocin was potent cytotoxic effect CCL-119 cells and the (IC$_{50}$) concentration. The value of crude bacteriocin after 24 hrs of incubation at 37 °C was 322.8 µg /ml.

Table 1 shows the diameter of inhibition zone of crude bacteriocin on pathogenic biofilm formation bacteria with different ammonium sulfate saturation

**Table 1: Inhibition zone diameter with different ammonium sulfate**

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Ammonium Sulfate Saturation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(0-20)%</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>11</td>
</tr>
<tr>
<td>Pseudo</td>
<td>20</td>
</tr>
<tr>
<td>E.coli</td>
<td>15</td>
</tr>
</tbody>
</table>

Figure 2 shows that the crude bacteriocin has moderate cytotoxic effect on MCF7 cell line, and the IC$_{50}$ after 24 hours of incubation at 37 °C was 614 µg/ml.

Figure 3 show that the crude bacteriocin has little cytotoxic effect on WRL-68 cells and the IC$_{50}$ after 24 hrs and 37 °C (1486 µg/ml).

From above, it is clear that the CCL-119 cells has high sensitivity toward crude bacteriocin than MCF-7 and human liver WRL-68.
The cell wall proteins of *L. lactis* have an obvious cytotoxic effect in growth inhibition of *Acute* myeloid leukemia cell line as well as Human Epithelial type 2 tumor cell lines *in vitro* depending on both concentration and time (14). Similar results recorded by (Vajihe Akbari, 2017), he found that bacteriocin produced by *lactococcus lactis* have cytotoxic effect on MCF7 breast cancer cell lines and slight cytotoxic effect on Human umbilical vein endothelial cells (HUVECs) normal cell line (15). Polysaccharides show less cytotoxic effect toward normal kidney (16).

The exact mechanism of anti-cancer effect of NISIN (Substance which is a mixture of related polypeptides) remains to be unexplained, studies have suggested several mechanisms including cell membrane disruption and pore formation that cause the altering to the level of intracellular ions, which lead to the changing in the trans membrane potential, and induct the cell cycle arrest and leads to cell apoptosis (17).

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**Conflict of Interests:** The authors declare that they have no conflict of interest

**Source of Funding:** Self–funding

**Ethical Clearance:** The researchers already have ethical clearance from College of Science, Mustansiriyah University, Iraq

**References**


New Bio-Therapeutic Candidate for Pancreatic Cancer

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Abstract

SIRT1 is NAD⁺-deacetylases, several histone and non-histone proteins which their involvement in metabolic processes, cell growth, apoptosis, and senescence are well known. Several Sirtuins targets are implicated in cancer. SIRT1 is both an oncogene and tumour suppressor, and it can act in this capacity depending on the kind of tissue and the cancer etiology. Subsequent studies in this field are going to make evident the exact function of SIRT1 at the cancer site and it is hoped that new chemotherapeutic functions of SIRT1 activators are going to be determined. In accordance with this, it is suggested that very selective ligands such as aptamer was created and investigated in various pancreatic cancer cell lines for the regulated activity of SIRT1. This study seeks to establish the therapeutic impact of the activator SIRT1 aptamer as a pharmacological model of pancreatic cancer by evaluating the impacts of activators SIRT1 (aptamer) on the growth of a series of human pancreatic cancer cell lines (AsPC-1, Capan-2, BxPC-3). Results gotten from in vitro cytotoxicity assays revealed that circular aptamer BAS inhibited the growth of pancreatic cancer cell lines [BxPc-3 (80%), Capan-2 (83%) and Aspc-1 (82.8%)] at 72h with IC₅₀ 0.55, 0.5, 0.76 µM respectively. Importantly, circular aptamer showed no reduction of cell viability on the non-pancreatic cancerous H6c7 cell line, implying it might be safe to non-cancerous tissue. Our results indicate that the SIRT1 activated by aptamer could a promising targeted therapeutic approach for pancreatic cancer.

Keywords: aptamer, pancreatic cancer, SIRT1.

Introduction

Pancreatic cancer is considered as a deadly tumor with no improvement in its prognosis regardless of the recent progress in cancer interventions. Many of the interventions for pancreatic cancers are associated with disappointing results. Though curative surgery is associated great benefits, it is hard to achieve in most advanced pancreatic cancers. Interventions such as conventional chemotherapy, radiation therapy, and even targeted chemotherapy have shown disappointing results. Therapies for target tumors by using monoclonal antibodies are the most thriving cancer therapies ¹; however, there are different limiting factors. These limiting factors include high costs and low penetration to tissue ². There is a need for the development of more efficacious and cost-effective targeted interventions. Aptamer (ssDNA, RNA or peptide oligomers) have been extensively studied for their therapeutic properties ³. Al –Sudani, 2017 was selected a circular aptamer against SIRT1 as used to treated many of cancer cell by activation of SIRT1 ⁴. Deacetylation of histones and non-histones proteins is by SIRT1. These histones and non-histone proteins are implicated in a variety of biochemical activities. These biochemical activities include metabolism, cell growth, apoptosis, and senescence ⁵. The function of SIRT1 in tumorigenesis and cancer advancement is not fully understood but it was stated that its function could be tissue-type and context specific ⁶. Pancreatic adenocarcinoma up-regulated factor (PAUF) is known to induce a swift spread of pancreatic cells by up-regulating b-catenin. This is based on the postulation that b-catenin could be a target molecule for pancreatic cancer therapy with a speculation that whether there is SIRT1 suppression of b-catenin in pancreatic cancer cells that express PAUF (Panc-PAUF). It is suggested that evaluation of such suppression could result in inhibition of the proliferation of these cells. The amounts of b-catenin protein and its transcriptional role in Panc-PAUF cells are (SIRT1 activator) siRNA improves b-catenin expression and transcriptional activity. Through the down-regulation of cylin-D1, activation of SIRT1 leads to inhibition of the growth of Panc-PAUF cells. Cyclin-D1 is a
target molecule of b-catenin. This project aimed to evaluate the therapeutic effect of the activator SIRT1 aptamer (BAS aptamer) as a pharmacological model for pancreatic cancer cells.

**Methodology**

**Cell culture**

Human pancreatic cancer cells: AsPC-1, Capan-2, and BxPC-3 were gotten from American Type Culture Collection ATCC (Middlesex, UK). Human pancreatic normal cells: H6c7 were bought from Kerafast (Boston, USA). Capan-2 cells was preserved in McCoy’s 5A Medium, AsPC-1 and BxPC-3 cells were stabilized in Roswell Park Memorial Institute-1640 (RPMI-1640) medium, all media were enhanced with 10% fetal bovine serum FBS and 1% L Glutamine as well as to 1% Penicillin-Streptomycin-Amphotericin B 100X as an antiseptic. H6c7 cells were maintained in Keratinocyte SFM + EGF + bovine pituitary extract (Invitrogen). Then they were supplemented with 1x antibiotic-antimycotic (Gibco). Cells were cultured in 75cm² flasks and incubation carried out in 5% CO₂/95% humidified air at 37°C. Once the cells reached 90% confluency, flasks containing Capan-2, BxPC-3, and AsPC-1 were kept under sterile conditions. The cells were subjected to washing with 5 ml of phosphate buffered saline solution (PBS) and then incubate for 2 min in trypsin solution at 37°C to permit cells to separate from the bottom of the flask. Addition of an equal volume of complete growth media was done, and the cell suspension was moved into a 50ml conical tube. Centrifugation of the cells was performed at 1200 rpm for 3 min. Separation of the supernatant was done, and the cell pellet underwent re-suspension in fresh supplemented growth media. Cells were counted under the microscope on a haemocytometer and use as required. The cells were stored at -80 °C for 24hrs and then were stored under liquid nitrogen.

**Cell viability by MTT assay**

The MTT assay was used assessing the effects of aptamer on pancreatic cancer cell viability. A 100 μl from all cells suspensions (Capan-2, BxPC-3, AsPC-1 and H6c7) were dispense into 96-well flat-bottom tissue culture plates at concentrations of 5 x 10³ cells per well and were incubated 24h under standard conditions; 4 x 10³ cells/well for 48h incubation, and 3 x 10³ cells/well for 72h incubation. After 24 h, the cells would have treated with 2.5 μM circular aptamer (BAS). After a recovery period 24h, 48h and 72h, the cell culture medium was removed and cultures was incubated with medium containing 30 μl of MTT solution (3 mg/ml MTT in PBS) (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) for 4h at 37°C. After 4h this medium was done with by gentle inversion and tapping onto paper. Control wells receive only 100 μl growth media. 100 μl of dimethyl sulfoxide (DMSO) was added to all wells, the plates were then kept at room temperature in the dark for about 15-20 min. The absorbance of each well was measured by multi-scan reader at a wavelength of 540 nm and correcting for background absorbance using a wavelength of 650 nm. IC₅₀ was evaluated by MTT assay at 72h after the cells exposure to aptamer. The concentration range used for circular aptamer (BAS) are, 0.0078, 0.0156, 0.0312, 0.625, 0.125, 0.25, 0.5, 1.00 μM.

**Immunofluorescence microscopy**

The seeding of the cells was performed at roughly 10,000 cells per well in 96-well clear bottom imaging tissue culture plates. Eighteen hours later, cells were treated with 1μM circular aptamer (BAS) and incubation done in 5% CO₂/95% humidified air at 37°C overnight, after that the washing of the cells was done thrice in PBS, fixing for 5mins at room temperature with Formalin 4%, washing 2 times in PBS, then will be permeabilised by 0.5% Triton X-100 for 5mins, washing 3 times with PBS; nonspecific binding was obstructed with 3% FBS for 1h at room temperature, then the blocking solution was removed, followed by an addition of 100 μl/well of Anti-Sirtuin1 antibody to the cells. After that incubation was done overnight at 4°C in a wet tray. The following day, cells will be washed thrice in PBS, and incubated for 2h in dark at room temperature with 1:2000 Alexa Fluor 546 goat anti-mouse IgG (AF546)/1% FBS in PBS. Thereafter the washing of the cells was done thrice in PBS. At the end, the preparation was treated with 10 μl mounting medium containing DAPI for staining cell nuclei. It was left for 1hr before the microscopic examination using fluorescence microscopy.

**Results**

**Cell viability by MTT assay**

The cell viability of circular aptamer, at 2.5μM concentration shows it is extremely active on the different pancreatic cancer cells up to a period of 72 hours. As demonstrated in figure 1, BxPC-3,Capan-2 and Aspc-1 cells demonstrated to be highly sensitive with an increase response dependent on time (cell death around
80%, 83% and 82.8% at 72h, respectively). Importantly, circular aptamer showed no reduction of cell viability on the non-cancerous H6c7, implying it might be safe to non-cancerous tissue. The IC50 of circular aptamer was very low concentration (0.55, 0.5, 0.76 µM) in BxPc-3, Capan-2, and Aspc-1 cells respectively.

Figure 1: Cell viability studies on different pancreatic cancer cell lines, at 24, 48 and 72h of incubation. All samples were run in triplicate, control is expressed as 100% viable cells. Cells were challenged with circular aptamer at 2.5µM. At 24h the cell viability was as: 79%, 28, 37, and 67 for Aspc-1, BxPc-3, Capan-2, and H6c7 respectively. At 48h the cell viability was as: 89%, 24, 19, and 56 for Aspc-1, BxPc-3, Capan-2, and H6c7 respectively. At 72h the cell viability was as: 88%, 20, 17, and 17.2 for Aspc-1, BxPc-3, Capan-2, and H6c7 respectively.

Determine the Location of Circular Aptamer by Fluorescence Microscopy

To determine the location of the circular aptamer, fluorescence microscopy was used in this experiment. The circular aptamer was labelled with green fluorescent, DAPI (4’, 6-Diamidino-2-Phenylindole, Dihydrochloride) was utilized for locating the nuclei and a Texas Red (sulfonyl chloride) antibody for SIRT1. The cells were dosed with circular aptamer at a low concentration to avoid cell death depending on their IC50. Thus, figure 2 showed that fluorescence microscopy is very useful tool for studying the location of circular aptamer in cancer and non-cancer cell lines. Cells treated with circular aptamer were brightly fluorescent and over-expression of SIRT1 as showed in figures 2. In contrast, there was no observable fluorescence signal from control H6c7 cells treated with the circular aptamer.

As a figure 2 shown the localization of SIRT1 in BxPc-3 and Capan-2 cells in the cytoplasm of these cells, while in AsPC-1 and H6c7 cells, the SIRT1 was localized in the nucleus. These results strongly suggested that this aptamer is highly affinity and selectivity binding with SIRT1 as the results of fluorescent imaging shown that when the aptamer present in this cancer cells it could be activated and increased the expression of the SIRT1 enzyme.

Figure 2: Fluorescence microscopy analysis of circular aptamer binding to SIRT1 enzyme in BxPC-3, Capan-2, AsPC-1 and H6c7 cell lines. Cells pretreated with 1 µM circular aptamer. Circular aptamer was labelled with green fluorescent, nuclei were stained with DAPI (Blue) and a Texas Red antibody for SIRT1 (Red). Fluorescence intensity were measured by luminometer microplate Readers, fluorescence measurement system with an excitation wavelength of 395, 359 and 596 nm and an emission wavelength of 509, 461 and 615 nm of GFP, DAPI and Texas Red respectively. Images were taken at a magnification of 100X.

Discussion

This study investigated oligonucleotide aptamer and its usefulness for anticancer drug discovery. Our study has provided information concerning the usage of MTT assay in assessing the inhibitory effect of SIRT1-aptamers on viability of BxPc-3, Capan-2, and Aspc-1 cell lines and non-cancer cells (H6c7). Our experiments using Aspc-1, BxPc-3, and Capan-2 cancer cell lines demonstrates that circular aptamer BAS has a growth suppressor characteristics. Kabra, (2010) was found that knockdown of SIRT1 raises the percentage of tumour advance by promoting cell spread, whereas over-expression of SIRT1 decreases tumour induction and expansion in nude mice 8. This result is in agreement with our suggestion that the pharmacological activation of SIRT1 by aptamer causes reduction of the rate of cell viability in Aspc-1, BxPc-3, and Capan-2 cell lines as shown in figure 1. Our results indicate that SIRT1 works as a context-dependent tumour suppressor. Also activation of SIRT1 causes suppression of tumour initiation and promotion of cell death. The anti-apoptotic role of SIRT1 is well known and it is rooted in the assumption that SIRT1 acts as an oncogene. However, it was revealed that transgenic mice over-expressing SIRT1 decreased the progress of neoplasia in the intestine caused by ApcMin mutation, this suggests a tumour inhibitory function of SIRT1 9. Many studies have pointed out that
SIRT1 could behave as an oncogene. This is possible because of the higher association of normal expression of SIRT1 level in specific tumors than normal tissue. In contrast, Kabra, (2010) established that SIRT1 levels are uneven in various phases of colon cancer tumours. It has been reported that SIRT1 has both oncogenic and tumour-suppressive functions. The expression of SIRT1 at high levels shows that SIRT1 is effective in inducing G1 arrest. Indeed, our results confirmed these previous suggestions that activators of SIRT1 by circular aptamer could have therapeutic potential as an anti-cancer target. Another condition is that activators of SIRT1 could affect cancer blocking impacts by promoting the growth-inhibitory impact of SIRT1 in cancer cell line, and revealed the decrease level of intracellular ROS production. Through direct deacetylation the inactivation of the p65 subunit of NF-κB is caused by SIRT1. Inhibition of NF-κB causes suppression of the iNOS (inducible nitric oxide synthase are a family of enzymes catalysing the production of nitric oxide (NO) from L-arginine) and thus could decrease the cellular ROS load. To advance our study the role of SIRT1, circular aptamer was used to study the activity of SIRT1 in (BxPc-3, Capan-2, and Aspc-1) cells. The results observed in this study are consistent with several research groups have reported that resveratrol which activator SIRT1 was inhibited much more cancer cells proliferation such as Caco-2, MCF-7, A549, U2OS, MDA-MB-468 and HepG2. Interestingly, it has been demonstrated that there is not effect to used SIRT1-aptamers on H6c7 cells viability, which indicating that circular aptamer have actively killing cancer cells without affecting normal cells. The IC_{50} of circular aptamer was very low concentration (0.55, 0.5, 0.76 µM) in BxPc-3, Capan-2, and Aspc-1 cells respectively. This low concentration of IC_{50} is very good results because the low dose of circular aptamer can suppress the spread of cancer cells. To study the localization of SIRT1 in these cells, fluorescence microscopy was used and the results were showed the different location of SIRT1 between cytoplasm and nuclei depending on the type of cells. As a figure 5 shown the localization of SIRT1 in BxPc-3 and Capan-2 cells in the cytoplasm of these cells, while in AsPC-1 and H6c7 cells, the SIRT1 was localized in the nucleus.

In Conclusion, the results provided here indicate that circular aptamer could be effective in treating pancreatic cancer because its properties of a growth suppressor for specific killing of the tumor cells only avoiding unpleasant side effects from damage to the rest of the body. Additional research works are required, for instance, the cellular mechanisms of action of SIRT1 in aptamer-mediated apoptosis still needs a lot of study.

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Ethical Clearance- Taken from Mustansiriyah University, Iraq.

Source of Funding- Self

Conflict of Interest - None

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Infanticides in Dakar: Medico-Legal Aspects

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Abstract

Infanticide is a crime that evokes emotion and misunderstanding in the general population. The overall objective was to assess the current situation regarding the killing of new-born children in Dakar. This is a cross-sectional, descriptive study spread over a 12-year period from 1 January 2004 to 31 December 2015. The study population was women accused of infanticide incarcerated in the prison and correctional facility of Liberté 6 and who had either been tried or who were awaiting trial during the study period. The 46 cases tried concerned 47 newborns and one mother having killed both her babies. The victims were found especially in the mother’s home (28 cases) For all women in our sample, the murder was motivated by personal, social and economic factors. The average age of the mothers was 25.5 years with the youngest being 17 years old and the oldest 41 years old. These detention periods ranged from 2 to 6 years. The average length of pre-trial detention was 3.26 years, The preventive strategies put in place in some countries are proof that prevention against these acts remains possible.

Keywords: murder, newborn, pregnancy, asphyxia

Introduction

Infanticide is a crime that evokes emotion and misunderstanding in the general population. Our desire to better understand this issue of maternal child murder stems from the magnitude of the phenomenon. The overall objective was to assess the current situation regarding the killing of new-born children in Dakar. The specific objectives were to describe the epidemiology of infanticides in the general population of the Dakar department; The aim was also to describe the socio-demographic, economic, cultural and psychopathological factors involved in the occurrence of this phenomenon and to propose strategies to prevent such acts.

Material and Method

The study took place in two sites in Dakar: The Court (Palais de Justice) and the Detention and Correctional Facility (Maison d’Arrêt et de Correction) of Liberté 6. As of 27 April 2016, there were about one hundred prisoners, including 26 women suspected of infanticide, who agreed to answer our questions. This is a cross-sectional, descriptive study spread over a 12-year period from 1 January 2004 to 31 December 2015. The study population was women accused of infanticide incarcerated in the prison and correctional facility of Liberté 6 and who had either been tried or who were awaiting trial during the study period. We included in this study women convicted of infanticide whose criminal records are exploitable and women accused or detained for infanticide at the Liberté 6 Detention and Correctional Facility and who responded to our questionnaire. The data was collected on the survey sheet. Data entry and analysis were respectively performed on Epidata Entry version 3.1 and Epidata Analysis software.

Results

The results which follow concern both convicted women and women accused (pre-trial detention) of infanticide. We identified 70 cases of infanticide over the period January 2004 to December 2015 in the Dakar region. Of the 70 cases recorded, we have a group of 46...
women who have been tried and sentenced and another group of 24 women who are in pre-trial detention or awaiting trial. In the latter group, the women concerned were incarcerated in the penitentiary establishment of Liberté 6. Due to the secrecy of the investigation, the study of the group of accused persons concerned only demographic, socio-economic and cultural data. The study of the convicted group covered demographic, socio-economic, cultural and forensic data. The 46 cases tried concerned 47 newborns and one mother having killed both her babies.

The facts were brought to the attention of the police authorities by denunciation of individuals in 35 cases and by judicial reporting of a hospital structure in 11 cases of which 8 of these were due to an immediate postpartum haemorrhagic complication and 3 due to suspicious death reports.

Of the 46 files available, almost all victims were found. In two cases, the bodies of the new-borns were never found. The victims were found especially in the mother’s home (28 cases), in a variety of places (closets, bedroom, backyard of the house), in garbage sites near the home (dustbins, rubbish bags or garbage dumps) in 8 cases, in deserted places (forest, bush) in 4 cases, and 4 bodies were discovered in the hospital after suspicious death reports.

The new-born’s found had been disposed of in a number of ways. Most victims were placed in plastic bags (88.6%) or wrapped in a cloth (9.1%). In one case, the victim was found naked.

In almost all cases, the women had confessed to their actions (95.7%). Only two of them did not recognize their act.

In the majority of the 44 cases, the women implicated admitted that they had acted alone. The complicity of family members was mentioned in two cases. The mother of one of the perpetrators was identified as an accomplice in one case and in the other, it was the father and the mother-in-law.

For all women in our sample, the murder was motivated by personal, social and economic factors. It was motivated by fear of dishonour in 25 cases (35.7%), despair in 14 cases (20%), social isolation in 10 cases (14.3%), extramarital paternity in 6 cases (8.6%), abandonment of the father in 8 cases (11.4%), social insecurity in 6 cases (8.6%) and for reasons not specified in 1 case (1.4%). None of the women in our sample had been previously arrested.

The average age of the mothers was 25.5 years with the youngest being 17 years old and the oldest 41 years old. The median was 25 years old, with a standard deviation of 5.3. At the time of the incident, 37 women (52.8%) were single and 20 women (28.6%) were divorced. Eleven women were married (15.7%) and 2 were widows (2.9%).

The women in our sample had a generally low level of education. The majority of these women - 51 cases (72.8%) - were uneducated. None of the women in our study sample had received sexual education. A very low level of knowledge about contraception was found in 2 women (2.9%) and 68 women disregarded contraceptive techniques.

Of these, 57 women were working as domestic workers (81.4%). 8 women (11.4%) were self-employed (food seller, shopkeeper, telecentre manager, seamstress). One woman was employed in the public service (garbage collector) (1.4%). Two women were students (2.9%) and two others were housewives (2.9%) at the time of the incident.

At the time of the incidents, 3 women were living alone (4.3%). Twenty-eight women lived with their children (40%) and they were not in a relationship. Other women lived with extended families (47.1%), or in couples (10%). For the latter, the husband was present in 3 cases (4.3%) and for the other 4, the husband had emigrated.

The women involved were almost all (67 cases) from economically disadvantaged backgrounds (95.7%). Only 3 of them came from an average economic background (4.3%).

The women involved had had an unhappy childhood marked by emotional deficiencies, a lack of communication and a climate of loneliness. They all came from unskilled families.

In the majority of cases, 68 female respondents had an unstable relationship (97.1%) with the alleged father of the victim at the time of the incident.

For the majority of women (n=54) in our sample, the pregnancy was the result of an extra-marital relationship (84.3%). Pregnancy occurred in marriage for 11 cases (15.7%), including 5 pregnancies attributed to the legal
spouse (7.1%) and 6 pregnancies resulting from an adulterous relationship (8.6%). The majority of women (n=65) had not informed their family or father about their pregnancy.

It was the first pregnancy and childbirth for 21 of the women (30%) and the other 49 women had already been pregnant at least once (maximum 6 pregnancies) and had at least 1 child alive at the time of the incident.

The majority of women (n=44) did not have any psychiatric disorders before the event. Two of them (4.4%) had in the past been followed for psychiatric disorders. One presented a table of a stabilized acute psychosis while for the other the mental pathology had not been diagnosed.

Regarding the notion of addictive behaviours, none of the women in our sample presented an addiction to any substance.

Of the 46 accessible files, age was not specified in 2 files and in the in 2 files where the bodies were not found, the age was not determined. As for the 43 cases where identification was possible, 41 cases (95.3%) were neonaticides and 2 cases were infanticides (4.6%). The sex remained undetermined in some victims (6 cases) due to the advanced state of decomposition of some bodies (2 cases), the fact that victims were never found (2 cases), and the fact that the sex was not specified in two cases. Among the victims whose sex was known (41 cases), it was more often than not a girl (24 cases) rather than a boy.

Childbirth occurred in almost all cases in the mother’s home (89.1%), either in the mother’s bedroom (52.2%) or in the toilet (30.4%). Rarely had it taken place outside: outside the mother’s bedroom (veranda, backyard of the house) (6.5%), in the hospital (4.3%) or in a deserted place (6.5%).

Death occurred most often in the mother’s home (89.2%), particularly in the mother’s bedroom (60.9%), in the toilet (28.3%) and rarely outside in a deserted place (10.9%).

Of the 46 accessible files, an autopsy had been performed in 25 cases. In the other 21 cases, it could not be done, 7 cases due to the advanced state of decomposition of the bodies and in 2 cases because the bodies of the newborns were never found. Almost all of the victims (23 cases) were viable and had breathed because the hydrostatic docimasia was positive. In 2 cases, the victims were declared non-viable. A congenital malformation anomaly with a type of transposition of large vessels was noted in 1 case. In the other victim, it was reported that the child probably did not breathe and that it was stillborn.

The cause of death was known in 22 of the 25 cases. Most deaths were due to asphyxia (12 cases) by suffocation: manual (1 case), in a plastic bag (2 cases), with a loincloth (1 case) or by burial in the ground (1 case). Manual strangulation (4 cases), or attachment to a piece of cloth (1 case) was also used. Submersion took place in cesspools (2 cases). The other causes of death were related to combined mechanisms (5 cases) by association between polytrauma and internal bleeding (2 cases), internal and external bleeding (2 cases) and internal bleeding with strangulation (1 case). Finally, death rarely occurred as a result of fatal injuries (1 case).

All women in the study either accused or awaiting trial had been subjected to pre-trial detention. Among the 46 cases that were judged, variations in the length of detention were noted.

These detention periods ranged from 2 to 6 years. The average length of pre-trial detention was 3.26 years, the median was 3 years with a standard deviation of 0.855. None of the women involved in our investigation had been placed in a psychiatric centre.

The final decision regarding the women involved in our study population was as follows: of the 46 women involved, 41 were sentenced to prison terms, while the other 5 were dismissed and the charges against them dropped. Sentences ranged from 6 months to 7 years. The most common sentence was 5 years of hard labour (31 cases).

**Discussion**

This typical profile is contrasted in our study. We found women with similar profiles but with certain specificities. Indeed, the young age reported as one of the common characteristics is neither validated in recent studies nor in our cases. Shelton et al and Tursz and Cook had found an average age of between 21.7 and 26 years, and in our study the average age was 25.5 years. In addition, Beyer et al have shown in their work that there are both young women and women in their 30s and 40s. The same is true for our cases. These women were in contrasting couple situations. As in our
study, these women had already had previous children or pregnancies and were predominantly employed as domestic workers.

The women involved in our study, as well as those described in a number of studies had no psychiatric history themselves, nor in their parent’s generation, and had no criminal record. As for the psychopathological profile of these women, it remains unknown in our cases because no expertise was carried out. But it is noted, with considerable consensus, that a psychiatric disorder affects only a minority of women who have committed an infanticide (neonaticide). Several authors reported in their work that the women concerned did not have an obvious psychopathological or psychopathic profile at the time of the crime.

In the literature, the common characteristic concerning women who commit this crime was an unwanted pregnancy hidden from close family and friends. This is the case in our study. This could be explained by the fact that these pregnancies occurred under conditions subjectively disadvantageous for these women. Tillier in his historical study reported that the women concerned buried the body or threw it into the bush. Neonaticide was traditionally considered in a sociological context but it is still relevant today. In the literature, as in our study, the neonaticide act was dominated by psycho-social factors associated with socio-cultural and economic constraints (fear of dishonour, despair, fear of social rejection, abandonment of the father, extramarital paternity, social insecurity).

Despite the introduction of new reforms, prolonged pre-trial detention continues to be a reality in prisons in Senegal. In neonaticide cases, very few studies focus on criminal decisions. The sentences for infanticide have varied widely throughout history and continue to do so, ranging from the greatest tolerance to the greatest rigour. Much more, juries and the court show leniency towards mothers who acknowledge the facts.

Many preventive strategies have been used around the world to reduce the number of neonaticides. Legislation is evolving slowly but not always in the direction of decriminalization of abortion.

Conclusion

Early infanticide is a crime that has existed since ancient times. In the Senegalese social context, particularly in Dakar, this crime appears to be the act of distress of a woman from a physically, economically and culturally vulnerable environment. She is faced with the stigmatization of an illegitimate birth against the backdrop of a lack of education, the inaccessibility of contraception, as well as the prohibition of abortion.

In our study, 70 cases were identified during the study period but only 46 trial cases were available. With regard to the social profile of these women, there is no typical, classical profile as described in the Anglo-Saxon literature. Despite the difficulties that neonaticide presents in terms of forensic liability, it is a largely avoidable tragedy. The preventive strategies put in place in some countries are proof that prevention against these acts remains possible. Multidisciplinary coordination between health and justice professionals as well as social actors would be necessary to set up preventive strategies and avoid such tragedies in the future.

Conflict Interest: I certify, Pr Mohamed Maniboliot Soumah, that this article is a personal work. It has not been proposed to another scientific magazine. This study is not sponsored by any organization. I submit this article Indian Journal of Forensic Medicine & Toxicology in conformity with the rights transferred to the journal.

Sincerely yours.

Source of Funding: This study is not sponsored by any organization.

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References


Changes in Some Urinary Variables After Potassium Citrate Therapy In Patients With Recurrent Urinary Stone Disease In Tikrit City, Iraq

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Abstract

Objective: to determine the effect of oral potassium citrate (k-citrate) on urinary calcium, magnesium, volume, and pH in patients with recurrent stone disease.

Patients & Method: Interventional clinical trial study was implemented from June 2016 to February 2019 in Tikrit city, Iraq. 94 patients with recurrent urinary stone disease were enrolled in this study. Oral k-citrate therapy was given to patients in a dose of 15 meq three times daily for 2 weeks. Patients instructed for 24 hours urinary collection before and after therapy. Urinary calcium, magnesium, volume, and pH were measured before and after therapy to examine the effect of k-citrate.

Results: mean age was 38.5 (SD 17.4) years. Male patients were 64% while 36% of patients were females. Mean urinary calcium level decreased significantly after k-citrate therapy, p value < 0.001. Mean urinary volume and pH increased significantly after therapy, p value < 0.01 and < 0.05 respectively. No significant change was identified in mean urinary magnesium level after therapy, p value = 0.14. No adverse effects related to k-citrate therapy were reported by patients during study period.

Conclusion: Urinary volume and pH increased significantly in patients after potassium citrate therapy. Also k-citrate therapy significantly produced marked decrease in urinary calcium level. No change was observed in mean urinary magnesium level before and after k-citrate therapy. No adverse effects related to k-citrate therapy were reported by patients during study period.

Keywords: Urinary calcium, Urinary magnesium, Urinary volume, Urinary pH, Potassium citrate.

Introduction

Renal stones have affected human beings since the earliest civilization. The etiology of stones remains hypothetical. Developments in the surgical treatment of urinary stones have overtaken our understanding of their causes. For patients, it is important to ensure complete metabolic evaluation that results in proper medical therapy and lifestyle changes to help decrease recurrent stone disease. Lacking such follow-up and medical involvement, stone recurrence rates can reach 50% within 5 years. [1]

The life time prevalence of renal stone disease is evaluated at 1% to 15%, with the likelihood of having a stone is different according to age, gender, ethnicity, and geographical location. [2] Hypercalciuria, hypocitraturia, hyperoxaluria, and hyperuricosuria are major metabolic disorders observed in patients with calcium containing stones. [3]

Potassium citrate is one of the most important therapies on the way to alkalinization of the urine and management of hypocitraturia. Potassium citrate is an
oral agent that increases urinary pH actively by 0.7–0.8 pH units. Classic dosing is 10-60 meq in three or four divided doses per day. Most studies support the reduction in stone recurrence rates for patients receiving citrate treatments. [4, 5]

Certain mechanisms are presumed for k-citrate to decrease stone formation. Citrate attaches calcium ions in the urine, creating a stable complex. It was suggested that this mechanism decreases accessible urinary calcium, consequently inhibiting calcium and oxalate precipitation. [6] Citrate as well diminishes the spontaneous nucleation of calcium oxalate. [7] The inhibitory action of magnesium is gained from its attachment with oxalate, which decreases oxalate and calcium oxalate supersaturation. [2]

Some randomized controlled trials of oral k-citrate have identified a drop in stone formation in patients with recurrent stones taking a variety of citrate medications. [4, 8, 9] In another one more study, no difference was found in stone recurrence. [10]

The aim of this study is to assess the consequence of oral k-citrate on urinary parameters like volume, pH, calcium, and magnesium in patients with recurrent stone disease in Tikrit city, Iraq.

Patients and Method

This interventional clinical trial study was performed during the period from June 2016 to the end of February 2019 in Tikrit city in Iraq. 100 patients with recurrent attack of urinary stone, recruited from Tikrit teaching hospital and private clinic, were included in this study. At the end of the study, 94 patients completed the study treatment, and 6 patients were lost to follow-up.

Patients with severe urinary tract infection, borderline renal function, diuretic therapy, patient with single functioning kidney, and pregnant women were excluded from this study. Patients instructed for 24 hours urinary collection before k-citrate therapy to get baseline measurement of urinary volume, pH, calcium, and magnesium. Oral k-citrate therapy was given to patients in a dose of 15 meq three times daily. Patients were encouraged to discontinue all medication during the study time. After 2 weeks of therapy, patients were instructed to collect urine for 24 hours for final assessments.

Urinary calcium was determined by semi-auto chemistry analyzer (expected values in 24 h in urine is 100-300 mg/24h, detection limit is approximately 0.16 mg/dL). Urinary magnesium was measured by semi-auto chemistry analyzer (expected values in 24 h in urine is 73-122 mg/24h, detection limit is 0.05 mg/dL). Urinary pH was estimated using a pH Meter.

All patients signed an informed consent to take part in the study, and the study was approved by ethical committee of Tikrit University, College of Medicine. All data were presented as mean and SD. Statistical analysis was implemented with paired student’s t test. A P value of less than 0.05 was regarded significant. Analysis was performed by IBM SPSS Statistics for Windows, Version 23.0.

Results

A final number of 94 male patients completed the study. The mean age was 38.5 (SD 17.4) years. Male patients were 64% and female patients were 36%. Table 1 shows that mean urinary calcium level decreased significantly after k-citrate therapy, p value <0.001. Mean Urinary volume and pH increased significantly after k-citrate therapy. No significant statistical difference was observed in mean urinary magnesium level before and after k-citrate therapy. No adverse effects related to k-citrate therapy were reported by patients during study period.

<table>
<thead>
<tr>
<th>Urinary Parameters</th>
<th>Before K-citrate Therapy</th>
<th>2 weeks After K-citrate Therapy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/day) Mean (SD)</td>
<td>288.4 (45.3)</td>
<td>212.7 (36.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Magnesium (mg/day) Mean (SD)</td>
<td>97.3 (32.5)</td>
<td>104.6 (26.3)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table (1) : Mean Urinary parameters before and after k-citrate therapy.
Discussion

Renal stone development is often regarded as nutritional or environmental disease, on the other hand hereditary or anatomic factors are substantial. Nearly 5% to 10% of the people of the western countries are believed to have formed a minimum one kidney stone by the age of 70 years, and the occurrence of urinary stones is escalating. For most stone categories, there is a male predominance. The mechanisms in charge of multiple recurrences of renal stones in only certain people are not completely explained. Reasons involved consist of urine flow that depends on fluid consumption, excretion of large amounts of stone constituents, the lack of a constituent or substances in the urine that prevent stone development, and urinary pH. [11]

The cornerstone of therapeutic approach for renal stone disease management was potassium citrate for many decades. The main mechanisms by which k-citrate inhibits stone formation are to make the constituents in urine more soluble, and increase inhibitory action against development of calcium containing stone. Potassium citrate causes marked increase in urinary levels of both potassium and citrate. [12] Urinary citrate is a potent inhibitor for stone formation. [4]

In this study, it was observed that urinary calcium level reduced significantly after potassium citrate therapy. Same result was reported by other studies [4, 5]. This result can be explained by the binding that occur between citrate and calcium in urine. Citrate and calcium in urine make stable complex that decrease the amount of urinary calcium needed for stone formation. [2] No significant change in urinary magnesium after potassium citrate therapy was identified in this study. This result can be attributed to the nonexistence of chemical interaction between potassium citrate and urinary magnesium.

Urinary volume of patients with recurrent stone disease was significantly increased after k-citrate therapy in this study. This finding is similar to the result of Robison and his followers in 2009 [13]. The increase in urinary volume can be explained by the suggested diuretic effect of potassium citrate that increased urinary volume in patients with recurrent renal stones.

Urinary pH was significantly increased in this study after the administration of potassium citrate therapy for patients with recurrent stone disease. Whitson and his followers in 2007 [14] found that no relation existed between potassium citrate therapy and urinary pH, and change in urinary pH was mainly caused by change in patients diet. The increase in urinary pH in this study can be explained by the increase in alkaline load caused by potassium citrate therapy.

Conclusion

Urinary volume and urinary pH increased significantly in patients with recurrent stone disease after potassium citrate therapy. Also potassium citrate therapy significantly produced marked decrease in urinary calcium level in patients with recurrent stone disease. No change was observed in mean urinary magnesium level before and after k-citrate therapy. No adverse effects related to k-citrate therapy were reported by patients during study period.

Conflict of Interest - (nil – There are “No Conflict of Interest”).

Source of Funding - By all

Ethical Clearance: Committee members are approved to perform a study about:

“Changes in some urinary variables after potassium citrate therapy in patients with recurrent urinary stone disease in Tikrit city, Iraq”

After discussion of study plan with researchers:
References


Synthesis and Characterization of Some Bis-Nitroaryl Pyrazolones and Estimation of Their Anti-Bacterial and Anti-Fungal Activities

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Abstract

A simple and efficient one-pot synthesis of bicyclic nitro pyrazolone derivatives in traditional alcoholic solvents under refluxing conditions, The chemical structure of the synthesized derivatives was substantiated by FT-IR, 1H NMR, 13C-NMR and CHNS analysis, All of these compounds of the series have been tested in vitro as antibacterial and antifungal agents against four types of organisms. The minimum and maximum inhibitory concentrations were determined against each organism.

Keywords: pyrazolone, one-pot synthesis, nitroaldehyde, antibacterial activity, antifungal activity.

Introduction

Pyrazoles are five membered ring compounds, containing three carbon atoms in addition to two adjacent nitrogen atoms. The keto (=O) derivatives of pyrazoles are called pyrazolones.1 Chemically, pyrazoles are known as 1,2-diazole and are becoming a desirable topic by reason of their diverse applications.2 Pyrazolone is a substantial group of compounds obtained from pyrazole. It possesses a five membered ring in addition to a lactam functional group and occurs in two isomeric forms: 3- and 5- pyrazolone.3

In recent years, the synthetic work of functionalized pyrazolones has caught the attention of organic chemists, by reason of their potent bioactivities.4

Compounds derived from pyrazolone have taken a major part in the pharmaceutical chemistry and have been used as important pharmacophores in the design of drug dosage forms too,5 as antitumor,6 antimicrobial, anti-inflammatory,7,8,9,10 anti-oxidant,11 antidepressant, anticonvulsant,12 analgesic,13 antihistaminic14,15,16,17 and so on.

pyrazolones are prepared by lengthy and often tedious methods because of the tautomerization that affects the pyrazolone ring.18 A special effort has been put into preparing this important moiety, a general procedure for the synthesis involves the condensation of hydrazine hydrate with ethylacetoacetate in commonest mixed solvents.19 The archetypal pyrazolone synthesis involves the condensation of a b-ketoester, such as ethylacetoacetate, with a hydrazine.20

The composition of a bicyclic pyrazolone via a previously synthesized pyrazolone ring is not usually a feasible method because of the tautomerization that affects the pyrazolone ring. Therefore, we contrived a step that is capable of generating both rings intramolecularly in a one-pot reaction.

Our continuous work in this field led us to plan a one-pot method to get combined bicyclic pyrazolone moieties in a good yield. In this paper, we hope to declare our comprehensive results in preparing bicyclic pyrazolone derivatives.

This paper describes in vitro screening results of the compounds in question for their antimicrobial activity, minimum and maximum inhibitory concentrations. Herein, we provide information about the synthesis and pharmacological assessment of bis pyrazolones.
Experimental

Apparatus measurements

The used of chemicals were synthesis of analytical reagents grade. Melting points was uncorrected and were taken throughout open capillaries, TLC used to check purity of compounds, (Shimadzu Infrared Spectrophotometer Fourier Transform FTIR-8400S) using KBr pallets were used to record IR Spectra,The recorded NMR peaks were on NMR bruker500 MHz; which recorded on δ scale (ppm) against TMS. The utilized solvent was DMSO (3.33-3.35). The elemental analysis was done by Euro Vector EA 3000A Italy.

Organic Preparations

Synthesis of 3-methylpyrazole-5-one (I)

Hydrazine monohydrate (80 % 6.25 g, 100mmole) in methanol-water mixture (1:1) in (20 mL) completely dissolved and in an ice bath was cooled to 0-5 C°. To the mixture in question, ethyl acetoacetate was added (13 g, 100mmole) dropwise with continuous magnetic stirring and the temperature was kept at 0-5 C° by external cooling. Then, (in 5-20 minutes) a white crystalline deposit separated. The solution was left to stand further at 5-15 C° for 30-40 minutes to complete the crystallization. The mixture is then filtered under vacuum system pump, washed by ice-cold water. Recrystallization by absolute ethanol gave short needles, melted at 220-222 C°. The ratio was 9.15 g, 93%. [21]

General procedure for the synthesis of pyrazolone derivatives (II-IV)

(2 mmole, 0.392 g) of pyrazolone derivative (I) with (1 mmole, 0.302 g) of nitro-derivatives benzaldehyde was put under reflux using ethanol (within 3 hr). until the completion of the reaction, the gained solid compound was extracted out, dried,recrystallized by absolute ethanol to get the products. The physical properties and yield are listed in Table 1, The TLC was used for monitoring reaction progress (ethyl acetate 3: 1 n-hexane). [22]

Biological activity

Examination the antibacterial and antifungal activity of synthesized compounds were conducted in plates method.

Standard solutions

Preparing a stock solution by solubilizing anappropriate amount of the USP Standard Reference of the used antibiotic, where appropriate in the specified solvent as in Table 2; and diluted to obtain the required concentrations. Stored at 2o-8o , and use within the period indicated. Stock solution raisegradually in concentrations, often in the ratio of 1:1.25. see table (2).

Table (1) Data of standard antibiotic.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Stock Solutions</th>
<th>first solvent</th>
<th>first concentration</th>
<th>Further Diluent</th>
<th>eventual concentration</th>
<th>Used for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neomycin</td>
<td>B.3</td>
<td>–</td>
<td>–</td>
<td>1 mg/mL</td>
<td>14 days</td>
<td></td>
</tr>
</tbody>
</table>

Table (2) Buffers

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Concentration of Dibasic potassium phosphate (g/L)</th>
<th>Concentration of Monobasic potassium phosphate (g/L)</th>
<th>Volume of 10 N potassium hydroxide (mL)</th>
<th>PH after sterilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer B.3(0.1 M, pH 8.0)</td>
<td>16.73</td>
<td>0.523</td>
<td>–</td>
<td>8.0 ± 0.1</td>
</tr>
<tr>
<td>Buffer B.6(10%, pH 6.0)</td>
<td>20</td>
<td>80</td>
<td>–</td>
<td>6.0 ± 0.05</td>
</tr>
</tbody>
</table>

* a Adjust the pH with 18 N phosphoric acid or 10 N potassium hydroxide
Results and Discussion

First, we obtained apyrazolone scaffold as the starting materials by tandem condensation and cyclization.[23] as depicted in Scheme 1.

Scheme (1) synthetic pyrazolones (I-IV)

The cyclization of ethylacetoacetate by hydrazine hydrate and bicyclic formation may proceed by the following mechanism. [24]

FT-IR Spectra

Pyrazolone (I), the starting material of this research, was synthesized by cyclic condensation which is characterized by TLC, melting points, FT-IR stretching bands indicated the appearance of the (N-H) at 3389 cm\(^{-1}\) and aliphatic (-C-H) at 2931 cm\(^{-1}\) and (-C=O) at 1680 cm\(^{-1}\) and (-C=N) at 1583 cm\(^{-1}\). The FT-IR spectrum of bicyclic pyrazolone (IV) shown appear of (NH) stretching absorption band at 3413 cm\(^{-1}\) and absorption band for aliphatic (-C-H) at 2920 cm\(^{-1}\) and (-C=O) at 1690 cm\(^{-1}\) and (-C=N) at 1585 cm\(^{-1}\) and absorption band for nitro group at (1489, 1309) cm\(^{-1}\).[25]

1HNMR and 13C-NMR spectra

The 1HNMR spectrum of compound (I), (in DMSO as a solvent) revealed the following distinguished chemical shifts: a singlet signal at \(\delta\) (2) ppm refers to aliphatic CH\(_3\) protons, singlet signal at \(\delta\) (3.2) ppm refers to methylene protons of the pyrazolone ring, the characteristic singlet signal at \(\delta\) (8.4) ppm refers to NH
proton. [25]

1H NMR spectrum for derivative (IV), revealed a singlet signal at δ 1.8 ppm, refers to aliphatic CH₃ protons, α-carbonyl protons of the rings as doublet at δ 3 ppm, the characteristic triplet signal of linker carbon protons at δ 4 ppm, singlet signal at δ 5 ppm refers to NH proton, the doublet of doublet signal between δ (8-9) ppm refers to para-substitution nitro group ring protons. [25]

13C-NMR spectrum for derivative (I) revealed C=O at δ 178 ppm, C=N at δ 144 ppm, methylene of the ring at δ 67 ppm and methyl group at δ 25 ppm. [25]

13C-NMR spectrum for derivative (II) revealed C=O at δ 177 ppm, C=N at δ 145 ppm, C-NO₂ of benzene ring at δ 135 ppm, the carbon cited as (1) at δ 133 ppm, the carbon cited as (2) at δ 131 ppm, the carbon cited as (3) at δ 128 ppm, the carbon cited as (4) at δ 121 ppm, the carbon cited as (5) at δ 120 ppm, the carbon flanked at α-carbonyl of pyrazolone ring at δ 55 ppm, the carbon of methyl group at δ 35 ppm, and the linker carbon at δ 27 ppm. [25]

13C-NMR spectrum for derivative (III) revealed C=O at δ 164 ppm, C=N at δ 150 ppm, C-NO₂ of benzene ring at δ 148 ppm, the carbons cited as (1) at δ 141 ppm, the carbon cited as (2) at δ 125 ppm, the carbon cited as (3) at δ 122 ppm, the carbon cited as (4) at δ 115 ppm, the carbon flanked at α-carbonyl of pyrazolone ring at δ 72 ppm, the linker carbon at δ 32 ppm and the carbon of methyl group at δ 26 ppm. [25]


4. Biological activity

In our study, new series of heterocyclic derivatives showed moderate to significant antibacterial activity when compared with standard drug Neomycin and powerful antifungal activity.

The minimum inhibition for synthesized compound (I) was (11 mg/mL) against Staphylococcus aureus (picture 1) while the maximum inhibition for synthesized compound (I) was (14 mg/mL) against Bacillus subtilis (picture 2) and the maximum inhibition for synthesized compound (I) was (16 mg/mL) against candida albicans (picture 3). The minimum inhibition for synthesized compound (II) was (11 mg/mL) against Staphylococcus aureus (picture 4) while the maximum inhibition for synthesized compound (II) was (12 mg/mL) against Bacillus pumilus (picture 5) and the maximum inhibition for synthesized compound (II) was (18 mg/mL) against candida albicans (picture 6). The minimum inhibition for synthesized compound (III) was (11 mg/mL) against Staphylococcus aureus (picture 7) while the maximum inhibition for synthesized compound (III) was (16 mg/mL) against Bacillus subtilis (picture 8) and the maximum inhibition for synthesized compound (III) was (17 mg/mL) against candida albicans (picture 9).

In general, all the synthesized compound revealed moderate to good antibacterial activity concerning fungi, all the synthesized compound revealed good activity against candida albicans in all testified concentrations. See table (4).
Table (4). Results of antimicrobial activity of gained compounds.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Bacillus subtilis (B)</th>
<th>Bacillus pumilus (E)</th>
<th>Staphylococcus aureus (S)</th>
<th>Candida albicans (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>st. 0.1 mg/mL</td>
<td>25 mg/mL</td>
<td>15 mg/mL</td>
<td>5 mg/mL</td>
</tr>
<tr>
<td>I</td>
<td>15</td>
<td>14</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>16</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>16</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>12</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

- Inhibition zone in (mm).

- St. “References Standard USP” = Neomycin (as Sulfate).
Conclusion

New gained compounds (I,II,III,IV) were obtained by simple refluxing, short reaction time, ease of working and good yield. All of the produced derivatives were detected by TLC, melting points, FT-IR, 1H-NMR, 13C-NMR and elemental analysis. Thesesynthesized derivatives (I-IV) were evaluated to test their antibacterial and antifungal activities by cylinder-plate method against various Gram positive bacteria and fungi (Candida albicans). All synthesized compounds have shown significant activity against Bacillus subtilis and Bacillus pumilus and against Candida albicans too, whilst towards Staphylococcus aureus they showed comparable activity with the standard drug (Neomycin) at the concentrations used.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

References


Assessment of the Correlation between the Salivary Flow Rate and Dental Caries Experience among Children with β-Thalassemia Major

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Abstract

Thalassemia constitutes a group of congenital blood disorders which characterized by a defect in synthesis of one or more globin chains of human hemoglobin molecule and the resultant microcytosis and hypochromia of the RBCs. It is of two main divisions, α- and β-thalassemia and several other subdivisions. The study group composed of 40 patient years previously diagnosed with β-thalassemia major attending the thalassemia center in Thi-Qar province/Iraq. The control group, matching the age and sex of the study group, and consisted from 40 child selected from a number of primary schools. Unstimulated salivary samples was taken from each subjects under standardized conditions. The mean value of flow rate of saliva was lower among β-thalassemias (0.466±0.024) than for controls (0.829±0.048). The (mean±SE) for the primary teeth (dmfs) in β-thalassemias (1.450±0.324) was higher than that for controls (1.250±0.808), this difference was not significant (P> 0.05). The (mean±SE) of the caries experience (DMFs) in β-thalassemias (6.850±0.782) was higher than for the control group (3.600±0.489).

Keywords: β-Thalassemia major, Salivary flow rate, Dental caries experience (dmfs/DMFs).

Introduction

Thalassemia is a very worldwide common autosomal and recessive genetic disorder with a large geographical incidence difference, it is a so severe and incurable disease because prevention is the only way to evade the disease [¹], Thalassaemias are caused by markdown or complete absent in the synthesis of one or more of the globin chains that constitute the hemoglobin (Hb) unit [²,³]. About 60,000 to 70,000 newly born children are born yearly with a severe form of thalassaemias around the world [⁴], and unfortunately most affected children are those who born in areas of low sources of income [⁵]. β-Thalassemia major is the most severe type of thalassemia and occurs due to a defect in the synthesis of β-globin chain [⁶].

Beta-homotetramers in α-thalassemia are more stable than alpha- homotetramers in β-thalassemia; therefore, in beta thalassemia α-homotetramers tend to precipitate priorly in the RBCs life span, producing marked RBCs haemolysis [⁷]. Few of the pro-erythroblasts beginning their maturation and can survive [⁸,⁹], the resultant few RBCs will bear an inclusion bodies that identified in the spleen, shortening the RBC lifespan and producing severe haemolytic anaemia [¹⁰]. The produced anaemia stimulates the production of erythropoietin stimulating hormone from the kidney and liver with a compensatory erythroid hyperplasia, but the marrow response is disrupted by ineffective erythropoiesis. [¹¹]. Later on, massive bone marrow expansion will happened and the end result is skeletal deformities [¹²].

In thalassemias, high caries index, pallor of oral mucosa, atrophic glossitis, sialadenitis, retained deciduous teeth, shortened and spiked roots of teeth and thinning of the lamina dura could be seen [¹³]. Oral health, on the other hand, can also affect child’s personality, knowledge, and social relationships [¹⁴].
Dental caries is a chronic disease resulted from a complex intercommunication of a mass of cariogenic oral microorganisms which grow on the teeth surfaces within the dental [15]. Dental caries is widely spread and can affecting all ages. The experience of dental caries could be modified by several important factors, including diet, age, gender, socioeconomic level and some medicines [16].

Saliva is a heterogeneous mixture of fluids mainly produced via the major and minor salivary glands and contains oral bacteria and food debris from the gingival crevicular fluid [17,18]. Saliva have an important role in the maintenance of a healthy oral environment through a variety of physicochemical and biological properties. Unstimulated (resting) saliva includes secretions that enter the mouth without any exogenous stimuli [19,20].

Flow rate is the most important salivary parameter affecting oral health status and it represents the rate of salivary secretion and it is expressed in milliliters per minute [21]. Flow rate is very important protective factor against dental caries throughout its washing and buffering effect [22], any minimization in the normal salivary flow rate can results in establishment of dental caries [23]. Al-Jobouri and Al-Casey [24] and Kataria et al. [25]

## Materials and Method

The total sample included in this study was (80) subjects. The study group was consisted of a (40) child suffering from β-thalassemia major and aged (11-12 years old). The control group, the non-thalassemic children, matching the age and gender of the study group, and consisted of a (40) child selected from the primary schools. An ethical approval was firstly obtained from the Ministry of Health and the Ministry of Education to perform the clinical examination and laboratory biochemical analysis. Also, a written consent form as well as patient information sheet were provided to each participant for gaining the acceptance of the child’s parents or his/her caregiver. Children having other diseases whose known to affect the experience of dental caries or the characteristics of saliva such as diabetes mellitus, were excluded.

The oral examination was performed under standardized conditions of oral health surveys (WHO 1997). During examination, a suitable chair is used with noticing that it supports the head of the child. Clinical examination was performed using plane mouth mirror and dental probe. The reported caries experience was based on the criteria suggested by Manjie et al. [26]. Unstimulated salivary samples was taken from each subjects under standardized conditions suggested by Navazesh and Kumar [27]. Statistical analyses were done using SPSS computer programme, version 21. Descriptive statistics including (the mean and SE) of each clinical variable were determined for all the subjects. Student t-test was used to compare the caries experience between the study and control groups. Pearson’s correlation coefficient (r) was used to assess and compare the correlations among the variables. (P>0.05) was considered statistically not significant. (P≤0.05) was considered statistically significant and (P≤0.01) was considered to be statistically highly significant.

## Results

Table (1) illustrates the results showed that the (mean±SE) in β-thalassemias (1.450±0.324) was higher than that for the control group (1.250±0.808), This difference was not significant (P> 0.05).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>dmfs</td>
<td>1.45</td>
<td>0.324</td>
<td>1.250</td>
<td>0.808</td>
</tr>
</tbody>
</table>

* Not significant difference (P> 0.05).
Table (2) illustrates the results showed that the (mean±SE) value in β-thalassemias (6.850±0.782) was higher than that for the control group (3.600±0.489). This difference was highly significant (P≤ 0.01).

Table (2): Dental caries experience by surfaces in permanent dentition among β-thalassemias and their controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>DMFs</td>
<td>6.85</td>
<td>0.782</td>
<td>3.600</td>
<td>0.489</td>
</tr>
</tbody>
</table>

** Highly significant difference (P≤ 0.01)

Table (3) Results showed that the salivary flow rate among β-thalassemias was lower among β-thalassemia patients than for their control subjects. This difference was highly significant (P ≤ 0.01).

Table (3): Salivary flow rate among thalassemia patients and their controls.

<table>
<thead>
<tr>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.466</td>
<td>0.024</td>
<td>0.829</td>
</tr>
</tbody>
</table>

** Highly significant difference (P ≤ 0.01)

Table (4) In primary dentition, results showed a weak negative, not significant correlation between the salivary flow rate and (dmfs) index in the study and control groups. In permanent dentition, results showed a weak negative, not significant correlation between the salivary flow rate and (DMFs) index in the study and control groups.

Table (4): Correlation coefficients of the salivary flow rate and the dental caries experience among thalassemia patients and their controls.

*NS = Not significant

Discussion

In the present study, the mean value of salivary flow rate was founded to be lower among β-thalassemias (0.466±0.024) than in their control subjects (0.829±0.048). This result was also documented previously by Norri [28] and by Al-Jobouri and Al-Casey [24]. This minimization in the salivary flow rate among the study group might be attributed to the fact that the salivary glands function in thalassemic patients can be affected directly by the excessive iron deposits,
and the resultant painful inflammation of the salivary glands, with either regular or diminished salivary flow [23]. However, Siamopoulou et al [29] concluded in their study that the difference in salivary flow rate between the two groups is not significant. These results were in disagreement with Luglie et al. [30] and Greenberg et al. [31] whose showed in their studies that salivary flow rate was similar in both study and control groups.

The present study also concluded that the mean value of caries experience by surfaces in primary dentition (dmfs) among β-thalassemias was higher than that recorded in their controls. This result was corresponding with Gomber et al. [32], Kaur et al. [33], Dhote et al. [34]. On the contrary, this result was disagree with Scutellori et al. [35], Qureshi et al. [36] and Arora et al. [37] who were concluded that there is no difference between the two groups.

Furthermore, the results of the present study revealed that the mean value of caries experience by surfaces in permanent dentition (DMFs) among β-thalassemias was higher than that recorded in their controls. This results was in agreement with Hattab et al. [38], Al-Raheem et al. [39] and Al-Hadithi [40], while it was in disagreement with the results of Scutellori et al. [35] and Arora et al. [37] who were founded a similar mean value between the two groups.

In present study, there was a negative correlation between salivary flow rate and caries experience. Salivary flow rate may play an important role in relation to dental caries in which the flow rate of saliva exert cleansing activity which is critical in the clearance of food remnants and bacteria [41]. It was documented that the caries experience is obviously increased when salivary flow rate is stunted and this fact sign that the chronic decrease in flow rate is a risk factor for dental caries initiation and progression [42]. In thalassemic patients, reduced salivary flow rate can help in colonization of cariogenic S. mutans, which may have a role in the higher caries experience. Al-Zaidi [43] has documented an indubitable correlation between oral mutans streptococci and dental caries in β-thalassemia patients.

However, the higher caries experience in β-thalassemias than the normal controls can be related to factors other than a reduced salivary flow rate. This could be explained on the idea that these patients are less concern with their oral health, their parents are more worried about the serious physical condition, they paying less care to the oral health care, and seeking professional oral care just when the child experience severe pain [23, 44].

Al-Wahadni [45] and Gomber and Dewan [32] concluded in their studies that skeletal changes as enlargement in maxillary arch that occur in thalassemias in could result in protrusion of anterior segment, increased space between upper and lower teeth, over-bite or open-bite which all could predispose to dental caries.

**Conclusion**

Dental caries experience was higher among beta thalassemias compared to the control group. The salivary flow rate among β-thalassemias was lower among β-thalassemia patients than for their control subjects. The reduced salivary flow rate detected in β-thalassemia major patients could be considered as a modifying factor for the increased experience of dental caries in these patients.

**Conflict of Interest:** The author has no disclosures to report.

**Source of Funding:** Self.

**Ethical Clearance:** Not required.

**References**


34. Dhote V., Thosar N., Baliga S, Evaluation of oral


Application of Risk Management Using HIRADC Method in Analytical Chemical Laboratory of University in Indonesia

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Abstract

The analytical chemistry laboratory is one of laboratories at Universitas Airlangga which is often used in conducting analytical chemistry practicum activities and research for lecturers and students. In this analytical chemistry laboratory there are various chemicals and activities that are at high risk of causing danger, especially the exposure to toxic chemicals that can lead to risk of poisoning in students and laboratory staff. The purpose of this study was to apply the HIRADC method as an effort to prevent the risk of accidents and work-related illness in laboratory users. This research method is observational by observing the source of danger and the control efforts that have been made. From the results of the identification of hazards that have been made it is known that there are 10 potential hazards that can cause 10 risks. After doing the risk assessment, it is known that there are 4 low risks, 2 medium risks, 2 high risk, and 2 extreme risks. Control efforts have been made to minimize any risks. The recommendations that can be given from this study are the provision of personal protective equipment (PPE) that is in accordance with the hazards in the laboratory as well as training on how to use the PPE.

Keywords : HIRADC, analytical chemistry laboratory, risk.

Introduction

Risk is a combination of the possibility and severity of an event. In the aspect of occupational safety and health (OHS), the risk is usually negative, such as injury, damage, or disruption of the operation. Risk management is an effort to manage hazards that have the potential to pose a risk to occupational safety and health to prevent accidents and illnesses caused by unwanted work in a structured and well planned manner1. HIRADC is one of many methods that hold an important part because it deals with prevention and control of hazards that are used as a reference in determining OHS program objectives and plans.

Government Regulation Number 50 of 2012 concerning the Implementation of Occupational Safety and Health System has explained that in preparing policies and formulating an OHS plan strategy, employers must at least conduct an initial review of OSH conditions which includes identification of potential hazards, assessment and risk control2. In OHSAS 45001: 2018 it is stated that organizations must establish, make, implement and maintain procedures for identifying hazards, assessing risks, and determining the control of hazards and risks required3.

According to the 2013 International Labor Organization (ILO) data, there are more than 250 million cases of workplace accidents every year and more than 160 million cases of work-related illness. From a number of these cases, there were 1.2 million workers who died from workplace accidents and work-related illness or were sick at work4. Based on data from the BPJS (Social Security Agency) on Employment, the number of occupational accidents in Indonesia is still high even though it has decreased every year. Meanwhile, until August 2017 there were 80,392 work accident cases5.

Workplace accidents can occur everywhere, including laboratories in educational institutions.
Some examples of accidents that have occurred in the laboratory include the fire that occurred at the Chemical Engineering Laboratory, Patras, Egypt on August 27, 2005, which was caused by short circuiting in the laboratory equipment. Another case was the accident that occurred at the Texas Tech University chemistry laboratory on January 7, 2010 due to violating the procedure for using a mixture of nickel hydrazine perchlorate chemicals in conducting research which resulted in one student losing three fingers, perforating the eyes, and experiencing burns in several body parts.

**Material and Method**

Based on the method of retrieval of data, this study is observational, the data is obtained by making observations and there is no treatment of the object of research during the study. Based on the analysis, this research is descriptive, namely describing the process without analyzing relationships between variables.

The population of this study was all laboratory assistant at the analytical chemistry laboratory of Science and Technology Faculty Universitas Airlangga, which amounted to 2 people. Sampling in this study uses the total sampling method, namely the sample used in the study is all members of the population. Variables in this study included hazard identification, risk assessment, and determining control. Data collected in this study only primary data obtained through interviews with laboratory assistants responsible for the analytical chemistry laboratory and observations.

**Findings**

Work activities at the analytical chemistry laboratory at Universitas Airlangga consist of two activities, namely practicum by students and preparing and cleaning practicum equipment by laboratory staff. The first stage in HIRADC is doing hazard identification. Hazard identification was using the Job Safety Analysis (JSA) method. Based on the results of identification of hazards that have been carried out, obtained the potential or source of danger that exists in practicum activities by students as well as preparation and cleaning of practicum equipment by laboratory assistants are 10 sources of danger that can cause 10 risks.

After identifying hazards from work activities in the laboratory, the next step is to carry out a risk assessment. The risk assessment includes risk analysis and risk evaluation. Risk analysis is carried out by qualitative methods by assessing aspects of opportunity or likelihood and the consequences or severity then evaluating the level of risk. Qualitative risk analysis refers to the Australian Standard / New Zealand Standard (AS/NZS 4360:2004).

Based on the results of the risk assessment carried out on all work activities there are 10 risks including 4 low risk level, 2 moderate risk level, 2 high risk level, and 2 extreme risk level. The results of the risk assessment carried out on activities in analytical chemistry laboratories will be used as a basis in determining appropriate risk control efforts.

The next step is to determine risk control efforts. In this stage, the hazards and risks that have been identified are analyzed to formulate the control strategies based on each risk category. The determining control used in this research refers to OHSAS 45001:2018 that contains elimination, substitution, engineering manipulation, administrative, and PPE.

**Hazard Identification**

Hazard identification is the process of checking each area and work activity to identify all possible hazards. Hazard identification can be done by several methods, including passive, semi-active, and proactive. In this study hazard identification is carried out by using a proactive method that is carried out as a preventive and repairing effort before the loss arises due to danger at work. One technique in the proactive method, namely by making a Job Safety Analysis (JSA) conducted by analyzing potential hazards in each work activity in an analytical chemistry laboratory.

In practicum activities by students there are several potential hazards, firstly there are fragments of laboratory equipment made of glass. Most of the tools used for practicum in chemical laboratories are made of glass because they have several beneficial properties, including translucency, are not easy to react with chemicals, and have a high boiling point so that it doesn't melt at heating below 100°C. However, equipment made of glass risks breaking when it falls and will cause a lot of broken glass scattered and can injure students and laboratory staff.

The second potential danger is the sitting position of students when recording and doing labs that are less...
ergonomic. The incompatibility between the dimensions of the chair and the size of the anthropometry of the user can lead to unnatural posture and risk of causing musculoskeletal disorders (MSDs). Chairs in the laboratory use chairs that are upright, small in size, and without backrest so that they don’t interfere with student mobility but with a chair like that can reduce comfort and make the posture less ergonomic.

The third potential danger is lighting in an inadequate laboratory room. The standard of lighting in the laboratory is 500 lux. Lighting in analytical chemistry laboratories that are less or more than 500 lux can risk causing eye fatigue in students. The fourth is the electricity source. The presence of an inadequate source of electricity and errors when using it can run the risk of short-circuiting and fire.

The fifth potential hazard is the chemicals used in the practicum are corrosive. In analytical chemistry labs, students often use H2SO4 (Sulfuric acid) and HCl (Hydrochloric acid). These materials can enter the body through inhalation pathways and contact with skin and eyes and will have an effect on health. If corrosive chemicals are inhaled it can risk causing irritation to the nose and throat and can interfere with lung function. When in contact with the skin it is at risk of causing damage to the skin and painful sores. When in contact with the eyes, there is risk of causing blindness.

The sixth potential hazard is inadequate laboratory temperature. The temperature of the room that is too high when practicing can cause the body to sweat a lot and will reduce comfort and concentration when doing practicum. In addition, health problems can occur such as the appearance of white spots (milia) in the area of the nose, eyes, and forehead. The temperature of the room in the laboratory is tried between 23-26°C and there is adequate ventilation and wide windows to provide healthy environment.

The seventh danger potential is the presence of nitrogen gas (N2) tube which is not given a protective chain to prevent from collapsing. Gas cylinders in a lab that are heavy and easily dropped when touched can risking the feet of laboratory workers and students causing injury to the legs. The eighth potential danger is the heat that comes from the heating process using methylated spirits fire. In the chemistry practicum, most of the heating process of materials or chemical solutions uses fire from methylated spirits that can be at risk of being touched by the hand when doing lab work and causing burns.

The second activity is to prepare and clean lab equipment by laboratory staff. In these activities there are two potential hazards, those are the presence of corrosive chemicals, such as H2SO4 and HCl. Corrosive chemicals can enter the body through inhalation pathways, contact with the skin, and eyes. Next is exposure to various chemicals continuously. Some chemicals can have a direct or short-term effect when exposed and some chemicals do not have a direct effect, but continuous exposure to various chemicals through inhalation and skin contact can risk negative effects on long-term health.

Risk Assessment

Risk assessment is the process of assessing the risk of each potential hazard that has been identified. In this study, risk assessment was carried out with a qualitative risk assessment method that refers to the AS / NZS 4360: 200412. In qualitative risk assessment a scale of risk level categories will be generated, namely extreme, high, medium and low risk. The purpose of risk assessment is to determine whether the risk is still at an acceptable level or not and requires risk control.
Table 1. Risk Assessment at the FST Analytical Chemistry Laboratory of Universitas Airlangga

<table>
<thead>
<tr>
<th>No</th>
<th>Activity</th>
<th>Source of Danger</th>
<th>Risk</th>
<th>Likelihood</th>
<th>Severity</th>
<th>Total Risk</th>
<th>Risk Level</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1.</td>
<td>Practicum by Students</td>
<td>Shards of laboratory equipment made of glass</td>
<td>Hurt</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Sitting position when recording and practicum</td>
<td>Musculoskeletal disorders (MSDs)</td>
<td>√</td>
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<tr>
<td></td>
<td></td>
<td>Lighting</td>
<td>Eye fatigue</td>
<td>√</td>
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<tr>
<td></td>
<td></td>
<td>Electricity</td>
<td>Short circuit, fire</td>
<td>√</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>The chemicals used in the lab are corrosive</td>
<td>Inhalation: irritates the nose, throat and the lungs</td>
<td></td>
<td>√</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skin contact: damage the skin and cause very painful sores</td>
<td></td>
<td></td>
<td>√</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AFFECTED BY EYES: can cause blindness</td>
<td></td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperature of the laboratory room</td>
<td>Excessive sweating can cause discomfort and health problems such as milia</td>
<td></td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>There is a gas cylinder (N2) that is not given a protective chain to prevent the tube from collapsing</td>
<td>Can overwrite report legs or practice and cause injury and damage to the laboratory floor</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat (heating process using methylated spirits fire)</td>
<td>Touched hands when doing practicum</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Prepare and clean lab equipment by laboratory staff</td>
<td>Corrosive chemicals (H2SO4 and HCl)</td>
<td>Inhalation: irritates the nose, throat and the lungs</td>
<td></td>
<td></td>
<td>√</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skin contact: damage the skin and cause very painful sores</td>
<td></td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AFFECTED BY EYES: blindness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Continuous exposure to various chemicals</td>
<td>various short-term and long-term risks that might be caused</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The results of the risk assessment conducted on all work activities have 10 risks including 2 extreme risk level, 2 high risk level, 2 moderate risk level, and 4 low risk level. Based on an experiment in pharmacy laboratory, spilled by chemicals such as HCl, Nitric acid, and Sulfuric acid become high-ranking risk and dangerous.

**Determining Control**

Risk control is the process of taking action to eliminate health and safety risks to the extent that they can be implemented fairly or rationally. According to OHSAS 45001: 2018 a risk control hierarchy is carried out by eliminating, substituting, engineering, administrative control, and using personal protective equipment (PPE).

At this laboratory, efforts have been made to control any risks, including substitution efforts by replacing several detached plugs with new electrical terminals to temporarily reduce short circuiting and fire risk. Technical engineering efforts by installing local and general exchanger to reduce the risk of discomfort and health problems due to excessive sweating due to hot rooms.

Administrative control is carried out with neat storage facilities and supervision by lecturers and laboratory assistants to reduce injury risk due to glass fragments, there are wooden seats to reduce the risk of MSDs, ceilings and walls painted in bright colors and adjustable lighting to reduce the risk of eye fatigue, and provided a heat-resistant clamp for the heating process to reduce the risk of getting heat from heating spirits. Control with PPE is done by providing medical masks and medical gloves to students and laboratory assistants to reduce the risk of irritation of the nose and throat, disruption of lung function, damage and injury to the skin, and blindness as a result of the danger of corrosive chemicals used in practicum. Meanwhile, the risk of injury and damage to the floor due to being crushed by a nitrogen gas tube has not been controlled.

**Conclusion**

Hazard identification has been done on activities in analytical chemistry laboratory. We can conclude from the average results that this laboratory contain low-risks activities. But although risk control efforts in analytical chemistry laboratories have been carried out, there are still risks with extreme and high categories, so that control efforts are deemed less effective and need improvement.

1. Extreme category risks can be done by provision of PPE in the form of acid resistant gloves and respirators, policy making about using PPE when in the laboratory, requiring students to have a personal respirator, and conducting hazardous socialization of each chemical by lecturers or laboratory assistants and for laboratory assistants are given training in the use of PPE and instill the importance of using PPE on laboratory.

2. High category risks can be done by replacing all electrical plugs that are detached to prevent the electrical wires from being pulled out and providing electrical terminals for each device that has high electrical power. In addition, refer to health risks of laboran due to continuous exposure to chemicals can be carried out periodically medical check-ups.

3. Medium category risks can be done by optimizing the existing exhauster and adding air conditioner to prevent hot temperature, also providing a protective chain tube and put nitrogen tubes in a safer place to prevent N2 tube become falling.

4. Low category risks can be done by giving first aid kit due to Minister of Manpower and Transmigration Regulation Number 15 of 2008. The MSDs risk can be done by providing a standardized seat in the laboratory and providing cushioning on the seat to reduce pain in the sitting bones. The eye fatigue risk due to inadequate lighting can be controlled by turning on all lights in the laboratory room when practicing and immediately replacing a damaged or dead lamp. And the risk of burned out when warming up with spirits can be monitored by the laboratory during the heating process and use gloves to reduce the risk of being exposed to fire heat.

**Funding:** Sponsored by University.

**Conflicts of Interest:** None.

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Study of Epidemiological Factors According to the Positive Response of IgG of Patients Infected With Blastocystis Hominis in Diyala Province, Iraq

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Abstract

Background: Blastocystis hominis (B. hominis) is the most common intestinal parasite in humans and many other animals. Infections with the organism are spread worldwide and some of them have been asymptomatic, acute symptomatic and chronic.

Materials and Method: 100 blood samples were collected from patients with B. hominis which were reviewing to some Hospital and health center in Diyala province during the period from October 2018 to March 2019. To determine the extent of IgG antibody responses in serum patients infected with B. hominis for depending on the chromatic changes resulting from the association of antigens with antibodies.

Results: The results of the current study showed the percentage of infection among males was (56.25%), which is higher than that of females (43.75%), and the age group (3-6) years among males showed the highest rate of infection (33.34%) and the lowest in age groups less from one year and (9-12) years groups at (11.11%). The rate of infection in the age groups (1-3) years and (6-9) years was (22.22%) and in the age group of (1-3) years was the highest incidence among female groups (42.86%), while the age group (3-6) years showed less than that (28.57%) and significant differences at the level of probability less than (0.05). The percentage of B. hominis infection increased among the rural population by (56.5%), male infected, (57.14%) female, while the proportion of males among the urban population was (44.44%) and females reached (42.86%), with a standard deviation of (1.600 ± 0.495) and (1.480 ±0.505) respectively.

Conclusions: The presence of immunoglobulin IgG in serum patients has been shown to stimulate the cellular immune response and be indicative of long-term immunity against pathogenic antigens.

Keywords: Blastocystis hominis, diagnosis, parasite, infection, patients.

Introduction

B. hominis is one of the most common protozoa intestinal parasitic diseases worldwide and a common infected among humans and animals [1]. It isolated from stool specimens appear as unicellular and it has multiple shapes, such as vacuolar, granular, and amoeboid [2]. It was considered as harmless yeast, but it is now getting acceptance as an agent of human intestinal disease especially under immunosuppressive conditions [3]. The extent research on B. hominis is transmission mechanisms, incubation period, epidemiology, and treatment options [4]. Its status as a true pathogen is controversial - while it has been found in patients with gastrointestinal symptoms with diarrhea or severe abdominal pain. Symptoms associated with human papillomavirus infection include: diarrhea, nausea, colic, abdominal distension, fever and chills. It is not proven to be the cause and many carriers are asymptomatic [5].

Diagnosis of B.hominis is used by clinical diagnosis of diarrheal symptoms and dehydration of the patient and laboratory methods. Direct microscopy of stool and cystic phase observation overlap with other causatives of diarrhea specially [6,7]. These data indicate that
B. hominis induces as well as modulates the immune response in intestinal epithelial cells, and we conclude that different pathophysiological events may occur during B. hominis infection \[8\]. The spread of disease is associated with poor hygiene, exposure to animals and the consumption of contaminated food or water \[9\].

Iraq has seen widespread outbreaks of gastrointestinal diseases. The Iraqi Ministry of Health and contributed many factors in the spread of the disease; including displacement of people to other places and water pollution. There are several studies that showed prevalence in different Iraqi governorates of the country. The rate of infection was recorded in 2013 (5.08\%) for (31) children in Dohook in northern Iraq suffers from gastrointestinal symptoms \[10\]. 28 (4.1\%) was infected with B. hominis from a total of 861 reviewers \[11\]. While in Muthana province in southern Iraq, the infection rate was 58 (45.67\%) out of 127 patients infected with B. Hominis \[12\].

The aim of the present study is to investigate the most important immunological changes in infected patients by measuring the IgG level, determining positive response rate, age and genders, and setting up patients.

**Materials and Method**

**Collection of samples**

The present study collected 100 cases with Blastocystis hominis within the period from October 2018 to March 2019, of 50 male and 50 female, ranged in age less one year to 12 years. It has diagnosed the disease process and determined by dermatologists at some Hospital and health center in Diyala province and the injury was diagnosed based on the clinical symptoms and distinct phenotypic traits of infections.

**Serological diagnosis**

Serological diagnosis was performed by measuring the IgG level in the serum of patients with B. hominis to determine the response of the positive in the body of the infected patients. Blood samples were collected from patients. Was withdrawn 5 ml of venous blood for each of the infected persons, therapists and control the use of a syringe, put blood samples in test tubes and let the blood to clot at room temperature (20-25) minutes and then the separation of the serum Centrifuge device speeds of (3000) rpm and then it is kept freeze-preserved models when (-20°C) until the subsequent immunological tests.

To find out the most important immunological changes associated with infection in patients with B. hominis, the study has included measuring levels of certain cellular dynamics in the serum of infected by and based on the principle of color change resulting from the correlation of quality IgG antibodies and measured color change resulting mediated.

**Statistical analysis**

Statistical analysis of the results of the current study using the Statistical Package for Social Sciences conducted (SPSS) metadata. Test was used T-test, variance analysis ANOVA, Chi-square, percentage, standard deviation and standard error in the present study to find moral differences between groups with infection Blastocystis hominis and there were significant differences (P < 0.05).

**Results**

The present study included 100 patients with B. hominis, 50 males and 50 females. The positive response rate of IgG for males was higher than that of females, which recorded positive response rate of (56.25\%) at (1.820±0.388) .While the percentage of response to the antibiotic in the serum infected females (43.75\%) at (1.860±0.351). Hence, the statistical analysis of this increase indicates that there isn’t a significant difference of statistical significance at the level the probability is less than 0.05 as shown in Table 1.

Table 2 shows that the injuries spread within a wide range of age groups. The age of those infected was less than 1 to 12 years. In males recorded age groups the (3-6) year age group had the highest IgG response rate at (33.34\%) and followed by tow the age groups (1-3) years and (6-9) years (22.22\%) and less than at (11.11\%) in tow groups less one year and (9-12) years age groups. Compared to the female group, the (1-3) years age group had the highest incidence of IgG infection and response 42.86\%.The lowest response among the age group of females was 0.00\% in the age group less than one year, so that no percentage is recorded. This is due to the visitors who visit the health centers at the time of collecting samples. While the age group 3-6 years by 28.57\% and less than the age groups (6-9) and (9-12) years by (14.29\%). Differences between the proportions of m and their response to IgG were statistically significant at the probabilistic level the level the probability is less than 0.05 as shown in Table 2.

The patients’ place of residence showed a clear
difference between their urban and rural residence, but did not constitute a significant difference of statistical significance at the probability of \( p > 0.05 \). The prevalence of IgG immunoglobulin was significantly higher among urban than in rural areas as Table 3 showed. The proportion of males was 55.56\% of respondents 5 patients of the total number are 20 cases, while females registered 57.143\%, with 4 patients of the total number of 26 cases. The response rate in the urban group of infected males was 44.44\% with 4 patients of 30 cases, less than 42.857\% in urban areas, with 3 patients of the total 24 cases.

### Table 1: Effect of the sex factor on the positive serotonin of the IgG antibody of Blastocyte hominis for children covered in the infection

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Positive response</th>
<th>Percentage</th>
<th>Mean±SD</th>
<th>Standard Error(SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>50</td>
<td>9</td>
<td>56.25</td>
<td>1.820±0.388</td>
<td>0.055</td>
<td>0.280</td>
</tr>
<tr>
<td>Girls</td>
<td>50</td>
<td>7</td>
<td>43.75</td>
<td>1.860±0.351</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>16</td>
<td>32%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Distribution of numbers and percentages of infection of Blastocystis hominis for the age of the infected and the extent of IgG response

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>Number</th>
<th>% Number positive response</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Grils</td>
<td></td>
</tr>
<tr>
<td>&lt;1-</td>
<td>5</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>1-3</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3-6</td>
<td>7</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>6-9</td>
<td>12</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>9-12</td>
<td>22</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>50</td>
<td>9</td>
</tr>
</tbody>
</table>

### Table 3: Effect of the living factor on the positive serotonin of the IgG antibody of Blastocystis hominis for children covered in the infection

<table>
<thead>
<tr>
<th>Residence</th>
<th>Number tested</th>
<th>Number positive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
</tr>
<tr>
<td>Rural</td>
<td>20</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>Urban</td>
<td>30</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>50</td>
<td>9</td>
</tr>
</tbody>
</table>

| mean±SD  | 1.600±0.495 | 1.480±0.505 |         |
| Stander Error(SE) | 0.699 | 0.714 |         |
Discussion

B. Hominis is a protozoan intestinal parasite disease that caused by the emergence of various symptoms, including diarrhea, nausea, abdominal cramps, bloating, excessive gas, and anal itching. The timescale of infection with the parasite can range from weeks to years [13]. The present study showed an increase in IgG and positive response rate in males, with (56.25%) infection rate and (43.75%) female infection as in the table (2). This may be due to the number of hospital-reviewed cases and some male health centers more than females, and therefore dependent on individual immunity. This is consistent with the findings of Nayef et al. (2011) which indicated the percentage of male (23.99%) height on females (16.27%) and attributed the reasons for this to the above, attributed the reasons to as exposure to the infection increases with the top of nutrition and increased funding [14]. The percentage of infection between females and males does not make any significant difference at the probability level (0.05), which is consistent with the results of Mahmood and Khudher (2016) indicating that there is no significant difference between the rates of infection of males and females and accounted for the percentage of infection B. hominis in Sammara city - Salah Al-Deen province was (9.09%) [15].

The study showed the effect of the age group in the incidence of B. hominis, whose age ranged between (> 1-12) years with a significant difference of (P < 0.05). As it was the highest rate of infection in both genus and is attributed to the lack of development of the immune system in the younger age groups compared to the large age and this is consistent with what Salman (2015), who pointed to a significant difference at level (P <0.05) between different age groups ranging from 6 months to over 61 years out of a total of (177) patients with B. hominis [16]. The main cause of diarrhea is B. Hominis among other pathogens, as explained by al-Kaissi and Majdi (2009), the highest proportion of who were 82 cases with B. Hominis isolated from 200 patients suffering from diarrhea by 41% [17]. Where the current study recorded the age group (3 – 9) years is the highest response rate positive for IgG among males infected with B. hominis, which amounted to (33.34 %) and the lowest was in the two age groups less than one year and (9-12) years is (11.11%). While the age group (1-3) years among females infected with the highest rate of (42.86%) and less than in the categories of age (6-9) years and (9-12) years is (14.29%), while the category less than a year does not register any case among females and this is due to patients reviewing hospitals and centers when collecting samples as showed in table (2).

The current study indicates that the housing factor has a difference in the percentage of IgG positive response for patients with B.hominis for both sexes but does not register any significant difference at the probability level (P >0.05). The positive response rate for patients with B. hominis who live in rural areas is higher than that of urban dwellers. This is due to the pollution of the environment and the environment of the rural inhabitants of B. hominis. They may be directly related to the reservoir stocks such as sheep, cattle, cats, dogs and other domestic animals. For rural males (55.56%) of respondents (5) patients of the total number is (20) cases, while females registered (57.143%), with (4) patients of the total number of (26) cases. The response rate in the urban group of infected males was (44.44%) with (4) patients of (30) cases, less than (42.857%) in urban areas, with (3) patients of the total (24) cases as showed table (3).

Conclusions

The present study conclude that the disease’s ability to stimulate the cellular immune response leading to the production of IgG that observed height in patients groups.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: Blood sampling was performed from October 2018 to March 2019 with the consent of the reviewing patients to participate in this study.

References

4. Levine, N. D. “Protozoan Parasites of Domestic


Risk Analysis of Occupational Disease and Accident in Environmental Health Laboratory

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Abstract
Risk analysis is a process to identify hazards by calculating the size of the risks and determining whether a risk is acceptable or not. The purpose of this study is to do hazard identification and risk assessment in environmental health laboratories at Faculty of Public Health at Universitas Airlangga. This study was included in an observational and cross-sectional study based on the time of his research. Primary data is obtained through laboratory observations and interviews with reports presented in the form of tables and diagrams then analyzed descriptively. The results of this study indicate that in environmental health laboratories Faculty of Public Health Universitas Airlangga there are 10 potential hazards with 12 risks out of 4 activities. Based on the results of the risk assessment, from 12 risks there are 5 types of risks with low risk category, 4 types of risk for with moderate risk category, and 3 types of risk with high risk category. The conclusion of this study is that the greatest risk level in environmental health laboratories is low risk category of 41.67%. While for the moderate risk category was 33.33% and the high risk category was 25%. Risks with high risk categories include power failure, fire, and explosion at a time caused by the flow of electric current and chemical reactions carried out in the laboratory.

Keywords: environmental health laboratory, hazard identification, risk assessment

Introduction
The laboratory is a place for conducting scientific research, experiments, scientific measurements or training. Environmental health laboratories are usually used to test food safety, solid waste, wastewater treatment, environmental exposure assessment, drinking water quality, radiation exposure assessment, and biomonitoring.¹ One of the aims of this laboratory is to provide services to the community related to provide information about the quality of the natural environment that is beneficial for citizens as well as their own natural environment.² Activities in the laboratory may have chance to cause work accidents and work-related diseases such as sampling, pipetting, pouring and mixing of materials for experiments. Occupational health and safety risks that can occur when conducting an activity in an environmental health laboratory are exposure to the use of chemicals, exposure to biological hazards such as microbes and experimental organisms, physical hazards such as non-ergonomic positions when conducting research, scratched materials and research equipment, fires, electrical zips and explosions.³

Based on accidental survey statistics in the laboratory at the junior high school laboratory in Hong Kong during the 2014/2015 there were 241 cases of accidents that occurred to students and 10 cases occurred to employees and a total of 280 cases of accidents in the laboratory such as scratches, burns on hands, heat burns, eyes spattered from outside materials, and exposure to chemicals.⁴ According to other data from the Berkeley Laboratory the number of accident cases occurred from October 2017 to May 2018 as many as 70 injury cases recorded and first aid were given, accidents in Berkeley’s laboratory such as non-ergonomic working positions when moving the material, loss of hearing function, transportation accidents, exposure to chemicals, splashes in the eyes, and falling objects.⁵ Other cases also occurred at the Laboratory of High Schools in the

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suburbs of Nashville, United States in 2018 as many as 17 people including students along with teachers who were teaching science experiments were experiencing fires due to the reaction of chemicals used.\(^{(6)}\)

Actually these work accidents can be prevented by risk analysis of activities carried out in environmental health laboratories. The identification activities and risk assessment are able to determine the various risks so that control efforts can later be carried out to reduce these risks so that accidents or health problems will not occur.\(^{(7)}\) Same as determined in ISO 45001, laboratories as workplaces that have potential hazards must be controlled so accidents will not occur. It is one of the international standards in occupational safety and health management systems that provides a framework for an organization to manage risks and opportunities to help prevent work-related injury and ill health to workers. The outcome of this system is to improve and provide a safe and healthy workplace. One of the provisions in ISO 45001 in clause 6, the company is required to do hazard identification and assessment of risk and opportunities proactively.\(^{(8)}\)

Therefore, based on the potential hazards that can occur in environmental health laboratories the authors are interested in conducting research related to Risk Analysis of Occupational Disease and Accident in Environmental Health Laboratory at the Environmental Health Laboratory of Universitas Airlangga. The general objective of this study is to identify the hazards, assess the risks and determine the level of risk at the environmental health laboratory of the Faculty of Public Health, Universitas Airlangga.

**Material and Method**

This study use an observational and cross-sectional research design then conduct interview with related party to Faculty of Public Health Universitas Airlangga environmental health laboratories. This research is included in descriptive research because this study does not make comparison or relation between variables and only describes a situation objectively. This research was conducted at the Environmental Health Laboratory, Faculty of Public Health, Universitas Airlangga. The variables to be examined in this study are hazard identification, risk assessment and risk level determination at Faculty of Public Health Universitas Airlangga Environmental Health Laboratory.

The data collected in this study are the primary data obtained through observation and interview which are used to find out the activities in the laboratory, the potential hazards that exist, the magnitude of the risks and the condition of the work environments. Data processing and analysis techniques are carried out based on data from observation and interview. Based on the data we will know potential hazards and the value of likelihood and severity, then they are analyzed using the risk assessment matrix table based on AS/NZS 4360:2004 Risk Management.\(^{(9)}\) The results of the analysis can determine level of risk of potential hazards with low, moderate, high, or extreme risk categories.

**Findings**

The environmental health laboratory is included in one of the laboratories owned by the Faculty of Public Health, Universitas Airlangga. It is functioned to support the learning process of public health study program. Environmental health laboratories have various potential hazards that can cause safety and health risks for laboratory users, workers and people in the vicinity of laboratory. Activities carried out at this place include the use of electrical power equipment and roll cables while the learning process, lectures and lab activities, the cleaning of the room after lab activities. In addition, there are several source of danger potentially cause harm to occupational health and safety such as electricity that is not in accordance with the MCB used, cable roll circuit, noise and hot temperature from outside, chemicals that are in direct contact with the skin and respiratory tract, eyes that exposed to foreign objects, sitting position, equipment and part of the room that can cause injury, chemical interactions/reactions, and blockages in water installations.

Based on the results of the study, it is known that there are three levels of risk that may occur in this laboratory, namely there are 3 high risks category, 4 moderate risks category and 5 low risks category. Activities that have a high risk are the use of equipment with electric power and excessive use of cable while the learning process with the risk of power failure and fire at any time as well as interactions or reactions between chemicals during lectures or labs which also pose a risk of explosions and fires in the laboratory that will cause harm to people in the location. The total risk of high risk category at this place is 10. Control can be done to reduce the level of risk is to minimize electricity use, manufacture cable lines/electrical installations, arrange the layout of chemicals in accordance with the MSDS,
obtain appropriate work procedures and use of adequate personal protective equipment (PPE).

### Table 1. Hazard Identification Table and Environmental Health Laboratory Risk Assessment

<table>
<thead>
<tr>
<th>No</th>
<th>Activities</th>
<th>Source of Damage</th>
<th>Risk</th>
<th>L</th>
<th>S</th>
<th>Total Risk</th>
<th>Risk Level</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Use of equipment with electric power when the learning process.</td>
<td>Electric current is not in accordance with the MCB used</td>
<td>Power failure and fire at any time</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>H</td>
<td>Minimizing electricity usage.</td>
</tr>
<tr>
<td>2.</td>
<td>The overuse of roll cable when the learning process.</td>
<td>Cable roll circuit.</td>
<td>Stumbled</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>L</td>
<td>Make grooves of roll cable that will be used from the front to the back of the room</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Can cause fire at any time.</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>H</td>
<td>Make grooves of roll cable that will be used from the front to the back of the room and minimize the electricity usage</td>
</tr>
<tr>
<td>3.</td>
<td>Lecture and practicum</td>
<td>Noise from outside room.</td>
<td>Interferes the concentration of learning process.</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>L</td>
<td>Install sound absorbers in the laboratory area</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The heat from outside room.</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>L</td>
<td>Arrangement of room ventilation and installation of air conditioner</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chemicals in direct contact with skin, inhaled.</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>M</td>
<td>Use latex mask and gloves.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Foreign substances get in the eye or exposed to chemicals.</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>L</td>
<td>Use the eye rinse or clean your eyes in the bathroom</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sitting position</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>M</td>
<td>Replace the wooden chair with an ergonomic chair (adjustable height with cushioning)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Equipment/interior parts that can cause harm.</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>L</td>
<td>First aid giving</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chemical interactions/reactions</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>M</td>
<td>Providing special storage and fume hoods that meet storage requirements</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Irritations of the skin, eyes and respiratory tract</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>M</td>
<td>Providing special storage and fume hoods that meet storage requirements</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Explosion and fire</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>H</td>
<td>Arranging the layout of chemicals in accordance with the MSDS sheet</td>
</tr>
<tr>
<td>4.</td>
<td>Laboran cleans the room after practicum.</td>
<td>Blockages in water installations.</td>
<td>Irritation of the skin and respiratory tract</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>M</td>
<td>Reconstruction of water installation channels Using PPE latex gloves and masks</td>
</tr>
</tbody>
</table>

**Hazard identification**

There are four main activities carried out in this environmental health laboratory, each of which has the potential to cause disruption to health and safety for example the use of equipment with electric power when the learning process takes place with a source of electrical hazards not in accordance with the MCB used causing the electricity failure and fire at any time, excessive use of chlorine cables during the learning process with hazardous sources of cable roll circuits that causing tripping and the occurrence of fire. Lecture
and practicum with sources of danger of noise and hot temperatures from outside of the room which is disrupting the concentration learning process. Direct contact with chemicals that can be inhaled and irritate the skin, respiratory tract and eyes. Sitting position which has a risk of causing symptoms of MSDs, equipment/parts of the room that can cause injuries that risk the body part of being scratched and slashed when using equipment, and the reaction between chemicals with a risk to irritate the skin, eyes and respiratory tract and cause an explosion as well as fire. Cleaning the room after practicum by laboratory assistant with a source of danger of blockages in water installations that cause waste disposal in water installations to irritate the skin and respiratory tract.

Risk Assessment

The risk assessment carried out at the environmental health laboratory is by determining the likelihood and severity based on the AS/NZS 4360:2004 risk management category.(9) The biggest risk is electricity failure and fire at a time with a source of danger of electricity that is not in accordance with the MCB used, fire due with the source of danger of a series of roll cable that is too much and irregular, explosions and fires due with the source of chemical reactions with the probability of each occurring is 2, the possibility of occurring is rare or the frequency of occurring is annual. The severity of 5 is causing death, very large financial losses, damage to equipment that causes the cessation of the activity. On all three risks, the total value of the risk that causes electricity to fail, fire, and the occurrence of an explosion of 10 with a high risk category requires control measures and consideration of resources to reduce the risk.

MCB(Mini Circuit Breaker) functions to disconnect electric current when the electricity network is overloaded. MCB is one of the safeguards so that the risks and dangers caused by excessive use of electricity do not occur. But if the use of the MCB is not in accordance with the standard of use are able to trigger a short circuit and even a fire at a time, if the use of the MCB is in accordance with the standards then there will be an electricity failure.(10) The use of a roll cable that exceeds the usage capacity has potential to cause electrical short circuit and fire. Whereas for explosions and fires due to the danger source of a chemical reaction can be caused by a reaction between chemicals with one another whose use is not accordance to material safety data sheet, certain chemicals cannot be used together or placed in the same place.(11) 

Risk control

Risk control can be done so that the risk level can decrease so that the risks for the occurrence of occupational health and safety disturbances can be minimized when conducting activities in an environmental health laboratory(12), namely by applying appropriate and established laboratory work procedures, minimizing electricity use by using electricity as needed, make grooves of roll cable that will be used from the front of the room to the back of the room, arrangement of layout and use of chemicals material safety data sheets (MSDS), in addition also provides a special place for chemical storage and fume hoods that are eligible for activities that related to chemical reactions and the use of PPE in accordance with the designation.

Applying appropriate and established laboratory work procedures aims to ensure that activities are carried out in accordance with the standards, organizational goals, and the effectiveness and efficiency of an activity. The existence of work procedures can be used as work guidelines for various interested parties and to determine work control measures as precisely as possible.(13) Minimizing the use of electricity with the necessary use so as not to exceed the load capacity that can risk triggering electrical short circuit and fire, in addition to make the cable roll grooves is aimed to arrange the cable neatly, not blocking the people, and minimizing the potential short circuit. The arrangement of the layout and use of chemicals is applied for the chemical according to the MSDS in order to avoid unwanted reactions that have the potential to harm people around the laboratory(11)

Conclusion

The hazards identified in the Faculty of Public Health Environmental Laboratory Universitas Airlangga are 10 potential hazards with 12 risks out of 4 activities which include the use of electrical power equipment during the learning process, lectures and practices, using roll cables during the learning process, and cleaning the room after practicum activities by laboratory staff. Based on the results of the risk assessment, there were 12 risks in 4 work activities with 5 types of risk in the low risk category, 4 types of risk for the moderate risk category, and 3 types of risk for the high risk category. Control measures that can be taken to reduce risk are by implementing work procedures, minimizing the use of electricity, arranging the cable neatly and not blocking the people, minimizing the potential short circuit,
arranging the layout and use of chemicals and applying MSDS and the use of PPE in the laboratory.

References


A Cadaveric Study on the Prevalence of Hartmann Pouch of Gallbladder with Relation to Gallstones in Basrah City

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³Department of Anatomy, Histology & Embryology / College of Medicine / Al - Mustansiriyah University

Abstract

Hartmann pouch is a dilatation of the wall of the gallbladder at the region between neck and the cystic duct, It is inconstant feature found as an anatomical variant and considered a frequent site for gall stone impaction and mucocele formation as a complication. Knowledge about anatomy of the pouch is important while performing surgery for cholecystectomy to avoid serious complications.

Objective: To identify the incidence and nature of Hartmann’s pouch in human gallbladder and the relation with gallstones for the growing importance and use of various invasive surgical techniques for gallbladder and extrahepatic biliary diseases.

Material and Method: The study was carried out in the department of Forensic Medicine at Basrah city, from Jan 2018 to Jan 2019. Two hundred fourteen gallbladders were obtained from cadavers that were under examination in the morgue. The gallbladders were examined morphologically to show Hartman pouch and gall stone existence and biopsies from the pouch were taken for histopathological examination.

Results: In the present study Hartman pouch was found in 4.7%, usually at older age group (> 40 yrs.). It was slightly higher in male than female and no significant association between Hartman pouch and gall stone presence. Histological picture showed normal histology of the three layers as seen in normal gallbladder

Conclusion: Hartman pouch is inconstant feature, more in male with no significant relation to gall stone, exist as an anatomical entity in our area of study.

Key words: Hartmann pouch, gallstones and percentage

Introduction

The gallbladder is a hollow pear shaped end diverticulum which stores and concentrates bile. It lies on the visceral surface of the right lobe of the liver and anatomically formed of neck, body and fundus [1,2]. Variations in the shape and size of the gallbladder is not uncommon [3,4] and should have attention through ultrasonic investigations and during laparoscopic cholecystectomy because the related Injuries may elevate the risk of morbidity and mortality[5-9].

One of these variations is Hartmann’s pouch, which was named after Henri Albert Hartmann who first described it in 1891 [10]. He recognized it as a dilatation on the ventral aspect in the junction of neck and cystic duct, has inconstant feature, variable in size and described it as a normal variant [11-14]. The Hartmann’s pouch may cabin the gallstones as common site [15]. A big Hartmann’s pouch may hide the cystic duct and the Calot’s triangle, which poses a major trouble during cholecystectomy [16-18]. It may also be complicated in associated pathological conditions responsible for adhesion between the neck and cystic duct. In addition, it
may cause chronic infections, which promote malignant changes later on [19].

Recently, Hartmann’s pouch is recognized more as an outcome of pathology in the form of dilatation or presence of stones, when the gallstones lodged within the Hartmann’s pouch result in mucocele formation [20]. Different data about the incidence of the pouch and gallstone relation were obtained from many articles, which makes the knowledge of relevant anatomy important for safer cholecystectomy.

**Aim**

To clarify the disagreements with regard to the incidence, nature and gallstones relation to the Hartman pouch and to obtain our own reference data because of inadequate information of this type of variant in our population of Basrah city which gives value to the present study.

**Material and Method**

The study was conducted from Jan 2018- Jan 2019. Two hundred fourteen human gallbladders were collected during routine postmortem examination in the morgue of the department of Forensic Medicine at Basrah city. Both gender were taken and age ranged from 10 to 79 years. The Ethical Review Committee at Health institute in Basrah city approved the study.

A Morphological examination was carried out on all gallbladders among the studied samples of unidentified bodies. Abdominal surgery for Crush injury to the abdominal organs specifically liver, biliary system dysfunction and suspected cases of poisoning were excluded. Specimens were divided in two groups, those with Hartmann’s pouch and those without. Gallbladders were opened for the presence of gallstones. (Fig 1 a and b ). The samples were divided according to age groups as in Table 1.
Table 1: Age distribution and Numbers

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>No of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>30</td>
</tr>
<tr>
<td>20 - 40</td>
<td>111</td>
</tr>
<tr>
<td>40 - 60</td>
<td>58</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>214</td>
</tr>
</tbody>
</table>

Three millimeter thickness tissue samples from Hartmann pouch were preserved in 10% NBF for routine histopathological processing to obtain sections of 4 micrometer thickness then stained with hematoxylin-eosin and examined under the light microscope by using Elica camera for photomicrography. Data analysis were done using SPSS v.20, and P value < 0.05 is considered significant with 95% confidence interval.

Results

1. Morphological study:

A total of 214 gallbladders underwent morphological examination, age range from 3 - 79 years with mean age 36.22 ± 14.94, Male / Female ratio = 2.3 / 1.

Hartmann Pouch was observed in 10 out of 214 specimens (4.7% incidence), while gallstones were found in 14 out of all gallbladders (6.5% incidence) as shown in Fig 2.

Regarding the relation of Hartman pouch to age groups. The pouch was detected mostly in the 40-60 years old age group (n=6) followed by the (> 60 years) group (n=3) with significant statistical difference (P=0.03) in comparison to the other age groups as shown in Figure 3.

![Figure 3: Relation of Hartmann pouch to age groups.](image)

In relation to gender, Hartmann pouch was significantly more in males than in females (P=0.032), but gallstones presence did not differ significantly between the studied male & female cases (P=0.919). (Fig 4).

![Figure 4: relation of gender to the incidence of Hartmann pouch & gallstones.](image)

In relation to the pouch presence, the incidence of gallstones was slightly but not significantly greater (P=0.0707) as shown in table 2.

<table>
<thead>
<tr>
<th>Presence of Hartmann pouch and gall Stones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallstones</td>
</tr>
<tr>
<td>present</td>
</tr>
<tr>
<td>absent</td>
</tr>
</tbody>
</table>

2. Histological study:

Histological examination to the neck area of the pouch reveals three layers: the mucosal layer in form of
villi lined with simple tall columnar cells, faint stained cytoplasm and basely placed nuclei; the lamina propria with loose connective tissue and diffuse lymphatic tissue. The fibromuscular layer and smooth muscle layer interspersed with layer of loose connective tissue rich in elastic fibers. The outer layer is a wide perimuscular loose connective tissue having numerous blood vessels, lymphatics, and nerves. The serosa is seen outside. This histological picture resembles the normal histology of gallbladder (Fig 5).

Fig 5: Different histological sections of Hartmann pouch showed layers of mucosa (M), lamina propria (LP), connective tissue (CT) with blood vessels (BV), muscular layer (ML) and serosa (S), (10X, 40X),
Discussion

Brunicardi 2010 [21] found a developmental curvature at the neck region of the gallbladder, which may enlarge to form the Hartmann pouch. Standring 2008, Moore and Dalley 2006 [22, 23] detected a dilatation located at the junction of the neck of the gallbladder and the cystic duct, which is identified as Hartmann’s pouch. This anatomical variation of the biliary system and its associated choleithiasis may result in serious intra operative and postoperative complications [24, 25].

The present study found Hartmann pouch in 10 out of total 214 gallbladders (4.6%) which agrees with many other articles regarding the incidence like Leena et al 2015 who gave 4% of Hartmann pouch over total specimen examined in south Keralites, Another study by Nuran et al 2012 identified the pouch in 4 cases out of 70 (5.7%) in Dhaka, Bangladesh while Raj et al 2017 found an incidence of 8-9%. These studies consider Hartmann pouch from anatomical point of view as infrequent feature but it disagree with Van Eijck et al 2007 who detected 51 Hartmann pouch out of 98 (52%) gallbladders examined which was much higher than the present study. he also considered Hartmann’s pouch as a morphological entity. Khan et al 2014 [26] registered also higher prevalence (72.5%) of population of Dhaka (Bangladesh). In addition, Hartmann pouch was observed in a slightly higher proportion (81%) in a group of chronic calculous cholecystitis patients. The current study noticed also that the pouch tend to be more frequent with increasing age (40-60 & above 60 years) which may be due to data sample (more adults and older patients than young). Slight significant difference regarding gender and Hartman pouch distribution was detected in this study (male have slightly elevated ratio which could be due to data sample distribution). These findings disagree with Futura and Kinifu 2001 [27] who noticed a significantly higher prevalence of kinking of the gallbladder and Hartmann’s pouch in the female subjects more than male subjects.

The gallstone formation is frequent worldwide and presented as a common clinical problem faced usually in daily clinical practice in Basrah city. Majority of biliary diseases are a complication of gallstones, in form of acute and chronic cholecystitis, obstructive jaundice, gallbladder empyema, acute cholangitis, acute pancreatitis, malignancy, etc [28]. Lakshmi [29] found a significant association between Hartman pouch and presence of stone which differ from the current results (No significant relation), it could be asymptomatic but it may present with clinical manifestation which increases as the pathology of adhesions between the cystic duct and the neck of the gallbladder exist.

The Hartmann pouch could be congenital or acquired, the true (congenital) diverticula may or may not be involved in the pathology within the gallbladder but it include all three histological layers of normal gallbladder. While the acquired type may result from a disease process and has little or no smooth muscle in the wall of gallbladder [30, 31]. This study found normal histology of Hartman pouch as that present in normal gallbladder. These findings distinguish Hartmann pouch as an anatomical entity in our area of study.

Conclusion

This study provides facts of the Hartmann pouch prevalence and nature, which is of value in clinical-surgical events to manage invasive procedures, therapeutics and diagnostics in this region. It is inconstant feature with low proportion in our population, found more at older age, may lodge gallstones and tend to be anatomical more rather than pathological. A good anatomical knowledge of these structures is required, especially when performing emergency room procedures. Further recommendation is to study the Hartman pouch by imaging ultrasound in population with larger sample size.

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Conflict of Interest: Nil

Source of Funding: Self - funding

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Evaluation of Root Canal Morphology of Permanent Maxillary Canine Using CBCT – A Cross-sectional Study

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Abstract

Background: A clear understanding and thorough knowledge of anatomy of human teeth is the utmost of importance to all dental procedures especially in endodontic and orthodontics treatment. This includes the study of root canal anatomy and its variations according to the race and gender.

Aim: The scope of the research was to determine the root and canal morphology of the maxillary canine in Indian population by analyzing cone-beam computed tomography (CBCT) images.

Material and Method: Forty-six CBCT images consisting of 20 males and 26 females were examined in this study and a total of 88 maxillary canines were analyzed. The root anatomy of each tooth was evaluated for the following parameters: the root canal patterns, the shape of the access cavity, the mesiodistal width of the crown and the distance from occlusal pit to the pulp chamber. The data was analyzed and compared and p-value of the data was considered 0.05 statistically.

Result: It can be concluded that a higher prevalence of maxillary canines have Type I root canal pattern in both gender. The most common shape of access cavity in male subjects was oval. Round access cavity was the most frequently observed in female subjects. Maxillary canine in male population also had greater mesiodistal crown diameter than female population.

Conclusion: The present study reported the normal anatomy and variations of maxillary canines in Indian population. The incidence of root canal morphology differed with gender.

Keywords: CBCT, maxillary canine, root canal morphology, Vertucci’s classification.

Introduction

Complete mechanical preparation of the root canal system plays an important role of a successful endodontic diagnosis and treatment. Nevertheless, many roots possess accessory canals and a wide variations of canal configurations which some are considered unusual1.

Universally, canines are referred as the cornerstone of the human teeth with the canine eminence on their labial portion of the teeth. This is because canines help in normal facial appearance at the corners of the mouth and also functionally, they are important for determination of intercuspal position through the canine guidance in dental occlusion2. Maxillary canines typically present with a single root and a single canal. The root is oval in cross section containing an oval cross-section canal. However, root canal configurations in maxillary canines may vary according to the race, sex and population. There are many studies from past to the recent on root canal morphology and configurations have been reported which varies with the respect to the race, gender and ethnicity. All these demonstrated the complexities of the entire root canal space and frequently reported that a single root canal exists as a single foramen3,4,5,6.
As the root canal morphology varies from tooth to tooth and its classification is much needed for communication, diagnosis and treatment planning, numerous classification for root canal morphology and its modifications have been proposed over the years which figures the total number of root, the number of canal in every root and the course of canal which confines the root. The earliest classification of root canal morphology was given by Weine FS et al in 1969 and followed by Vertucci FJ in 1984. Vertucci FJ demonstrated configuration for three canals, after he studied the root canal morphology of maxillary first premolar using clearing technique. His classification contains eight types of root canal system and is the most commonly used in most of the studies. Aminsobhani et al reported a significance number of mandibular canines had more than one root canal in Iranian population. Caucasian population was identified with only Type 1 canal in maxillary canines and additional canal configuration (1-3-4-1) was found in Turkish population.

It is well known that there are many methods can be done to study the root and canal morphology, which includes computed tomography (CT) scanning, cone beam computed tomography (CBCT) canal staining and tooth clearing techniques, conventional radiography techniques, contrast medium-enhanced radiography and sectioning. Cone beam computed tomography (CBCT) has become a successful tool to examine the root canal anatomy and has been widely used nowadays. It had been introduced in dentistry since the year of 1990 for endodontic purpose. CBCT works in the cone-shaped beam of radiation in a single 360° rotation and it is more advantageous than CT scanning due to its improved accuracy, low radiation dosage and lesser scanning time. Neelakantan et al. also supported that this technique is considered as the gold standard of identification root canal morphology. This study aims to determine the root and canal morphology of the maxillary canine in Indian population by analyzing cone-beam computed tomography (CBCT) images.

**Material and Method**

**Sample Collection**

The sample size was determined based on the G Power analysis. It was calculated by using prevalence of 0.05% and the precision (d) is 0.05. The power analysis indicated that 76 sample size were required. In order to increase power of this study, 86 maxillary permanent canines were examined.

\[
\text{Sample size} = \frac{Z_{1-\alpha/2}^2 \cdot p(1-p)}{d^2}
\]

Here

- \(Z_{1-\alpha/2}^2\) is the standard normal variate. As in majority of studies p values are considered as 0.05 so, 1.99 is used in formula
- \(p\) is the expected proportion in a population based on previous studies and the value is 0.05
- \(d\) is the absolute error or precision and it is 0.05

\[
\text{Sample size} = \frac{1.99^2 \times 0.05(1-0.05)}{0.05^2} = 76
\]

A total sample of 46 CBCT images consisting of 20 males and 26 females were examined in this study and a total of 86 maxillary canines were analyzed. The root anatomy of each tooth was evaluated for the following parameters: the root canal patterns, the shape of the access cavity, the mesiodistal width of the crown and the distance from occlusal pit to the pulp chamber. The criteria selections are presence of erupted maxillary permanent canines, complete formed root apices and those with free of deep caries, fracture and root canal fillings.
Scanning Procedure

CBCT images were obtained in the Saveetha Dental College, Department of Oral and Maxillofacial Radiology and the software used was SICAT Galileo Viewer version 1.9. Tomography sections of 0.3 mm axial planes and magnification was 1 : 1 were created. Serial axial CBCT images were examined by carefully rolling the toolbar from the pulp chamber to the apex.

The shape of access cavity at cementoenamel junction (CEJ) was evaluated in cross sectional view and Jou et al.’s classification was used15. The root canal pattern of maxillary permanent canines was classified based on Vertucci’s classification. Mesiodistal width of crown structure in mesiodistal planes was measured based on three levels; incisal third, middle third and apical third. The distance between occlusal pit and pulp chamber was also measured in this study.

Results

Root Canal Pattern

According to the present study, the most prevalent root canal configuration of maxillary canine in both gender were Type I (male 75% and female 79.2%). Maxillary canines in male patient had 15% of Type II and equally 5% of Type III and Type V. In female patients, their other root canal patterns were Type IV (8.3%), Type V (8.3%) and Type VI (4.2%). Both gender did not have root canal morphology Type VII and VIII. Present results are tabulated in Table 1 and Table 2 shows comparison with earlier studies on maxillary canines.

Shape of Access Cavity at CEJ

In male patients, a relatively highest percentage of the shape of the access cavity was oval (40%) and other shapes include; round (35%), long oval (20%) and flattened (5%). The most common shape of access cavity of maxillary canines in female patients was found to be a round shape (54.2) and the least was long oval (8.3%).

Mesiodistal Width of Crown

The average of mesiodistal width of crown structure in male patients was higher than female patients with the respect of incisal third, middle third and apical third.

Distance Between Occlusal Pit and Pulp Chamber

Present study showed maxillary canines in male patients (mean = 4.67) had longer distance between occlusal pit and pulp chamber than in female patients (mean = 4.17).

Table 1 : Root canal patterns in maxillary permanent canine of both gender in %

(n = 86)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
<th>Type V</th>
<th>Type VI</th>
<th>Additional Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>75</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>79.2</td>
<td>0</td>
<td>0</td>
<td>8.3</td>
<td>8.3</td>
<td>4.2</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2 : Root canal patterns in maxillary permanent canine in %

<table>
<thead>
<tr>
<th>Authors</th>
<th>Techniques</th>
<th>Number of teeth</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
<th>Additional Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertucci [10]</td>
<td>Clearing and staining</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sert and Bayirli [11]</td>
<td>Clearing and staining</td>
<td>100</td>
<td>91</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pineda and Kuttler [18]</td>
<td>Radiographs</td>
<td>260</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3: Shape of the access cavity at CEJ in %

<table>
<thead>
<tr>
<th>Gender</th>
<th>Round</th>
<th>Oval</th>
<th>Long Oval</th>
<th>Flattened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>35</td>
<td>40</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>54.2</td>
<td>16.7</td>
<td>8.3</td>
<td>20.8</td>
</tr>
</tbody>
</table>

Table 4: Average value of mesiodistal width of crown structure

<table>
<thead>
<tr>
<th>Gender</th>
<th>Parameter</th>
<th>Crown Diameter (Mesiodistal Width, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Incisal Third</td>
</tr>
<tr>
<td>Male</td>
<td>Mean</td>
<td>6.73</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.77</td>
</tr>
<tr>
<td>Female</td>
<td>Mean</td>
<td>6.60</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Table 5: Average value of distance between occlusal pit and pulp chamber

<table>
<thead>
<tr>
<th>Gender</th>
<th>Parameter</th>
<th>Distance Between Occlusal Pit and Pulp Chamber (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Mean</td>
<td>4.67</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.90</td>
</tr>
<tr>
<td>Female</td>
<td>Mean</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Figure 1: Vertucci’s classification of root canal system [16]
Discussion

Most of the methodologies to study root canal anatomy in the past such as periapical radiographs, clearing and demineralizing method and surgical operating endoscopy are invasive procedures as they are able to alter the actual canal morphology of the root. Based on a test done by Nguyen, it was proven that CBCT is an accurate measurement tool that can be used for measuring mesiodistal diameter of unerupted teeth. Neelakantan et al. also said in his study that CBCT is the gold standard in the determination of root canal configuration since its technology overcomes some drawbacks of conventional radiography as relatively high-resolution, very thin and high contrast that is able to eliminate distortion, superimposition and artifacts of the imaging.

For centuries, a great number of researchers have been studying the morphology of root canal especially in human teeth and they occasionally concluded that the system of root canal morphology have always been varies in regards to the ethnicity, gender and also population. Most reported earlier studies include the population of America, Sudan, Chinese, Sri Lanka, Uganda and Turkey. The variation of root canal morphology extremely is challenging in endodontic and orthodontics management. Endodontists bear huge responsibilities to clean these canals from any microbial biofilms with a proper mechanical preparation and use of irrigants. All these can only be achieved with a thorough knowledge and comprehension of the root canal anatomy thereby reducing the number of post treatment failures.

In the current study, the most common root canal pattern in both male and female Indian are Type I (75 % and 79.2 % respectively). Similar findings were found by Vertucci (100 %), Sert and Bayirli (91 %) and Pineda (100 %) in maxillary canines (Table 2). The second most common root canal in the maxillary canine of male was a Type II (15 %), followed by Type III and Type V, both in 5% of the samples. In the present study, the second most commonly occurring canal pattern in maxillary canines of female population was Type IV and Type V at 8.3% and followed by Type VI in 4.2% of the samples. Previous study10,11,19 did not report the presence of Type V and Type VI canal morphology in...
the maxillary canine, but in the present study both types of canal pattern were seen in both gender.

The various shape of access cavity of maxillary canine is tabulated in Table 3. In maxillary canine of male population 14 teeth had round access cavity, which is at 35% of the samples. 16 teeth had oval access cavity, which makes up the most commonly (40%) occurring shape of access cavity in male population. Other shape include long oval access cavity at 20% and the least was flattened access cavity at 5%. In female population, the most commonly found was round access cavity at 54.2% (26 teeth), followed by 20.8% of flattened access cavity. Based on the report of Somalinga and Raghu20, the most common shape of the access cavity was oval, found in 94 teeth with the percentage of 37.6%. 66 teeth of maxillary canine had flattened access cavity, amongst which 59% of the teeth had more than one canal. Somalinga and Raghu also reported that a flattened shape of access cavity might have more than one canal pattern in both maxillary and mandibular canine.

Based on the result of present study, male population (mean value = 4.67mm) showed a greater distance between occlusal pit and pulp chamber than female population (mean value = 4.17mm). Precaution should be taken into notes during access opening preparation of canals in order to prevent perforation. The average measurements of crown diameter of male and female subjects at incisal third were 6.73mm and 6.6mm respectively. In male subjects, it was 6.79mm at middle third and 5.7mm at apical third. Meanwhile, the average crown diameter of female population was 6.13mm at middle third and 5.18mm at apical third. For this study, comparison was made and one can differ that male subjects highly has greater mesiodistal width than female subjects. This is very helpful in replacement of prosthetic teeth in the cross section of population.

**Conclusion**

The present study reported the normal anatomy and variations of maxillary canines in Indian population. The incidence of root canal morphology differed with gender. CBCT is truly a useful device for endodontic diagnosis and treatment. It can be concluded that a higher prevalence of maxillary canines have Type I root canal pattern in both gender. The most common shape of access cavity in male subjects was oval. Round access cavity was the most frequently observed in female subjects. Maxillary canine in male population also had greater mesiodistal crown diameter than female population.

**Ethical Clearance** – All datas were taken from examination of outpatients of Saveetha Dental College and Hospital

**Source of Funding** – Saveetha Dental College and Hospital

**Conflict of Interest** – Nil

**References**

Relationship between Radiographic Evaluation with Age and Gender – A Radiographic Study

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Abstract

Background: Forensic odontology plays an important role in identification of man-made or natural disasters event which restricts identification through conventional methods. Various approaches have been specified which helps in the identification of the post-mortem remains. One such is the identification of the morphological features of the human skull and mandible. These features may help in sex differentiation. The aim of this study is to evaluate the relationship between radiographic evaluation with age and gender.

Materials and Method: A retrospective study was done with a total of 250 panoramic radiograph image. The images of 150 males and 100 females were selected with the range of age from 20 to 70 years old and divided into group 1 (20-45 years old) and group 2 (46-70 years old). Three parameters were evaluated from the panoramic radiograph which were gonial angle, antegonial angle and mental foramen against the age and gender. Statistical analysis was done for hypothesis testing.

Results: The results of this study suggests that there is no significant difference seen in the gonial angle and antegonial angle with relation to the age (p≥0.05). However, the relationship between age and mental foramen is highly significant (p≤0.01). With regards to gender, all the three parameter shows highly significant results with (p≤0.01).

Conclusion: In conclusion, the three parameter assessed in this study can be used for gender differentiation but not for age estimation which were highly significant. As for age estimation, the distance of mental foramen and tangent line of inferior border of mandible can be assessed.

Keywords: Antegonial angle, gonial angle, mental foramen, panoramic, radiographic evaluation

Introduction

An event which causes total destruction to the human body may require a group of specialist which deals with post-mortem identification. This team would include a forensic odontologist. Forensic odontology is a branch of dentistry which in the interest of justice, deals with the proper handling and examination of dental evidence, and with the proper evaluation and presentation of dental finding (1). This specialised branch of dentistry helps in identification of human remains if the other conventional method such as fingerprints and visual recognition are not possible (1).

The skeletal remains play an essential role in identification of gender. Most common skeletal parts used in the previous study were the skull and mandible. However, mandible which is the almost dimorphic, largest and strongest bone contributes to the significant difference in identifying gender among the human remains (2, 3). Mandible is made up of compact bone which makes it durable and highly resistant to fracture(4).
Apart from that, chronological age assessment is also an important factor in identifying human remains. Several factors may affect the morphological changes in the mandible which are the dental status and age. Some anatomical structure which might be affected are gonial angle, antegonial angle, condyle and ramus.

Dentofacial radiographs plays a significant role in identifying anatomical landmarks of the maxilla and mandible. Various intraoral and extra oral radiographs are available. However, the most commonly used radiograph in the assessment of anatomical landmarks in the mandible are the orthopantomography (OPG) or panoramic radiograph. This radiograph shows a whole view of the dentofacial tissues of the maxilla and mandible (5). Besides, the use of OPG in forensic anthropology which helps in the comparison of ante-mortem and post-mortem radiographs is one of the cornerstones of positive identification of human remains(4).

The aim of this study is to evaluate the relationship between the radiograph evaluation with age and gender. The radiographic evaluation includes the gonial angle, antegonial angle and mental foramen.

**Materials and Method**

This retrospective study was conducted in Department of Oral Medicine and Radiology at Saveetha Dental College and Hospital, Chennai, India. This study was approved by the institutional ethical committee of the college and informed consent was taken from all patients who were included in the study. A total of 250 panoramic radiographs were taken from the department. A few inclusion and exclusion criterias were assessed before including the radiographs in this study. The inclusion criteria was a clear radiographic image showing the anatomical landmarks of the gonial angle, antegonial angle and mental foramen. The exclusions criteria were any fractured mandible, mixed dentition, blurred radiographic images and micrognathia.

In this present study, three parameters were assessed which were the gonial angle, antegonial angle and mental foramen. Gonial angle is the angle between the distal ramus of mandible and lower border of the mandible. The angle can be determined by tracing two line intersecting from the distal ramus of mandible and lower border of mandible. Next, antegonial angle is an angle formed at the deepest antegonial notch at the lower border of the mandible. It can be measured by tracing a line parallel to the antegonial region. Third parameter was the mental foramen. It was measured by drawing a line tangent to the lower border of the mandible and a perpendicular line from the inferior most mental foramen was drawn along the tangent.

The panoramic radiographs were divided into two category: age and gender. For the first category, the age was divided into two groups. Group 1 consists of patient age from 20-45 years old and group 2 consists of patient age from 46-70 years old. Based on gender, the patients were divided into male and female. Statistical analysis was done by using t-test to test the hypothesis.

**Results**

Table 1 shows the result of this study which suggests that there are no significant differences seen in the gonial angle and antegonial angle in relation to the age (p≥0.05). However, the relationship between age and mental foramen is highly significant (p≤0.01). In regards of gender, all the three parameter shows a highly significance results with (p≤0.01) as shown in Table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age</th>
<th>Mean ± Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonial angles</td>
<td>Group 1</td>
<td>122.38 ± 7.27</td>
<td>≥0.05</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>123.15 ± 8.92</td>
<td>≥0.05</td>
</tr>
<tr>
<td>Antegonial angle</td>
<td>Group 1</td>
<td>165.83 ± 7.49</td>
<td>≥0.05</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>165.06 ± 7.47</td>
<td>≥0.05</td>
</tr>
<tr>
<td>Mental foramen (mm)</td>
<td>Group 1</td>
<td>10.60 ± 1.79</td>
<td>≤0.01</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>11.33 ±1.08</td>
<td>≤0.01</td>
</tr>
</tbody>
</table>
The relationship between age and the radiographic evaluation shows that as the age increases, the more obtuse the gonial angle become. There is slight increase of gonial angle in group 2 with 123.15 ± 8.92 compared to group 1 with 122.38 ± 7.27. Meanwhile, there is no significance difference seen in the antegonial angle in both age groups. As for mental foramen, the distance is greater in group 2 compared to group 1 (11.33 ±1.08 and 10.60 ± 1.79).

Table 2 Shows the relationship between radiographic evaluations with gender

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gender</th>
<th>Mean ± standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonial angle</td>
<td>Male</td>
<td>121.05 ± 8.74</td>
<td>≤0.01</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>124.89 ± 6.28</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Antegonial angle</td>
<td>Male</td>
<td>164.60 ± 7.99</td>
<td>≤0.01</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>167.04 ± 5.44</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Mental foramen (mm)</td>
<td>Male</td>
<td>11.09 ± 1.62</td>
<td>≤0.01</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10.61 ± 1.51</td>
<td>≤0.01</td>
</tr>
</tbody>
</table>

This present study shows that male has a smaller gonial angle compared to female (121.05 ± 8.74 and 124.89 ± 6.28). To compare the antegonial angle, female has a greater antegonial angle compared to male (167.04 ± 5.44 and 164.60 ± 7.99). However, for the distance of inferior border of mental foramen to the tangent line of inferior border of mandible shows a slight differences between male and female (11.09 ± 1.62 and 10.61 ± 1.51).

Discussion

Based on the results of this study, there was no statistically significant difference between age and gonial angle. However, there was increase in gonial angle as the age increase. In the study done by Upadhyay et al, there was a significant decrease in the mean gonial angle as the age advances but the results were not statistically significant to be reliable (6). Meanwhile, in the study done by Bhardwaj et al., Kasat V et al., and Shendakar AT et al., suggested that there were changes of the gonial angle from more obtuse to less obtuse and become more obtuse as the age advances (7-9). Apart from that, another study done by Sicher H et al, suggested that edentulous patient will have a greater gonial angle due to the disuse atrophy and tooth loss (10). In the study done by Carolina et al, there was significant difference between age and gonial angle in which the gonial angle decreases and stop from 21 years old onwards (11).

According to gender, many studies suggested that the gonial angle were greater in female compared to male which was similar to this present study (≤0.01). In the study done by Burak Apaydin et al. and Revant H. Cole et al. a statistically significance difference were found between male and female patients (p≤0.05) in which female has greater gonial angle than male (12, 13). However, in the study done by Upadhyay et al., Raustia AM and Salonen mam and Ceylan et al, there were no statistically significance between gender and gonial angle (6, 14, 15). Usually, male will have a greater mean angle of 3°-5° due to the large anatomical mandible (16). Nevertheless, the reason why female has a greater gonial angle is due to the masticatory forces act on the mandible (7). On average, male will have a greater masticatory forces which results in smaller gonial angle. The rotation of the mandible which is different between male and female may also affect the gonial angle. Female will have a downward and backward rotation of the mandible whereas male will have a forward rotation of mandible (12, 17).

The next parameter assessed was the antegonial angle. This study suggested that the antegonial angle in female was greater than male with a statistically significant result (≤0.01). The result was comparable with the study done by Bhardwaj et al and Burak Apaydin et al which showed that antegonial angle was greater in female which was highly statistically significant (≤0.01) (7, 12). Antegonial region is situated at the lower of mandible which is exposed to bone resorption which in turn decrease the antegonial angle and antegonial depth. The reason of the differences between male and female may be due to the hormonal changes. Testosterone and oestrogen affecting the bone metabolism of male and female respectively (7).

The relationship between the age and antegonial angle in this present study was not statistically significant (≥0.05) which was comparable to the study done Ghosh et al. Only a slight changes seen between group 1 and
Mental foramen is an anatomical landmark which is usually seen in the second premolar and first molar region. In this study, there was a statistically significant changes seen in the distance of mental foramen from the tangent line of the inferior border of the mandible (≤0.01) in relation to age and gender. As the age increases, the distance increases. Usually in the adults, the mental foramen is nearer to the inferior border but as the age advances, the mental foramen moves upward closer to the alveolar border due to tooth loss and bone resorption (19). In the study done by Bhardwaj et al, Amorin M et al, Kasat V et al and Shendarkar et al suggested that there were no statistically difference between ages (7-9). However, with regards of gender, this study showed that the distance from mental foramen to the tangent line of inferior border of mandible is higher in male than female (≤0.01) which is consistent with the study done by Burak Apaydin et al and Thakur et al (12, 20). Meanwhile in the study done by Prabhu et al, there was no statistically significant between mental foramen and gender (≥0.05) (21).

**Conclusion**

In conclusion, the three parameter assessed in this study can be used for gender differentiation but not for age estimation as gender differentiation alone was highly significant. As for age estimation, the distance of mental foramen and tangent line of inferior border of mandible can be assessed. Further study should be done using advanced modalities device to reduce the limitation of this study.

**Conflict of Interest**: Nil

**Source of Funding**: Nil

**References**


Corrosion of Orthodontic Metallic Brackets Immersed in Solutions of Salt and Spices in Artificial Saliva

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Abstract

Background: Orthodontic treatment is commonly use to treat dental malalignment, especially orthodontic braces as it help to realigned teeth to the normal position as well as for aesthetic purpose. As orthodontic braces is a choice of orthodontic treatment various kind of orthodontic braces had been invented based on the material used, adhesives systems as well as their strengths to bring about tooth movement. As it is placed in the oral cavity, the aggressive environments of the oral cavity, will lead to corrosion of the orthodontic brackets. This may affect it physical properties and clinical performance.

Aim: To study the corrosion of orthodontic brackets that immersed in the solution of artificial saliva and spices.

Materials and Method: Stainless steel orthodontic brackets were used for this studies. Each bracket was placed in the container contain artificial saliva and other agents. Several types of aqueous solutions were prepared for this study by using different agents (spices). They were pure artificial saliva, salt (sodium chloride), black pepper, turmeric and mixture of all of the spices along with salt. After 24 hours, surface changes was seen under the optical microscope.

Results: Based on the study, orthodontic brackets surface immersed in the controlled medium, revealed that more corrosion was seen in the salt medium when compared to pure artificial saliva. More pitting also was seen in the salt medium of the controlled group. Based on the surface analysis of the orthodontic brackets in the studied groups, demonstrate that more corrosion and pitting was seen in the aqueous solution of mixture of artificial saliva with salt and spices and less pitting and corrosion was seen in the turmeric solution.

Conclusion: Based on the study, the finding suggest that the corrosion behaviour of the orthodontic brackets is influenced by the presence of a salt (NaCl) and other spices. Salt and black pepper are responsible for the corrosion of the orthodontic brackets, while turmeric capable in reduction or slowing the process of corrosion.

Keywords: Corrosion of brackets, spices, orthodontic treatment

Introduction

Orthodontic treatment is commonly use to treat dental malalignment, especially orthodontic braces as it help to realigned teeth to the normal position as well as for aesthetic purpose. Early approach for orthodontic treatment will help to provide a better outcome. As orthodontic braces is a choice of orthodontic treatment various kind of orthodontic braces had been invented based on the material used, adhesives systems as well as their strengths to bring about tooth movement. This will help to provide a good results and also patient comfort and aesthetic through the dental treatment. Example of dental brackets include metal brackets, titanium brackets, ceramic bracket and plastic brackets (aesthetic) [1].

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Among these brackets, metal brackets is commonly used for orthodontic treatments due to their strong shear strength and also its successful results in clinical orthodontic study. However, when orthodontic brackets are placed inside the oral cavity, pitting and corrosion may occur as they are exposed to the components of the saliva as well as the food that accumulate in the oral cavity. Generally, saliva composed of inorganic salts which mainly chlorides and phosphates, along with other components like organic acids, enzymes, bacterial and also gastric secretions [2]. These mixture of saliva along with the food intake (especially spices), will create an aggressive environment for the orthodontic braces, which in return may results to degradation. Even though, they are manufacture to become resistant against the mechanical stress and also degradation cause by the active forces and corrosive environment [3].

This aggressive environments of the oral cavity, will lead to corrosion of the orthodontic brackets. Variety of dietary sources like spices, common salt; sodium chloride (NaCl), fruit juices and carbonated drinks also could be one of the factors which promote the corrosion of the brackets [4]. therefore, diet plays an important role in causing the corrosion of the metal appliances that placed in the oral cavity [5]. Corrosion of this dental materials in oral cavity tend to cause tooth discolouration, local pains or allergic reactions due to released of the metals ions into the organisms present in oral cavity [3,6]. Apart from that, corrosion of the metal parts can also affect their biomechanical properties along with the appliance efficacy [7].

On the orthodontic brackets surfaces, pitting corrosion are commonly seen. It is the most destructive type of corrosion which seen in the orthodontic appliances placed in the oral cavity [8]. It caused localised type of corrosion which can be scattered, isolated or closed together in forms of holes or pits within the metal. It will result to rough like appearance on the surface of the appliance. This pitting usually occur primarily within the crevices or other protected areas on metal surfaces that exposed to ward the corrosive agents or environment [4]. Therefore, this study was done to evaluate the relationship between the corrosion of the orthodontic brackets in artificial saliva with salts and spices.

### Materials and Method

#### Artificial Saliva

Materials that bring used in the study were mainly orthodontic brackets, artificial saliva and also spices. Orthodontic brackets used for the study are stainless steel (Libral Traders). The artificial saliva used was Wet mouth ICPA, which is manufactured artificial saliva for wetting purposed. It commonly indicated for the xerostamia patient or patient on oral therapy. It composed of water, glycerine, cellulose gum, sodium saccharin, parabens and also flavouring agents.

#### Salt and Spices

Several types of aqueous solutions were prepared for this study by using different agents (spices). They were pure artificial saliva, salt (sodium chloride), black pepper, turmeric and mixture of all of the spices along with salt. Salt which is sodium chloride (NaCl) was used as the promoter of corrosive agent in this study, while numeric act as inhibitor of the corrosion. The chemical compositions of the spices used in this study were showed in Table 1.

<table>
<thead>
<tr>
<th>Spices</th>
<th>Scientific Name</th>
<th>Chemical Compositions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black pepper</td>
<td>Piper nigrum</td>
<td>Crystalline alkaloids, piperine, piperetine, terpenes, β-caryophyllene, limonene, sabinene, β-pinene, myrcene, p-cymene and oxidated constituents [5]</td>
</tr>
<tr>
<td>Tumeric</td>
<td>Curcuma longa</td>
<td>Diaryl heptanoid (curcuminoid, curcumin), sesquiterpenes (zingiberene), ketones and monoterpenes [5]</td>
</tr>
</tbody>
</table>
Surface analysis study

Initially, this study was conducted by preparing the aqueous medium, followed by immersing the orthodontic brackets in the prepared aqueous solutions as shown in Figure 1. The orthodontic brackets was placed in the closed container for about 24 hours. After 24 hours, surface analysis was done in order to identify the rate of corrosion of the orthodontic brackets immersed in the artificial saliva with salt and other spices solutions. Surface analysis was done by using the optical microscope.

Results

Artificial saliva and artificial saliva with sodium chloride medium were controlled variables of the study, which used as reference mediums. Whereas, turmeric, black pepper and mixture of the spices were used as the studied solutions. Based on the visual observation of the orthodontic brackets surface immersed in the controlled medium, it revealed that more corrosion was seen in the salt medium when compared to pure artificial saliva. More pitting also was seen in the salt medium of the controlled group.

Based on the surface analysis of the orthodontic brackets in the studied groups, demonstrate that more corrosion and pitting was seen in the aqueous solution of mixture of artificial saliva with salt and spices as shown in Figure 6. Figure 3 reveals less pitting and corrosion was seen in the turmeric solution. The corrosion and pitting of the orthodontic brackets were arranged in the ascending order as mention below.

1. Turmeric
2. Artificial saliva
3. Salt
4. Artificial saliva and black pepper
5. Mixture of artificial saliva, salt, turmeric and black pepper

Table 2: pH of solution medium immediately after preparation and after 24 hours.

<table>
<thead>
<tr>
<th>MEDIUM</th>
<th>pH of solution immediate after preparation</th>
<th>pH of solution after 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial saliva</td>
<td>7.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Salt</td>
<td>7.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Turmeric</td>
<td>6.2</td>
<td>6.5</td>
</tr>
<tr>
<td>Black pepper</td>
<td>6.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Mixed of spices and salt</td>
<td>6.5</td>
<td>6.9</td>
</tr>
</tbody>
</table>
Discussion

Variety of spices used in food have lots of benefits and medical used. In general, both tumeric and black pepper are having anti oxidants property, which allowed them to scavenge the free radicals produced inside the body. However, when they are consumed in a large amount it may cause corrosion to the orthodontic appliances which placed inside the oral cavity due to their aggressive nature especially the black pepper. Salt is also one of the ingredients which commonly used in food, as flavour enhancer. However, high intake of salty food should be avoided especially during the orthodontic treatment, as it can promote the corrosion of the orthodontic brackets, due to formation of acid. This acid is formed due to release of chloride ions from the salt with all available hydrogen ions in the oral cavity.

Released of the chloride ions from the salt also cause negative effect on the formation of the passive layer on the metal surface. Passive film is a protective film that formed on the orthodontic alloy which can be spontaneously forms or reformed in air or under the wet conditions, in the presence of oxygen. Oxygen allow this film to form and maintain it consistency. However, in the presents of acidic or chloride ions, it may cause alterations on the passive film, which can promote corrosion of the orthodontic brackets. This chloride ion will combine with a metal ion to form metal chloride, which lead to dissolution and propagation of the latter in autocatalytic manner. As the release of this metal ions increased, this will result to increase in the pH of the saliva, which in turn increase the rate of corrosion of the orthodontic brackets.

Other spices like black pepper and chilly powder also may cause corrosion of the orthodontic brackets, due to their aggressive nature. Adding of the black pepper to the artificial saliva enhance the aggressive property of the artificial saliva also through chloride ions, which promote corrosion of orthodontic brackets. Pitting is one of the common localised corrosion which seen on the surface of the metallic orthodontic brackets. It usually form due to the chloride ions, which break down the protective thin oxide film and cause rapid dissolution of the underlying metal and result to localise pitting formation. Thus, it may cause formation of holes, if the solution are too aggressive. Therefore, mixture of artificial saliva with salt and black pepper show the most corrosion in compare to other aqueous solutions.

Although, some spices can cause corrosion of the orthodontic brackets, some spices may inhibit or slowing the process of corrosion of orthodontic brackets like turmeric and coriander. The components of turmeric may have favoured in delayed of the corrosion of the orthodontic brackets. They will allow for prevention of adsorption of chloride ion, which is the responsible agent to cause pitting and corrosion of the orthodontic brackets. Therefore, aqueous solution of turmeric show the least corrosion on surface analysis min compare to other mixtures.

Thus, a proper diet should be educate to patient undergone the orthodontic treatments, as corrosion of the orthodontic appliances may affect the physical properties and its mechanical performance for a success orthodontic treatments.

Conclusion

Based on the study, the finding suggest that the corrosion behaviour of the orthodontic brackets is influenced by the presence of a salt (NaCl) and other spices. Corrosion can cause weakening of the orthodontic brackets and leading to mechanical failure of the orthodontic treatment, as it may affect the physical and chemical properties of the materials. Salt and black pepper are responsible for the corrosion of the orthodontic brackets, while turmeric capable in reduction or slowing the process of corrosion. Therefore, a proper diet should be instructed to patient undergone orthodontic treatment, in order to prevent failure of the treatment and also promote the success of the treatment.

Ethical Clearance - Not required
Source of Funding- Self
Conflict of Interest - Nil

References


Cheiloscopy: The Study of Lip Prints in Sex Identification between Indian and Malaysian Population

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Abstract

Background: The study of lip prints, is commonly applied in forensic odontology and it is known as cheiloscopy. The term cheiloscopy was derived from Greek words, in which “cheilos” means lip and “scopy” means to examine. In general, cheiloscopy is define as a method of identifying an individual based on the arrangement of lines seen on the red part of the lips or a science dealing with the various line appearing on the red part of the lips. The pattern of wrinkles and grooves that are present on the lips has distinct characteristic features of an individual which are almost similar to the fingerprints. It also will not change throughout the life. Therefore, it can be used for personal identification and sex determination.

Aim: To investigate lip print pattern in males and females and to evaluate them between Malaysian and Indian population.

Materials and Method: There were 40 participants of both gender. 20 of them are from the Malaysian population and another 20 people are from the Indian population. Each participant was instructed to clean their lips with a clean white cloth, before taking the lip print. This was followed by the application of dark coloured lip stick on lips by using the lip stick applicator. Cellophane tape was used to take the negative impression of the lip pattern. The glued portion of the cellophane tape was placed on the lips by giving a light pressure beginning from the centre of the lips till the corner of the mouth. Then, the impression was transferred to a clean white paper and seen under the magnifying glass.

Results: Type I lip pattern was commonly seen in Indian population, followed by type III, II and IV. Whereas for Malaysian population, type III was most common followed by type I, II and IV. There was significant difference between both population. In this study, it showed that type I was the most common lip print pattern seen in both Indian and Malaysian population for females. Whereas for male, type III was more common in Malaysian population, and both type III and IV were more common in Indian population.

Conclusion: There was no significant difference in both population according the general distribution of lip print pattern. There was no correlation or similarity of lip print patterns between each participants in both population. Thus, cheiloscopy can be used for sex determination and also personal identification.

Keywords: Lip prints, Cheiloscopy, Malaysian population and Indian population

Introduction

Each of every human beings is very unique, as each of the individuals have different personality along with their facial and body features. These special features can be used to distinguish one individual from another. Generally, fingerprints and DNA coding are commonly used in personal identification especially in the forensic field, as this information can be easily obtained and
different for each individual. This is also similar to the lip prints. The pattern of wrinkles and grooves that are present on the lips has distinct characteristic features of an individual which almost similar to the fingerprints [1]. It also will not change throughout the life [2]. Even though, inflammation, trauma or disease like herpes may occur, there is possibility for the pattern on the lips to recover to normal pattern [3].

In fact, smoking and age factors also does not influence the alteration or changes of lip pattern [4]. Therefore, it can be used for personal identification and sex determination. However, under certain circumstances like genetic deformities, surgical marks or maybe due to their occupation like blowing musical instruments [5], alteration on the lip patterns may occur. It is also similar to fingerprints which can be lose due to works or injury to the finger. Thus, it may be a disadvantage in certain cases and in those cases under unavoidable circumstances, where other alternative like DNA is required for further investigations.

The study of lip prints, is commonly applied in forensic odontology and it is known as cheiloscopy. The term cheiloscopy was derived from Greek words, in which “cheilos” means lip and “scopy” means to examine [6]. In general, cheiloscopy is define as a method of identifying an individual based on the arrangement of lines seen on the red part of the lips or a science dealing with the various lines appearing on the red part of the lips [7,8]. This involves the examination of the normal lines and fissures in the forms of wrinkles and grooves present in the transition area in between the inner labial mucosa and the outer skin of the lips [9]. The wrinkles and grooves that form on lip, contribute to different type of lip patterns, which differs for each individual.

In a crime scene investigation, a study of lip prints is very useful as they can be used for crime analysis and can be used to identify and rule out the suspect of the crime. Even the sex of the suspect can be analysed and determined. The lip prints evidence obtained from the crime scene should be preserved and the lip prints of suspects should be obtained to ruled out the criminal. For personal identification, cheiloscopy is useful as the lip prints may have been preserve in photograph, which can be applied in individual identification. This proves that Cheiloscopy has an equivalent value for personal identification and sex determination in forensic similar to other types of forensic evidences [10]. Therefore, this study was conducted to investigate lip print pattern in males and females and to study between Malaysian and Indian population.

Materials and Method

The study was conducted based on Indian and Malaysian populations of both populations with the average age values of 19 to 22 years old. There were 40 participants involved in this study, in which 20 of them are of Indian population and remaining 20 participants are Malaysian population of both sex. Consents of individuals were obtained for this study. During this study, the lips of the participants should be free from any injuries or deformities which may alter the lip print patterns during the study. Cracked or chapped lip also should not be seen on the lip of the participants, as it may result to distortion of lip print pattern. Therefore, the lip should be smooth with no other irregularities seen on the lips.

Each participants was instructed to clean their lips with a clean white cloth, before taking the lip print. This was followed by the application of dark coloured lip stick on the participants lips by using the lip stick applicator for a smooth and a proper coverage of the lips. Cellophane tape was used to take the negative impression of the lip pattern. The glued portion of the cellophane tape was placed on the lips by giving a light pressure beginning from the centre of the lips till the corner of the mouth. Then, the impression was transferred to a clean white paper and seen under the magnifying glass. The lip prints obtained were kept, with respect to name and sex of the participants in each population. Each lip print impression was recorded and analysed for the sex determination and comparison between Indian and Malaysian population.

The classification of lip pattern was analysed according to the classification of lines on lips which was proposed by Suzuki and Tsuchibashi in 1970 [11,12]. They were divided into six types based on the shape and grooves presence on the lips. The lips were examined based on the majority of lines or grooves seen on the entire part of the lips.

- Type I: Clear-cut vertical grooves or line that run across the entire lips.
- Type I’: Straight groove that disappear half way into the lip, instead of covering the entire part of lip or similar to type I, but do not cover the entire lip.
• Type II: Grooves that fork in their course or branched grooves (branching Y-shaped pattern).
• Type III: Criss-cross pattern or intersected grooves
• Type IV: Reticular grooves.
• Type V: Grooves that do not fall into any of the categories and cannot be differentiated morphologically (Undetermined).

Next, for the sex of the individuals other types of classification was used, which was introduced by Vahanwala et al [13,14]. The sex determination based on the lip prints was done as follows. The results were tabulated and analysed.
• Type I, I’ pattern dominant: Female
• Type I and II patterns are dominant: Female
• Type III pattern present: Male
• Type IV showing varied patterns: Male

Results

The study revealed that there were no two lip prints matched or identical with each other, which approved the uniqueness and differentiation of lip print of each individual. Based on the Table 1, type I (40%) lip pattern was commonly seen in Indian population, followed by type III (25%), II (20%) and IV (15%). Whereas for Malaysian population, type III (40%) was most common followed by type I (35%), II (20%) and IV (5%). There was significant difference between both populations.

According to Vahanwala et al classification of lip print pattern, sex determination was done in general for both population. It was found that 19 individuals were correctly diagnosed as females (Type 1 and 2) and 17 individuals were correctly identified as males (type 3 and 4), based on the lip prints obtained. Only one participant was misdiagnosed as females and three participants were wrongly identified as males, as shown in Graph 1. This proved that type I and type II of lip prints were common in females, while type III and IV were common in males.

Table 1: Comparison of Lip Print Pattern between Indian Population and Malaysian Population

<table>
<thead>
<tr>
<th>Types of Lip Print Pattern</th>
<th>Population</th>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian</td>
<td>Type I</td>
<td></td>
<td>8 (40%)</td>
<td>4.00</td>
<td>4.24</td>
</tr>
<tr>
<td></td>
<td>Type II</td>
<td></td>
<td>4 (20%)</td>
<td>2.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Type III</td>
<td></td>
<td>5 (25%)</td>
<td>2.50</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>Type IV</td>
<td></td>
<td>3 (15%)</td>
<td>1.50</td>
<td>2.12</td>
</tr>
<tr>
<td>Malaysian</td>
<td>Type I</td>
<td></td>
<td>7 (35%)</td>
<td>3.50</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>Type II</td>
<td></td>
<td>4 (20%)</td>
<td>3.50</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Type III</td>
<td></td>
<td>8 (40%)</td>
<td>4.00</td>
<td>4.24</td>
</tr>
<tr>
<td></td>
<td>Type IV</td>
<td></td>
<td>1 (5%)</td>
<td>0.50</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Next, based on Table 2, it showed the classification of lip print pattern based on sex of each populations. It showed that type III (70%) was more common in Malaysian population, while in Indian population both type III (40%) and IV (30%) were more common. However for females type I was the most common lip print pattern seen in both Indian (70%) and Malaysian (50%) population. Although, there was no significant difference in sex identification for both populations, the use of lip print pattern in sex determination was proven and can be applied for any situation where lip print pattern is available and can be used. There was also no significance difference based on the distribution of lip print pattern of both populations.

Table 2: Classification Lip Print Pattern based on Gender

<table>
<thead>
<tr>
<th>Population</th>
<th>Gender</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian</td>
<td>Female</td>
<td>7 (70%)</td>
<td>2 (20%)</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>1 (10%)</td>
<td>2 (20%)</td>
<td>4 (40%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Malaysian</td>
<td>Female</td>
<td>5 (50%)</td>
<td>4 (40%)</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>2 (20%)</td>
<td>0 (0%)</td>
<td>7 (70%)</td>
<td>1 (10%)</td>
</tr>
</tbody>
</table>

**Discussion**

Lip prints are considered to be one of important tools for personal identification as well as sex determination, as it has an equal value as the finger prints. In some cases it also can be used for finger prints or bite prints replacement or as supportive evidence. This study was carried out based on the cheiloscopy method, to describe the diversity of lip print patterns and its application in sex determination. Through the application of lip print
classification by Suzuki and Tsuchibashi as the standard classification, the results obtained showed compliance based on proposed groups by them. However, in general the lip print pattern was not focusing on a single type or group, but it is a combination of different types [15], which made the lip pattern appear different and unique for each person.

On top of that, the way the lip muscles relax to produce a particular pattern also give uniqueness to the lip print, as stated by Sivapathasundharam et al in 2001 [16,17]. Even for twins, although their lip print pattern may appear similar, but they are not exactly identical to each other [18]. Therefore, lip print can be used for personal identification, and even can be used to differentiate people in different populations.

In a crime scene, lip prints are usually found and can be easily collected as evidence for crime analysis. If the lip prints are invisible, it can be visualised by making impression over the pattern by using different type of powder like aluminium powder to give a clear visibility of the lip print. However, a lipstick print is more visible and can be easily traceable. This type of lip print shows distinct features and is difficult to be removed. Thus, they are known to be the persistent lip prints [19].

In addition to this, presence of minor salivary glands and sebaceous glands at the vermilion borders and edges of lip, along with hair follicles and sweat glands in between also will lead to secretion of oils and moisturization, which result to formation of latent lip print. This will enhance the formation of lip print pattern on objects such as glasses [20]. Hence, lip print pattern can be acknowledged for personal identification and also sex determination.

In this study, it showed that type I was the most common lip print pattern seen in both Indian and Malaysian population for females. Whereas for male, type III was more common in Malaysian population, and both type III and IV were more common in Indian population. The results obtained in our study was found similar with studies of Vahanwala et al [13], Preethi et al [19] and Shilpa et al [21]. However, the result obtained was opposite with the study done by Kiran et al [14], as they stated that type II pattern was common in both males and females, which was also obtained from study by Deepa et al [15]. There, were no lip print pattern similar or identical with each other observed in the study. This was also proven in another study.

Conclusion

In conclusion, type I was the most common lip print pattern seen in both Indian and Malaysian population for females. Whereas for male, type III was more common in Malaysian population, and both type III and IV were more common in Indian population. There was no significant difference in both population according the general distribution of lip print pattern. There was no correlation or similarity of lip print patterns between each participant in both population.

Ethical Clearance: The lip prints results were obtained with individuals consent from students of Saveetha Dental College, Saveetha University Chennai, India

Source of Funding- Self

Conflict of Interest - Nil

References

[8]  DineshshankarJ, GanapathiN, Yoithapprabunath


Type 2 diabetes mellitus (T2DM) is one of the metabolic diseases characterized by hyperglycemia and includes individuals who have relatively insulin deficiency and insulin resistance. Helicobacter pylori (H. pylori) is a spiral-shaped bacterium, infects 50% of the world’s people, and inhabits in the human gastric epithelium, causing many diseases. It has been suggested that H. pylori infection is more frequent among T2DM patients. Aim of this study is to evaluate the levels of interferon gamma (IFN-γ) in Iraqi patients with type 2 diabetes mellitus infected by H.pylori bacteria. Anti-H.pylori IgG and IFN-γ concentrations were measured by enzyme-linked immunosorbent assay (ELISA). The study includes 140 patients, divided into 3 groups, which are a group of patients infected by T2DM and H.pylori (T2DMHp+ve), a group of patients infected by T2DM but not by H.pylori group (T2DMHp-ve), and the third group includes patients infected with H.pylori only (Hp), in addition to apparently healthy control group (AHC). Our study revealed significantly decreasing in serum IFN-γ in both T2DMHp+ve group (P˂0.05) and T2DMHp-ve group(P˂0.01), compared with an apparently healthy control group(AHC). Also, this study shows decreased IFN-γ level in patients with Hp group compared with healthy control but without significant difference. Also, the concentration of IFN-γ in T2DMHp+ve is slightly more than that in T2DMHp-ve group.

Key words: T2DM, Anti-H. Pylori IgG, IFN-γ

Introduction

Diabetes mellitus (DM) is one of the metabolic diseases characterized by increased blood sugar (hyperglycemia) due to defects in insulin secretion, insulin action, or both (1). By the year 2030, DM will be the 7th principal cause of death according to the World Health Organization (2). Some pathogenic processes are associated with the development of diabetes. These processes include destroying the beta cells (β-cells) of the pancreas with subsequent insulin deficiency, and others that cause resistance to insulin activity (3).

Type 2 diabetes (T2DM), accounts for about 90–95% of all diabetes cases. This form includes individuals who have relatively insulin deficiency and peripheral insulin resistance (4). T2DM is the primary cause of death in only 13% of mortality cases, whereas the rest 87% is due to diabetes-associated complications—including infections (5).

Patients with diabetes are found to have a reduced cytokine response to acute infections, and the immune system induces inflammation in type 2 diabetes mellitus (T2DM) and triggers insulin resistance along with diabetic complications (6, 7).

Helicobacter pylori (H.pylori) is a microaerophilic, gram-negative and spiral-shaped bacillus. It infects 50% of the world’s people, although most infected individuals have no clinical symptoms (8, 9).

H. pylori are capable to attack the stomach lining, and causes gastritis, peptic ulcer, gastric lymphoma, and gastric adenocarcinoma. It is adapted to survive in the acidic and harsh nature of the stomach which has an ability to change the stomach pH so they can survive (10, 11). It is indicated that the worsen glycemic and metabolic control rises the rates of H. pylori infections and in the same time, this pathogen is considered as one of the common problems of diabetic patients with
Interferon-gamma is a key immunoregulatory protein which plays a major role in both innate and adaptive immune response. The biological effects of IFN-γ are widespread. IFN-γ although may be implicated in protection versus H.pylori infection but in a long-term infection, IFN-γ may play a vital role in gastric inflammation (13).

The aims of the current study is to evaluation of IFN-γ concentrations in diabetic patients infected by H.pylori, diabetic patients non-infected with H.pylori, non-diabetic patients infected by H.pylori, in comparison with apparently healthy control.

Materials and Method

Samples Collection

Ninety eight blood specimens were collected from suspected individual’s revealed positive result by using H.pylori IgG rapid test. ELISA IgG test indicated that only (92) patients have H.pylori infection. 3 ml of venous blood were taken from patients and control group using 5 ml disposable syringes under sterile condition. The specimens were collected in gel tubes and left for 30 minutes until clotted at room temperature. The tubes were centrifuged at 3000 rpm for 5 minutes, and then the serum is divided into 3 equal parts in Eppendorf tubes and stored in (-40°C) until it was used.

Rapid Anti-H.pylori Test

Helicabacter pylori rapid test is a lateral flow chromatographic immunoassay based on the principle of the double-antigen sandwich technique.

Diagnosis of Anti-H.pylori IgG by ELISA

The human body produces IgG when infected by H.pylori. This test was performed according to Human H.pylori ELISA Kit (14).

Method principle

The surface of microplate wells is coated with a target protein and incubated with a primary antibody to the target protein, followed by a (secondary antibody) versus the primary antibody. After washing, the microplate well-bound enzyme activity is estimated with the microplate reader at 450 nm.

Interferon Gama (IFN-γ) Determination

Determination of Interferon Gama (IFN-γ) was performed according to the protocol of Human IFN-γ ELISA Kit (15).

Principle of the assay

This kit is based on the sandwich technique. Anti IFN-γ monoclonal antibodies were coated on microtiter well. Calibrators and samples react with the capture monoclonal antibody and with a monoclonal antibody labeled with HRP (sandwich formation). After incubation and washing, the bound enzyme-labelled antibody is measured through a chromogenic reaction by added TMB solution.

Results and Discussion

Distribution of the groups

This study includes 140 individuals divided into 3 groups including patients with diabetes mellitus type 2 infected by H.pylori (T2DMHp+ve) were 45 cases, patients with diabetes mellitus Type 2 not infected by H.pylori (T2DMHp-ve) were 48 cases and non-diabetic patients infected by H.pylori (Hp) were 47 cases. In addition to these groups, there is an apparently healthy control group (AHC) were 20 cases. According to the Specialized Center for Endocrinology and Diabetes-Baghdad/Al-Rusafa, diagnosis of T2DM was performed, while infections with H.pylori were determined initially by antibody rapid test as a screening test, then followed by ELISA as a confirmatory test.

Serum Anti H.pylori IgG levels

A. Rapid test

Ninety eight from 140 individuals gave positive results in rapid anti-H. pylori IgG as screening and qualitative test, and when confirmed by using ELISA method, only 92 of individuals gave positive result while all negative results in the rapid test were corresponding to ELISA results. So, the sensitivity of Anti- H.pylori rapid test were 100%,and the specificity was 87.5%, as shown in table 1. IgG Ab against H.pylori typically appears approximately 21 days after infection and can remain for a long period beyond eradication (16).

The low specificity of the immunoassays is may be due to cross-reaction between prepared crude antigen containing proteins with antibodies directed against
other bacteria (17).

**Table 1: Comparison of Anti-H. pylori IgG results between the rapid test and ELISA.**

<table>
<thead>
<tr>
<th>Method</th>
<th>ELISA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Results</td>
<td>Positive</td>
<td>Negative</td>
<td>Total Result</td>
</tr>
<tr>
<td>H. Pylori Rapid test</td>
<td>Positive</td>
<td>92</td>
<td>6</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Total results</td>
<td></td>
<td>92</td>
<td>48</td>
<td>140</td>
</tr>
</tbody>
</table>

Relative sensitivity: 100%, Relative specificity: 87.5%

B. Using ELISA method

Table 2 revealed a highly significant difference (P<0.001**) of serum anti-H. pylori IgG in T2DMHp+ve group were 41.6980±4.85127 and Hp group were 45.0590 ± 5.132 AU/mL in compared with a control group were (3.12 ± 0.211). Also, this study showed non-significant differences between T2DMHp-ve and healthy control group.

**Table 2: Anti H. pylori IgG serum levels, detected by ELISA test in the study groups**

<table>
<thead>
<tr>
<th>Type of groups Anti-H. pylori IgG (M±SE)</th>
<th>Type of groups Anti-H. pylori IgG (M±SE)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHC (3.1264±0.211)</td>
<td>T2DMHp+ve (41.6980±4.851)</td>
<td>0.001**</td>
</tr>
<tr>
<td></td>
<td>Hp (45.0590±5.13277)</td>
<td>0.001**</td>
</tr>
<tr>
<td></td>
<td>T2DMHp-ve (3.0212±0.13873)</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N.S</td>
</tr>
</tbody>
</table>

AHC: apparently healthy control; T2DMHp+ve: a group with type 2 diabetes mellitus infected by H. pylori; Hp: group infected with H. pylori; T2DMHp-ve: type 2 diabetes mellitus non infected by H. pylori; M±SE: Mean ± Standard error; ** highly significant P<0.01; N.S: Non-significant P>0.05.

The ELISA is an easy, cheap, effective and reliable immunological test, an alternative to UBT (Urea breath test) for detection of H. pylori infection (18, 19). Also, this study agreed with Mansour (2) who confirmed elevated the immunoglobulin (IgG) during infection with H. pylori. The immune system of the host produces antibodies (Abs) against H. pylori immediately after bacterial gastric colonization. The immunologic tests developed for detection of immunoglobulin g (IgG) antibodies that specific for H. pylori in the serum of patients.

The types of ELISA are more suitable for diagnosis of H. pylori infection in epidemiological studies of peoples. Different antigen preparations have been prepared in coating ELISA wells, in these methods: crude antigens, such as (whole cell extracts, glycine extracts, sonicated cell extracts), heat-stable antigens and recombinant antigens. Antibody levels remain in the blood for a long time. The best performance of these tests is dependent on specificity for detection of (local) H. pylori strains in each country by choosing antigens for coating the (ELISA) wells. Serological tests are recommended in many studies for epidemiological studies, and they based on the many reasons: rapid, inexpensive, availability, and easy in use, but it is not recommended for the confirmation of H. pylori elimination after treatment (20).
Comparison of IFN-γ levels among the study groups

The level of IFN-γ in patients in the study groups (T2DMHp+ve, Hp, T2DMHp-ve, and healthy control group) were (2.83 ± 0.29, 3.85 ± 0.65, 2.65 ± 0.28, and 4.07 ± 0.58 IU/mL) respectively.

Data in the table 3, revealed decreased significantly in serum IFN-γ in both T2DMHp+ve group (P<0.05) and T2DMHp-ve group(P<0.01) in compared with an apparently healthy control group(AHC).

Also, this study shows decreased IFN-γ level in patients with Hp group compared with healthy control but without significant differences between them. Also, it has demonstrated that the concentration of IFN-γ in T2DMHp+ve is slightly more than that in T2DMHp-ve group.

Table 3: Serum interferon gamma levels in the study groups

<table>
<thead>
<tr>
<th>Type of groups</th>
<th>M±SE of IFN-γ (IU/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHC (4.0738±0.58197)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2DMHp+ve (2.8319 ± 0.29659)</td>
<td>0.05*</td>
<td></td>
</tr>
<tr>
<td>Hp (3.8584 ± 0.65944)</td>
<td>0.8 (N.S)</td>
<td></td>
</tr>
<tr>
<td>T2DMHp-ve (2.6520 ± 0.28704)</td>
<td>0.01**</td>
<td></td>
</tr>
</tbody>
</table>

AHC: apparently healthy control; T2DMHp+ve: a group with type 2 diabetes mellitus infected by H.pylori; Hp: H.pylori infected group; T2DMHp-ve: type 2 diabetes mellitus non infected with H.pylori; M±SE: Mean ± standard error; *significant; ** highly significant P<0.01; N.S: Non-significant P>0.05.

Type 3 diabetic patients obviously had a decreased capacity of IFN-gamma production by a down-regulated production of cytokine molecules in the endoplasmic reticulum of both (CD4 and CD8) T-lymphocytes compared to healthy individuals (21). Many studies agreed with our result that decreased IFN-γ in T2DM patients (22-25). But disagree with Amin and his co-workers (26), who found elevated IFN-γ concentration in T2DM and that indicates T-cells activation against infection.

IFN-γ may be suppressed by H.pylori as a strategy to escape immune destruction through interference with IFN-γ signaling and that may cause permanent infection by H.pylori in gastric epithelial cells (27). The expression of cholesterol-α-glucosyl transferase (cgt) by H.pylori reduces cholesterol concentrations in infected gastric epithelial cells which result in blocks IFN-γ signaling, allowing H.pylori to escape the inflammatory response of the host (28).

In 2015, Yang and his coworkers (29) agreed with this study who found non-significant differences of IFN-γ in H.pylori infected persons compared with the control group. In contrast, different studies disagreed with this study (30, 31) who revealed that raised production of lipopolysaccharides, may trigger innate inflammatory processes which associate with increased inflammatory cytokines, also Abdollahi (13) and Bimczok(32) showed increased IFN-γ concentration in H.pylori infected patients and that indicates T-cells activation against infection ,and the IFN-γ maintains mucosal inflammation and may stimulate disease development to gastric ulcer.

Conclusions

1. Concentrations of serum interferon gamma (IFN-γ) decreased significantly in type 2 diabetic patients whether infected or non-infected by H.pylori. Also, slightly decreased IFN-γ level in H.pylori infected patients who are non-diabetic.

2. Anti-H.pylori IgG rapid test is important for the primary diagnosis of H.pylori infection, although ELISA IgG gives more sensitivity and specificity for confirmation of this infection.
Conflict of Interests: The authors declare that they have no conflict of interest.

Source of Funding: Self-funding

Ethical Clearance: The researchers already have ethical clearance from College of Science, Mustansiriyah University, Iraq.

References


Detection of ESBLs CTX-M Gene of *E. coli* Isolated from Clinical Cases in Maysan Province

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Abstract

The antimicrobial resistance are a worldwide increasing and important problem in health care domain. ESBLs represent a main group of β-lactamases enzymes that mostly produced by gram negative bacteria, and give resistance to β- lactam antibiotics, thus the detection of these enzymes are very important for optimal care of patients. The aim of this study was to determine the antibiotic profile with the prevalence of CTX-M gene producing *E. coli* isolates which recovered from clinical cases by phenotypic and genotypic methods. A total of (291) clinical samples (urine, wound swabs, blood and seminal fluids) were included in this study. All bacterial isolates were subjected to the cultural, microscopical, and biochemical examinations methods, confirmed by API 20E and Vitek 2 system. Where the results revealed that 105 of isolates were identified as *E. coli*. Antibiotic sensitivity was performed by using disk diffusion methods. Investigation of extended spectrum β-lactamase(ESBL) production for isolates was performed using Initial screening and double disc synergy method(DDST). The results showed that most isolates showed high resistance to β-lactam and Cephalosporins antibiotics and vast majority of isolates were resistant to a minimum of three classes of antibiotics, which indicate that identified *E. coli* were multidrug resistant and ESBLs producer. While all isolates were sensitive to Imipenem and Amikacin. PCR technique was performed to detect ESBLs blαCTX-M gene, the results revealed that (100%) of *E.coli* isolates carried this gene.

**Keyword:** *Escherichia coli*, Antibiotic Resistance, ESBL, CTX-M gene.

Introduction

β-lactam antibiotics are the most common agents used for treatment of many bacterial infections caused by Gram-negative bacteria, but resistance versus these antibiotic groups occurred rapidly worldwide.[1] Extended spectrum beta-lactamas(ESBLs) are defined as enzymes produced by certain bacteria that are able to hydrolyze extended spectrum cephalosporin, they are therefore effective against beta-lactam antibiotics such as ceftazidime, ceftriaxone, ceftaxime and oxyiminomonobactam.[2]ESBL-producing organisms are mostly resistant to other classes of antibiotics.[3] Therefore Patients who infected with ESBL producing organism not only have an increased risk of treatment failure and some times lead to death, but also require more health-care resources.[4]The CTX-M enzymes are belong to group of class (A) ESBLs that in general exhibit much higher levels of activity against cefotaxime and ceftriaxone than ceftazidime.[5]Additionally, the CTX-M enzymes have been recognized as the most prevalent among *Enterobacteriaceae* especially in *E.coli*.[6]

Materials and Method

Bacterial samples collection and identification:

A total of 291 samples, were collected from different clinical cases (urine, wound swabs, blood and seminal fluid) from main Hospitals in Maysan Province during the period from October 2018 to the end of September 2018. Clinical samples immediately transferred to laboratory and culture according to the standard methods, the positive culture were identified by conventional techniques and confirmed by using API 20 E system and using VITEK®2 GN kit (BioMe’rieux, France) then the identified *E. coli* stored for molecular
Antimicrobial susceptibility testing

Susceptibility testing was determined by the agar disk diffusion method, and interpreted according to CLSI guidelines. The following antibiotic disks were used: Ampicillin 10 μg, Pipracillin 100 μg, Amikacin 30 μg, Amoxicillin + Clavulanic acid 20/10 μg, Oxacillin 1 μg, Imipenem 10 μg, Ciprofloxacin 5 μg, Ceftazidime 30 μg, Cefotaxime, 30 μg, Ceftriaxone 30 μg, Sulfamethoxazole+Trimethoprim 1.25/23.75 μg, Cefpodoxime 10 μg, Aztreonam 30 μg, Gentamicin 10 μg, Norfloxacin 10 μg, Nitrofurantoin 300 μg, Cefepime 30 μg, Cefoxitin 30 μg, (Bioanalyse, Turkey).

Phenotypic detection of ESBLs

All β-lactamase producing bacterial isolates were tested for ESBL production by initial screening test according to CLSI. The isolates showing resistance to one or more 3rd generation cephalosporins (3GCs) were tested for ESBL production by Double Disc Synergy Test (DDST).

Extraction of Bacterial Chromosomal DNA

DNA extraction was done toward all E. coli isolates according to Presto™Mini gDNA Bacteria Kit protocol (Geneaid, Taiwan). The integrity of extracted DNA was tested using Agarose Gel Electrophoresis. The chromosomal DNA then subjected to monoplex PCR.

Molecular detection of bla_CTX-M Gene Using PCR Technique

The protocol used depending on manufacturer’s instructions (Bioneer, South Korea). The specific primer for bla_CTX-M gene have been chosen according to[10,11] where F: CGCTTTGCGATGTCAG, R: ACCGCGATATCGTTGGT, which yield a product 550bp. PCR Program was performed as following: (1) cycles of (5 min.) initial denaturation at (94°C), followed by (34) cycles of denaturation at (94°C), (1 min.) annealing at (55°C), (1 min.) extension at (72°C), (1 min.) and a final extension step of (5 min.) at(72°C), then 5μl of amplified PCR product were loaded to the agarose gel wells with standard molecular weight of DNA ladder (Bioneer, South Korea) and the Electrophoresis were ran as described by[12].

Results and Discussion

Isolation and identification of Bacterial Isolates

The results of this study showed that among 291 clinical samples 235 gave positive growth and 105 (44.7%) were identified as E.coli. As compared with other studies, our findings were compatible with study[13] where the result was (44.8%), also in India by[14] and in Southwestern Uganda by[15] who obtained results very similar to our results that were (44%) for both. While the results were contrary to the findings of many researchers as[16,17] E.coli is the most gram negative bacteria found in clinical laboratories samples including the majority of urinary, wound, blood and peritoneal isolates.[18]

The antibiotic susceptibility pattern

The resistance patterns of E.coli towards various antibiotics were determined using disc diffusion method. Data in (Figure 2) exhibited that isolates of E. coli have the highest level of resistance to Ampicillin where up to (98.1%) were resist to this antibiotic, followed by (96.2%) for Pipracillin and (93.3%) for Ceftazidime, Cefpodoxime and Oxacillin, and (92.4%) for Cefepime, and the resistance to Cefotaxime was (94.3%), while to Amoxicillin/Clavulanic acid the percent of resistance was (90.5%). The resistance to the Ceftriaxone was (87.6%), (86.7%) for Cefoxitin, Aztreonam (81.9%) and (68.6%) for Trimethoprim/Sulfamethoxazole while the resistance was (53.3%) for Gentamicin. Whereas the resistance to antibiotic is less than a half for Norfloxacin, Nitrofurantion and Ciprofloxacin (46.7%), (35.2%) and (34.2%) respectively. Moreover this study recorded that all isolates (100%) were sensitive to Imipenem antibiotic followed by (90.5%) for Amikacin. Where these results were corroborated with findings of previous reports[19] which concluded that E. coli showed high resistance to Ampicillin and Pipracillin, also study conducted by[16] showed high resistance rates of E.coli to aztreonam, amoxicillin-clavulanic acid, which compatible with present study. Whilst[20] showed high resistance to third generation (Cephalosporins). The observed high resistance rates in most antibiotic may be due to uncontrolled consumption, consequence of easy access to inefficient and cheap antibiotics moreover could be justified by insufficient adherence to guidelines for infection control as well as inappropriate use of antibiotics. The members of Enterobacteriaceae has many mechanisms of resistance to β-lactam antibiotics.
like loss of porin and efflux pumps etc, however, β-lactamases enzyme most common and clinically significant mechanism of resistance to β-lactam antibiotics among these bacterial family. The most effective β-lactam antibiotics against E. coli was Imipenem where (100%) of E. col were susceptible to this antibiotic, followed by Amikacin. Similar pattern of results were obtained by which appeared high degree of effectiveness of these antibiotics against E. coli, also corresponded with the recent study for who considered the antibiotic Imipenem and Meropenem should be preferred drugs for E. coli infection isolated from clinical samples.

**Phenotypic Detection of ESBLs**

ESBLs have been found worldwide and they are considered a leading causer of drug resistance in many Enterobacteria. One hundred and five of E.coli isolates in present study were examined phenotypically and genotypically for ESBL production. The findings of screening test concluded that (82.9%) of E. coli were suspected to be ESBLs producers whereas the results of Double disk synergy test was (21.8%) as showed in figures (3 and 4). The results of screening test were similar to local studies of who found that screening test was positive in (100%) of E. coli. The high occurrence of ESBLs producing E. coli obtained in this study was probably due to the consumption of large amount of third-generation cephalosporins, trend of self-medication and the extensive prophylactic misuse of antimicrobials by Iraqi patients and physicians. Additionally a number of previous studies have showed the low prevalence of ESBLs producing E. coli when used confirmatory test which in concordance with present study such as the study implemented by where the results were (23.8%) and (29.8%) respectively. Many researcher such as concluded in their studies that there is incompatible between the results of the phenotypic observation and genotypic analysis and showing that the presence of ESBL-associated β-lactamase genes may be undetected when using the conventional phenotypic approach and mutation in these unexpressed genes may be the leading cause to ESBL antibiotic resistance, suggesting that the unexpressed and undetected genes may serve as reservoir for ESBL genes, therefore phenotypic screening should not be used as monitoring of ESBLs because there had been studies showing the discrepancy in the phenotypic and genotypic detection.

**Molecular Detection of ESBL Genes**

The CTX-M type β-lactamases constitute a novel group of enzymes that have a typical ESBL resistance phenotype, are capable of hydrolyzing broad-spectrum cephalosporins and are inhibited by clavulanic acid, They also confer a high level of activity against ceftazidime. As shown in figure (5) the results of molecular study revealed that (100%) of E. coli carry the CTX-M gene with amplified product 550 bp. The findings were directly in line with previous studies as who reported that majority of CTX-M ESBL producers were E. coli. The CTX-M was considered as a common type of ESBLs that detected at Asia, Europe, North and South America among multidrug-resistant E.coli. The important reason for the prevalence of CTX-M β-lactamases gene in Maysan Province may be due to the use of certain
third generation cephalosporins which led to high resistance in our study. Whereas, the results of current study went beyond previous reports\cite{19,32,33} that results were\( (56.7\%)\), \( (28.9\%)\), \( (10\%)\) respectively.

**Conclusion**

In this study isolated *E.coli* showed high levels of resistance to most antibiotics especially Beta-lactam group and considered as multidrug resistant bacteria. Furthermore, *E. coli* produced CTX-M in high rate of occurrence reached to \( (100\%)\). This terrifying situation with the spread of ESBL isolates highlights the need to adopt strict antibiotics using in internal hospitals to assess the effect of high resistance in bacteria and to take steps to reduce this resistance.

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**Ethics of Experimentation:** Permission to conduct this study was issued by the Health institutional committee at Al-Sader Teaching, Birth and Child, and Al-Zahrawi Hospitals, Maysan province; and the samples were taken from patients under the supervision of professional health care workers.

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Impact of Electronic Games on the Behavior of Children and their Academic Achievement upon Schools in Mosul City

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Abstract
The research aims at finding the effects of electronic games on the behavior of children and finding the relationship between the demographic information and the impact of these games on their behavior and educational achievement. A descriptive and analytical study of the effects of electronic games on the behavior of 60 children in the schools of Mosul city, conducted for the period from Feb.2019 to March 2019. (Non-probability) The sample was chosen according to us of phone or computer and other smart devices for the purpose of playing. Boys and girls equally chosen after fill a questionnaire which was used with a semi-guideline for collecting information. Results of the study revealed that time taken by the students to play a daily or intermittent basis is less than two hours, at 45.7%, and that higher than who spend one to two hours are 32.6% and those who spend more than two hours are 21.6%. Students playing electronically at the age of 3-6 are 19.5%at 7-8 years at 43.5% and, at 9 years are over 37.0%.

Yes, No, sometimes are answers of questionnaire.χ² value was less than p<0.05. The study showed differences between males and females in use of games. The study recommends Ministry of Higher Education, with assistance and participation of Ministry of Education, should develop an educational program for parents about electronic games and their harm to children.

Key words: Effect of electronic games on children, Damage electronic games.

Introduction
Children often have an inclination to play instinctively; definition play is an important and necessary activity in their lives because it reveals my inner self and personality, as well as emotional and other hidden aspects. Through play, personality is formed, and their emotional, social, The studies and psychologists have confirmed that play enhances the personality of the child, as well as is one of the basic means of learning in the present time, especially in the past, the concept of play was related to physical and motor activities only [1]. Today and the scientific and technological are developed in way that has been accompanied by changes in the concept of play than it was in the games, which attracted the attention of all age groups in the community [2].

On the other hand, these games helped the child how to deal with modern font engineering science such as computing machine internet and electronic devices, and other modern and modern applied science introduced child technology cosmos and virtual world [3]. It also brands him more determination to achieve success and win and achieve ambition his victory in Plot and his insistence on win generate. This will achieve success and win, which affect his ambitions for hereafter and his finding to achieve his goals and planning, liveliness [4]. This is what we explored through this study often parents do not want video games for their children because they think its wasting time. Some experts believe that these games are possible to have been spoiled by the brain [5, 6]. Violent media also play a role and easily blame violent video games as the reason why some teens turn violent or engage in aggressive anti-social behavior [7]. “According to the survey of national institute on media and family in the United States, find that 92% of children and adolescents between 2-17 old playing video games” [5]. Terri K. and Susan C (2013) said that because of the different types of electronic games that children engaged in various forms, such as television and electronic games
on the Internet, video and computer, iPod, movies, magazines, newspapers. The media has a big role that does not affect the children negatively or positively, according to the pictures displayed and the information contained therein which are not useful to children or always in their interest [6].

AL-Gamidy A.,(2010) state Some evidence of video games that reduce the size of boys and girls, or among researchers who participated in the US Senate, “found violent video games to increase violent behavior by 13% -22% for awareness”. A study by Stanford University researchers found that it is time to reduce violence with violent games and television programs that encourage violent behavior to 50 hours a week, reducing physical violence to 40% in third and fourth grade. The objective of the study is finding effect of electronic games on children’s behavior and Discover association between children’s behavioral problems and the educational level in school. The effects of the electronic games among school age children on health status [2].

Methodology

A descriptive and analytical study on behavior of children in the schools of Mosul city was conducted for the period from February 2019 to Murch 2019. A sample (non-probability) was chosen of 60 students for the schools from which the sample was taken. For boys and AbiDharGhaffari and for girls in the city of Mosul left coast. The sample was chosen according to the controls: children who use the phone or computer and other smart devices for the purpose of playing boys and girls equally after taking their consent and help them fill out the questionnaire for the specific research. A questionnaire was used in the study with a semi-guideline for collecting information. The for data collection is used as a tool questionnaire, which includes the following: Part 1: This part contains information about biographical data which is consisted of 6 items which include Age, sex, educational level, the number of student in same classroom, the number of students in the school, the sample size and the date of test done.

Part 2: This part consisted of 19 questions about electronic games, how they are used, the times, periods, and effects that these games cause on children’s health, their educational level, and the behaviors used by children using these games.

Statistical analysis

The research data were analyzed by descriptive statistical analysis using SPSS p<0.05

Results

Results of statistical analysis of the questionnaire (the impact of electronic games on the behavior of children and their academic achievements).

Section One

Conduct descriptive statistics on students’ information such as age, duration of student play, and duration of the electronic games.

Table (1): Frequency, percentages and averages of the data of the study members

<table>
<thead>
<tr>
<th>Items</th>
<th>F</th>
<th>%</th>
<th>Mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Hours of daily play</td>
<td>&gt;2 h</td>
<td>21</td>
<td>45.7</td>
</tr>
<tr>
<td></td>
<td>1-2 h</td>
<td>15</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>More 2 h</td>
<td>10</td>
<td>21.6</td>
</tr>
<tr>
<td>Age of started electronic games</td>
<td>3-6 y</td>
<td>9</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>7-8 y</td>
<td>20</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td>&lt;9 y</td>
<td>17</td>
<td>37.0</td>
</tr>
</tbody>
</table>

Section two:

Understanding and analyzing the opinions of the students participating in the study focusing on questionnaire (the effect of electronic games on children’s behavior and educational achievement).

The questionnaire was divided into a three-dimensional scale as follows:

Table (2): Estimation scale according to the triangular Likert scale

<table>
<thead>
<tr>
<th>Response</th>
<th>Weighted average</th>
<th>Intervals</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>1 to 1.66</td>
<td>0.66</td>
<td>High</td>
</tr>
<tr>
<td>Sometimes</td>
<td>1.67 to 2.33</td>
<td>0.66</td>
<td>Average</td>
</tr>
<tr>
<td>No</td>
<td>2.34 to 3</td>
<td>0.66</td>
<td>Low</td>
</tr>
</tbody>
</table>

The following is an analysis of the views of the study members on the focus of the questionnaire
Table 3: Analysis of the responses to the first axis: average, L: Low, H: high

<table>
<thead>
<tr>
<th>Item</th>
<th>Yes</th>
<th>%</th>
<th>No</th>
<th>%</th>
<th>S. time</th>
<th>%</th>
<th>mean</th>
<th>SD</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 play on your phone or computer?</td>
<td>28</td>
<td>46.7</td>
<td>14</td>
<td>23.3</td>
<td>18</td>
<td>30</td>
<td>1.83</td>
<td>0.867</td>
<td>A</td>
</tr>
<tr>
<td>2 feel weak in focus and attention?</td>
<td>18</td>
<td>39.1</td>
<td>14</td>
<td>30.4</td>
<td>14</td>
<td>30.4</td>
<td>1.91</td>
<td>0.839</td>
<td>A</td>
</tr>
<tr>
<td>3 having difficulty in memorizing and studying during this period?</td>
<td>20</td>
<td>43.5</td>
<td>13</td>
<td>28.3</td>
<td>13</td>
<td>28.3</td>
<td>1.85</td>
<td>0.842</td>
<td>A</td>
</tr>
<tr>
<td>4 Do you feel weak social relationships with family and friends?</td>
<td>7</td>
<td>15.2</td>
<td>23</td>
<td>50</td>
<td>16</td>
<td>34.8</td>
<td>2.20</td>
<td>0.6</td>
<td>A</td>
</tr>
<tr>
<td>5 feeling heavy in head and headache after you finish playing</td>
<td>6</td>
<td>13</td>
<td>25</td>
<td>54.3</td>
<td>14</td>
<td>32.6</td>
<td>2.22</td>
<td>0.696</td>
<td>A</td>
</tr>
<tr>
<td>6 Do you wear glasses?</td>
<td>8</td>
<td>17.4</td>
<td>33</td>
<td>71.7</td>
<td>4</td>
<td>8.2</td>
<td>1.96</td>
<td>0.595</td>
<td>Av</td>
</tr>
<tr>
<td>7 Do you feel pain in your back or muscles?</td>
<td>5</td>
<td>6.5</td>
<td>25</td>
<td>54.3</td>
<td>17</td>
<td>39.2</td>
<td>2.35</td>
<td>0.640</td>
<td>L</td>
</tr>
<tr>
<td>8 Do you plan to leave the electronic games and devote to study in the future?</td>
<td>24</td>
<td>52.2</td>
<td>14</td>
<td>30.4</td>
<td>8</td>
<td>17.4</td>
<td>1.65</td>
<td>0.766</td>
<td>H</td>
</tr>
<tr>
<td>9 Have you ever been advised to leave the games altogether?</td>
<td>37</td>
<td>80.4</td>
<td>7</td>
<td>15.2</td>
<td>2</td>
<td>4.3</td>
<td>1.24</td>
<td>0.524</td>
<td>H</td>
</tr>
<tr>
<td>10 Do you fight with your friends for the games?</td>
<td>22</td>
<td>47.8</td>
<td>21</td>
<td>45.7</td>
<td>3</td>
<td>6.5</td>
<td>1.59</td>
<td>0.617</td>
<td>H</td>
</tr>
<tr>
<td>11 Are you trying to break down the property of others?</td>
<td>11</td>
<td>23.9</td>
<td>54</td>
<td>65.4</td>
<td>7</td>
<td>8.7</td>
<td>1.85</td>
<td>0.556</td>
<td>A</td>
</tr>
<tr>
<td>12 Are you trying to hurt your friends without hurting you?</td>
<td>4</td>
<td>8.7</td>
<td>38</td>
<td>82.6</td>
<td>4</td>
<td>8.7</td>
<td>2.0</td>
<td>0.422</td>
<td>A</td>
</tr>
<tr>
<td>13 Do you respond to physical abuse with the strongest of them?</td>
<td>24</td>
<td>52.2</td>
<td>12</td>
<td>26.1</td>
<td>10</td>
<td>21.7</td>
<td>1.70</td>
<td>0.813</td>
<td>A</td>
</tr>
<tr>
<td>14 Do you prefer the electronic games wrestling and fighting?</td>
<td>14</td>
<td>30.4</td>
<td>21</td>
<td>45.7</td>
<td>11</td>
<td>23.9</td>
<td>1.95</td>
<td>0.742</td>
<td>A</td>
</tr>
<tr>
<td>15 Do you think of harming those who are older than you?</td>
<td>1</td>
<td>2.2</td>
<td>42</td>
<td>91.3</td>
<td>3</td>
<td>6.5</td>
<td>2.04</td>
<td>0.295</td>
<td>A</td>
</tr>
<tr>
<td>16 Are you subjected to violence and beatings by parents at home?</td>
<td>7</td>
<td>15.2</td>
<td>17</td>
<td>37</td>
<td>22</td>
<td>47.8</td>
<td>2.33</td>
<td>0.732</td>
<td>L</td>
</tr>
<tr>
<td>17 Do you cheat classmates?</td>
<td>7</td>
<td>15.2</td>
<td>30</td>
<td>65.2</td>
<td>9</td>
<td>19.6</td>
<td>2.04</td>
<td>0.595</td>
<td>A</td>
</tr>
<tr>
<td>18 Do you think about leaving school and prefer games to study</td>
<td>4</td>
<td>8.7</td>
<td>36</td>
<td>78.3</td>
<td>6</td>
<td>13</td>
<td>2.04</td>
<td>0.460</td>
<td>A</td>
</tr>
<tr>
<td>19 Have you ever thought of stealing to buy a favorite game?</td>
<td>2</td>
<td>4.3</td>
<td>40</td>
<td>87</td>
<td>4</td>
<td>8.7</td>
<td>2.04</td>
<td>0.462</td>
<td>A</td>
</tr>
<tr>
<td>Total</td>
<td>19482</td>
<td>11946</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows the frequency and percentage of respondents’ responses to the questionnaire (the effect of electronic games on children’s behavior and educational attainment). The first paragraph, which states: “Are you subjected to violence and beating by parents at home?” With an average of 2.33 and a standard deviation of 0.732 of 17 answers (no) and 22 answers (sometimes) out of 60 which corresponds to the answer (no) and low level as in table (2) and came last in the paragraph that states 1.24 and a standard deviation of 0.524 at a high level, which corresponds to the yes (in the Lycert triangular scale of Table 2). This indicates that the majority received tips for leaving the electronic games altogether.

The weighted mean weight of the entire questionnaire was 1.948 and a measurement deviation.

(0.1446), which corresponds to the answer (sometimes) by reference to the scale. This indicates that the reality of the effect of electronic games on children’s behavior and achievement is average for the target population in the study. The following figure shows the averages of the sample around the sections of the questionnaire.

Section three:

Know the relationship between respondents’
responses to the questionnaire and the gender variable (male and female).

I used the $\chi^2$ of independence to determine the extent of a statistically significant relationship between two variables. This type of test is applied if both variables have class data representing the community.

Table (4): Results of the Kay box test to identify the relationship between respondents’ answers and sex variables

Table 4 shows the responses of male and female students and the value of the tables and the significant value of the questionnaire. The table shows a statistically significant relationship between the answers of male and female students to the following questions: Do you play electronic games on the phone or computer? During this period, you feel heavy in the head and headache after you finish playing.

Do you feel the pain in the back or the muscles, do you prefer the electronic games wrestling and fighting, do you cheat colleagues in the class and the moral value of the test of $\chi^2$ in these questions less than the value of moral = p value 0.05 and the value of $\chi^2$ less than the value of the scale at df 2. The reason for this is that most children who use electronic games affect the concentration and attention and length of play cause them fatigue and head pain because of the length of play while the responses of male and female respondents did not record any statistical significance. The reason for this is that most males prefer violent electronic games such as wrestling and fighting, which develop aggressive behavior and harm their peers or close relatives without cause. Males are more than females at 19.49%. 

Discussion

This study deals with The impact of electronic games on the behavior of children and their academic achievement in schools table (1) Frequency, percentages and averages of study data table shows the majority that 79% of males play: approximately half of boys toys 45% of them play and spend one to two hours by 32.6% and for more than two hours by 21.6%, as opposed to females. In my opinion the type of games and parents life style have a very significant impact on the behavior of the child used for those games. “It is important to emphasize how video games affect children’s learning processes and adolescents, as well as their effects on the educational process in general. First thoughts on this subject in the United States as early as 1978, the line was established Look for learning-driven, including”[1]. This is Research spread somewhat in the 1980s, especially in the late 1990s. That time continued, scientific Search may already be links between video games and different worlds of the human began to examine the links between playing video games and Complex social processes that, in contemporary [5], so video games, as any other technological device, are simply media through which children people engage in numerous activities. Or, seen from another land site, they are no more than another feature with symbolic, economic, and technological attribute in a complex residential district circumstance that is constantly subjected to an intense and accelerated process of change[6].

“Kids who regularly play videogames are at slightly increased risk for developing attention problems at school”[8]. In my opinion this result due to; most sample playing games was boys and they use electronic games more than girls and it effect on their behavior.

The study found that most children have been advised to leave the electronic games and this indicates the desire of the people to leave these games because of the negative impact on them. The results showed that the majority of children are exposed to violence in their own way by parents as well as have knowledge about the damage to the games on their health and school level. We also have a large percentage of students who are fighting their friends because of the games and this is evidence of the growing aggressive behavior they have. This indicates that the effect of electronic games on children’s behavior and achievement is average for the target population in the study [9].

I used the $\chi^2$ of independence to determine the extent of a statistically significant relationship between two variables. The study said that current research has no clear evidence continuously links exposure to violence in video games. Aggression or violence in societies, but other studies and research are necessary to assess impacts on vulnerable subgroups that are at risk as children [3].

The study has shown that prolonged eye sight on the small screen affects the health of the eye as well as prolonged use of the computer has a negative impact on the health of the joints because of the length of the sitting period for playing Do you feel the pain in the back or the muscles, do you prefer the electronic games
wrestling and fighting, do you cheat colleagues in the class) results was less than the value of the scale at the df \[2\]. The reason for this is that most children who use electronic games influence the concentration, attention and length of play cause them fatigue and head pain due to the length of the playing time, while the responses of male and female respondents did not record any statistical significance. The reason for this is that most males prefer violent electronic games such as wrestling and fighting, which develop aggressive behavior and harm their peers or close relatives without cause [10&4]. We also have a large percentage of students who are fighting their friends because of the games and this is evidence of the growing aggressive behavior they have. We also note the differences between boys and girls in playing electronic games, as boys were more likely to be counted boys are more than girls. Two major trends can be observed towards the impact of electronic games; these games expose children to serious risks and these risks include injuries arising from repeated stress obesity, social isolation and visual stress, and in the long term caused the destruction of psychological and emotional growth mental health of children. The results showed that students have difficulty in studying and understanding when playing for long periods during the day and this is evidence of the impact of these games on the level of academic achievement with some differences between females and males [11] And that the majorities who play the games suffer from headaches in the muscles pain if they sit for long periods [7].

The second trend is that electronic games have a positive impact on learning and education, It helps develop image reading strategies and increases the use of attention and speed strategies in processing Information and effectiveness in problem solving, and this shows the argumentative hypothesis posed by many researchers in science Psychology, sociology, and educational science on the impact of these games on behaviors in children [9].

Overall the boys more effects electronic game usage compared to girls. Statistically significant difference was also observed for electronic game addiction and age groups the results showed that the duration of the children’s play has a negative impact on their health and that the majority feels a lack of concentration and attention. Also, the majority of them have been advised to leave these games. The growing aggressive behavior in some was due to violence by parents at home. While the responses of boy and girl respondents did not record any statistical significance. The reason for this is that most males prefer violent electronic games such as wrestling and fighting, which develop aggressive behavior and harm their peers or close relatives without cause. Males are more than females that the majorities who play the games suffer from headaches in the muscles pain if they sit for long periods. This is evidence of the impact of games on health, behavior and learning achievement.

The authors think that the Ministry of Higher Education, with the assistance and participation of the Ministry of Education, should develop an educational program for parents about electronic games and their harm to children. As well as, monitor their children constantly and identify specific times during the day without affecting their studies and social relationships.

Source of Funding– Self

Ethical Clearance – Not required

Conflict of Interest: None

References


effects-of-videogames


Hazard Identification, Risk Assessment, and Determining Control (HIRADC) Method in a University Laboratory in Surabaya, Indonesia

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Abstract

Risk management is a system owned by every organization, including university laboratory, to prevent losses. Amongst the methods of risk management that are usually applied is HIRADC method, which is a systematic stage to identify hazards, to assess and analyze risks, and to plan controls measures according to the existed risks. This research is observational research which aims to describe the phenomena. There are several stages in this method, namely hazard identification, risk analysis, and determining control. The data is collected by conducting observation before doing data matching with laboratory worker. The research results indicate that from five activities performed in the Laboratory of Histology Anatomy at Faculty of Medicine Universitas Airlangga, such as experiments by the medical students, dry cadaver shaping, new cadaver shaping, cadaver maintenance and the goods burning by the laboratory worker, there are 16 hazards discovered, with two extreme-risk hazards and three high-risk hazards. The controls and recommendation provided are giving socialization about the importance of wearing PPE (rubber gloves, safety goggles, and laboratory coat), providing explanation about the work to the worker as well as explanation about the laboratory SOP and providing the fire extinguishers.

Keywords: Risk Management, HIRADC, Laboratory

Introduction

Every workplace, including university laboratory, carries its own hazards and risks of occupational accidents. Based on the book authored by Ramli (2010) entitled “Pedoman Praktis Manajemen Risiko dalam Perspektif K3 OHS Risk Management”, hazard is all that includes problems or actions that lead accidents or damages to humans or other disturbances\textsuperscript{(1)}. According to ILO (International Labour Organization), every year the number of occupational accidents reach more than 250 million accidents at work and 160 million workers suffer from occupational illnesses\textsuperscript{(2)}.

Risk management must be owned by every organization, including university to protect it from all things that can cause accident, injury or losses. As mentioned in ISO 45001, risk can be explained as the terms of a combination of the severity caused by the events and its likelihood of adverse events. Risk management can be defined as the total procedure related with identifying an existing hazard, assessing the risk, arranging control measures, and reviewing the outcomes\textsuperscript{(3)}.

HIRADC (Hazard Identification, Risk Assessment, Determining Control) in ISO 45001 is listed in the planning section, as a procedure resolve the risks which consists of several stages of activity, specifically hazard identification, risk assessment, and determining control\textsuperscript{(4)}. Hazard identification is a process of identifying the hazards that may occur in a workplace. The types of hazards are physical, chemical, biological, psychosocial, or ergonomic hazards and also be caused by the workers, which also known as at-risk behavior.

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Risk assessment as referred from ISO 45001 is an activity to assess the level of the risk from identified hazards as well as to review the effectiveness of the existing control program. Risks that are assessed by the results of the likelihood and severity will be matched to the risk matrix to determine the level of each risk and to priorities identified hazards.

Table 1. Likelihood Level by AS/NZS 4360: 1999

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Almost certain</td>
<td>Expected to occur in most circumstances</td>
</tr>
<tr>
<td>4</td>
<td>Likely</td>
<td>Will probably occur in most circumstances</td>
</tr>
<tr>
<td>3</td>
<td>Possible</td>
<td>Might occur at some time</td>
</tr>
<tr>
<td>2</td>
<td>Unlikely</td>
<td>Could occur at some time</td>
</tr>
<tr>
<td>1</td>
<td>Rare</td>
<td>May occur only in exceptional circumstances</td>
</tr>
</tbody>
</table>

Table 2. Severity Level by AS/NZS 4360: 1999

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
<th>Example detail</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Insignificant</td>
<td>No injuries, low financial loss</td>
</tr>
<tr>
<td>2</td>
<td>Minor</td>
<td>First aid treatment, on-site release immediate contained, medium financial loss</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Medical treatment required, on-site release contained with outside assistance, high financial loss</td>
</tr>
<tr>
<td>4</td>
<td>Major</td>
<td>Extensive injuries, loss of production capability, off-site release with no detrimental effects, major financial loss</td>
</tr>
<tr>
<td>5</td>
<td>Catastrophic</td>
<td>Death, toxic release off-site with detrimental effect, huge financial loss</td>
</tr>
</tbody>
</table>

Table 3. Risk Matrix Scale (Level of Risk) in AS/NZS 4360

<table>
<thead>
<tr>
<th>Likelihood</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>5 (Almost Certain)</td>
<td>H</td>
</tr>
<tr>
<td>4 (Likely)</td>
<td>M</td>
</tr>
<tr>
<td>3 (Possible)</td>
<td>L</td>
</tr>
<tr>
<td>2 (Unlikely)</td>
<td>L</td>
</tr>
<tr>
<td>1 (Rare)</td>
<td>L</td>
</tr>
</tbody>
</table>
Determining risk control usually uses the hierarchy of control to establish priority order for the types of action to be used to minimize or control risks. There are five stages in Hierarchy of Control ANSI Z10: 2005 in Djatmiko (2016): (5):

1. Elimination is the most effective way to control the risk, to eliminate potential hazards.
2. Substitution to replaces hazardous materials, process or equipment to become more harmless.
3. Engineering Control by modifying machine/equipment or workplace to become safer.
4. Administrative Control to secure workers from exposure by implementing SOP, training, working hours, etc.
5. Personal Protective Equipment (PPE) such as helmet, safety glasses, mask, gloves, earplug, work shirt and safety shoe is the most ineffective way to control and reduce the impact of hazards.

Material and Method

This research is an observational research and then the data are analysed descriptively, which is aimed to delineate a phenomenon. The method of risk analysis applied in this research is HIRADC method in accordance with AS/ NZS 4360: 1999 and ISO 45001: 2018. There are several steps mentioned in this research before, namely hazard identification, risk assessment, and determining control. The data collected is qualitative data in form of description, words or behaviour that were observed. Researchers completed the data collection from hazard identification by making an observation in the Laboratory of Histology Anatomy in Faculty of Medicine, Universitas Airlangga. Then, the researchers give the score in the risk assessment based on the likelihood and severity level. After that, the researchers match and check it with the laboratory worker. After completing a risk assessment, the researchers start to plan the control activity to reduce the risks that are likely to occur in the laboratory.

Findings

Hazard Identifications

Hazard identification is determined by the activities conducted in the Laboratory of Histology Anatomy. The Laboratory of Histology Anatomy contains five main activities, such as experiments conducted by the students, dry cadavers shaping by the laboratory worker, the used goods burning, new cadavers shaping and cadaver maintenance by the laboratory worker. The chemical substances used in cadaver handling that are likely to be hazard sources are 70% alcohol, formaldehyde, and glycerin.

In the Laboratory of Histology Anatomy, risks identified is mostly related to chemicals and psychology because of the cadaver. The two extreme-risk level risks found caused by the exposure of formaldehyde in a new shaping of cadaver and cadaver maintenance by the laboratory worker. There are three high-risk level risks identified, namely fire that used for goods burning, inconvenience and scare that affect laboratory psychology in new cadavers shaping and cadaver maintenance. Eight moderate-risk level risks are fingers injuries from sharp laboratory equipment, Musculoskeletal Disorders (MSDs) because of the sitting position, blisters from boiled water, skin and eyes irritation caused by 70% alcohol, glycerine in new cadaver shaping and cadaver maintenance, and skin diseases from Candida sp. and Epidemophyton sp., fungi while making new cadavers and maintaining cadavers. And three low-risk hazards are injuries by glass-made laboratory equipment, diseases transmission and eyes fatigue.

In fact, formaldehyde as the primary chemical substance is often used in cadaver handling (new cadaver shaping and cadaver maintenance). The students and the laboratory worker can expose by the chemical substances to the respiratory, eyes or skin and they can experience the inconvenience because of the cadaver during the laboratory activities.

Experiments Conducted by the Medical Students

Based on the findings in Laboratory of Histology Anatomy, the experiment usually uses the cadaver. Formaldehyde is a main chemical substance that can potentially cause any issue on Occupational Safety and Health in laboratory. The students can easily smell the formaldehyde in the air when entering the laboratory because of the cadaver. They may be exposed continuously to the formaldehyde for 2-3 hours in the laboratory and they feel the pain in the throat and shortness of breath and formaldehyde has an odor that makes many students feel unpleasant during the anatomy experiments. Moreover, the effect of formaldehyde exposure to the students are likely possible to irritate the
According to the previous finding by Raja & Bahar (2011), it was affirmed that during the gross anatomy dissection, the main exposure to formaldehyde vapor and contact with formaldehyde can cause several effects. The common effects of exposure to formaldehyde are skin disorders, congenital malformations, ocular irritation, cancer risk, ingestion-related gastrointestinal effects, inhalation-related upper airway irritation and bronchial asthma (6).

Cadaver Handling (Dry Cadaver Shaping, New Cadaver Shaping and Cadaver Maintenance) and The Goods Burning by the Laboratory Worker

From the research in Laboratory of Histology Anatomy, the laboratory worker is continually exposed to the chemical substances during the cadaver handling and the goods burning activity. The hazards from this activity can be classified to chemical, psychology and biological hazards. The chemical hazards in Cadaver Handling are alcohol 70 %, formaldehyde, and glycerin. The use of formaldehyde which is the fundamental substance to shape new cadavers and to maintain the existed cadavers surprisingly becomes the main hazard source in the Laboratory of Histology Anatomy.

Additionally, the potential hazard that may occur during the existed cadaver maintenance is extreme due to the fact that the maintenance is a routine activity every two weeks and also the shaping of a new cadaver is done once every two or three months. The manual handling process of formaldehyde may increase the risk of formaldehyde spill and spark to the skin or eyes. And when handling the cadaver, the laboratory worker doesn’t wear PPE properly. The laboratory worker experienced discomfort in breathing, eye and nose nerves irritation, and sore throat which known as the symptoms from formaldehyde exposure.

Formaldehyde is a colorless, flammable gas with a pungent odor used as a preservative and a corrosion inhibitor that are highly irritating to the nose and toxic if swallowed. If the skin contacts with formaldehyde, it can cause severe injury accompanied by drying, cracking, and scaling (7). The International Agency for Research on Cancer (IARC) has classified formaldehyde in Group 1 as a carcinogenic agent for human and someone is exposed formaldehyde will suffer from nasopharynx cancer (8).

This is in accordance with the research initiated by Jalles Dantas de Lucena, et al. which also took place in a Laboratory of Anatomy. In result, they discovered that 70.3% of the respondents were in discomfort due to unpleasant and disturbing smell from the use of formaldehyde. Furthermore, the formaldehyde exposure is followed by the symptoms, such as excessive lacrimation, red eyes, nasal congestion, and respiratory disorder which continuously happen in the laboratory (9).

Other potential hazards in the Laboratory of Histology Anatomy are having a nightmares and stress due to the works with the dead body that can affect laboratory worker’s psychology because of the inconvenience and scare. Afterwards, other activity is burning the goods with fire that carried out inside the laboratory and without providing fire extinguishers. This can be a high-risk level since the severity and impact can cause enormous losses both for laboratory and university.

Risk Assessment

The risk assessment is arranged to present the risk analysis and suggestions for the evaluation stage, which later will be considered by the organization to decide what control measures that effectively control the risk. In this stage, the assessment by giving scores to hazard ratings is needed by the Likelihood (the possibility of the risk occurrence) and the Severity (the consequences of the risks) in accordance with AS/NZS 4360 standard (10).

There are two extreme-level risks on Laboratory of Histology Anatomy that caused by cadaver handling. The exposure of formaldehyde in new cadavers shaping has the level of likelihood of 4 and in cadaver maintenance has the level of likelihood of 5 which means that the possibility of hazard occurrence will probably and expected to occur in most circumstances. This happens because formaldehyde is a main chemical used in cadaver handling activities. In this two cadaver handling activities the Severity level of 5. This can be said so due to the fact that formaldehyde may cause toxic release with detrimental effect to the respiratory tract, eye and skin.

In risk assessment process, it is noticed that the total risk of the exposure of formaldehyde in new cadavers shaping is 20 and 25. Based on the result of the risk assessment, the exposure of formaldehyde is categorized as extreme risk. It means that the control and consideration of resources are immediately needed to
reduce risks based on the priority in controlling the risks.

And three high-level risks on Laboratory of Histology Anatomy which also need to be noticed are fire on goods burning, inconvenience and scare that affect laboratory psychology in new cadavers shaping and cadaver maintenance. The fire risk of goods burning has the level of likelihood of 2 which means could occur at some time and the severity of 4 means can cause extensive injuries, loss of production capability, major financial loss with the total risks is 8. Both in new cadavers shaping and cadaver maintenance, psychology hazard has the level of likelihood of 3 which means might occur at some time and severity level of 3 means medical treatment required with the total of the risk is 9. Based on the result of the risk assessment, the exposure of fire and psychology hazards are categorized as high risk that need to be controlled so the risk not become more dangerous or change to extreme-risk level risks.

**Risk Control**

Determinant control is performed to reduce risk level to minimize the risks that can affect Occupational Safety and Health when conducting activities in the Laboratory of Histology Anatomy Universitas Airlangga. In accordance with hierarchy of control, risk controls need to be carried out to decrease the occurrence of occupational accidents in laboratory based on the hazards and risks that have been identified are analyzed to arrange the control measures by the priority of each risk level.

Moreover, the authors provide several recommendations to reduce the extreme-risk the engineering control which can be applied is by increasing the number of the ventilation. Laboratory worker needs to check the smell and temperature in the laboratory and open the ventilations immediately when strong formaldehyde odor was found. The next control is by giving socialization about the importance of wearing Personal Protective Equipment (PPE) while handling the cadaver or chemical substances, such as rubber gloves and laboratory coat, safety goggles and mask to avoid direct contamination with hazardous substances that can cause irritation and burns. And providing fire extinguishers when burning the goods in the laboratory. Therefore, the risk of psychological hazard while handling the cadavers can be done with adapting to the work, providing further explanation regarding the job and undertake the work together while handling the cadaver shaping at least with 3 people in a team. Providing the explanation about the laboratory SOP to the students and academicians who are involved in the laboratory activities and also providing the fire extinguishers when burning the goods in the laboratory.

**Conclusions**

Based on the research that has been done, it can be concluded that at Laboratory of Histology Anatomy has a low to extreme-risk potential. There are 16 risks found with two extreme-hazard from formaldehyde exposure and three high-risk hazards that need to be controlled. The controls and recommendations that can be provided are giving socialization about the importance of wearing PPE (rubber gloves, safety goggles, and laboratory coat), providing the explanation about the work to the worker as well as explanation about the laboratory SOP to the academicians who are involved in the laboratory activities and providing the fire extinguishers.

**Acknowledgement:** The authors acknowledge Mr. Sumain as the laboratory worker of the Laboratory of Histology Anatomy, Universitas Airlangga, for providing information regarding the research of HIRADC Method of Occupational Safety and Health.

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**Ethical Clearance:** Ethical Clearance is taken from Health Research Committee, Faculty of Public Health, Universitas Airlangga, Indonesia.

**References**


Investigating Relationship between Positive Organizational Behaviors and Organizational Commitment among Nurses at Emam Khomeyni Hospital in Jiroft, Iran, in 2017: A cross Sectional Study

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Abstract

Introduction: One of the most important indicators of development and excellence of an organization is the presence of committed and loyal manpower. Thus, this study aims to investigate the relationship between positive organizational behaviors and organizational commitment among nurses working at Emam Khomeyni Hospital of Jiroft, Iran in 2017.

Method: This study is correlative-descriptive. The statistical population consisted of all the nurses working at Jiroft Hospital and they were all selected as samples. The data were analyzed using SPSS v25. Independent t-tests were applied to investigate the relationships between demographic specifications, organizational behavior, and organizational commitment, while correlation coefficient was employed to study the relationship between the main variables and Regression test and Pearson Correlation Coefficient were used to examine the relationship between organizational commitment and organizational behaviors and its dimensions.

Results: the results indicated that there was an inverse and significant relationship between organizational behavior and organizational commitment in general (p<0.001) and in emotional dimension (p<0.001) and normative dimension (p<0.01) in particular. Moreover, a positive and significant relationship was observed between positive organizational behavior and organizational commitment (p=0.03).

Conclusion: since positive behaviors are of great importance for performance evaluation systems among nurses and, at the same time, can affect a variety of their attitudes, including organizational commitment, it is suggested to improve nurses’ understanding of positive organizational behavior and organizational commitment to allow them to have better opportunities to better provide services in their workplace.

Keywords: Positive Organizational Behavior, Organizational Commitment, Nurses

Introduction

Formerly, approaches of many organizational behavior psychologists were focused on employees’ weaknesses and their behavioral pathology to introduce suitable solutions to reduce the weaknesses, with their abilities and positive aspects not being considered to a considerable degree. But, with the emerson of psychology movement, this approach was reoriented toward positivism and emphasis on positive behavioral aspects, bringing up positivist organizational behavior approaches [1-3].

The new approach believes that individuals’ strengths and desirable qualities can be considered by avoiding leaders’ continuous focus on weaknesses and weak performances of individuals to improve their positive behaviors, thereby improving individual and
organizational performance \cite{4, 5}. Positive physiologic approach is a term that covers the study of positive emotions, positive individual characteristics, and healthy and strong organizations, including family \cite{6, 7}. The goal of such approaches is to identify the structures and methods that finally result in human wellbeing. Hence, factors that provide individuals’ with compatibility to their lives’ pressures and threats as much as possible are the most fundamental structures of the approaches. Relying on individuals’ strengths, positive psychology helps them improve their individual and organization performance \cite{4, 8}. Pundits and experts have proposed various components investigating positive psychology but four of them were the most agreed by them: 1) optimism, 2) self-efficacy, 3) tolerance, and 4) hope \cite{9}.

Organizational commitment is a concept that attempts to achieve the nature of attachment created by individuals in an organization. In fact, organizational commitment reflects the extent of an individual’s compatibility and attachment to the organization \cite{10}. Organizational commitment is the link between individual and the organization, which is shown by three factors: strong belief and accepting the organization’s objectives and values, high willingness toward the organization, and high willingness to stay at the organization. In view of some psychologists, commitment is a complicated multidimensional structure. Hence, a variety of definitions have been proposed by experts for commitment \cite{11}.

Meyer and Allen defined organizational commitment as a physiologic state that indicates willingness to, requirement for, or necessity of providing a service in an organization. They introduced a 3-dimensional model of organization commitment, which is composed of emotional commitment, continuous commitment, and normative commitment \cite{12}. Organizational commitment can be a good indicator to show the effectiveness of an organization. Authorities must indicate high interest in an individual and not consider them as an organization member but respect them as an individual because they will have better performance if they are viewed as humans and unique identities \cite{13}.

Given the importance of organizational commitment among nurses, this study attempts to investigate and identify problems that create responsibility, loyalty, and commitment toward an organization among the staff. Identifying factors influencing organizational commitment, better performance is enabled by making modifications and changes to create commitment.

Method

This study is descriptive-correlational aiming to investigate the relationships between psychologically positive organizational behaviors and organizational commitment components among nurses working at Emam Khomeyni Hospital of Jiroft, Iran in 2017. The statistical population consisted of all the nurses at the hospital. To collect data, demographic specifications questionnaire was used. At the same time, the questionnaire in Lutanz et al. (2007) was employed to measure positive organizations behaviors and the questionnaire introduced by Allen and Meyer in 1990 was used to measure organizational commitment.

The standard questionnaire proposed by Lutanz et al. (2007) consisted of 20 questions, 8, 2, 4, and 6 questions of which were concerned with self-efficacy, tolerance, optimism, and hope, respectively. Each question was evaluated on 5-point Likert scale. Lutanz et al. (2007) reported the reliability of the questionnaire to be 90%. Hoveyda et al. \cite{1}(1) reported the validity of the questionnaire as 70%.

The questionnaire introduced by Allen and Meyer in 1990 consists of 8 questions that measure emotional, continuous, and normative dimensions \cite{12}.

To control the validity of the positive organizational behaviors and organizational commitment questionnaires, they were given to a number of experts to make some modifications. Cronbach alpha was used for reliability. Alpha was obtained to be 86%, 91%, 63% for self-efficacy, hope, and tolerance. It was also calculated to be 82%, for the total of the organizational commitment questionnaire (87%, 78%, and 96% for emotional commitment, continuous commitment, and normative commitment, respectively). The data were analyzed using SPSS v25. Amount, mean, standard deviation, and percentage were employed for the descriptive section. After the normality of the data distribution was ensured, independent sample t test was performed to investigate the relationships between the demographic specifications, organizational behavior, and organizational commitment. At the same time, correlation coefficient was used to explore the relationship between the main variables and regression test and Pearson correlation coefficient were applied to study the relationships between organizational commitment, organizational behavior, and its dimensions. A significance level of 0.5 was considered for all the tests.
Results

210 nurses participated in this study. Individual information of the nurses, including age, gender, marital status, educational degree, and work expertise, is given in Table 1.

The mean score of the study units in the organizational commitment questionnaire was 71.81 of 115. Given the confidence level of 95% for the obtained mean score, the number of the study units was between medium and high. To determine to which dimensions the study units were more committed, repeated measure analysis and paired sample t test were employed. It was found that emotional commitment, continuous commitment, and normative commitment were significantly different (p<0.05). Paired sample t test revealed that emotional commitment and continuous commitment were almost the same among the participants, while there was a significant difference between emotional commitment and normative commitment (p<0.05) and between continuous commitment and normative commitment (p>0.05). Considering the mean scores obtained for each organizational commitment dimension, it can be said that normative commitment was lower than emotional commitment and continuous commitment (see Table 2).

The mean score obtained from the organizational behavior questionnaire was 48.88 of 100. Considering the confidence level of 95% for the mean scores, the commitment of the respondents was between medium and high (see Table 3).

The results showed that the mean score of organizational behavior for the respondents has no significant difference based on the demographic specifications (p<0.05). The mean score of organization commitment of male respondents was significantly higher than females (p=0.01). But no significant difference was observed based on other demographic specifications (p> 0.05) (see Table 4).

The obtained Pearson correlation coefficient revealed that there was a significant relationship between organizational behavior and organizational commitment in general, and between emotional dimension and normative dimension in particular. The relationship between organizational commitment and organization behavior in continuous dimension was positive and significant.

Table 1 Frequency Distribution and Percentage of Individual Information

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (Percentage)</th>
<th>Mean and Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>48 (22.86%)</td>
<td>29.86.7</td>
</tr>
<tr>
<td>26-30</td>
<td>80 (38.10%)</td>
<td></td>
</tr>
<tr>
<td>31-35</td>
<td>46 (21.90%)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>36 (17.40%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45 (21.43%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>165 (79.57%)</td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>164 (78.10%)</td>
<td></td>
</tr>
<tr>
<td>Non-married</td>
<td>46 (21.90%)</td>
<td></td>
</tr>
<tr>
<td>Educational Degree</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma</td>
<td>35 (16.60%)</td>
<td></td>
</tr>
<tr>
<td>Bachelor and higher</td>
<td>175 (83.40%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Statistical Indicators Obtained from Organizational Commitment Questionnaire

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Confidence Level 95% for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Emotional Commitment</td>
<td>24.94</td>
<td>6.83</td>
<td>34.01</td>
</tr>
<tr>
<td>Continuous Commitment</td>
<td>26.17</td>
<td>6.33</td>
<td>25.29</td>
</tr>
<tr>
<td>Normative Commitment</td>
<td>20.67</td>
<td>5.63</td>
<td>19.88</td>
</tr>
<tr>
<td>Organizational Commitment (Total)</td>
<td>71.81</td>
<td>14.25</td>
<td>70.06</td>
</tr>
</tbody>
</table>
Table 3 Statistical Indicators Obtained for Positive Organizational Behavior

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Confidence Level 95% for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Self-efficacy</td>
<td>15.32</td>
<td>4.83</td>
<td>14.64</td>
</tr>
<tr>
<td>Tolerance</td>
<td>9.82</td>
<td>4.43</td>
<td>9.34</td>
</tr>
<tr>
<td>Optimism</td>
<td>9.88</td>
<td>3.57</td>
<td>9.38</td>
</tr>
<tr>
<td>Hope</td>
<td>13.86</td>
<td>3.13</td>
<td>13.43</td>
</tr>
<tr>
<td>Organizational Behavior (Total)</td>
<td>48.88</td>
<td>12.1</td>
<td>46.79</td>
</tr>
</tbody>
</table>

Table 4 Comparison of Organizational Behavior Scores and Organizational Commitment Scores based on Demographic Specifications

<table>
<thead>
<tr>
<th>Demographic Specification</th>
<th>Number Mean</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Deviation</th>
<th>p-value</th>
<th>Organizational Behavior</th>
<th>Organizational Commitment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>25</td>
<td>48</td>
<td>51.2</td>
<td>6.02</td>
<td>0.6</td>
<td>47.1</td>
<td>12.94</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>26-30</td>
<td>80</td>
<td>50.4</td>
<td>10.3</td>
<td></td>
<td>70.21</td>
<td>12.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31-35</td>
<td>46</td>
<td>53.9</td>
<td>9.4</td>
<td></td>
<td>71.74</td>
<td>11.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>3</td>
<td>47.6</td>
<td>8.7</td>
<td></td>
<td>69.03</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>45</td>
<td>45.6</td>
<td>10.6</td>
<td>0.3</td>
<td>74.71</td>
<td>10.08</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>165</td>
<td>49.8</td>
<td>9.1</td>
<td></td>
<td>75.05</td>
<td>13.12</td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td>Married</td>
<td>164</td>
<td>47.8</td>
<td>8.3</td>
<td>0.3</td>
<td>70.03</td>
<td>9.09</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Non-married</td>
<td>46</td>
<td>52.5</td>
<td>8.5</td>
<td></td>
<td>71.72</td>
<td>10.06</td>
<td></td>
</tr>
<tr>
<td>Educational Degree</td>
<td>Diploma</td>
<td>35</td>
<td>48.1</td>
<td>10.12</td>
<td>0.4</td>
<td>72.72</td>
<td>13.00</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Bachelor and higher</td>
<td>175</td>
<td>46.58</td>
<td>11.02</td>
<td></td>
<td>69.03</td>
<td>12.08</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Today, given the extensive role of organizations in social lives of humans, manpower has become more salient than before. Concerning the first three objective of the study, results are provided in tables. Regarding the relationship between organizational commitment and psychologically positive organizational behaviors, demographic information only indicated a significant relationship between organizational commitment and gender, with no significant relationships being revealed for other items. The mean organizational commitment was higher among the males than among the females. This result was consistent with Jahangir et al. [14]. Moreover, mean emotional commitment in Sajedi et al. was higher among males than among females, which was statistically significant.

In Bahri et al. [15], no significant relationship between organizational commitment and gender was observed, but there was a significant relationship between organizational commitment and marital status, which is not consistent with our results. The higher organizational commitment obtained for males was perhaps due to the fact that males are often responsible for their families’ financial requirements. Thus, it is more important to them to keep their job and promote, ensuring their income. This responsibility is mostly focused on workplace and they rarely consider their home management, while women are more responsible in home. Hence, men are more responsible toward their organizations. Indeed, more studies with more male and
females respondents are required to approve it. In the current study, organizational commitment was obtained to be 71.81% in general and 24.97%, 26.17%, and 20.67% in emotional dimension, continuous dimension, and normative dimension, respectively, which is at a medium level. There are many studies consistent with this study. For example, Sajedi [14], Jahangir [16], and Mahmoudi [17] reported organizational commitment among nurses to be at a medium level. An overview on statistical analysis results shows that the effects of positive psychological capacities on organizational commitment are very high. In other words, there is a positive and significant relationship between psychologically positive capacities and organizational commitment. But, as the multiple regression results indicate, the role of hope in increasing staff organizational commitment is more salient and significant. These findings are in an agreement with Joseph et al. (2007) who studied the relationship between psychologically positive capacities and work-related outcomes and concluded that hope and tolerance, among the other psychologically positive capacities, have increasing role in the emergence of organization commitment [4]. This is also consistent with Luthans study [4].

With respect to the relationships between hope and the three organizational commitment dimensions, the relationship between hope and emotional commitment is more significant than with the other two dimensions. This is consistent with Joseph study [4]. Concerning the relationships between optimism and the three organizational commitment dimensions, our results are consistent with Hodge study [18].

These findings demonstrate that continuous commitment plays a more salient role than the two other organizational commitments in individuals’ optimism. For the relationship between tolerance and the three organizational commitment dimensions, it was observed that tolerance can take a more effective step toward organizational commitment, which provides individuals’ with more compatibility to different conditions and meets their organizations requirements. This finding is consistent with other studies [4, 18, 19].

**Conclusion**

In overall, a higher efficiency and effectiveness of organizations depend on their manpower efficiency and effectiveness. Hence, it is a priority for organizations to move their staff toward hope, optimism, and tolerance, thereby increasing organizational commitment among them.

**Acknowledgment:** The authors would like to thank all the participants and experts who helped to collect information.

**Ethical Clearance:** Taken from Jiroft university of medical science committee

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**


Alcoholic Extract of Shilajit as Anti Protein Denaturation, Anti Blood Hemolysis, and Anti Microbial

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\textsuperscript{1}Mustansiriyah University/ College of Science/ Biology Department, \textsuperscript{2}Former Associate Professor, University of Baghdad, Iraq

Abstract

Shilajit is a natural material found mostly in the Himalayas (India), formed by the slow decomposition of certain plants by the action of microorganisms. It is an effective and extremely safe dietary supplement, and potentially able to prevent several diseases. Shilajit alcoholic extract was prepared by dissolving 10 grams of it in 200 mL of 95% ethanol. FTIR and other chemical procedures were used to detect the presence of Phenols, Alkaloids, Terpenoids, Tannins, Proteins Carbohydrates and, Steroids. Different concentrations of the extract were prepared and antimicrobial activity against several positive and negative gram stains, anti-protein denaturation and anti-blood cell hemolysis. Investigation results show that the nanoparticles were effective in inhibiting protein denaturation of albumin. Proteins denaturation refers the cause of inflammation, while the maximum inhibition of albumin was 87% observed at 300 μg/ml of Shilajit alcohol extract compared with aspirin at the same concentration. The results of inhibiting hemolysis at 300μg/ml in comparing with standard drug Diclofenac sodium 300μg/ml resulted in good protection against the damaging effect of heat solution

Keywords: Shilajit, anti blood hemolysis, phenol, alkaloid, anti protein.

Introduction

Shilajit is a brownish-black powder from high mountain rocks, particularly in the Himalayans Mountains (India), Russia, Tibet (China), Afghanistan, and recently in South America (Chile). Shilajit has been known and used for centuries as medicine, and as anti-aging compound. Lately, additional properties was found in Shilajit that is, to increase physical ability and to support human health\textsuperscript{(1-3)}. It is also used to treat chest problems, diabetes mellitus, nervous disorders, immune disorders, obesity, kidney disorders, asthma, gall stones, painful and bleeding piles, liver, fermentative dyspepsia, worms, renal and bladder calculi, nervous debility, sexual neurasthenia, hysteria, fainting, female infertility, joint pains, wounds, ulcers and skin diseases\textsuperscript{(4, 5)}.

Shilajit has demonstrated good inhibition against viral enzymes and anti HIV activity it is available in tablet form in medicines such as Abana, Cystone and Diabecon. It is also available in syrup form as Evecare and Geriforte. Variation in the quality of shilajit humus (both chemical and biological) and the factors that cause variations in shilajit humus are: (i) altitude and the nature of shilajit-bearing rocks; (ii) atmospheric conditions (e.g. alternate wetting and drying); (iii) pH and moisture content of the rock source; and (iv) activity of the rhizospheric microorganisms and their exo-enzymes\textsuperscript{(7)}.

Current knowledge on the phytochemical screening and antimicrobial activity of shilajit is sparse and thus there remains a wide gap in our knowledge of it and thus it needs to be explored\textsuperscript{(8)}.

In this article researchers Shilajit alcoholic extract was prepared. FTIR and other chemical technique were used to detect the presence of Phenols, Alkaloids, Terpenoids, Tannins, Proteins Carbohydrates and, Steroids. Different concentrations of the extract were prepared and antimicrobial activity against several positive and negative gram stains, anti-protein denaturation and anti-blood cell hemolysis. Investigations were carried out.
Materials and Method

Alcoholic extract

10 grams of the shilajit powder were add to 200 ml of 95% ethanol; stirred well on hot plate for 15 min, and kept for ten day at 28 °C, then filtered, dried and was kept at 4 °C (8).

Detection of Terpenoids

Terpenoids content was determined as was described by Oyagi and his co-workers (11). Peel extract powder (0.5 g) was mixed with 10 ml 90% methanol then 2 ml of chloroform and 3 ml of sulphuric acid were added and mixed well. Reddish brown color indicates the presence of terpenoids.

Detection of Tannins

Tannins were measured according to procedure mention by Oyagi and his co-workers (11) by adding (0.5 g) of peel extract to 10 mL distilled water then 2% of FeCl₃. A blue-green color appeared indicated tannins.

Detection of Proteins

Protein content was measured by an assay as described by Oyagi and his co-workers (11). Violet color appearance suggests the presence of amino acids and proteins.

FTIR spectroscopy

The spectra of Fourier transform infrared generated by the radiation of electromagnetic absorption in the frequency range 500 to 4000 cm⁻¹. The absorption and intensity of different active functional group indicate geometry features of these groups. FTIR spectra were taken using Shimadzu model.

Antibacterial activity assay

The antibacterial activity was determined by agar disc diffusion Oyagi and his co-workers (12). Agar plates were inoculated with 0.1 ml broth culture of tested organisms and was spread with sterile an L-shaped rod glass spreader. Whatman No. 1 filter paper of 5 mm diameter were impregnated with different concentration of crude extracts and dried in a hot air oven at 60 ºC for 5 min. The disc in the center of agar plate which impregnated with sterile distal water was used as control.

Denaturation inhibition of albumin

Human albumin (1%) was incubated at 37 °C for 20 minutes, heated at 51 °C and then the albumin was cooled. The turbidity was measured at 660 nm by UV Visible Spectrophotometer. Denaturation percent was determined according to the following equation:

Detection of membrane RBCs stabilization

Human blood cells centrifuged by 3000 rpm for
(10) min, washed the precipitate with normal saline then re-suspended to (10%) v/v in normal saline, the suspension was incubated at 56 °C by water bath for 30 min, centrifuged at 3000 rpm for five minutes, and the absorbance was measured at 560 nm. The Percentage of haemolysis inhibition calculated using the following formula:

**Statistical Analysis**

Data were expressed as a mean values ± SD by the statistical software package SPSS (version 16).

**Results and Discussion**

Shilajit consist of different groups of active components, Table 1 shows Shilajit components.

**Table 1: Shilajit active component**

<table>
<thead>
<tr>
<th>No.</th>
<th>Component</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total phenol</td>
<td>+++++</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoids</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>Tannin</td>
<td>+++++</td>
</tr>
<tr>
<td>5</td>
<td>Protein</td>
<td>+++++</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates</td>
<td>++++</td>
</tr>
<tr>
<td>7</td>
<td>Steroids</td>
<td>+++</td>
</tr>
</tbody>
</table>

Flavonoids are secondary metabolites, it protect cell from degradation, stress, and act as anti-cancer and anti-viral molecule. Phytoalexins, detoxifying agents, reduce toxic effects and stimulants. Recent research indicated that active components can be nutritionally helpful by triggering the production of natural enzymes that fight disease, such as cancers, heart disease, and age-related degenerative diseases (13).

**FTIR**

The band at 1181 cm\(^{-1}\) can be attributed to C=O stretching indicating the presence of polysaccharide or polysaccharide like compounds, at wavenumber 1411 cm\(^{-1}\) (O-H) bending indicating the presence of alcohols or carboxylic acid. A peak at 2930 cm\(^{-1}\) stretch is referred to aliphatic (C-H). The peak at region of 1613 cm\(^{-1}\) (aromatic C=C double bond) figure 1.

![FTIR spectra](image1.png)

**Figure 1: FTIR spectra**

Table 2 shows that different bacterial species exhibited different sensitivities towards the extract of shilajit. The sensitivities of bacterial species against phenolic compounds showed more activity against gram positive bacteria compared to gram negative bacteria under this study. These variations in inhibition may be because of differences in the composition and structure surface between Gram positive and Gram negative bacteria (14).

**Table 2: The mean of inhibition zone of the aqueous extract of shilajit against certain bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>10</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>12</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>8</td>
</tr>
<tr>
<td>Pseudomonas euroginosa</td>
<td>-</td>
</tr>
</tbody>
</table>

Maximum inhibition of albumin was 87% observed at 300 μg/ml of shilajit alcohol extract compared with aspirin (which exhibit 91%) at the same concentration figure 2. Each value represents the mean ± SD. All values showed significant results when it compare with control p<0.01. This result investigated that nanoparticle was effective in inhibiting protein denaturation of albumin. Proteins denaturation refers to cause of inflammation (15).
In this study, protein denaturation was used for detection mechanism of the anti-inflammatory activity.

Figure 2: Results of albumin denaturation inhibition of shilajit extract

**Heat Induced Haemolysis**

Figure 3 show the results activity of inhibiting haemolysis at different concentrations of shilajit. The effective concentration was 300μg/ml in comparing with standard drug Diclofenac sodium 300μg/ml resulted in good protection against damaging effect of heat solution.

Figure 3: Result of anti hemolysis effect of shilajit extract

**Acknowledgment:** Authors would like to thank the staff in Life Science, Biology department in College of Science/ Mustansiriyah University, for their cooperation to complete this research.

**Conflict of Interests:** The authors declare that they have no conflict of interest

**Source of Funding:** Self–funding

**Ethical Clearance:** The researchers already have ethical clearance from College of Science, Mustansiriyah University, Iraq

**References**

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A Stereomicroscopic Analysis of Dentinal Micro Cracks after Root Canal Preparation Using Four Different Rotary Instruments

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2Department of Conservative Dentistry, College of Dentistry, Al-Mustansiriyah University, Baghdad, Iraq

Abstract

A variety of single-engine driven files and kinematics have been introduced to improve the clinical performance of NiTi rotary files. The purpose of this in vitro study was to measure and compare the incidence of dentinal defects after root canal preparation with different single file systems.

Keywords: Root canal preparations, Root canal instrumentation, Tooth cracks

Introduction

During and after chemomechanical root canal preparation with rotary instruments that aimed to remove infected soft and hard tissue through the enlarged root canal; [1] the root canal wall can be harmed with the development of dentinal defects in the form of dentinal cracks or craze line which serves as localized sites of increased stress. [2]

Through the application of repeated tension via occlusal forces or when further clinical procedures are required such as post placement, these dentinal defects may have the potential to develop into vertical root fracture (VRF).[3,4,5]

Most shaping systems are categorized as to whether the file has active or passive cutting edges, a fixed or variable taper along its active portion, or a more traditional or unique cross-sectional design [6]. In addition to that whether the file has benefited from heat treatment that improves flexibility and the resistance to cyclic fatigue [7]. Also described whether it has a centered or offset mass of rotation, works with a rotary or reciprocation, whether the clockwise (CW) and counter clockwise (CCW) angles are equal or unequal [8,9,10]. A single file technique in conjunction with a novel reciprocating movement of unequal bidirectional angles that complete a full forward rotation of 360 degrees after four 90-degree cutting cycles of reciprocation can start and fully complete the preparation of a canal to a perfect shape [11].

It operates at 350 rpm speed in 170ºCCW and 50ºCW direction and completes 360º, torque 5 Ncm in 3 cycles, also it has parallelogram cross-section with two cutting edges at apical end [12].

F 6 Sky Taper (Komet Brasseler GmbH and Co., Lemgo, Germany) is a NiTi system that performs root canal treatment with continuous clockwise rotation, speed 300 rpm and torque 2.2 Ncm. It is available in five different sizes (20, 25, 30, 35 and 40) with a constant taper of 0.06 with S-shape section [13].

When using this instrument alone. It performs root canal preparation with continuous rotation at speed 800 rpm and torque 1 Ncm [14].

AF Blue R3 (Shanghai Fanta Dental Materials Co., Ltd ) is a rotary AF- R wire- 3- files- system suitable for curved canals, it performs root canal preparation with a reciprocating motion. It operates at 300 rpm speed, torque 2.6 Ncm in 150ºCCW and 30 º CW direction with 06 taper design and improved file flexibility while still retaining the cutting efficiency [15].

Root sectioning at different levels and inspection through a digital stereomicroscope allows direct inspection of dentinal defects on the root surface and provides information regarding the extension pattern and direction of cracks [16, 17].

To the best of our knowledge, little information has been found regarding the incidence of dentinal defects
resulting from the use of AF Blue R3, and F6 Sky Taper systems. Thus, the purpose of this study was to measure and compare the incidence of dentinal root defects after root canal shaping with four automated file systems: Wave One Gold, AF Blue R3, F6 Sky Taper and XP-Endo Shaper. The null hypothesis was that there would be no significant difference in dentinal defects amongst the studied groups.

**Materials and Method**

Seventy-five freshly extracted human mandibular premolars with approximately similar bucco-lingual and mesio-distal. The teeth were disinfected in a 0.1% thymol solution at room temperature for 24 h and were kept in purified filtered water until they were used [19]. Savannah, GA USA) under copious water coolant approximately 17mm from the apex to the facial CEJ [20]. The patency of the canal was ensured before and after instrumentation [21]. All the roots were imbedded in its simulated socket in impression materials [22].

Seventy-five root samples were randomly divided into five groups.

**Group I:** Specimens were instrumented by Wave One Gold (25 \ 0.07), length 25mm in a reciprocating movement.

**Group II:** Specimens were instrumented by AF Blue R3 (25\0.06), length 25 mm in a reciprocating movement using endodontic micromotor (speed: 300rpm, torque: 2.6Ncm).

**Group III:** Specimens were instrumented by F6 Sky Taper (25\0.06), length 25 mm in a rotation movement at WL with gentle in-and out- motion using endodontic micromotor (speed: 300rpm, torque: 2.2Ncm).

**Group IV:** Specimens were instrumented by XP-Endo Shaper (30\0.04), length 25 mm in a rotation movement at WL with gentle in-and out- motion using endodontic micromotor (speed: 800 rpm, torque: 1Ncm).

**Group V:** Specimens without instrumentation (control group).

The glide path was performed using size #15 K-file (DentsplyMaillefer) up to the working length,. A total amount 12 ml of 1% NaOCl was used per canal [23, 24].

A final flush of 5 ml of distilled water was administered to the prepared specimens to remove the remnant debris and irrigating solutions inside each canal. All root canals were dried with a sterile paper points to ensure that a complete instrumentation was achieved [25].

All the root samples were sectioned perpendicularly to the long axis in 3, 6, and 9 mm slices from the root apex [26, 27] using diamond disc (0.1 mm) under water cooling to avoid heating and to minimize smearing [28].

‘Defect’ was defined if any craze lines, microcracks, or fractures were present in root dentin or extending to outer root surface [18].

**Results**

Table 1 : The distribution of incidence of dental defects by the experimental groups at each level (apical, middle, and coronal) is shown in Table (1).

<table>
<thead>
<tr>
<th>Total group</th>
<th>Status</th>
<th>Apical level (3mm)</th>
<th>Middle level (6mm)</th>
<th>Coronal level (9mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Group I (Wave One Gold)</td>
<td>Defects</td>
<td>6</td>
<td>40%</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No defects</td>
<td>9</td>
<td>60%</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>100%</td>
<td>15</td>
</tr>
<tr>
<td>Group II (AF Blue R3)</td>
<td>Defects</td>
<td>2</td>
<td>13.33%</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No defects</td>
<td>13</td>
<td>86.67%</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>100%</td>
<td>15</td>
</tr>
<tr>
<td>Group III (F6 Sky Taper)</td>
<td>Defects</td>
<td>5</td>
<td>33.33%</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>No defects</td>
<td>10</td>
<td>66.67%</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>100%</td>
<td>15</td>
</tr>
</tbody>
</table>
Table (1): Number and percentage of dentinal defects by the experimental groups at each level (n=15)

<table>
<thead>
<tr>
<th>Group (XP-Endo Shaper)</th>
<th>Defect</th>
<th>1</th>
<th>6.67%</th>
<th>1</th>
<th>6.67%</th>
<th>0</th>
<th>0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No defects</td>
<td>14</td>
<td>14</td>
<td>93.33%</td>
<td>14</td>
<td>93.33%</td>
<td>15</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>15</td>
<td>100%</td>
<td>15</td>
<td>100%</td>
<td>15</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (2): Chi-Square test for the incidence of dentinal defects among different groups at the apical level (3mm). p>0.05 Non-Significant (NS), p<= 0.05 Significant (S)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Defect</th>
<th>No Defect</th>
<th>Total</th>
<th>Chi-Square test</th>
<th>d.f.</th>
<th>P-value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave One gold</td>
<td>6</td>
<td>9</td>
<td>15</td>
<td>11.77</td>
<td>4</td>
<td>0.02</td>
<td>S</td>
</tr>
<tr>
<td>AF Blue R3</td>
<td>2</td>
<td>13</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F6 SkyTaper</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XP-EndoShaper</td>
<td>1</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Chi-Square test for the incidence of dentinal defects among different groups at the middle level (6mm)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Defect</th>
<th>No Defect</th>
<th>Total</th>
<th>Chi-Square test</th>
<th>d.f.</th>
<th>P-value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave One gold</td>
<td>4</td>
<td>11</td>
<td>15</td>
<td>6.818</td>
<td>4</td>
<td>0.211</td>
<td>NS</td>
</tr>
<tr>
<td>AF Blue R3</td>
<td>1</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F6 SkyTaper</td>
<td>3</td>
<td>12</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XP-EndoShaper</td>
<td>1</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Chi-Square test for the incidence of dentinal defects among different groups at the coronal level (9mm)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Defect</th>
<th>No Defect</th>
<th>Total</th>
<th>Chi-Square test</th>
<th>d.f.</th>
<th>P-value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave One gold</td>
<td>2</td>
<td>13</td>
<td>15</td>
<td>4.285</td>
<td>4</td>
<td>0.404</td>
<td>NS</td>
</tr>
<tr>
<td>AF Blue R3</td>
<td>1</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F6 SkyTaper</td>
<td>2</td>
<td>13</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XP-EndoShaper</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (5): Chi-Square test for the incidence of dentinal defects among different level of Wave One Gold group

<table>
<thead>
<tr>
<th>Wave one cold</th>
<th>Defect</th>
<th>No Defect</th>
<th>Total</th>
<th>Chi-Square Test</th>
<th>d.f.</th>
<th>p-Value</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical level</td>
<td>6</td>
<td>9</td>
<td>15</td>
<td>2.727</td>
<td>2</td>
<td>0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Middle level</td>
<td>4</td>
<td>11</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronal level</td>
<td>2</td>
<td>13</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

During the root canal instrumentation using engine driven instruments, high stress concentration in the wall of the root canal system is applied. This may increase the risk of dentinal damage predisposing the tooth to vertical root fracture [29].

Dentinal defects and then root cracking are a complex procedure because it is affected by many factors such as the design feature of the file used and its kinematics in addition to the alloy from which the instrument was manufactured. Since the primary aim of chemomechanical root canal preparation is to decrease the bacterial load and prepare the root canal for obturation, at the same time; the preservation of tooth structure and increase its resistance to fracture that lead to a long-term survival rate to the tooth [30]. The length of the canal and lower overall stresses, also to facilitate comparison of the results with several previous studies in which similar teeth had been used [27,31].

Adorno et al., found that apical cracks occurred in 50% of mandibular premolars after root canal preparation [32].

The teeth samples were selected from young adult patients because aging result in changes in the dentinal microstructure which in turn change the mechanical behavior of dentin resulting in average reduction of its strength predisposing it to crack growth and dentinal defects [33, 34]. Unlike the use of SEM that requires section treatment which may change the actual status of the tissue [35].

Although high resolution micro CT scans method is conservative and non-destructive, it is a complex procedure that lasts for an hour or more, this may increase dehydration of the samples resulting in spontaneous cracks in dentin [36].

The control group after sectioning was not shows any dentinal defects. This finding comes in line with several studies [4,18].

The largest number of dentinal defects promoted by Wave One Gold instruments may be related to its high level of flexibility due to the heat treatment of NiTi alloy and its parallelogram-shaped cross section [37]. During root canal preparation [40], which agrees with previous reports [16, 18]. However; reciprocating movement could prevent the continuous rotary force and constant torque that are applied to the walls of the canal [41].

The occurrence of dentinal defects is independent on the kind of the instrument used. The experimental groups varied in their design, cross section, tip design and taper but similar only in size (#25) of the tip, therefore; the null hypothesis of the present study has been partially accepted.

**Conclusions**

Within the scope of this in vitro study, it can be concluded that Wave One Gold system generated the significantly higher incidence of dentinal defects in mandibular premolars compare to the other tested file systems. While XP-EndoShaper system generates the lowest incident of defects in the rotating file systems compare to F6 SkyTaper file systems. The highest incidence was at the apical level in all of the tested groups. Additional researches may be required to assess these instruments on crack propagation and the fracture
resistance of the root canal treated teeth.

References

Conflict of Interest – Nil

Source of Funding - Self

Ethical Clearance – Not required


[38]. FKG Dentaire SA. XP-Endo shaper: the one to shape your success. Available at: http:// www.fkg.ch


Studying Effective Factors on Overweight and Obesity in High School Students in Erbil

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Abstract

Introduction: Overweight and obesity are explained as excessive fat depositions in human body. They have become one of the serious public health problems of the twenty-first century in low income countries. The prevalence of overweight and obesity in children is increasing worldwide, and currently 1 child in 10 is overweight or obese. The aim of this study was to assess overweight and obesity and associated factors in high school students in Erbil.

Method: It was a cross-sectional study in which 1,000 students in the age range 16-18 years of Erbil, Iraq in 2016-2017 were randomly selected. Data were gathered by a questionnaire. Data were analyzed using SPSS version 22 by appropriate statistical tests.

Results: Our results showed that some effective factors such as Computer games, watching TV, and irregular sleep (P-value<0.05) were the known factors affecting obesity/overweight in this study.

Conclusion: This study revealed that there are many factors involved in emerging over weight, such as overeating, eating snacks just before bedtime, and eating between meals especially between dinner and bed time, having lunch/dinner outside at restaurants and eating rice frequently, taking a nap during the day and watching TV/play video games/computer games.

Key words: overweight, obesity, high school, students

Introduction

Overweight and obesity can be explained as excessive and abnormal fat depositions in human body. They are major risk factors for several diet-based non-communicable diseases like dyslipidemia, cardiovascular diseases (CVD), and type II diabetes mellitus. About 2.8 million deaths and 35.8 million (2.3%) of global Disability Adjusted Life Years (DALYs) are caused by overweight or obesity.

The prevalence of overweight and obesity in children is increasing worldwide, almost 1 child in 10 is overweight or obese. Results of a study in United State suggest that obese adolescents are likely to stay obese into adulthood, and among individuals who were obese as adolescents, incident of severe obesity was 37.1% in men and 51.3% in women.

In lower- to middle-income countries, obesity co-exists with under nutrition where most overweight and obese children being concentrated in urban areas and presents serious social and psychological impacts. The prevalence of overweight in Iraq is experiencing a shift from underweight to overweight along with rapid socioeconomic and nutritional transition particularly in their area population. While, Africa is experiencing a shift from underweight to overweight along with rapid socioeconomic and nutritional transition particularly in their urban population. This transformation comes with increased access to energy-dense foods and less...
strenuous jobs resulting into many people having a positive energy balance and hence becoming overweight or obese.\textsuperscript{8,9}

It should be noted that 44\% of the diabetes burden, 23\% of the ischemic heart disease burden and between 7\% and 41\% of certain cancer burdens are attributable to overweight and obesity\textsuperscript{10,11}.

According to increase prevalence of overweight in high school children, the aim of this study was to assess the overweight in high school students and its relationship with lifestyle for the first time in Erbil, Iraq.

**Method**

It was a cross-sectional study which was conducted from the 20\textsuperscript{th} of September 2016 to the 10\textsuperscript{th} of March 2017. Target population were the students of grade 10, 11 and 12 of public schools (between 16-18 years of age) in Erbil city of Kurdistan region of Iraq.

The sample was collected by multistage cluster sampling technique. Schools were divided into 6 groups according to the municipalities of Erbil city, to cover all quarters of the city. Schools were randomly selected according to the number of students in each region. The total number of selected schools was 32 distributed in the 6 municipalities. Of the 36,777 students in these schools, 1,000 students were randomly selected. From each school a single class was selected randomly; For Data collection from each class was attended by the counseling specialist of the school.

**Questionnaire Design:**

A questionnaire which was designed by the Centers for Disease Control and Prevention (CDC) was modified and used as data collection\textsuperscript{9}. The questionnaire had three parts including demographic information, habits and life style of the students and socio-economic status (SES) that was calculated taking into consideration age, education and occupation of the father, car ownership and house ownership, according to the formula designed by Omer and AL-Hadithi \textsuperscript{10} and They were asked to choose between low SES, middle SES, and high SES which is one of the best types of descriptions. For better understanding; the questionnaires were adapted to each school’s language of instruction (local Kurdish and also to Arabic language).

After collecting the questionnaires, Data were examined using statistical package for social sciences (SPSS) version 22 (SPSS Inc. Chicago, IL) for windows. Data were analyzed through the use of simple descriptive statistical data analysis approach and inferential data analysis approach. Chi-square test of association was used to assess the association between the prevalence and several risk factors and Fisher’s exact test was used instead of the Chi square test when the expected count of more than 20\% of the cells of the table was less than 5. P value of ≤ 0.05 was considered as statistically significant.

**Ethical Consideration**

This study followed the tenets of the Declaration of Helsinki and written informed consent was obtained from all patients. All students were assured that their participation in the study was voluntary; they were informed about the purpose of the study; their anonymity and confidentiality were assured. Also an official permission from Erbil General Directorate of Education and from schools’ administrations to collect data was obtained. The study was approved by the Ethics Committee of the college of medicine of the Hawler medical university.

**Result**

This cross sectional study was done on 1000 high school children. Among them were 572 women and 428 men with an average age of 16.94±0.79. The tables of 1 to 5 show the prevalence of overweight and obesity by different factors.

**Table 1. Prevalence of overweight and obesity by history of diseases in the family.**

<table>
<thead>
<tr>
<th></th>
<th>Prevalence of overweight &amp; obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Overweight father</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>825</td>
</tr>
<tr>
<td>Yes</td>
<td>175</td>
</tr>
</tbody>
</table>
Table 1. Prevalence of overweight and obesity by history of diseases in the family.

<table>
<thead>
<tr>
<th>Condition</th>
<th>No</th>
<th>Yes</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes father</td>
<td>885</td>
<td>115</td>
<td>1000</td>
<td>0.449</td>
</tr>
<tr>
<td>Heart Problems father</td>
<td>988</td>
<td>12</td>
<td>1000</td>
<td>&gt; 0.999*</td>
</tr>
<tr>
<td>Overweight mother</td>
<td>806</td>
<td>194</td>
<td>1000</td>
<td>0.173</td>
</tr>
<tr>
<td>Diabetes mother</td>
<td>888</td>
<td>112</td>
<td>1000</td>
<td>0.040</td>
</tr>
<tr>
<td>Heart Problems mother</td>
<td>993</td>
<td>7</td>
<td>1000</td>
<td>&gt; 0.999*</td>
</tr>
<tr>
<td>Overweight brother</td>
<td>962</td>
<td>38</td>
<td>1000</td>
<td>0.043</td>
</tr>
<tr>
<td>Diabetes brother</td>
<td>997</td>
<td>3</td>
<td>1000</td>
<td>&gt; 0.999*</td>
</tr>
<tr>
<td>Heart Problems brother</td>
<td>998</td>
<td>2</td>
<td>1000</td>
<td>0.510*</td>
</tr>
<tr>
<td>Overweight sister</td>
<td>974</td>
<td>26</td>
<td>1000</td>
<td>0.340</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>300</td>
<td>1000</td>
<td>(30.0)</td>
</tr>
</tbody>
</table>
Table 2. Prevalence of overweight and obesity by knowledge and opinion about obesity.

<table>
<thead>
<tr>
<th>Do you think that your parents are overweight or obese?</th>
<th>N</th>
<th>No.</th>
<th>(%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>744</td>
<td>222</td>
<td>(29.8)</td>
<td>0.850</td>
</tr>
<tr>
<td>Yes</td>
<td>256</td>
<td>78</td>
<td>(30.5)</td>
<td></td>
</tr>
<tr>
<td>Do your parents have Diabetes?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>694</td>
<td>207</td>
<td>(29.8)</td>
<td>0.599</td>
</tr>
<tr>
<td>One of them</td>
<td>159</td>
<td>49</td>
<td>(30.8)</td>
<td></td>
</tr>
<tr>
<td>Both of them</td>
<td>33</td>
<td>13</td>
<td>(39.4)</td>
<td></td>
</tr>
<tr>
<td>I don’t know</td>
<td>114</td>
<td>31</td>
<td>(27.2)</td>
<td></td>
</tr>
<tr>
<td>Do you think that you are overweight or obese?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>748</td>
<td>153</td>
<td>(20.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>252</td>
<td>147</td>
<td>(58.3)</td>
<td></td>
</tr>
<tr>
<td>Do you think that obesity in adolescents is a health risk?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>469</td>
<td>141</td>
<td>(30.1)</td>
<td>0.967</td>
</tr>
<tr>
<td>Yes</td>
<td>531</td>
<td>159</td>
<td>(29.9)</td>
<td></td>
</tr>
<tr>
<td>Have you heard about the bad effect of overweight and obesity?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>258</td>
<td>89</td>
<td>(34.5)</td>
<td>0.067</td>
</tr>
<tr>
<td>Yes</td>
<td>742</td>
<td>211</td>
<td>(28.4)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>300</td>
<td>(30.0)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Our study showed that the overweight and obesity was significantly more in the age 16 and female students who were almost in class 11. A study by Taresa Kisi Beyen showed that More than half of the respondents 423 (53.5%) were females. The sex specific prevalence of overweight was similar with findings from Nigeria of which 0-8.1% males and 1.3-8.1% females were overweight. Ghana and Uganda (10.4% girls, 3.2% boys were overweight while 0.9% females and 0.5% males were obese) and Raichur district, India of which 6.17% of students were overweight in the year 2007. Our finding was in line with study done in South Africa, Jordan. The possible reason for this could be; girls spent most of their time at home and their movement from place to place are much restricted due to cultural influence than boys which result in more sedentary life.

The results showed that the students had history of such disease in their family: Diabetes mother and Overweight brother. Another interesting result was that most of them did not think that they were overweight or obese.

Family history is a consistent and independent risk factor for many common chronic diseases, and professional guidelines usually include the use of family history to assess health risk, initiate interventions, and motivate behavioral changes. Family history is a consistent and independent risk factor for many common chronic diseases, and professional guidelines usually include the use of family history to assess health risk, initiate interventions, and motivate behavioral changes.
between children and adults may lead to different manifestations of the disease.\textsuperscript{19}

Early onset of severe obesity increases the risk of long-term obesity and metabolic complications.\textsuperscript{20-23} Overweight and obesity from childhood to adulthood have been related to an increased risk of T2DM, AH, dyslipidemia, and carotid-artery atherosclerosis.\textsuperscript{24} Therefore, an early, multidisciplinary approach (pediatric, endocrinological, nutritional) in overweight and obese children is necessary to reduce the development of metabolic complications. IR, previously considered a problem in adulthood, becomes a serious issue also in children.

We concluded that there are many factors involved in emerging overweight, such as family history of diabetes and obesity and unawareness of being obese.

Conflict of Interest: Not

Ethical Clearance: The study was approved by the Ethics Committee of the college of medicine of the Hawler medical university

Source of Funding: Myself

References


Removing Methods of Ultracide Residues in Imported Apple Peels in Iraqi Local Markets

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Abstract

The residues of ultracide insecticide used in controlling apple insects may have public health problems. This work was designed to find much effective method that capable of removing as much as possible of such residues from imported apple peels available in local markets. Imported apple samples were collected during winter 2018 from different local markets and divided into four subsamples as physical treatments where the first was left unwashed apple, the second was washed by tap water, the third was washed with saline solution and finally apple sample washed by tap water with acetic acid. Apple peels of all examined samples were left to dry in an oven at 85°C for 24 h and then powdered. 2.5 g from each sample was received 12.5 ml distil water to estimate the insecticide residues by using gas chromatography. It has been found that unwashed apple peels had the highest ultracide insecticide level followed by that washed by tap water while the peels washed by saline water was obviously free from the insecticide residues.

Key words: Ultracide residues, apple peels, tap water, saline water, acidic water.

Introduction

Crop plants may severely suffer from many diseases that are induced by different kinds of organisms such as viruses, fungous, herbs, insects, rodents and other organisms. Such crop diseases would cause significant reduction and economic losses. From early ages, farmers have tried to prevent such diseases and improve crop growth by using other biological species that can control plant pathogens [1-3].

In modern agriculture, biocides in general and pesticides in particular are very efficient materials to control crop diseases and enhancing agricultural production and therefore it is used worldwide. However, such insecticides particularly those contain phosphorous and intensively used with vegetables and fruits are concerned the most toxic material that may cause severe health problems to infants and teenagers [4-6].

The randomly use of various insecticides by farmers without scientific and official surveillance in terms of trade name, type and recommended doses suggested by producing companies may lead to real public health troubles since most of these insecticides are very toxic for both the health and the environment [7,8] where such disaster related to the fungicide was occurred in Iraq during seventeenth of last century with wheat grains treated with organ mercury [9].

Organophosphorus compounds are very toxic (LD50-25) especially ultracidine pesticide which affects cholinesterase due to increasing exposure and causes reducing of the enzyme level in the blood resulting in syndromes of muscarinic, nicotinic and central nervous system damage [10,11].

The application of such compounds as agricultural insecticides being very effective in protecting various edible plant crops from different insect species [12,13] where such edible plants have large surface area capable of taking significant quantities of these insecticides [14] such as peach, apple, cherry, ground berries, grape, spinach, potato, lattice and other vegetables.

Most of these oregano phosphorene insecticides such as parathion, malathion, dipterex and ultracide may form significant public health and environmental threats where several studies have reported the inhibition of choline enzyme at higher doses [15,16].

However, handling and removing such pesticide residues was received much attention being very
significant public phenomena \[17-21\] which needs to be investigated thoroughly and to find a proper removing method that should be accessible particularly at every home.

This work was designed to find a usable way to remove the residues of ultracide insecticide from imported apple where apple samples were collected from local markets and subjected to lab tests.

**Material and Method**

Imported apple samples were collected from local markets during winter 2018 and these samples were divided into four subsamples and subjected residues removing test where the first sample was left unwashed while the second was washed thoroughly by only tap water. The third sample was washed by diluted saline water at concentration of 1% for one minute and the forth sample was by acidic water which was tap water mixed with acetic acid at concentration of 1%. However, each treatment was replicated three times.

All apple samples after being treated as explained above were peeled and the peels placed in electric oven at 85 C° for few minutes then moved out and left to cool under room temperature. Dried peel apple samples were grinded by electric grinder and 2.5 g from each peel powder was placed in 250 volumetric flasks and received 12.5 ml distil water (1:5) and shacked for 30 min. Ultracidine pesticide residues was isolated by using chromatography/Mass Spectrometry-GC/MS following the method of previous study \[22\].

**Results and Discussion**

All obtained results were presented for unwashed, tap water, saline water and acetic acid water washed respectively of examined apple samples (Figures of analysis not shown).

It has been found that the residues of ultracidine insecticide in apple peels of unwashed sample had highest sharp peak by the value of 87.376 among other treatments. Also, there is another peak with a value of 62.621 due to reactions of unknown materials.

The residues of organophosphorin compounds in apple peels may be accumulated in human body up to lethal dose causing severe damage of various tissues and organs and also affect cell divisions \[18\].

In case of apple sampled washed by tap water, it was found the ultracidine insecticide residues were less than those left unwashed and had two peaks where the first peak was highest sharp peak was 12.23 while the second peak was 86.77

Again, the residues of such phosphoric compounds may be accumulated up to the lethal dose having severe effects as explained previously. the ultracidine insecticide residues in apple peel after being washed by saline water.

Apparently, there was no insecticide residues in the apple samples washed by saline water but there was a small peak due to unknown material reaction. However, it seems that saline water has totally destroyed all toxic phosphorene compounds.

Finally, the ultracidine insecticide residues were totally removed from apple sample washed by acidic water (acetic acid and tap water).

The obtained results suggest that the better effective methods of removing any quantity of residues of the ultracidine insecticide are washing such apples by using either saline water or acidic water that are available at all homes.

Several previous works examining similar pesticide residues removing methods were carried out worldwide. Previous work \[23\] has used dissolved ozone to get rid of four pesticides from vegetable which were methyl-parathion, parathion, diazinon and cypermethrin and found that low level (1.4 mg/l) of dissolved ozone was capable to oxidize up to 90% of all these pesticides except parathion within 5 minutes. Similar method (dissolved ozone) was followed by a work \[24\] carried on by using low concentration of dissolved ozone to remove four residues of four pesticide species and recorded a removing rate up to 99% within 30 minutes but it has reported that such removing rate was affected by temperature, concentration of applied ozone gas and preparing dissolved ozone concentration. In another study \[25\], five species of solutions were applied to remove blended organophosphorus pesticide residues from chines cabbage such as glycyrrhiza-mung bean, garlic juice, sods-salt, tap water and washed rice water and reported that soda-salt solution was the best removing solution while washed rice water showed very poor effects. Another study \[26\] has examined the impacts of using four washing methods (tap and ozone waters, ultrasonic cleaning and boiling) on 16 pesticide residues.
from raw strawberries and found that within less than 5 minutes, boiling technique had the highest removing rate followed by ultrasonic cleaning and ozonized water while tap water had the lowest removing rate which were 92.9 %, 91.2%, 75.1% and 68.1% respectively.

Apparently, it seems that several solutions can be used to remove pesticide residues from fresh fruits and vegetables where some of them such as ozonated, saline and acidic waters are capable of removing rate up to 90%. Nevertheless, such ability may be affected by certain variable such temperature, solution concentration and pesticide residues quantity as suggested by previous study [24]. So, the current results are supported by those of other works mentioned above but it necessary to examine other variables particularly temperature that may have significant effect on these methods used in this study.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


Evaluation of Benzene Threshold Value in Benzene Exposed Work Environment: Case Study at Ciputat Gas Station

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Abstract

Benzene is dangerous chemical compounds from products General Fuel Filling stations (gas stations) and is one component in gasoline with 1-5% carcinogen content. The purpose of this study was to determine the limits of safe concentration for benzene in the work environment of gas stations in Ciputat. This type of research is an observational, cross-sectional and descriptive study. The population in this study were all workers at one gas station in the Ciputat region. The sampling technique is the total population, so the sample is 27 workers. The design of the study begins with the collection of secondary data related to work processes which include chemicals in the work area and the number of workers involved. Furthermore, primary data collection was carried out related to the concentration of benzene in the workplace air, length of work time, and worker weight. In addition, secondary data was collected in experimental animals, namely the weight of white mice. The type of data in this study is primary data, obtained through questionnaires and observations as well as measurements of benzene in the air of the respondent’s workplace.

The measurement results of the concentration of benzene in the gas station in Ciputat in point 1 and point 2 are 0.58 mg / m3 or 0.18 ppm so that the concentration is still below the Threshold Value (TLV) according to Minister of Manpower Regulation Number 5 of 2018. Based on the manual calculation for safe limits of benzene concentration obtained 0.085 mg / m3 or 0.026 ppm. Control recommendations are to consume the CYP2E1 enzyme contained in beef liver and salmon which serves to reduce the level of benzene in the body, use the appropriate Personal Protective Equipment (PPE) in the form of half mask respirator with an organic vapor cartridge, and plant a number of ornamental plants that can absorb and reduce benzene concentrations such as Boston and Golden Photos 1.

Keywords: Benzene, safe concentration, workers gas stations.

Introduction

Benzene (C6H6) is an aromatic compound in the form of clear liquid at room temperature. This compound has a double bond and a saturated bond in the group with a resonating structure2. Benzene is one of the most applicable chemicals. Many uses of benzene are very useful for everyday life. The most important use of benzene is as a solvent and as a raw material for making other aromatic compounds which are benzene derivative compounds3.

Benzene is widely used in the rubber industry, oil refining, shoe factories, chemical plants and other oil-related industries including age fueling stations (gas station). The General Fuel Filling Station (gas station) is a facility or place that provides and sells various types of fuel oil for all types of vehicles. According to the Agency for Toxic Substances and Disease Register (ATSDR), hazardous and toxic chemicals contained in the oil content are benzene, toluene, xylene, ethylene, TPH (Total Petroleum Hydrocarbon), and Polycyclic Aromatic Hydrocarbon (PAHs). Of the six chemicals, benzene exposure has a very serious health impact.4
Individuals at risk of being exposed to benzene directly are workers at the gas station itself. Benzene exposure in gas station workers is mainly through inhalation or respiratory pathways. Gas station workers are continuously exposed to benzene because they are in the environment that emits benzene originating from the fuel pump engine when refueling, the fuel storage warehouse and the exhaust from the vehicle during the refueling queue.

Benzene has acute and chronic effects for exposed individuals. Acute toxic symptoms of benzene are suppression of the exposed central nervous system. While chronic and recurring chronic benzene exposure, even in low concentrations, can cause a variety of blood disorders from anemia to leukemia, a malignant disease that is irreversible and fatal. This chronic poisoning can result in non-cancerous effects and the effects of cancer.

In Hayat’s (2012) study, the results of benzene measurements at two points in the Ciputat region gas station were 0.58 mg/m³ or 0.18 ppm. The results are still below the threshold value (TLV) of 0.5 ppm (Minister of Manpower Regulation 5 of 2018). Although the concentration of benzene in the Ciputat region gas station is below the NAV, however, gas station workers exposed to benzene will still have a carcinogenic effect. A similar study at the Pancoranmas Depok gas station by Salim (2012), benzene concentrations at these sites had yields below the TLV but the gas station workers had a risk of carcinogenic effects at the lifetime duration of exposure.

In Hayat’s (2012) study, the results of benzene measurements at two points in the Ciputat region gas station were 0.58 mg/m³ or 0.18 ppm. The results are still below the threshold value (TLV) of 0.5 ppm (Minister of Manpower Regulation 5 of 2018). Although the concentration of benzene in the Ciputat region gas station is below the NAV, however, gas station workers exposed to benzene will still have a carcinogenic effect. A similar study at the Pancoranmas Depok gas station by Salim (2012), benzene concentrations at these sites had yields below the TLV but the gas station workers had a risk of carcinogenic effects at the lifetime duration of exposure.

Saridewi and Tualeka conducted research on safe (C safe) concentrations in the gas station area. The results of the study obtained a value of 0.03 as a safe concentration of benzene. Based on the research, the author will determine the limits of the safe concentration of benzene in different gas station areas, namely in the Ciputat gas station. In this study also the determination of RFC was also based on the conditions of workers in the study area which included weight and height.

**Material and Method**

This type of research is an observational, cross-sectional and descriptive study. The population in this study were all workers at one gas station in the Ciputat region. The sampling technique is the total population, so the sample is 27 workers. The design of the study begins with the collection of secondary data related to work processes which include chemicals in the work area and the number of workers involved. Furthermore, primary data collection was carried out related to the concentration of benzene in the workplace air, length of work time, and worker weight. In addition, secondary data was collected in experimental animals, namely the weight of white mice. The type of data in this study is primary data, obtained through questionnaires and observations as well as measurements of benzene in the air of the respondent’s workplace.

The research variables were benzene concentration in the workplace, worker body weight, worker height, respiration rate of workers, length of day working, body surface area, weight of white mice, body surface of white mice, highest dose of toxin without effect on experimental animals (NOAEL), Km factor in animals (Animal Km), Km factor for workers (Human Km), safe dose limit for workers (SHD), and benzene concentration in safe air for workers (C is safe).

Data analysis in this study was carried out by using quantitative data analysis manually to determine the safe concentration of benzene for workers in the Ciputat region gas station.

**Findings Experimental**

**A. Characteristics of Try Animals and Surface Area of Animals (White Mice)**

Toxicity is the ability of a chemical substance or compound to cause damage when it comes to sensitive internal parts or surfaces. According to Saridewi and Tualeka, the implementation of a toxicity test using experimental animals is a white mouse. In general, the human response to toxicity is qualitatively similar to that of animals, so this fact forms the basis of extrapolation from animal to human data.

In table 1, the characteristics of experimental animals in the form of white rat body weight are displayed.
Table 1. Distribution of Characteristics of Try Animals (White Mice)

<table>
<thead>
<tr>
<th>Try Animals (White Rat)</th>
<th>W (kg)</th>
<th>BSA (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1405</td>
<td>0.024165</td>
</tr>
<tr>
<td>2</td>
<td>0.1405</td>
<td>0.024165</td>
</tr>
<tr>
<td>3</td>
<td>0.1410</td>
<td>0.024223</td>
</tr>
<tr>
<td>4</td>
<td>0.1410</td>
<td>0.024223</td>
</tr>
<tr>
<td>5</td>
<td>0.1395</td>
<td>0.024050</td>
</tr>
<tr>
<td>6</td>
<td>0.1415</td>
<td>0.024165</td>
</tr>
</tbody>
</table>

Based on the data of white rat body weight, it can be calculated the body surface area of white mice using the following formula

BSA animal = 0.09 W^{0.67}

Description:

BSA : Body Surface Area / body surface area (m²)

W : Weight / weight (kg)

B. Characteristics of Workers, Worker’s Body Surface Area, and Worker’s Respiratory Rate

The characteristics of workers in this study include body weight and working time from 27 workers in the area Ciputat regional gas station. Based on Table 2, it is known that the highest body weight of workers in the Ciputat area gas station area is 80 kg, the lowest weight is 44 kg, and the average body weight is 57.73 kg. The duration of work in a day is 8 hours. Whereas for height use the average value of Indonesian adult male height which is 159 cm.

Based on data on workers’ body weight and worker height, the body surface area and the respiratory rate of workers can be calculated using the following formula.

1. The surface area of the worker body

BSA = \sqrt{W \cdot h / 3600}

Description:

BSA : Body surface area (m²)

W : Weight (kg)

2. Worker’s respiratory rate

BR = 5.3 ln - 6.9 / 24

Description:

BR : Breathing rate (m³/ hour)

W : Weight (kg)

Table 2. Distribution of Characteristics of Workers, Employee Respiratory Rate and Length of Working Time at Ciputat Regional Gas Station

<table>
<thead>
<tr>
<th>Worker</th>
<th>W (kg)</th>
<th>H (cm)</th>
<th>BSA (m²)</th>
<th>BR (m³/hour)</th>
<th>t (hour/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>159</td>
<td>1.41</td>
<td>0.55</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>159</td>
<td>1.88</td>
<td>0.68</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>159</td>
<td>1.39</td>
<td>0.55</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>159</td>
<td>1.63</td>
<td>0.62</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>159</td>
<td>1.76</td>
<td>0.65</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
<td>159</td>
<td>1.77</td>
<td>0.65</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>159</td>
<td>1.63</td>
<td>0.62</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>159</td>
<td>1.49</td>
<td>0.58</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>70</td>
<td>159</td>
<td>1.76</td>
<td>0.65</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
<td>159</td>
<td>1.47</td>
<td>0.57</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>70</td>
<td>159</td>
<td>1.76</td>
<td>0.65</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>159</td>
<td>1.49</td>
<td>0.58</td>
<td>8</td>
</tr>
<tr>
<td>13</td>
<td>64</td>
<td>159</td>
<td>1.68</td>
<td>0.63</td>
<td>8</td>
</tr>
<tr>
<td>14</td>
<td>60</td>
<td>159</td>
<td>1.63</td>
<td>0.62</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>67</td>
<td>159</td>
<td>1.72</td>
<td>0.64</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>48</td>
<td>159</td>
<td>1.46</td>
<td>0.57</td>
<td>8</td>
</tr>
<tr>
<td>17</td>
<td>54</td>
<td>159</td>
<td>1.54</td>
<td>0.59</td>
<td>8</td>
</tr>
<tr>
<td>18</td>
<td>50</td>
<td>159</td>
<td>1.49</td>
<td>0.58</td>
<td>8</td>
</tr>
<tr>
<td>19</td>
<td>63</td>
<td>159</td>
<td>1.67</td>
<td>0.63</td>
<td>8</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
<td>159</td>
<td>1.76</td>
<td>0.65</td>
<td>8</td>
</tr>
<tr>
<td>21</td>
<td>55</td>
<td>159</td>
<td>1.56</td>
<td>0.60</td>
<td>8</td>
</tr>
<tr>
<td>22</td>
<td>74</td>
<td>159</td>
<td>1.81</td>
<td>0.66</td>
<td>8</td>
</tr>
<tr>
<td>23</td>
<td>65</td>
<td>159</td>
<td>1.69</td>
<td>0.63</td>
<td>8</td>
</tr>
<tr>
<td>24</td>
<td>57</td>
<td>159</td>
<td>1.59</td>
<td>0.61</td>
<td>8</td>
</tr>
<tr>
<td>25</td>
<td>55</td>
<td>159</td>
<td>1.56</td>
<td>0.60</td>
<td>8</td>
</tr>
<tr>
<td>26</td>
<td>47</td>
<td>159</td>
<td>1.44</td>
<td>0.56</td>
<td>8</td>
</tr>
<tr>
<td>27</td>
<td>69</td>
<td>159</td>
<td>1.75</td>
<td>0.65</td>
<td>8</td>
</tr>
<tr>
<td>Average</td>
<td>59.89</td>
<td>159</td>
<td>1.62</td>
<td>0.61</td>
<td>8</td>
</tr>
</tbody>
</table>
The results of the analysis of the calculation of body surface area and worker respiratory rate according to Table 2 shows that the average body surface area of workers is 1.62 m² and the average respiration rate of workers is 0.61 m³/hour.

C. Benzene Concentration

The results of the measurement of benzene concentration at both points in the Ciputat region gas station showed the same results, namely 0.58 mg / m³ (0.18 ppm)

Table 3. Distribution of Benzene Concentration in Ciputat Regional Gas Station

<table>
<thead>
<tr>
<th>Benzene Concentration (ppm)</th>
<th>Location Measurement of</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.18</td>
<td>Point 1</td>
<td></td>
</tr>
<tr>
<td>0.18</td>
<td>Point 2</td>
<td></td>
</tr>
</tbody>
</table>

Based on the results of measurements carried out, the concentration of benzene in the working environment of the Ciputat gas station is 0.58 mg / m³ or 0.18 ppm. This benzene concentration is below the threshold value of 0.5 ppm in accordance with the provisions of the Minister of Manpower Regulation Number 5 of 2018 concerning Occupational Safety and Health at the Work Environment. However, the concentration of benzene is above the Minimum Risk Level (MRL), the level of benzene inhalation exposure determined by ATSDR, i.e. for acute exposure (≤14 days) = 0.009 ppm, moderate exposure (15-364 days) = 0.006 ppm, and chronic exposure (≥365 days) = 0.003 ppm.

D. Animal Km and Human Km

The first step to determine the safe dosage of toxin for workers is by calculating Animal Km and Human Km.

1. Animal Km

\[ \text{Animal Km} = \frac{W_{\text{animal}}}{BSA_{\text{animal}}} \]

Description:
Animal Km: Km factor in animals
W: Experimental animal weight (white mouse)

2. Human Km

\[ Km = \frac{W_{\text{human}}}{BSA_{\text{human}}} \]

Description:
Human Km: Km factor in human/worker
W: worker weight
BSA: Body Surface Area worker

The results of the calculation are Human Km shown in Table 5. Based on Table 5, the Human average Km for Ciputat region gas station workers is 36.70.
Table 5. Calculation Results Human Km in petrol stations Workers Ciputat Region

<table>
<thead>
<tr>
<th>Workers</th>
<th>Human KM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.92</td>
</tr>
<tr>
<td>2</td>
<td>42.56</td>
</tr>
<tr>
<td>3</td>
<td>31.56</td>
</tr>
<tr>
<td>4</td>
<td>36.86</td>
</tr>
<tr>
<td>5</td>
<td>39.81</td>
</tr>
<tr>
<td>6</td>
<td>40.09</td>
</tr>
<tr>
<td>7</td>
<td>36.86</td>
</tr>
<tr>
<td>8</td>
<td>33.65</td>
</tr>
<tr>
<td>9</td>
<td>39.81</td>
</tr>
<tr>
<td>10</td>
<td>33.31</td>
</tr>
<tr>
<td>11</td>
<td>39.81</td>
</tr>
<tr>
<td>12</td>
<td>33.65</td>
</tr>
<tr>
<td>13</td>
<td>38.07</td>
</tr>
<tr>
<td>14</td>
<td>36.86</td>
</tr>
<tr>
<td>15</td>
<td>38.95</td>
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<td>16</td>
<td>32.97</td>
</tr>
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<td>17</td>
<td>34.97</td>
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<td>19</td>
<td>37.77</td>
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<td>20</td>
<td>39.81</td>
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<td>35.29</td>
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<td>25</td>
<td>35.29</td>
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<tr>
<td>26</td>
<td>32.62</td>
</tr>
<tr>
<td>27</td>
<td>39.52</td>
</tr>
<tr>
<td>average</td>
<td>36.70</td>
</tr>
</tbody>
</table>

E. NOAEL

one objective of the research activities in the field of toxicology is to be able to evaluate the safety of a substance. To determine the safe limit of the concentration of a chemical begins with the toxicity test determining the highest dose without causing effects on experimental animals or No Observed Adverse Effect Level (NOAEL).

The results of the research by Swaen et al. (2010) state that benzene NOAEL is 3.0 mg/m³ or equivalent to 0.022 mg/kg which is allowed from the calculation of formulas as follows.

\[
NOAEL \text{ benzene} = \frac{3 \times 0.00013 \times 8}{0.1405} = 0.022 \text{ mg/kg}
\]

F. Safe Human Dose

\[
SHD = NOAEL \times \frac{W_{\text{human}}}{BSA_{\text{human}}}
\]

Based on this formula, the calculation of SHD obtained from the NOAEL value, the average animal Km, and the average human Km is:

\[
SHD = 0.022 \times \frac{\text{data}59.89}{36.7} = 0.003 \text{ mg/kg}
\]

G. Limit Safe Benzene Concentration

Determination of safe limits of benzene concentration in the working environment of Ciputat region gas stations using formulas (William, 1985; Soemirat, 2003; Davis, 1991) the following.

\[
C_{\text{safe}} = \frac{(SHD) (W)}{(\delta) (BR) (t)} \text{ mg/m}^3
\]

To convert units of mg/m³ to ppm the following formula is used.

\[
C_{\text{safe}} = \frac{# \text{ mg/m}^3}{(MW)} \times 24.5 \text{ ppm}
\]

Description:

C is safe: concentration of toxin in the air is safe for workers (mg/m³)

SHD: Safe Human Dose (mg/kg)

W: Weight (kg)

\(\delta\): % of substances absorbed by the lung

BR: Human respiratory rate (m³/hour)

t: Duration of working time (hours)

MW: Molecular Weight / Molecular Weight

Based on the above formula, the results of calculating the safe concentration of benzene in the gas station in Ciputat region obtained from the value of SHD, average body weight, a percentage of substance absorption, average respiration rate of workers and the average length of work time are:

\[
C_{\text{safe}} = \frac{(0.003) (59.89)}{(50\%) (0.61) (8)} = 0.085 \text{ mg/m}^3
\]

\[
C_{\text{safe}} = \frac{0.085 \times 24.45}{78.11} = 0.026 \text{ ppm}
\]

The results of calculating safe limits in the air for workers above can be used to predict the concentration of toxins in the air a safe work environment for workers if
there is no determination of the Threshold Value (William, 1985 in Tualeka, 2013), and for comparison with NAV which has been determined by various institutions both by the Ministry of Manpower and Transmigration, the National Standardization Agency, ACGIH, NIOSH and OSHA.

**Conclusion**

The results of measuring the concentration of benzene in the gas station in Ciputat in point 1 and point 2 were 0.58 mg/m³ or 0.18 ppm so that the concentration was still below the Threshold Value according to Minister of Manpower Regulation Number 5 of 2018. Based on manual calculations for safe limits benzene concentration is obtained 0.085 mg / m³ or 0.026 ppm. TLV evaluation is a comparison of the results of the above calculation with TLV Benzene. According to Minister of Manpower and Transmigration Regulation Number 5 the Year 2018 which is 0.5 ppm. Based on manual calculations, safe limits benzene concentration in Ciputat gas station was bellow the TLV. So that the benzene threshold value is not feasible and needs to be revised.

Control recommendations are to consume the CYP2E1 enzyme contained in beef liver and salmon which serves to reduce the level of benzene in the body, use the appropriate Personal Protective Equipment (PPE) in the form of half mask respirator with an organic vapor cartridge, and plant a number of ornamental plants that can absorb and reduce benzene concentrations such as Boston and Golden Phothos.

**Conflicts of Interest:** All authors have no conflicts of interest to declare.

**Source of Funding:** The source of this research costs from self.

**Ethical Clearance:** This research was approved by the Institutional Ethics Board of Airlangga Surabaya University. All subjects received complete information about the procedure and purpose of this study, each subject before the study signed an informed consent form.

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A Complete Perusal of Polluter Pays Principle “Incorporation and Application In India”

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Abstract

Since long it’s a common practice to pay for the destruction and management of destruction caused by one, may it be destruction to property, life, nature etc. However for each kind of destruction there are specific laws and regulations enacted by the parliament. Apart from the laws there are few fundamental truths that act as the base or foundation for a system or in other words some theorems that have numerous special applications across a widespread area which are otherwise known as principles. Polluter pays principle is one of such preventive and remedial theorem applied to people causing environmental damage. This research work focuses on how the polluter pays principle was incorporated in India and emphasizes on its judicial application in various perspectives in India only. This paper starts with the introduction of the principle by “OECD (Organization for Economic Cooperation and Development)” in 1972 and further tracing its movement through the extended version of the principle, being enshrined under principle 16 of the Rio Declaration (UNCED) in 1992, being adopted by the European Community, being adopted as an extension of the rule of Absolute Liability in Torts and finally coming to how it started being applied to, by the Indian Judiciary. This article analyses the judgments pronounced in various cases by the Indian Courts relating to Polluter pays principle.

Keywords: OECD, UNCED, Torts, Absolute Liability, Strict Liability, Sustainable Development, EAP (Environment Action Program).

Sustainable Development and Polluter Pays Principle:

Today India has a variety of legislative measures, executive policies and programs to deal with environmental prevention and protection. In 1992, government of India adopted the well settled Polluter Pays Principle and the Doctrine of Sustainable Development as policy programs on matters relating to control of pollution. One of the major doctrine of sustainable development is the “Polluter Pays Principle, which recognizes that the polluter should pay all the environmental damage created and the burden of proof in demonstrating that a technology or process or product is safe for environment should lie with the developer not the general public.” As culled out from the Bruntland report named “our common future” the major features of the sustainable development includes polluter pays principle.

In Bruntland Commissions Report[1]:

The “World Commission on Environment and Development” or the “Bruntland Commission” in its report “Our Common Future” suggested that, the environmental costs of economic activity can be paid in an internalized manner by the enterprises. Which meant the payment may be in the form of investment made by the enterprises in order to prevent the damages caused or to restore the unavoidable damages like rehabilitation of man, afforestation, rehabilitation of land, or compensating the victims of the damage caused by such enterprises etc.

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OECD (Organization for Economic Cooperation and Development) in 1972: OECD introduced the Polluter Pays Principle in 1972 and “the Guiding Principles Concerning International Economic Aspects of Environmental Policies under OECD stated that the polluter is to be held responsible for the environmental damage and pollution caused by such polluter.”

**EXTENDED VERSION OF POLLUTER PAYS PRINCIPLE:**

- **OECD IN 1989:** OECD suggested to extend the polluter pays principle to include the costs of accident prevention and for internalizing the damages caused by such accidents.

- **OECD JOINT WORKING PARTY ON AGRICULTURE AND ENVIRONMENT:** The OECD, Joint Working Party on Agriculture and Environment stated, extended version of polluter pays principle as: “…the polluter should be held responsible for environmental damage caused and bear the expenses of carrying out the preventive measures or paying for damaging the environmental state where the consumptive and productive activities causing the environmental damage are not covered by property rights.”

**RIO DECLARATION (UNCED) IN 1992:** In 1992 the guidelines for sustainable development were laid down in the Rio Declaration which aimed at utilization of the resources by the present generation without compromising the needs of the future generations. In furtherance of such aim the Rio Declaration enshrined the polluter pays principle under “Principle 16 of the Rio Declaration” which stated that the polluter should bear the cost of pollution.

**EUROPEAN COMMUNITY IN 2002:** In 2002 the European Community adopted the first EAP(Environment Action Program), thus following the Polluter Pays Principle of Organization for Economic Cooperation and Development.

**POLLUTER PAYS PRINCIPLE IN TORTS:** In this case of M.C.Mehta v.Kamal Nath and Ors[2], the court held that, “pollution is a civil wrong and thus it’s a tort committed against the community and any such person who is guilty of causing pollution has to bear the damages or compensation in order to restore the environment.” In torts the principle of Strict Liability or the rule in Rylands v. Fletcher[3] stated that, “any person in the course of non natural use of land is deemed to be responsible for the accumulation on it of anything that is likely to cause harm if it escapes and is liable for the interference with the use of land of another which results from the use of such thing that escapes from his land. However this principle of strict liability is subject to exceptions(plaintiffs fault, Act of God, Act of Third Party, Consent of the Plaintiff, Act done by Statutory Authority).” Whereas the principle of Absolute Liability is not subject to any exceptions and is thus considered to be the improvement of the principle of strict liability since in M.C.Mehta v. Union of India[4] the court held that “the rule in Ryland v. Fletcher laid down in 19th century did not meet the needs of the modern industrialized society therefore the need to recognize a new rule was necessary and the rule of absolute liability was laid. Under this rule of Absolute Liability the court pointed out the duty is absolute and non-delegateable and the enterprise cannot escape liability by stating that he has taken all reasonable care and that no negligence was there on his part. The polluter pays principle is said to be an extension of the principle of absolute liability since the rule of absolute liability is invoked regardless of whether or not reasonable care was taken in the dangerously inherent activity taking place due to the escape of the hazardous thing and such person is liable to compensate the victims of such dangerous activity,adding to it in polluter pays principle the polluter is also liable to pay the cost of repairing the damage caused to the environment.”

It can be said that the strict liability, absolute liability and polluter pays principle are interlinked with each other.

**FEW REMARKABLE DOCTRINES AND PRINCIPLES PROPOUNDED BY THE INDIAN JUDICIARY:** Below given are the list of the major doctrines and principles propounded by the Supreme Court of India and the corresponding case law in which it was propounded:
DOCTRINE AND PRINCIPLES: | CASE IN WHICH IT WAS PROPOUNDED:
---|---
1. DOCTRINE OF ABSOLUTE LIABILITY (laid down in M.C.Mehta Case) | BHOPAL GAS LEAK CASE (UNION CARBIDE CORPORATION V. U.O.I AIR 1990 SC 273) (1st applied here; laid down in M.C.Mehta Case)
4. PUBLIC TRUST DOCTRINE | M.C.MEHTA V. KAMAL NATH & OTHERS (1997) 1 SCC 212.

Polluter Pays Principle and The Indian Judiciary:

- **Indirect Recognition And Application of Polluter Pays Principle (M.C.Mehta v. Union of India)[8] (Oleum Gas Leak Case):** The Supreme Court of India declared that “we need to evolve new principles and lay new norms which could adequately deal with the new arising problems in a highly industrialized economy.” This case was also popularized because in this case the court formulated the principle to measure the liability of industries that are involved in hazardous or inherently dangerous activities. It was stated that in order to measure the liability in such cases “the larger and more prosperous the enterprise, the greater must be the amount of compensation payable by it for the harm caused on account of an accident in the carrying on the hazardous or inherently dangerous activity by the industry or enterprise.” In this case the industry (Shri Ram Food and Fertiliser Corporation of Delhi) was asked to deposit a sum of rupees 35 lakh in a bank and guarantee of rupees 15 lakh in the court so as to be paid as compensation to the persons who claim and prove to be the victims of the Oleum Gas Leak Case. Thus this innovative remedy evolved in this case which was considered to be the indirect recognition and application of the Polluter Pays Principle.

- **Case where the Polluter pays principle was first applied and defined (Indian Council for Enviro Legal Action v. Union of India)[6]:** In this case it was stated that, “the polluter is liable to pay the cost of the individual sufferers as well as the cost of redemption of the damaged environment which is considered to be a part of the sustainable development[7]. Thus here the polluter pays principle means the absolute liability for harming the environment which shall extend to compensating the victims of such pollution caused as well the costs incurred for restoration of the environmental degradation caused.”

- **Vellore Citizens Welfare Forum v. Union of India (AIR 1996 SC 2715):** Here the Supreme Court declared two principles as part of the environmental jurisprudence of India namely: The Precautionary Principle and The Polluter Pays Principle.

- **A.P.Pollution Control Broard v. Prof. M.V. Nayadu (AIR 1999 SCW 434.[8]:** In this case the Supreme Court declared that, “the polluter pays principle and the precautionary principle have become a part of the environmental law of the country.”

- **Taj Trapezium And Polluter Pays Principle, M.C.Mehta:** In this case “the court gave its order based
on the precautionary principle and the polluter pays principle as was defined in the vellore citizens welfare forum case and also stated that the poluter pays principle is an essential feature of Sustainable Development.”

**Conclusion**

As we found that more and more principles are being created to mitigate the lacunas that remained unseen in the previous principles or doctrines, similarly the polluter pays principle came up to mitigate the damage being caused to the environment which wasn’t compensated for in strict or absolute liability. However the measurement of the damage caused to the environment has no absoluteness due to lack of an imposed mechanism to measure such damages caused. Thus the amount of compensation charged for restoration of the environment remains inadequate at times and are in requirement of some more effective provisions to be beneficial in the long run.

**Ethical Clearance:** Not required, as the research article is based on environmental pollution and its protective principles. The research is doctrinally undertaken.

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**

2. M.C.Mehta v. Kamal Nath and Others, 1 SCC 388 (Supreme Court of India 1997).
Risk Assessment, Risk Management, and Risk Communication at Drug Industries PT. Kimia Farma (Persero) Tbk. Plant Bandung

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Abstract

There have been many accidents and occupational diseases caused by weak risk management efforts. Risk management can be carried out by starting with a risk assessment. Risk assessment is an important aspect of occupational health and safety. The pharmaceutical industry has a high risk of occupational health and safety. This study aims to identify the level of occupational health and safety risks and provide control recommendations. This research is descriptively using the Job Safety Analysis (JSA) method. The results of the identification show that from 19 work steps 10 of which include high risk, 8 including moderate risk and 1 low risk. The hazards identified include chemical, physical, ergonomic and fire hazards. High risks include hazards originating from chemicals as raw materials for making drugs. Several control measures have been taken, but to ensure worker health and safety, additional efforts should be made such as noise suppression personal protective equipment, special masks for use in chemical hazards and work accident emergency response procedures. Risk communication at the drug company is going well.

Keywords: Risk Assessment, Risk Management Risk Communication, Job Safety Analysis, Drugs Industries.

Introduction

In Indonesia, the number of accidents due to work is still fluctuating, recorded the number of work accidents from 2011-2014 the highest number of accidents occurred in 2013 with 35,917 cases of work accidents (in 2011 there were 9,891 cases; in 2012 there were 21,735 cases, and 2014 occurred 24,910 case). The provinces with the highest number of work accident cases in 2011 were Banten and Central Kalimantan. In 2012, Jambi and Maluku Provinces. In 2013 the provinces of Aceh and North Sulawesi. In 2014 were the provinces of South Sulawesi and Riau¹.

Whereas in the case of Occupational Diseases (PAK) from 2011-2014 the trend has decreased even though in 2012 and 2013 it has increased. In 2011 the number of PAK in Indonesia reached 57,929 cases, in 2012 60,322 cases and in 2013 increased again to 97,144 cases, then in 2014, it began to decline to 40,696 cases. The provinces with the most PAK prevalence in 2011 were Central Java Province, in 2012 North Sumatra, South Sumatra and West Java, 2013 Banten Province, and in 2014 were the Provinces of Bali, East Java, and South Sulawesi. The data shows that efforts to prevent accidents and occupational diseases are still not optimal¹.

Efforts to prevent work-related accidents and diseases can be carried out with a number of approaches, namely, approaches to weaknesses in the human element, such as the selection of employees properly and knowledge building or training. Approach to weaknesses in hardware or production equipment through the design, maintenance of production equipment and planning of the work environment. Approach to all levels of management by conducting equitable distribution of tasks, and determining the implementation of risk assessments².

Risk assessment is a systematic effort to identify opportunities for accidents and occupational diseases. Through risk identification efforts we can find out the characteristics of risks from hazards so that it is easier to make security efforts to avoid accidents. One method that can be used to identify risk is the Job Safety Analysis (JSA) method. JSA is a risk identification method by
reviewing and assessing the risk of each stage of the work performed.

Job Safety Analysis (JSA) is a process of identifying hazards and risks based on each stage in a work process that has the potential to cause serious harm before an accident occurs. Determine how to control hazards or reduce injury rates and make written documents that can be used for hazard information.

Benefits of Using JSA

1. Provide individual training in safety and efficient work procedures.
2. Provide information about hazards in the workplace.
3. Hazard information is described based on work stages so that it is more detailed.
4. Provide risk control alternatives based on the results of risk identification in the workplace.
5. Accurate absences, workers compensation becomes cheaper and increases productivity.

The risks identified must be immediately controlled, risk control efforts are known as risk management. Risk management is a systematic effort to determine the best actions in conditions of uncertainty. Risk management is an activity which contains elements: systematic identification, analysis, improvement, monitoring, and communication against risks.

The exchange of information between interested parties about the circumstances, magnitude, and importance of risk control. risk communication itself is included in the risk analysis process.

PT. Kimia Farma (Persero) Tbk. Plant Bandung, is one of the State-Owned Enterprises (BUMN) engaged in the production of drugs or pharmaceuticals. In an effort to guarantee the supply of drugs on the market, this company provides 3 parts of the production. Particularly for production I, producing solid drug preparations in the form of tablets and caplets.

Based on the visits made to the production section I, there are some potential hazards that can lead to accidents and occupational diseases. During the production process, workers make physical contact either through inhalation or dermal with chemicals as raw material for making drugs. The chemical ingredients for the manufacture of drugs, namely ethyl alcohol, dextrose anhydrous, amyllum maydis, and microcrystalline cells have the potential to cause health problems such as irritation, respiratory disorders, and nervous system disorders.

The purpose of this study is to conduct occupational health and safety risk assessments in the Production Section I of PT. Kimia Farma (Persero) Tbk. Plant Bandung using the Job Safety Analysis (JSA) method.

Material and Method

This study used a descriptive observational research design. The danger is a potential hazard, risk refers to actual danger. Risks can be identified through direct observation at the production site.

The study was conducted in the Production Section I of PT. Kimia Farma (Persero) Tbk. Plant Bandung. The risk assessment method uses the JSA with the following risk assessment steps:

1. Select the place to be analyzed
2. Describe the stage of work
3. Identify the various hazards and risks that exist in each step of the job, and identify various possibilities that have the potential to become accidents;
4. Risk assessment (likelihood and severity)
5. Categorize risk (risk assessment matrix)
6. Provide control recommendations

Risk Management is carried out by selecting control options that are in accordance with the characteristics of the risk. Evaluation of available controls, so that it can provide additional control recommendations. Data on risk communication is obtained by direct observation at PT. Kimia Farma (Persero) Tbk. Planting Bandung. Risk communication can generally be observed through the media, company management meetings with workers and local stakeholders.

Table 1. likelihood

<table>
<thead>
<tr>
<th>Value</th>
<th>Description</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Almost Certainly Happens</td>
<td>Can occur at any time under normal conditions, such as traffic accidents.</td>
</tr>
<tr>
<td>B</td>
<td>Often occur</td>
<td>Occurs several times in a certain period of time, such as a train accident.</td>
</tr>
<tr>
<td>C</td>
<td>Can occur</td>
<td>Risk can occur but not often, falling from a height at the construction site.</td>
</tr>
<tr>
<td>D</td>
<td>Sometimes</td>
<td>Sometimes it happens, for example, the leakage of nuclear installations or other machines.</td>
</tr>
<tr>
<td>E</td>
<td>Rarely</td>
<td>Can occur under certain conditions, such as people being struck by lightning.</td>
</tr>
</tbody>
</table>

Source: 3

Table 2. Severity

<table>
<thead>
<tr>
<th>Value</th>
<th>Description</th>
<th>Detailed Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not significant</td>
<td>Losses do not cause harm or injury to humans</td>
</tr>
<tr>
<td>2</td>
<td>Small</td>
<td>A minor injury, a small loss, and no serious impact</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Severe injuries, do not cause disability, moderate financial losses.</td>
</tr>
<tr>
<td>4</td>
<td>Great</td>
<td>Causes severe injury and permanent disability, heavy financial losses.</td>
</tr>
<tr>
<td>5</td>
<td>Disaster</td>
<td>Can stop the company’s activities forever.</td>
</tr>
</tbody>
</table>

Source: 3

Table 3. Risk Level Matrix

<table>
<thead>
<tr>
<th>Likelihood</th>
<th>Not significant</th>
<th>Small</th>
<th>Moderate</th>
<th>Great</th>
<th>Disaster</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>High</td>
<td>High</td>
<td>Extreme</td>
<td>Extreme</td>
<td>Extreme</td>
</tr>
<tr>
<td>B</td>
<td>Moderate</td>
<td>Moderate</td>
<td>High</td>
<td>Extreme</td>
<td>Extreme</td>
</tr>
<tr>
<td>C</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
<td>Extreme</td>
<td>Extreme</td>
</tr>
<tr>
<td>D</td>
<td>Low</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
<td>Extreme</td>
</tr>
<tr>
<td>E</td>
<td>Low</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>
Information:

<table>
<thead>
<tr>
<th>Risk level</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme Risk</td>
<td>Activities may not be carried out or continued. If it is not possible to reduce risk because resources are limited, then activities may not continue.</td>
</tr>
<tr>
<td>High Risk</td>
<td>Activities may not be carried out until the risk has been reduced.</td>
</tr>
<tr>
<td>Moderate Risk</td>
<td>The action is needed to reduce risk, but the costs needed must be carefully calculated and limited.</td>
</tr>
<tr>
<td>Low Risk</td>
<td>The risk is acceptable, additional controls are not needed. But monitoring must still be carried out.</td>
</tr>
</tbody>
</table>

Source: 3

Findings

a. Risk Assessment

There are 9 stages of work then clarified into 19 job descriptions. The first stage is the weighing of raw materials carried out at the weighing center. The weighing process has 3 job descriptions, opening drums containing medicinal raw materials, weighing drug-making materials and manual lifting process. The risks identified are, workers inhale the raw materials of drugs that can cause shortness of breath. Workers sitting too long can cause Lob Back Pain (LBP), lifting the manual can cause LBP and Thoracic Outlet Syndrome (TOS). All work descriptions at this stage are included in the high risk.

The second working stage is a wet granulation process, in this stage described into 4 job descriptions. Climb the ladder of the granulation machine, insert the solvent of water or organic alcohol into the machine, hold the granulation results into a plastic barrel and there are operators as supervisors. The risks identified include, workers can experience injury, dizziness, shortness of breath and eye irritation. Two job descriptions are included in the high-risk level and the other 2 are moderate risk levels.

The third working stage is the drying process, there are 2 job descriptions in the drying process. Enter the raw material into the dryer with the average temperature of the 70°C machine and the manual lift process. The risk identified at this stage is that workers can become dehydrated due to exposure to a hot work climate, and workers can experience fatigue, LBP, TOC due to the position of non-ergonomic apes. The level of risk in this stage is included in the low and moderate levels.

The fourth working stage is sieving, there is one job description in this process. Workers enter mixtures that have previously been dried into the sieving machine. The risk identified in this process is LBP due to work position when overseeing the sieving process for too long sitting and not ergonomic. The level of risk in this job description is included in the level of moderate risk.

The fifth working stage is dry granulation, in this stage, there are 3 job descriptions. Workers put the mixture into a dry granulation machine, pour additives in powder or liquid form and bring the results of dry granulation to the drug printing section. The risks identified are workers can experience LBP, shortness of breath nervous system disorders, fatigue, and TOS. Two work descriptions are included in the level of moderate risk and 1 other is high-risk level.

The sixth working stage is drug printing, in this stage, there are two job descriptions. First Enter the ready-made material into the printing press. The risks identified are the risk of shortness of breath and nervous system disorders due to workers being too close to the reach of the dust collector. The level of risk in this job description includes high risk. Both operators process to monitor printing machines. The identified risks are workers experiencing varicose veins and fires from electrical installations. The level of risk includes high risk.

The seventh working stage is in coating the drug, there is one job description at this stage. Workers put drugs into the coating machine. The risks identified are workers at risk of experiencing varicose veins due to too long standing when watching the machine. Included in the median risk level.

The eighth stage is the primary packaging. There are 2 job descriptions at this stage. First, the workers send the drug into the engine, the identified risks are that workers can experience hearing loss due to noise produced by the primary packaging machine. Second, workers control the packaging results. The risks identified include that workers can experience PBP as a result of too often sitting in a non-ergonomic position. This risk is included in the level of moderate risk.
The ninth working stage is secondary packaging. There is one job description where the worker enters the drug into the box. The risk identified is that workers can experience varicose veins due to too long standing when packing. The level of risk in this job description is included in the high-risk level.

b. Risk Management

Based on the risk assessment above, it shows that of the 9 work stages and 19 job descriptions, 10 descriptions or 52.6% are at high risk for Occupational Safety and Health. The weighing and printing stage is a part of which all work descriptions are high risk. The remaining 8 descriptions or 42.1% are medium risks and 1 description or 5.3% low risk of Safety and Occupational Safety.

Referring to the results of the risk assessment that there are several job descriptions that have a high risk, the activities may not be carried out until the risk has been reduced or controlled. The purpose of risk control is to avoid workers from the worst consequences that can cause loss of work time and worker productivity.

Some stages of the production run the risk of causing health problems. Identified ergonomic risks such as errors in manual handling, excessive physical loads, and posture incompatibility with work stations, risk causing muscle disorders.9

Research conducted by Wahyu, P.D and Tualeka, A.R., 2013 in one of the welding industries in East Java, Indonesia explained that there were still residual risks despite risk control. So that the residual risk assessment needs to be done so that it can further determine additional risk control recommendations10.

PT. Kimia Farma (Persero) Tbk. Plant Bandung has carried out several efforts to control identified risks. The effort is a combination of technical control, administrative and use of personal protective equipment. controls that have been carried out between, providing masks, making Standard Operating Procedures, providing chairs for workers, working rooms equipped with refrigeration equipment, providing lightweight fire extinguishers, and efforts to maintain workers’ fitness with gymnastics every morning.

Such control is still not enough to reduce some of the identified risks. Some controls must also be carried out, such as conducting safety talk, safety inspections, stretching work intervals, giving awards and reprimands, providing accident and fire emergency response procedures, exchanging with colleagues who are already tired and weak and providing earplugs.

c. Risk Communication

PT. Kimia Farma (Persero) Tbk. Plant Bandung applies OHSAS 18001: 2007 as work safety and health management system. One form of implementation of the system is communication, participation, and consultation11.

Risk communication is the exchange of information about the magnitude of health risks in the work environment. The forms of application of risk communication carried out in the company include, meetings between the entire corporate structure include workers in communicating occupational health and safety policies. Risk communication media are installed such as posters and banners. In addition to these efforts, PT. Kimia Farma (Persero) Tbk. Plant Bandung routinely holds meetings with workers on the National Occupational Health and Safety commemoration. This meeting was held so that there was an exchange of information between workers and managing the company.

Conclusion

Based on the results of the study it can be concluded that of the 9 work stages and 19 job descriptions, 10 descriptions are at high risk for Occupational Safety and Health. 8 description is the moderate risk, and 1 description low risk. Risk management has been carried out, but additional control is needed so that the risk of illness and occupational accidents can be reduced. The process of risk communication works well, risk communication involves management and workers. Communication media have been installed, and information exchange with routine workers is carried out.

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: The study was approved by the institutional Ethical Board of The Bhakti Kencana Institute of Health Science.
All subjects were fully informed about the procedures and objectives of this study each subject prior to the study signed an informed consent form.

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Work Safety Risk Assessment at Container Load Unloading Jobs at PT. Pelabuhan Indonesia II (Persero) Palembang Branch 2017

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Abstract

Loading and unloading of container goods is carried out using cranes and truck containers as a means of transportation besides facilitating activities, which can also lead to the risk of workplace accidents. In 2014 there was a work accident in Kuningan Jakarta, workers were crushed by a container during the loading and unloading process. Container loading and unloading is a routine activity carried out at PT. Pelabuhan Indonesia II (Persero) Palembang Branch. This activity involves tools that can cause accidents such as being hit by a container and hit by a truck head, therefore a risk assessment is carried out on container loading and unloading work. The purpose of this study was to analyze occupational safety risk assessments on container loading and unloading work. This study uses a qualitative research design, using Job Safety Analysis (JSA) for the risk identification process, the US / NZ 4360: 1999 semi-quantitative risk assessment table for risk analysis and semi-quantitative risk level Cross (1988) for risk evaluation. The results of the study indicate that container loading and unloading work consists of the preparation stage, the operation phase of the QCC and the stage of moving containers. The risks identified based on the stage of work are overtaken by containers, falling from heights, collisions between head trucks, falling lifts, hit by a lock lock, getting hit by a truck head, falling into the river, collisions between QCCs and electric shock. The hazards included in the acceptable risk category include being crushed by repair equipment, hands pinched, tripping, slipping lubricants and head banging. The suggestion of this research is that all activities in the field should be carried out in accordance with the applicable regulations and supervision should be carried out more specifically in the use of PPE and conduct periodic health checks on workers.

Keywords: Risk Assessment, Container Loading and Unloading, Job Safety Analysis.

Introduction

Occupational Health and Safety (K3) is a compulsory program that exists in every workplace. Its implementation has spread widely in almost every industry sector, its main goal is to reduce the number of accidents, and reduce the risk of being dangerous1.

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The number of occupational accidents in the world is in quite alarming conditions, every year two million people die and 270 million people are injured due to workplace accidents that occur throughout the world2. The development of workplace accidents in developing countries is also very high, including Indonesia, this is because developing countries have many labor-intensive industries, so that more workers are exposed to potential hazards3.

Workplace accidents are unwanted events that have an impact on someone or material damage, which are the result of contact with kinetic, electrical, chemical, heat and other sources4. Most (85%) accidents are caused by human factors with unsafe actions. Unsafe action is an action that can endanger the workers themselves
and others who can cause accidents that can be caused by various things such as not using PPE, not following work procedures, not following work safety regulations and not working carefully, where out of every 300 unsafe actions, there will be 1 (one) accident resulting in the loss of workdays.

The port is one of the workplaces that have a high risk of work accidents. Every year accidents caused by lifting at the port occur. Based on the statistics of the Marine Industrial Accident, the Hong Kong Maritime Department in 2016 had occurred as many as 76 cases of workplace accidents in the loading and unloading section port. The work accident consisted of 60 minor accident cases, 15 serious accident cases and 1 fatal accident case.

One of the work accident incidents that occurred during loading and unloading was the breaking of the RTG wayer trolly while carrying out container loading and unloading from the ship which caused the death of the RTG operator assistant on October 21, 2017. In addition, in the past two years there has been a work accident at JICT Jakarta is related to loading and unloading containers such as damage to spreader tools and falling over on maintenance and container cars which have deteriorated during the appointment process.

Goods container loading and unloading activities at the port have a high risk. Based on accident data obtained from the Maritime Department of Hong Kong, the incidence of accidents related to cargo handling / loading and unloading in Hong Kong is quite high. In 2006 there were 302 work accident cases related to container loading and unloading. In 2007 there were 240 work accident cases related to container loading and unloading. In the following year, from 2008 to 2010 each recorded 220 cases, 176 cases and 157 cases each year. The incidence of these accidents is seen to decline every year, but the decline is not accompanied by a decrease in the risk of death which reached its peak in 2010 as many as 6 deaths due to workplace accidents related to container loading and unloading.

The 24-hour bongkarmuat service that involved humans and machines definitely caused the danger of accidents such as dropping slings at the time of appointment, spreaders falling on head truck operators, falling containers on TKBM or head truck operators, TKBM falling into the river until collisions between QCC. Risk assessment is deemed necessary to minimize the incidence of accidents.

### Material and Method

This study uses a qualitative research design, using Job Safety Analysis (JSA) for the risk identification process, the US / NZ 4360: 1999 semi-quantitative risk assessment table for risk analysis and semi-quantitative risk level Cross (1988) for risk evaluation.

The population in this study were procedures / jobs in container loading and unloading activities carried out at PT. Pelabuhan Indonesia II (Persero) Palembang Branch.

Primary data in this study is in the form of data from field observations, as well as data from interviews with key informants and informants. Observation data is used to identify risks and get a description of the dangers in loading and unloading work at PT. Pelabuhan Indonesia II (Persero) Palembang Branch.

Secondary data in this study were in the form of library research, company profile, work instructions for planning container loading and unloading activities, work instructions for controlling loading and unloading activities, container service procedures for loading and unloading of PT. Pelabuhan Indonesia II (Persero) Palembang Branch and review of documents related to research.

Data obtained from the results of observations and interviews were analyzed using semi-quantitative risk analysis techniques to determine the level of risk and evaluated at each step of the work and involved workers.
in carrying out the assessment. In addition, data analysis based on observations is also done by confirming the observations in the form of photos of work procedures, known as photovoice. Furthermore, the data will be validated using triangulation of sources, methods, and data to test the credibility of the research results.

Discussion

The results of the research conducted during the container loading and unloading work at PT. Pelabuhan Indonesia II (Persero) Palembang Branch shows that there are 12 risks with very high risk levels. These risks are grouped into 4 types of risks, namely being crushed by the container, collision between the head of the truck, falling from a height, and the elevator falling.

The risk of overwritten containers is in several work steps. Work steps that have the risk of being hit by containers include, among others, lasing or unlasing containers, opening a twist lock on containers, head truck operators waiting for cargo, giving signals to TKBM and recording container numbers done by tally. In a day this work is usually done once so that the exposure value is given 6 (Frequently).

Another risk that has a very high level is the risk of being hit by a truck head when the head truck operator waits for the load of goods, the operator should not be allowed to get off the vehicle, but the operator comes down from the head truck and runs on the truck track. This can lead to the risk of an accident being hit by a vehicle that will exit or go to the QCC in front of it to load containers, therefore a 6 probability value. Consequences of 25 (very serious) because if the head truck operator is hit then it can cause injury and even permanent disability and exposure 3 (occasionally). The risk of getting hit by a truck head is not only for the operator. The risk of being hit by a truck head can also occur at the stage of signal loading and unloading by the TKBM to the head truck operator and at the stage of recording the container number by tally. The probability value 6 (Likely) is because the TKBM and tally are right next to the head of the truck and stand in the truck path when giving sinya and recording containers, this can cause TKBM and tally to be hit by trucks that will pass, moreover the activities are carried out at night. What consequences if TKBM or tally gets hit is the value is 25 (very serious) because it can cause injury to permanent disability. The expiration value is 3 (Occasionally) because if the sprider is lifting the container on the TKBM ship and tally will move to the safe lane provided.

Collisions between head trucks can occur when the activity of the head truck operator takes the truck to the truck or carries a load to the stacking field, the reason is that the head truck operator is not vigilant when driving a vehicle so that it strikes a vehicle in front of it and this is likely to occur because every cargo the container earns 2000 rupiah so the head truck operator ignores safety, the probability value is 6 (likely). The consequence is 15 (serious) because if there is a risk accident the occurrence can occur is a serious injury such as a broken bone, this activity takes place several times because after the head truck brings the container from the dock to the stacking field it will return to the dock so that the exposure value is 6 (frequently).

The next risk is included in the very high category, which is falling from a height. Falling from a height can cause mild to severe injuries such as fractures and even death of workers. This risk can occur at TKBM which opens a twist lock on a stack of containers that reaches 12 M. The twist lock is a prism-shaped locking iron located in four container corners, so that the container stack does not sway or move position. To open a twist lock on a container that is in the topmost stack TKBM must go up above the container and be on the edge of the container. Based on the results of interviews with workers, there has been a work accident because the TKBM is in an unhealthy condition and loses balance which causes the TKBM to fall from a height so that the possible value is 10 (continuous) severity that can occur if the TKBM falls permanently so the consequence value is 25 (Very serious). The exposures value is 6 (frequently) because this activity is done repeatedly. The same results were obtained from the study of Makomulamin and Safitri (2017), which stated that the danger of falling from a height occupies a high risk level with a score of 15, the possibility of occasional occurrence and severity is severe injury, broken bones, disability and death which means necessary carried out promptly by the company.

Conclusion

The results of risk identification in container loading and unloading work at PT. Pelabuhan Indonesia II (Persero) Palembang Branch has 36 risks. Preparation stage: hit by a head truck, crushed repair equipment, hand pinched, fell into a river, tripped, hit a twist lock throw, slipped lubricant, hit the head, crushed the container, fell from a height and collided between the head truck.
QCC operation: tripping, the elevator falls, hands pinched on the elevator door, tripping, head banging, hands pinched swift limit, head hit, collision between QCC and electric shock. The stage of moving containers: getting hit by a head truck, being hit by containers, tripping, slipping and traffic accidents.

The results of the risk analysis obtained illustrate that in container loading and unloading jobs there are 12 risks with very high risk levels which are grouped into the risk of being hit by containers, falling from a height, collisions between head trucks and lifts falling. 1 risk with the risk level of priority 1, namely the supervisor is exposed to a twist lock throw, 2 risks with a substantial level of risk, 5 risks with a level of risk of priority 3 are risk of hit head, collision between QCC, pinched hands and electric shock, and 16 risks with acceptable risk levels that is overwritten by repair equipment, tripping, slipping lubricant head hit and pinched hands.

The results of the risk level obtained are divided into 5, namely very high, priority 1, substantial, priority 3 and acceptable.

The results of the risk evaluation indicate that there are still dangers included in the category of risk level (existing level) non-acceptable or still need improvement in the implementation of work and improvement in the implementation of risk control. Besides that, it is also found that hazards including acceptable level levels or risk control efforts that have been carried out are considered to have reduced the level of risk to acceptable limits, although the intensity of activities that pose a fixed risk should be reduced to a minimum. These hazards include pinched hands, slippery areas of the ship due to oil spills or lubricants, Laying a twist lock that is careless on the road, Laying mechanical equipment carelessly so that it falls from above the head.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Ethical Clearance:** The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

**References**

Correlation of Smoking and National Institutes of Health Stroke Scale (NIHSS) in Acute Ischemic Stroke Patients

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Abstract

Background: Ischemic stroke is the most common type of stroke with 87.00% prevalence of atherosclerosis as one of the causes of cerebral vascular lumen occlusion. Smoking is known as a modifiable risk factor for stroke. The process of atherosclerosis leads to a neurological deficit resulting in impairment of the patient. One of the scales used to assess impairment is the National Institutes of Health Stroke Scale (NIHSS).

Objectives: To know the correlation between smoking status and functional degree of acute ischemic stroke patients as measured by NIHSS.

Method: The subjects were 48 patients. The study design was cross-sectional and clinical sampling consecutive admissions and an acute ischemic stroke image imaging. Smoking status data was obtained through anamnesis at the time the patient was hospitalized supported by the testimony of the immediate family. NIHSS values were obtained on the first day of hospitalization. Data analysis used chi-square test and logistic regression.

Result: Sex (p = 0.001) and smoking (p = 0.013) were variables that had significant correlations with NIHSS. There was no association between smoking and the functional degree of acute ischemic stroke patients measured by NIHSS, (p = 0.57) with Adjusted Odds Ratio 1.5 (CI 95% 0.35 - 6.9).

Conclusion: There was no significant association between smoking with functional degree of acute ischemic stroke patients as measured by NIHSS.

Keywords: NIHSS, Acute ischemic stroke, Smoking status

Introduction

A stroke is a focal or global neurologic deficit that suddenly occurs over 24 hours unless it dies before 24 hours or surgery is performed, in the absence of other causes other than cerebral vascular disorders ¹. The prevalence of stroke in Indonesia has increased from 8.3 each mile in 2007 to 12.1 per mil in 2013. Ischemic stroke is the most common type of stroke with the prevalence of 87.00% ². Ischemic stroke is classified based on the mechanism of etiopathogenesis: Large Artery Atherosclerotic Stroke (LAA), cardioembolic stroke (CE), small artery occlusion or lacunar stroke (LAC), the stroke of other determined etiology and stroke of undetermined etiology ³.

One of the risk factors that increase the incidence of stroke is smoking behavior ⁴. Indonesia is ranked one in the world for the number of male smokers over the age of 15 (66.00%) ⁵. Exposure to secondhand smoke triggers many pathological effects in the endothelium, such as oxidative stress, platelet activation, trigger coagulation cascades and interfere with fibrinolysis ⁶,⁷. Smoking causes poorer functional outcomes at 3 months after the onset of ischemic stroke compared to non-smokers ⁸,⁹.

The scale to measure the functional deficit of acute ischemic stroke is NIHSS (National Institutes of Health Stroke Scale) ¹. Smoking is said to be associated with...
higher NIHSS scales in small vessel occlusion\textsuperscript{10}, while other studies produce different assumptions by using the same measurement tool\textsuperscript{11}. NIHSS has a specificity of 0.90 (CI 95%, 0.86-0.94), sensitivity of 0.71 (CI 95%, 0.64-0.79) and good accuracy of 0.83 (CI 95%, 0.79-0.87) in predicting post-stroke clinical outcomes\textsuperscript{12}.

How to know someone’s smoking status is with interviews that have sensitivity and specificity by 79.00\%\textsuperscript{13}. This method is known not invasive and does not require special techniques also cheap. The current studies on the relationship between smoking and functional degrees in acute ischemic stroke patients in Indonesia are not present. This study is expected to help clinicians to be more aggressive in controlling risk factors for stroke associated with the process of atherosclerosis, especially smoking, so as to reduce the degree of disability stroke patients.

\textbf{Method}

The subjects of the study were acute ischemic stroke patients in Dr. Soetomo General Hospital and treated in Seruni A and B rooms, from February to July 2017. The inclusion criteria of having the first attack of ischemic stroke and willing to participate the research. The exclusion criteria were having a history of brain tumors, brain infection, and head trauma as well as unclear or unreliable smoking status. The design used was cross-sectional study with a sample size of 24 patients using the technique of sampling consecutive admission. Patients were interviewed about smoking status and NIHSS scale measured on the first day of treatment.

Prior to the identification of the subject, the researcher conducted the ethical test (191/Panke.KKE/III/2017) in Dr. Soetomo General Hospital Surabaya, Indonesia. Primary data collection can be basic patient characteristics (age, sex, education level, smoking history, duration of smoking and number of cigarettes consumption each day) and clinical characteristics (history of brain infection, head tumor, head trauma, hypertension and diabetes mellitus). History of brain infection, brain tumor, and head trauma is evidenced by interviews, physical examinations, and radiology. Hypertension has a systolic standard of ≥ 140 mmHg and/or diastolic ≥ 90 mmHg according to the JNC 7 criterion and is measured by a sphygmomanometer. DM has a standard ≥ 200 mg/dL for laboratory examination. Measurement of functional deficits of acute ischemic stroke with NIHSS scale. Acute ischemic stroke patients were divided into 2 groups, i.e., NIHSS <5 were included in mild criteria and NIHSS ≥ 5 were included in moderate-severe criteria. The correlation between smoking status and functional grade of acute ischemic stroke patients was calculated and analyzed by chi-square statistic test and logistic regression with SPSS (SPSS, Inc., Chicago, IL).

\textbf{Results}

\textit{Clinical Characteristics and Basic Subject Research}

The mean age of the study subjects was 58.9 ± 12.3 years, with the youngest age of 27 years and the oldest 86 years old. The minimum random blood sugar level-maximum was 85 and 915. The systolic maximum values were 90 and 240. The diastolic maximum values are 60 and 140 (Table 1).

\textbf{Table 1. Clinical Characteristics of Research Subject}

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ±SD (N=48)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td>58.9 ± 12.3</td>
<td>27 – 86</td>
</tr>
<tr>
<td>GDA (mg/dL)</td>
<td>173.6±138.4</td>
<td>85 - 915</td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>158.8 ± 32.1</td>
<td>90 - 240</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>92.3 ± 16.6</td>
<td>60 - 140</td>
</tr>
</tbody>
</table>

The majority of subjects were male (64.60\%) with the highest number age was ≤ 60 years (68.70\%). Most subject education history (52.00\%) was higher education level (Senior high school and Bachelor degree). The majority of nonsmoker subjects (58.30\%). In smokers, most smokers for> 20 years (90.00\%) and cigarette consumption per day were 10-19 cigarettes (65.00\%) (Table 2).
**Table 2. Basic Characteristics of Research Subject**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n=48)</th>
<th>Percentase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>35.40</td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>64.60</td>
</tr>
<tr>
<td>AGE GROUPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 60 y/o</td>
<td>33</td>
<td>68.70</td>
</tr>
<tr>
<td>&gt; 60 y/o</td>
<td>18</td>
<td>37.50</td>
</tr>
<tr>
<td>Education Level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>23</td>
<td>48.00</td>
</tr>
<tr>
<td>Advanced</td>
<td>25</td>
<td>52.00</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>20</td>
<td>41.70</td>
</tr>
<tr>
<td>Non-smoking</td>
<td>28</td>
<td>58.30</td>
</tr>
<tr>
<td>Smoking Duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-9 y/o</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>10-20 y/o</td>
<td>2</td>
<td>10.00</td>
</tr>
<tr>
<td>&gt;20 y/o</td>
<td>18</td>
<td>90.00</td>
</tr>
<tr>
<td>Smoking per-day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-9 cigarettes</td>
<td>5</td>
<td>25.00</td>
</tr>
<tr>
<td>10-19 cigarettes</td>
<td>13</td>
<td>65.00</td>
</tr>
<tr>
<td>&gt;20 cigarettes</td>
<td>2</td>
<td>10.00</td>
</tr>
</tbody>
</table>

**Independent Variable Correlation and NIHSS Score**

Subjects of the study were male who had moderate-weight NIHSS 90.50%, it was greater than those with mild NIHSS (44.40%). The difference was statistically significant (p = 0.001), with Crude Odds Ratio of 11.875 (CI 95%, 2.297-61.396). In the age category, the majority of subjects aged ≤60 years, who had moderate-severe NIHSS were 57.10%, and it was smaller than those with mild NIHSS (77.8%) also were not statistically significant (p = 0.112).

Subjects with primary education (Elementary school - Junior high school) with moderate-severe NIHSS were 57.10% that greater than those with mild NIHSS (40.70%). In DM variables, those with moderate NIHSS were 28.60%, it was greater than those with mild NIHSS (22.20%). These two variables were also not statistically significant (p = 0.201) and (p = 0.431).

In hypertension variables, the study subjects who had moderate-severe NIHSS were 81.00%, smaller than those with mild NIHSS (88.90%) and were not statistically significant (p = 0.68). In smoking variables, NIHSS with moderate-weight was 62.00% greater than those with mild NIHSS (26.00%). The difference was statistically significant (p = 0.013), with Crude Odds Ratio of 4.64 (CI 95% 1.35-15.9) (table 3).
Table 3. Correlation of Independent Variables (Clinical and Basic) and Dependent (NIHSS)

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>NIHSS (n=48)</th>
<th>p-value</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium-Severe (%)</td>
<td>Mild (%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>90.50</td>
<td>44.40</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9.50</td>
<td>55.60</td>
</tr>
<tr>
<td>Age</td>
<td>≤ 60 y/o</td>
<td>57.10</td>
<td>77.80</td>
</tr>
<tr>
<td></td>
<td>&gt; 60 y/o</td>
<td>42.90</td>
<td>22.20</td>
</tr>
<tr>
<td>Education Level</td>
<td>Primary</td>
<td>57.10</td>
<td>40.70</td>
</tr>
<tr>
<td></td>
<td>Advanced</td>
<td>42.90</td>
<td>59.30</td>
</tr>
<tr>
<td>DM Statuses</td>
<td>Yes</td>
<td>28.60</td>
<td>22.20</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>71.40</td>
<td>77.80</td>
</tr>
<tr>
<td>Hypertensi Status</td>
<td>Yes</td>
<td>81.00</td>
<td>88.90</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>19.00</td>
<td>11.00</td>
</tr>
<tr>
<td>Smoking Status</td>
<td>Smoking</td>
<td>62.00</td>
<td>26.00</td>
</tr>
<tr>
<td></td>
<td>Non-smoking</td>
<td>38.00</td>
<td>74.00</td>
</tr>
</tbody>
</table>

**Multivariate Analysis**

In bivariate analysis, there was a significant correlation between functional degree measured by NIHSS and sex variable also smoking status. Therefore, the analysis was continued with logistic regression. After multivariate analysis logistic regression was obtained p = 0.57 with Adjusted Odds Ratio 1.5 (CI 95% 0.35 - 6.9) meaning that there was no correlation between smoking status and NIHSS score.

**Discussion**

The average age was elderly (> 60 years) with the majority being male. This is consistent with studies suggesting that males have a higher risk of stroke than female and sex is one of the unmodifiable risk factors for stroke. The well-documented modifiable stroke risk factors include hypertension, smoking, diabetes, dyslipidemia, atrial fibrillation, asymptomatic carotid stenosis, hormone replacement therapy, nutrition and physical inactivity. Smoking was believed to cause the development of atherosclerosis by initiating endothelial lesions through the production of oxygen radicals or by direct toxic effects of the element of cigarette smoke.

Smoking was associated with higher NIHSS scores in small vessel occlusion. But other studies stated that smoking was not associated with good functional outcomes after acute ischemic stroke. Both studies used NIHSS as an indicator of clinical outcome.

NIHSS was a tool used to measure the functional degree of stroke. In this research, the maximum values of NIHSS were 2 and 19. The mean of NIHSS was 5.56 ± 3.8. The subjects were divided into 2 groups, those with
mild neurologic deficit were 56.30% and the group with the severe neurological deficit was 43.80%. This study compared to NIHSS scales in the smokers, diabetes, and hypertension groups. There was no association between diabetes and poor NIHSS on the first day of treatment. This was similar to other studies that there was no significant difference in the severity of stroke at baseline treated as assessed by NIHSS between patients suffering from DM and non-DM 15. While hypertension was also not associated with NIHSS that consistent with other studies which suggest that elevated blood pressure during the onset of ischemic stroke was indicated the mild stroke 16.

There were two statistically significant variables (p <0.05) that were sex and smoking. However, in the logistic regression analysis for both variables, there was no association between smoking and NIHSS. Differences in the results of the multivariate analysis with bivariate analysis results can be overcome by the addition of the number of research subjects.

Oxidative stress could cause disorders of cerebral blood vessel metabolism that resulting in vascular lesions with forms of lacunar stroke, white matter hyperintensity, and microbleeds. Oxidative stress possibly triggered by hypertension, diabetes, aging, and smoking 17. The neurologic deficits in ischemic stroke patients who smoked in this study were mostly mild, as they may be caused by small vessel disease with clinical forms of lacunar stroke.

Although there was no significant association between smoking and the functional degree of acute ischemic stroke was assessed with NIHSS, the majority of the subjects were smoking. Although the functional degree of stroke in this study was mostly mild, smoking has been recognized as a risk factor for stroke so anti-smoking campaigns must still be aggressive for the primary prevention of ischemic stroke.

**Conclusion**

Based on the sex and smoking variables, it had a significant relationship with the functional degree measured by the NIHSS scale. In a multivariate analysis, there was no significant association between smoking status and functional degree of acute ischemic stroke patients as measured by NIHSS. Further research with more objective measures such as CO levels in expiratory air and blood levels of cotinine was also worth considering.

**Ethical Clearance:** The study has been approved by ethical committee (191/Panke.KKE/III/2017) in Dr.Soetomo General Hospital Surabaya, Indonesia.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding

**References**


Risk Assessment, Risk Management, and Risk Communication in the Carpet Industry: PT. ‘X’ Pandaan. East Jawa

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Abstract

There are many work-related accidents and diseases caused by weak risk management efforts. Risk management can be done by starting with a risk assessment. Risk assessment is an important aspect of occupational health and safety. The garment textile manufacturing industry has a high risk of occupational health and safety. This study aims to identify the level of occupational health and safety risks and provide control recommendations. This research was descriptive using the Job Safety Analysis (JSA) method. The results of the identification show that out of the 4 work stages and 19 job descriptions, 10 descriptions or 52.6% are at high risk for Occupational Safety and Health. Identified hazards include chemical, physical, ergonomic and fire hazards. High risks include hazards from chemicals as raw materials for the process of making carpets. Several control measures have been taken, but to ensure the health and safety of workers, additional efforts must be made such as personal protective equipment, special masks for use in chemical hazards and work accident emergency response procedures. Risk communication in drug companies is running well.

Keywords: Risk Assessment, Risk Management Risk Communication, Job Safety Analysis.

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Introduction

Today’s industrial development in Indonesia is very rapid, which is characterized by free trade, increasing information, communication and transportation technology. Globalization has encouraged the growth of industries in various sectors by implementing various machinery technologies and production equipment and the use of various materials. This has an impact, especially on labor in the form of work accident risks. OHS problems have become a serious problem in our country and still lack attention. As a result, work accident cases in Indonesia are increasing every year. Workplace accidents are an unexpected event because in the event of a work accident can cause injury, injury and even death to workers.

Based on BPJS Employment data, there are 157,313 work accident cases throughout 2018, the number comes from several categories, including in the category of work accidents are traffic accidents on workers’ trips to the workplace, and trips back from work to residence, work accidents are not it only causes death, material loss, morale and environmental pollution, but can also affect productivity and people’s welfare. Workplace accidents also affect the human development index and national competitiveness. The East Java (East Java) Manpower and Transmigration Office (Disnakertrans) claims that there were 21,631 cases of work accidents throughout 2017. That number rose by around 200 cases compared to the previous year.

Occupational accidents and work-related diseases not only cause material losses and fatalities and health...
problems for workers but can disrupt the production process as a whole and even damage the environment which ultimately affects the wider community. Whereas in the case of Occupational Diseases (PAK) from 2011-2014 the trend has declined even though in 2012 and 2013 it has increased. In 2011 the number of PAKs in Indonesia reached 57,929 cases, in 2012 60,322 cases and in 2013 increased again to 97,144 cases, then in 2014 began to decline to 40,696 cases. The provinces with the most prevalence of PAK in 2011 were Central Java Province, in 2012 North Sumatra, South Sumatra and West Java, 2013 Banten Province, and in 2014 were the Provinces of Bali, East Java and South Sulawesi. Data shows that efforts to prevent accidents and occupational diseases are still not optimal.

Efforts to prevent work-related accidents and diseases can be carried out with a number of approaches, namely, approaches to weaknesses in human elements, such as selecting employees properly and developing knowledge or training. Approach to weaknesses in hardware or production equipment through design, production equipment maintenance, and work environment planning. Approach to all levels of management by conducting a fair distribution of tasks, and determining the implementation of risk assessments.

Risk assessment is the process of evaluating risks caused by hazards, taking into account the adequacy of the controls owned, and determining whether the risk is acceptable or not. One component of risk management is risk assessment. The stages of risk assessment are identification of hazards, risk assessment, risk control and residual risk assessment.

One method that can be used to identify risks is the Job Safety Analysis (JSA) method. JSA is the identification of risk methods by reviewing and assessing the risk of each stage of work performed. JSA itself is a method that studies a job to identify hazards and potential incidents related to each step, and is used to develop solutions that can eliminate and control hazards. The implementation of JSA has benefits and benefits as follows:

1. Can be used to provide training or training on work procedures more safely and efficiently
2. Providing training to new workers / employees.
3. Provide Pre-job instruction on jobs that are not permanent.
4. Review the job procedures after an accident.
5. Conduct a study of work to enable improvement in work methods.
6. Identify what safeguards need to be used while working.
7. Increase work productivity and positive behavior regarding safety.

The implementation of the JSA must be carried out proactively where the focus of the JSA implementation refers to the inspection of work and not the workers who carry out the work. JSA can be used as a response to an increase in injury or illness, but the process of identifying hazards and determining the necessary precautions must be carried out through the process of planning and organizing the stages of work.

PT. ‘X’ Pandaan is a company engaged in and producing textiles and garments. For the garment consists of departments, from some of them: patter / marker, cutting, sewing / knitting, finishing, pressing, quality control, packing, and deliveries. Workers at PT. ‘X’ Pandaan, work a day for 8 hours starting at 07.30 s / d 16.30 and resting at 12.00 - 13.00. Within a week they work for 6 days and 1 day off. There are several divisions within the company, including cutting, sewing cloth, sewing, mats, foam or sponges, making mattresses, laminates, packaging, and drivers. Workers at PT ‘X’ Pandaan, the majority have tenure over 5 years and above.

Based on visits made to PT ‘X’ Pandaan, there are several potential hazards that can cause accidents and occupational diseases. During the production process, workers make physical contact either through inhalation or dermal with chemicals as raw material for making production. Chemicals for lamination, namely ethyl alcohol, xylen benzene and toluene which are contained in glue that have the potential to cause health problems such as irritation, respiratory problems, and nervous system disorders.

The purpose of this study is to conduct occupational health and safety risk assessments in the Production Section I of PT. ‘X’ Pandaan, uses the Job Safety Analysis (JSA) method.

Material and Method

This study used a descriptive observational research
design. The danger is potential danger, the risk refers to the actual danger. Risks can be identified through direct observation at the production site. The research was conducted at PT. ‘X ‘Pandaan. East Java. The risk assessment method uses the JSA with the following risk assessment steps:

Select the place to be analyzed

1. Explain the stages of work
2. Identify various hazards and risks at each step of the work, and identify the various possibilities that have the potential to become accidents;
3. Risk assessment (likelihood and severity)
4. Categorize risk (risk assessment matrix)
5. Provide control recommendations

Risk Management is done by selecting control options that are in accordance with the characteristics of the risk. Evaluation of available controls, so as to provide additional control recommendations. Risk communication data is obtained by direct observation at PT. ‘X ‘Pandaan. East Java. Risk communication can generally be observed through the media, company management meetings with workers and local stakeholders.

Assessment of potential hazards identified by risk hazards through analysis and evaluation of risk hazards intended to determine the amount of risk taking into account the possibility of occurrence and the magnitude of the consequences. From the results of the analysis it can be determined the ranking of risk values so that risk assessment can be carried out which has a significant impact on the company and the risk is not important.

Table 1. Scale of “Probability”

<table>
<thead>
<tr>
<th>Level</th>
<th>Criteria</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Insignificant</td>
<td>There are no losses, the material is very small</td>
</tr>
<tr>
<td>2</td>
<td>Minor</td>
<td>Minor injuries require p2k3 treatment to be handled directly at the scene, moderate material losses</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Missing workdays, requiring medical treatment, material losses are quite large.</td>
</tr>
</tbody>
</table>

Table 2. Scale of “Severity”

<table>
<thead>
<tr>
<th>Level</th>
<th>Criteria</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Insignificant</td>
<td>There are no losses, the material is very small</td>
</tr>
<tr>
<td>2</td>
<td>Minor</td>
<td>Minor injuries require p2k3 treatment to be handled directly at the scene, moderate material losses</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Missing workdays, requiring medical treatment, material losses are quite large.</td>
</tr>
<tr>
<td>4</td>
<td>Major</td>
<td>Injuries result in defects or loss of bodily functions in total material loss</td>
</tr>
<tr>
<td>5</td>
<td>Catastrophic</td>
<td>Causing a huge material disaster</td>
</tr>
</tbody>
</table>

Table 3. “Risk Matrix” Scale

<table>
<thead>
<tr>
<th>Possibility</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
</tr>
<tr>
<td>3</td>
<td>L</td>
</tr>
<tr>
<td>2</td>
<td>L</td>
</tr>
<tr>
<td>1</td>
<td>L</td>
</tr>
</tbody>
</table>

The results of the risk assessment will be the basis for risk control. Control (control) of hazards in the work environment is an action taken to minimize or eliminate the risk of workplace accidents through elimination, substitution engineering control warning system administrative control and personal protective
Findings

A. Risk Assessment

There are several stages in the production step in this company, including the measurement of the desired pattern cutting babahan, sewing materials, then filling the required filling material, as well as the lamination stage. The risks identified are, material workers result from cutting powder, which can cause shortness of breath. Workers who sit too long can cause Lob Back Pain (LBP), manual lifting can cause LBP and Thoracic Outlet Syndrome (TOS). All job descriptions at this stage are included in the high risk.

The next step is a series of results from cutting patterns, where the results of the pattern cuts were carried out by using a sewing machine. The risk identified at this stage is that workers experience eye fatigue when supervising the desired stitch pattern, and sitting too long can also cause low back pain. All job descriptions at this stage are included in the high risk.

The next step is filling the material in the form of a dacron to produce the shape according to the desired pattern. The risk identified at this stage is that workers experience inhalation of the remainder of the dacron which is not used because it is in the vicinity of the stack, after which the droron is flying as a result of lifting or transferring the results of filling. Risks identified include, workers can experience injuries, dizziness, shortness of breath and eye irritation. Two job descriptions are included in the high risk level and the other 2 are moderate risk levels.

The next stage is sewing all the patterns that have been filled by the dacron. All results are filled in sewing in such a way as to get the results obtained. The risk identified at this stage is that workers experience eye fatigue when supervising the desired stitch pattern, and sitting too long can also cause low back pain. All job descriptions at this stage are included in the high risk.

The last stage is packaging the products using lamination, where all the processed products are put into a specially provided plastic. The risk identified at this stage is that workers experience inhalation of the material contained in the plastic, workers can experience injury, dizziness, shortness of breath and eye irritation. All job descriptions at this stage are included in the Medium risk.

B. Risk Management

Based on the risk assessment above, it shows that of the 4 work stages and 19 job descriptions, 10 descriptions or 52.6% are at high risk for Occupational Safety and Health. The weighing and printing stage is the part where all the job descriptions are high risk. 8 descriptions of the rest or 42.1% are moderate risks and 1 description or risk is 5.3% lower than Safety and Occupational Safety.

Referring to the results of the risk assessment that there are several descriptions of tasks that have a high risk, these activities may not be carried out until the risk has been reduced or controlled. The purpose of risk control is to avoid workers from the worst consequences that can lead to loss of work time and worker productivity.

Some stages of production run the risk of causing health problems. Ergonomic risks are identified such as errors in manual handling, excessive physical burden, and posture incompatibility with the work station, the risk of causing muscle disorders.

Research conducted by Wahyu, P.D and Tualeka, A.R., 2013 in one of the welding industries in East Java, Indonesia explained that there were still residual risks despite risk control. So that residual risk assessment needs to be done so that it can further determine additional risk control recommendations.

PT. ‘X ‘Pandaan. East Java has made several efforts to control the risks identified. This effort is a combination of technical control, administration and the use of personal protective equipment. control that has been carried out between, providing masks, making Standard Operating Procedures, providing chairs for workers, work rooms equipped with refrigeration equipment, providing lightweight fire extinguishers, efforts to maintain workers’ fitness with gymnastics every morning, and providing a dining room to avoid contamination the ingredients in the production room enter the workers’ food.

Such control is still not enough to reduce some of the risks identified. Some controls must also be made, such as conducting safety talks, safety inspections, extending work intervals, giving awards and reprimands, providing fire and emergency response procedures, exchanging with tired and weak colleagues and providing personal protective equipment for work locations such as that is.
C. Risk Communication

PT. ‘X ‘Pandaan has not implemented OHSAS 18001: 2007, but Komitmet Top Management of work safety is very high as evidenced by the presence of occupational safety health officials in the company where it is a work safety and health management system. One form of system implementation is communication, participation and consultation12.

Risk communication is the exchange of information about the magnitude of health risks in the work environment 6. The forms of the application of risk communication carried out in the company include, meetings between all company structures including workers in communicating occupational health and safety policies. Risk communication media are installed such as posters and banners. Besides this effort, PT. ‘X ‘Pandaan East Java regularly holds meetings with workers on the National Occupational Health and Safety warning. This meeting is held so that there is an exchange of information between workers and company managers. And always do safety talk before work.

Conclusion

Based on the risk assessment above, it shows that of the 4 work stages and 19 job descriptions, 10 descriptions or 52.6% are at high risk for Occupational Safety and Health. The weighing and printing stage is the part where all the job descriptions are high risk. 8 descriptions of the rest or 42.1% are moderate risks and 1 description or risk is 5.3% lower than Safety and Occupational Safety. Risk management has been carried out, but additional control is needed so that the risk of illness and workplace accidents can be reduced. The risk communication process works well, risk communication involves management and workers. Media communication has been installed, and information exchanges with routine workers are carried out.

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: This research was approved by the institutional Ethics Board of Airlangga Surabaya University. All subjects received complete information about the procedure and purpose of this study, each subject before the study signed an informed consent form.

References

Coal Dust and Acute Respiratory Infections in South Kalimantan PT ‘X’ Coal Mining Workers

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¹Students at Department of Occupational Health and Safety, Faculty of Public Health, Airlangga University, Surabaya, East Java, Indonesia; ²Department of Occupational Health and Safety, Faculty of Public Health, Airlangga University, 60115 Surabaya, East Java, Indonesia

Abstract

Occupational illness is a disease caused by a person’s work or work environment. This disease is caused by the actions of someone who is unsafe (unsafe act) and unsafe condition (unsafe condition) in carrying out his work activities. The unsafe act is an act of someone who deviates from the rules of security standards that have been set in doing work. While unsafe conditions are conditions that can endanger workers. Acute respiratory infection is an acute inflammation of the upper and lower respiratory tract caused by infection with microorganisms, bacteria, viruses, and rickets, without or accompanied by pulmonary parenchyma. Factors that affect a person affected by ARI are environmental factors, individual characteristics, and worker behavior. Environmental factors include air pollution (air pollution due to industrial output and smoke from burning fuel). Dust particles that can cause acute respiratory problems from industrial products that pollute the air such as coal dust, cement, cotton, asbestos, chemicals, toxic gases, dust in rice mills (organic dust).

Keywords: dust, ARI, work-related diseases

Introduction

Occupational Diseases are diseases caused by work and the work environment. PAK risk factors include Physical, chemical, biological or psychosocial groups in the workplace. These factors in the work environment are the main causes and determine the occurrence of occupational diseases. Other factors such as individual vulnerability also play a role in disease progression among exposed workers¹.

Disease suffered by employees in relation to work both risk factors because of the conditions of the workplace, work equipment, materials used, production processes, work methods, company waste, and production results².

The risk factors that can cause PAK are as follows³:

1. Physical group
   a. Noise can cause interference with hearing through Non-induced hearing loss
   b. Radiation (radioactive rays) can cause blood and skin abnormalities
   c. High air temperatures can result in heat strokes, heat cramps, or hyperpyrexia. While low temperatures can result in frostbite, trench foot or hypothermia.
   d. High air pressure can cause caisson disease
   e. Lighting that is not enough can cause eye fatigue. High lighting can lead to accidents

2. Chemical groups
   a. Dust can result in pneumoconiosis
   b. Steam can cause metal fume fever, dermatitis and poisoning

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c. gas can lead to CO and H2S poisoning
d. Solutions can cause dermatitis
e. Insecticides can cause poisoning

3. Group injection
a. Anthrax
b. Brucella
c. HIV / AIDS

4. Mental group

It can be caused by bad working relationships or monotonous work conditions that cause boredom.

The international body of the International Labor Organization (ILO) suggests 34% of the causes of deaths caused by occupation are cancer, 25% are accidents, 21% are respiratory diseases, 15% are cardiovascular diseases, and 5% are caused by other factors. Acute respiratory infection (ARI) is a major health problem as evidenced by the prevalence of ARI in Indonesia as much as 25.5% (range: 17.5% - 41.4%) with 16 provinces including prevalence above the rate national and pneumonia as much as 2.1% (range: 0.8% - 5.6%)\(^4\).

ARI is the main cause of infectious morbidity and mortality in the world. Nearly four million people die from ARI every year, 98% of which are caused by lower respiratory tract infections. Very high mortality rates in infants, children and the elderly, especially in countries with low and middle income per capita\(^5\).

Acute respiratory infection (ARI) is an acute inflammation of the upper and lower respiratory tract caused by infection with microorganisms or bacteria, viruses, and rickets, without or accompanied by inflammation of the lung parenchyma. The factors that influence a person’s risk of developing ARI are factors environment, individual characteristics, and worker behavior. Environmental factors include air pollution (cigarette smoke, air pollution due to industrial results and smoke from burning fuel for cooking with high concentrations). Individual factors such as age, sex, and education level can also influence the risk of susceptibility to ARI. Worker behavior includes smoking and the use of masks\(^6\).

Indonesia is one of the countries that are rich in minerals, including gold, silver, copper, coal, oil, and gas. The coal mining industry can increase the country’s foreign exchange but massive exploitation and use of natural resources by ignoring the environment results in short-term and long-term health impacts. The issue of energy sources is also becoming the government’s main focus in relation to rising oil prices. Indonesia’s coal reserves are larger than oil and natural gas reserves so the government began to see coal as an alternative energy source\(^7\).

Coal dust is a complex mixture of various minerals, trace metals and organic materials with different degrees of coal particulates. Research by Nullolli et al. Revealed an increase in the number of people with asthma in children who live near or far from open coal mining locations. This indicates that the disease due to coal dust is related to the nature of dust that is easily carried away by the wind. Air pollution due to processing or the results of the coal mining industry will have a negative impact on the lungs of workers and communities around the mining area. Respiratory diseases that generally arise due to exposure to coal dust particles namely decreased air quality to a level that endangers the health and ultimately leads to an increase in respiratory disease disorders such as ARI\(^7\).

Hasnur Group’s coal mining business is currently handled by two subsidiaries, namely PT Energi Batubara Lestari which has reserves of 80 million Metric Ton, and PT Bhumi Rantau Energi has reserves of 200 million Metric Ton, located in Rantau, Tapin Regency, South Kalimantan. In addition, there are several coal projects currently under exploration (IUP Exploration). Hasnur also has special coal terminals located on the Putting River and Sungai Salai, South Kalimantan and a special port in Pendang, Central Kalimantan\(^8\).

Material and Method

This study uses the Analytical Survey method with the Case Control approach which is an observational study that assesses the relationship of exposure to dust levels with people exposed to dust and those not exposed to dust, then compares the frequency of exposure to both groups.

This research was conducted in boiler units (cases) and filling units (control) in June 2017. The samples used were 40 people, namely 20 people from the boiler unit (control) and 20 people from the filling unit using a total sampling technique.
Data analysis used in univariate and bivariate analysis. Univariate analysis using frequency distribution and bivariate analysis using the Chi-Square test with a significance level of 0.05. If the value of \( p \leq 0.05 \) then Ho is accepted and if the value of \( p > 0.05 \) then Ho is rejected.

**Findings**

The measurement results of coal dust levels in the boiler unit are 2.2 mg / m³ and the unit filling is 0.9 mg / m³, so the results of these measurements on the boiler unit the dust content still exceeds the NAB and in the filling unit the dust is below the NAB, based on NIOSH (2011), that the NAB standard for coal dust in the permitted work environment is 2 mg / m³.

Based on the filling in questionnaires conducted on the boiler unit workforce, the results of 20 respondents who experienced ARI were 13 people (65%) and those who did not experience ARI as many as 7 people (35%) and in the unit filling workforce obtained results from 20 respondents who experiencing ISPA as many as 5 people (25%) and those who did not experience IspA as many as 15 people (75%).

From the results of measuring the levels of coal dust in the workplace environment and distributing questionnaires regarding ISPA on the workforce of PT. ‘X’ boiler units and filling units in South Kalimantan. Then performed a statistical test with the Chi-Square test using the SPSS version 21 computer program. For windows, the results were obtained regarding the levels of coal dust against ISPA in the workforce in the boiler unit of PT. ‘X South Kalimantan, with a value of 0.011 so the value is \( p <0.05 \), which means that the result is significant. Thus it can be stated that there is an effect of the level of coal dust on ARI on workers in the boiler unit of PT. ‘X’ South Kalimantan.

**Conclusion**

Occupational illness is a disease caused by a person’s work. The cause of this disease can be caused by unsafe acts and unsafe conditions. The unsafe act is an act of someone who deviates from the rules that have been set and can cause harm to themselves and others, one of which is ISPA⁹.

Risk factors for ARI are due to pollution, poor environmental conditions, for example, air pollutants, family member density, humidity, cleanliness, season and temperature. Some other factors are age, gender, smoking behavior, working period, length of exposure and use of masks that function as protective devices from dust¹⁰.

Dust exposure can cause acute respiratory problems, one of which is an industrial product that can pollute the air such as coal dust, cement, cotton, asbestos, chemicals, toxic gases, dust in rice mills (organic dust) and others. Various factors influence the emergence of diseases or disorders of the airways due to dust¹¹.

There is a relationship of dust to the incidence of acute respiratory infections in coal mining workers.

**Conflicts of Interest:** All authors have no conflicts of interest to declare.

**Source of Funding:** The source of this research costs from self.

**Ethical Clearance:** This research was approved by the Institutional Ethics Board of Airlangga Surabaya University. All subjects received complete information about the procedure and purpose of this study, each subject before the study signed an informed consent form.

**References**

1. Indonesia R. Presidential Decree No. 22 of 1993 concerning diseases arising from work relations. Jakarta: President of the Republic of Indonesia; 1993.


Enhancement the Antimicrobial Activity of Disinfectants by the Purified Lipase from *Coronobacter Dublinensis*

Alaa Naseer Mohammed Ali¹, Sahira Nsayef Muslim²

¹Department of Biology, College of Science, Mustansiryiah University, Baghdad, Iraq

Abstract

There are many different commercial disinfectants purchased, choosing of effective product is a prime target to ensure its decontamination efficiency. Thus we screened *Coronobacter dublinensis* isolates to produce lipase enzyme with using of vegetable oil like olive oil as inducer of lipase production in the medium. The lipase was purified by using two-step purification procedure consisting of ethanol precipitation and ion exchange chromatography with a yield of 48.3%. The lipase has an important role in enhancement of disinfectant activity. Since the combination between this disinfectant and lipase led to increase the activity of disinfectant to higher levels and the width of inhibition area enlarged to 28 mm against *Acinetobacter* sp. followed by *P. aeruginosa* and *S. aureus* with 25mm, respectively. So that we can conclude that lipase has excellent activity for enhancement of the commercial disinfectants purchased in decontamination activities in the laboratory and hospital environments.

Keywords: Lipase, *Coronobacter dublinensis*, disinfectants

Introduction

*Cronobacter* genus composes of a different groups of Gram negative and facultative anaerobic rods that belongs to Enterobacteriaceae family and closely related to *Enterobacter* and *Citrobacter* (1,2). It is includes seven opportunistic pathogenic species: *Cronobacter sakazakii, Cronobacter malonaticus, Cronobacter muytjensii, Cronobacter turicensis, Cronobacter dublinensis, Cronobacter universalis* and *Cronobacter condimenti* (1,3).

The organism has association with severe blood infections (sepsis), neonatal infections; necrotizing enterocolitis, septicaemia and meningitis which may be lead to fatal effect. However, these infections with *Cronobacter* are rare and occur in all age groups (2,3).

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis of ester bonds in water insoluble lipid substrates, acting at the interface between the aqueous and the organic phases (4). They are produced by animals, plants and microorganisms. Microbes have an ability to produce of lipases with high yields in comparison with the animal and plants, this return to that they are more common than animal and plant sources, easier in extraction and more commercialization in their involvement in enzymatic reaction (5,6). Lipases from microorganisms (bacterial and fungal) are the most used as biocatalysts in biotechnological applications and organic chemistry (7,8).

Disinfectants are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects. They are an essential part of infection control practices and aid in the prevention of laboratory and nosocomial infections (9). Many gram negative bacteria, gram positive bacteria and yeasts leads to nosocomial infections (10). As there are many different commercial disinfectants purchased, choosing of effective product is a prime target to ensure its decontamination efficiency.

The aim of this research was to detect the lipolytic activity in *Coronobacter dublinensis* and purification of lipase from *Coronobacter dublinensis* isolated from hospital environment clinical samples and using of lipase for enhancement of the commercial disinfectants purchased in decontamination activities in the laboratory and hospital environments.

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Materials and Method

Bacterial isolates and Identification

In this study, we obtained 3 Coronobacter dublinensis isolates from blood samples of patients. All the laboratory procedures were performed at central public health laboratories of Teaching Laboratories / Medical city, Baghdad during July/2015. The phenotypic identification was confirmed by using of the morphological testes and by Vitek-2 system (Bio-Merieux, France).

Lipolytic activity on media

Lipase indicator plates were prepared in 1 liter by addition of 10 g peptone, 5g NaCl, 0.1g CaCl₂, 1ml olive oil, 10g agar-agar and 0.5g Congo red. The lipase activity was indicated by the formation of clear halos around the colonies (11).

Purification of lipase

Coronobacter dublinensis extracellular lipase was purified by a modification of the method (12). The cells were grown in the mineral growth medium (MGM) contained (in g/L): NaH₂PO₄ 12, KH₂PO₄ 2, MgSO₄·7H₂O 0.3 and CaCl₂ 0.25. Ammonium sulphate at 1% (w/v) and castor oil at 2% (v/v) were used as nitrogen and carbon sources, respectively (11) and incubated at 30°C in shaking incubator for 18-24h. The supernatant was carefully removed after centrifugation at 10000xg for 30min at 4°C and filtered through 0.22µm Millipore filters then lipase activity in supernatant was assayed. The supernatant was treated with ethanol at ratio of saturation 60% then the mixture was centrifuged and the lipase activity was assayed for the precipitate after dissolving in 20mM Tris buffer, pH=8. The supernatant was dialyzed against the same buffer.

The supernatant was loaded on diethyl aminoethyl (DEAE)- sephadex A-25 column (2.5 by 25cm) that has been equilibrated with 20mM Tris buffer, pH=8. The column was washed with 5 to 10 volumes of the above buffer. The lipase was eluted with a gradient elution from 0.1-0.5M NaCl solutions. The fractions (5ml) were collected and assayed for lipase activity.

Lipase assay

Lipase assay was conducted by a modified method based on (13). About 30mg of p-nitrophenyl palmitate (pNPP) was dissolved in 10ml of 2-propanol at 60°C and added to 90ml of 100mM potassium phosphate buffer (pH= 7.0) containing 10mM MgSO₄ to yield a final concentration of 1mM pNPP.50µl of sample was added to the substrate solution to give a final volume of 2.5ml, the solution was incubated for 15min at 37°C and the absorbance was measured at 410nm. One unit of lipase activity was defined as the amount of enzyme that produced an absorbance at 410 nm equivalent to 1µmol of p-nitrophenol in one min under the assay conditions.

Results

Bacterial isolates and identification

In this study, three Coronobacter dublinensis isolates were collected from blood samples of patients. These isolates were diagnosed by using many morphological and biochemical tests.

Lipolytic activity on media

The aims of this study was to screen the capability of Coronobacter dublinensis isolates to produce lipase enzyme. Therefore, we used olive oil as vegetable oil for determination of Coronobacter dublinensis harboring...
lipase activity. so the first step of the screening process, we analyzed for the hydrolytic action of *Coronobacter dublinensis* isolates. It is an easy, fast and economy test to act on solid media created on the optical examination of plates having one of the vegetable oils and presence of a clearing zone around the colony edges. The results revealed that all 3 *Coronobacter dublinensis* isolates have the ability to hydrolyze the olive oil but in different levels as shown in (figure-1). Therefore, Thus we can conclude that *Coronobacter dublinensis* had an ability to produce lipase in the medium by using olive oil as inducer.

**Figure 1: Lipolytic activity for *Coronobacter dublinensis* isolates**

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**Purification of lipase**

Mineral growth medium (MGM) with 2%castor oil was chose as the best medium for lipase production because it composed of only inorganic nitrogen sources and some minerals. In *Coronobacter dublinensis* 2, the castor oil was used as a sole carbon source for lipase production. A two-step purification program consisting of the precipitation by ethanol and ion exchange chromatography by DEAE-sephadex A-25 column were used to obtain a highly purified lipase from *Coronobacter dublinensis*2. Table (1) showed the details for each purification step. 60% saturation for cooled ethanol solution was added to the crude extract and led to rise of the specific activity to 4.8 U/mg and revealed 2.5 fold of purification with 55.7% lipase recovery before the dialysis. Ion exchange chromatography by DEAE-sephadex A-25 column was the second purification step. When lipase solution was passed through DEAE-sephadex A-25 column and eluted with NaCl solutions(0.1 - 0.5M), two peaks of protein appeared in the eluted fractions with one peak of lipase activity located in the second protein peak (figure-2). Fold of purification was 11.2 in this step with 48.3% recovery.

**Table 1: Purification of lipase from *Coronobacter dublinensis***

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Size (ml)</th>
<th>Protein conc. (mg/ml)</th>
<th>Lipolytic activity (U/ml)</th>
<th>Specific activity (U/mg)</th>
<th>Purification (fold)</th>
<th>Total activity</th>
<th>Total Recovery(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>60</td>
<td>32</td>
<td>62</td>
<td>1.9</td>
<td>1</td>
<td>3720</td>
<td>100</td>
</tr>
<tr>
<td>Ethanol precipitation</td>
<td>25</td>
<td>17</td>
<td>83</td>
<td>4.8</td>
<td>2.5</td>
<td>2075</td>
<td>55.7</td>
</tr>
<tr>
<td>DEAE-Sephadex A- 75</td>
<td>15</td>
<td>5.6</td>
<td>120</td>
<td>21.4</td>
<td>11.2</td>
<td>1800</td>
<td>48.3</td>
</tr>
</tbody>
</table>
Figure 2: Purification of lipase from Coronobacter dublinensis using ion exchange on DEAE-Sephadex A-75 column.

Effect of lipase on the efficiency of disinfectants

The results showed that any one of each type of disinfectant had antimicrobial activity against all the tested microorganisms. The type one of disinfectant revealed higher effectiveness toward Acinetobacter sp. with diameter 23mm followed by 22mm for P. aeruginosa while lower effectiveness against Candida albicans. But in the combination with the purified lipase the effect increased and showed higher level toward Acinetobacter sp. followed by P. aeruginosa and S. aureus since the diameter of inhibition zone was 26mm for Acinetobacter sp. and 25mm for P. aeruginosa and S. aureus (table 2).

In the case of using the second disinfectant, this disinfectant had stronger effect against gram positive and negative bacteria and lower effect toward Candida albicans. On the other hand, the combination between this disinfectant and lipase led to increase the activity of disinfectant to higher levels and the diameter of inhibition zone became 28 mm against Acinetobacter sp. (figure 3) followed by P. aeruginosa and S. aureus with 25, respectively, and lower diameter was 22 mm against Candida albicans. According to these results we can conclude that the lipase has an important role in enhancement of disinfectant activity.

Table 2: Diameter of inhibition zones for different strains in plates in presence of disinfectants with and without lipase

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diameter of inhibition zone for disinf.1 (mm) at different con. (mg/ml)</th>
<th>Diameter of inhibition zone for disinf.1 (mm) at different con. + 5.6 (mg/ml)</th>
<th>Diameter of inhibition zone for disinf.2 (mm) at different con. + 5.6 (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Acinetobacter sp.</td>
<td>23</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>22</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>21</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>S. aureus</td>
<td>21</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>19</td>
<td>18</td>
<td>17</td>
</tr>
</tbody>
</table>
Discussion

Most Cronobacter spp. have been associated with human infections in newborn and infant infections, causing meningitis, necrotizing enterocolitis (NEC) and bacteremia or sepsis (16,17).

There is distribution of Lipolytic germs in flora and about 20% of them are lipase creators (16). Lipase production from Microbacterium sp. showed that the bacterial growing and lipase creation got to upper level after 48 hours at the start of stationary phase (17) and lipase activity in a late stationary phase was decreased and this can be owed to the presence of proteases in media of the culture. In contrast, Staphylococcus warneri produced higher level of lipase after 24 hours and at the beginning of the stationary phase (18), the parameters of growth such as optimum temperature, pH and enzyme specificity affected on lipase production (19).

Ethanolic precipitation with alginate led to rapid and almost completely harvest of lipase from the cell-free culture medium by (about 95% of the original exolipase activity was coprecipitated) (20). An ammonium sulphate precipitation trailed with Sephadex G-100 column chromatography led to clean of an extracellular lipase from Microbacterium sp. with a complete harvest of 20.8% and fold purification equal to 2.1 (21).

The isolation source, the type of culture medium that was used for growth of bacteria and the procedure that was used for purification of enzyme have effect on the enzyme activity, fold of purification and the recovery of purified enzyme (22). Lipases are used generally in house, dishwashers and modern laundry. They generally working as additives to cleaners, as they are added to the cleaners mostly in mixture with proteases and cellulases (9), besides to other enzymes such as amylases, peroxidases and oxidases. The hydrolysis of fatty stains by lipase to 15 hydrophilic parts that make these stains easily removed in comparison with non-hydrolyzed stains (21). Lipases should be both thermophilic (30-60°C) and alkalophilic (pH 10-11) add to a wide substrate specify to be able to hydrolyze fats of numerous compositions and a suitable chemical addition in cleaners and keeping their action in the presence of the numerous workings of clothes wash powder formulations like surfactants and proteases. In addition, they should have (10). The combination of genetic and protein engineering enhanced these properties of lipases in addition to their application in laundry, dish washing, contact lenses cleaning, degradation of organic wastes on the surface of exhaust pipes and toilet bowls, etc (23).

Conclusion

Disinfectants have limited levels in decontamination. The lipase has an important role in enhancement of commercial disinfectants purchased activity to achieve acceptable level of decontamination.

Acknowledgement: The Authors would like to thank Mustansiriyah University (http://uomustansiriyah.edu.iq/) Baghdad, Iraq for its support in the present work.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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Estimation the Antibiotics Activity Against Vaginal Pathogenic Microorganisms in Pregnant Ladies with Early Rupture Membrane and their Fetal Outcome

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²Iraq/University of Sumer / College of Science / Department of Pathological Analyses

Abstract

Background: The Preterm Labor: is the mean reason of the (80%) from the neonatal intrapartem, postpartum morbidity and mortality. Recently, vaginal infections have been related to high risks of PPROM and PROM. However, preterm labor and subsequent neonatal bad outcome can be avoid through several protective actions. Methods: This study was prepared through the period from January to December 2018 at Babylon Province, were for estimate the effect of vaginal infections in patients pregnant women with premature rupture membrane, the samples were 100 pregnant women, 50 were vaginal infected (as patients) and 50 were no (as controls). Results. Shows significant increasing of the fetal weight, while significant decreasing of the Maternal age and duration time in the patients, also elevation in the percentages of C/S and PROM in patients when comparing all these with controls. The pathogenic microorganisms; were CONS (coagulase negative staphylococcus aures) are major insulated organism followed by Candida, and Bacteriod spp., and to less extent were Staph., Proteus and Bacteriod. The Cifixum, Ampicillin, Clotrimazol and Metronid were appropriate antibiotics used for treatment of the vaginal infections.

Keywords: Premature Rupture Membranes (PROM), Preterm Premature Rupture Membranes (PPROM), F. Outcome, Antibiotics and Pathogenic Microorganisms.

Introduction

Preterm delivery is the reason of (80%) from illness and death of the neonates, this occur earlier to 37 weeks of gestation, the premature rupture membrane is the natural rupture membrane and happen after 37 weeks from gestation before active labor, the preterm prelabour rupture membrane is the sobontanous split of the membrane and take place between (28 to 37) weeks from gestation without active labor (¹). Time from premature rupture membrane to usual active labor proposes occur within (24) hours of membranes rupture and (65%) of pregnant women go into usual active labor, in addition, the rise of the pathogenic microorganisms along the vagina will eventually infect the amniotic sac and fluid and this leads to the rupture of the sac and premature labor (²).

The genital infections and inflammations among the pregnant ladies lead to membranes rupture, the maximum serious consequence of the preterm, membranes rupture, preterm premature and rupture of membranes is frequently related with the infections of the maternal and the results that assessed showed the (12%) from perinatal deaths are directly or indirectly related to PROM, however, the all reasons of PPROM and PROM are not recognized, but a variety of conditions have been joined to vaginal infections, and the vaginal infections within ladies in age of reproduction were connected with amplified dangers to premature rupture of membranes (²⁻⁶).

The microbial toxins are produced after connection of pathogenic microorganism to specific receptors on the membrane and this lead to change in tensile power for amniotic membranes and it result inflammations then PPROM formation (⁷). These lead to increase metalloproteinase production, which responsible for collagen degradation and causes rupture of the membrane (⁷⁻⁸). The fact that collagen is the main component membrane which maintain the tensile power for amniotic membranes. Though, infection formed by going up pathogenic microorganisms may happen
owing to PPROM [9-10]. The microorganisms which were generally existing in the flora of vaginal, comprising group E. coli, Staph. aurous and B streptococci organisms and others which lead to bacterial vaginosis, secrete proteases & damage collagen then the amniotic membranes resulting in PROM [11-12]. Intrauterine infection had proved for up to (65%) from patients [9-10].

Additional modern information advocate several attack amniotic sac from the bloodstream after dissemination from distant places, e.g. from the gastrointestinal tract [13]. The single greatest threat to infant with preterm delivery, PPROM and PROM is respiratory distress syndrome (RDS). Prematurity is concomitant with approximately 60% of perinatal mortality [14]. There is increased incidence of perinatal mortality which may be owing to RDS, sepsis and asphyxia. Other reasons of death were cord accidents, intracranial hemorrhage and necrotizing enterocolitis. Incompetent blood brain barrier creates them more predisposed to brain damage. Since preventive methods can prevent the above phenomena, taking these facts into our attention.

However, several species of bacteria colonize both reproductive and gastrointestinal tracts, and have ability to colonize of the vagina and cause infections [15]. There are several types of antibiotics which include Ciprofloxacin, Norfloxacin, Gentamycin, Ampicillin, Cotrimoxazole, Amoxyccillin, Tetracyclin, Erythromycin and Clindamycin, these antibiotics have different mode of activity against vaginal infected bacteria and these bacteria include E. coli, S. aureus, Pseudomonas, Enterobacter, Klebsiella, Proteus and Group B streptococci [16]. Many antimicrobials have been used against pathogenic bacterial vaginosis, such as Metronidazole types and/or Clindamycin [17].

**Materials and Method**

A. The Patients:

The 100 pregnant women have been collected through the period from Jan/2018 to Sep/2018 at Public Teaching Babylon Hospital of Maternity and Children Babylon Province. Were 50 with vaginal infection (as patients) and 50 were no (as controls).

B. Identification of the pathogenic Microorganisms:

The swabs of vagina have been taken from control and patients groups, then send to the microbial unit for identification of the pathogenic microorganism. This were involved growing of swabs microorganisms on several type of the selective and differential media, as well as through use of several biochemical tests.

C. Detection of the antibiotics susceptibility:

Detection antibiotics activity against pathogenic microorganisms which were isolated from vaginal swabs were carry out on the Moller Hinton Agar medium through the use of several type of the antibiotics. This method involved incubated of the pathogenic microorganisms which were isolated from vaginal swabs with antibiotics discs on this medium and then measure the inhibition zone after 24 h.

D. Statistical analysis:

The results of this study were analyzed through the use of the SPSS version 20 program. This used for calculated the Mean, Standard Error, Standard Deviation and determine the least significant difference (LSD) between the study groups.

**Results**

Table (1) shows a significant elevation of the fetal weight in the patients women when comparing with the control ladies, whereas significant decreasing of the Maternal age and duration time in the patients ladies when comparing with the control women. Table (2) shows an increasing in the numbers of F. outcome (normal) at all three time categories in the control women. also shows an elevation in the numbers of F. outcome (RDS) at time category (0-8) hour as well as F. outcome (Sepsis) at time categories (9-17) and (18-26) hour in the patients women. Table (3) shows a number of the pathogenic microorganism isolates were identified in the infected vagina of the patients group, as well as appeared the used antibiotics types have activity with different modes against these the pathogenic microorganism.

Figure (1) shows an elevation in the percentages of C/S and PROM in patients when which comparing with control. Figure (2) shows a percent of the pathogenic microorganism isolates were identified in the infected vagina of the patients group, the largest number and percent were equal to (85) and (34%) respectively, while the smallest number and percent were equal to (3) and (2%) respectively, of Staph., Proteus and Bacteriod, in summation of the all disease
cases. And figure (3) shows the used antibiotics types have activity with different modes of action against pathogenic microorganism.

**Table 1. Significant differences and comparison between Fetal Wt., M. age., G A and Duration time of the Patients and Controls Women**

<table>
<thead>
<tr>
<th>Case Study</th>
<th>Study Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>Mean Differences</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Wt. (gm.)</td>
<td>Patients</td>
<td>2214.000</td>
<td>796.807</td>
<td>112.685</td>
<td>263.000*</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2477.000</td>
<td>829.199</td>
<td>117.266</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. age (years)</td>
<td>Patients</td>
<td>26.300</td>
<td>4.682</td>
<td>0.662</td>
<td>3.640*</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>29.940</td>
<td>4.752</td>
<td>0.672</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G A (wks)</td>
<td>Patients</td>
<td>33.160</td>
<td>4.127</td>
<td>0.583</td>
<td>1.700</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>34.860</td>
<td>3.692</td>
<td>0.522</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (hrs.)</td>
<td>Patients</td>
<td>8.120</td>
<td>7.397</td>
<td>1.046</td>
<td>5.740*</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>13.860</td>
<td>4.993</td>
<td>0.706</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant differences according to LSD system at P-Value equal to 0.05

Figure 1. Percentage of the C/S, NVD, PROM and PPROM in Patients Women as well as Normal Women.
### Table 2: Comparison between the number of the control and patients women according to duration time.

<table>
<thead>
<tr>
<th>Cases</th>
<th>women groups</th>
<th>Number of women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(0 - 8) hrs.</td>
</tr>
<tr>
<td>F. Outcome (Dead)</td>
<td>Control</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>2</td>
</tr>
<tr>
<td>F. Outcome (Sever Prem)</td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>3</td>
</tr>
<tr>
<td>F. Outcome (Normal)</td>
<td>Control</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>16</td>
</tr>
<tr>
<td>F. Outcome (RDS)</td>
<td>Control</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>20</td>
</tr>
<tr>
<td>F. Outcome (Sepsis)</td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3: Number of pathogenic microorganisms type associated with each disease states in Patients Women and antibiotic Susceptibility modes.

<table>
<thead>
<tr>
<th>Diseased state</th>
<th>Number of present microorganism types</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCOS</td>
<td>Staph.</td>
</tr>
<tr>
<td>F. Outcome</td>
<td>RDS</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>S.P.</td>
</tr>
<tr>
<td></td>
<td>Sepsis</td>
</tr>
<tr>
<td></td>
<td>Dead</td>
</tr>
<tr>
<td>Prev. Prate</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>RM</td>
<td>PROM</td>
</tr>
<tr>
<td></td>
<td>PPROM</td>
</tr>
<tr>
<td>Parity</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Mode</td>
<td>NVD</td>
</tr>
<tr>
<td></td>
<td>C/S</td>
</tr>
<tr>
<td>Antibiotics Sensitivity</td>
<td>Cifixum</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
</tr>
<tr>
<td></td>
<td>Clotrimazol</td>
</tr>
<tr>
<td></td>
<td>Metronid</td>
</tr>
</tbody>
</table>
Discussion

In this study we performed vaginal swabs of the pregnant ladies with PPROM and PROM then send to the microbiological unit lab for detection of the vaginal pathogenic microorganisms and their antibiotic sensitivity were assessed. Found the more prevalence pathogenic microorganisms were associated with virginal infection are CONS then candida and more less prevalence pathogenic microorganisms are Staph., Proteus and Bacteriod. In addition, the neonatal outcome were identified. There is good connection between micro flora of the lower genital tract and organism developed in liquor or blood of neonates with early onset sepsis. In reviews study of the McDonald et al (18) and Das et al (19), who showed that the infection was three times more common in pregnant women patients with rupture of membranes before 37 weeks of gestation than when fetal
membranes ruptured at term. In our study early rupture membrane before labor higher in patient term group (72%) while lesser in patient preterm group (58%). The (60%) of the study group had high vaginal infection in preterm pregnant while (72%) of the study group had high vaginal infection in term pregnant that may explain the higher percentage in PROM group. The study was done by Sharma who demonstrated the E. coli bacteria were the most commonest pathogenic microorganism isolated (20). And the studies of the Das et al (19) (44%), Raunt et al (21). Whereas the study of the Hussein who found the Proteus spp. and Enterobacter spp. were more causes of the UTI Infection (22). In our study the CONS which the most commonest bacteria were isolated with the number and percent of the isolates were 17 (34%) respectively, and second most common microorganism isolated was Candida albicans with the number and percent of the isolates were 11 (22%) respectively. In a study done by Lanier et al, the incidence of chorioamnionitis after PROM is 20% (23).

In the present study there was no case of clinical chorioamnionitis because we gave short trial for labor for pregnant with rupture membrane to prevent such complication so take rapid decision of interventions i.e all the patients in the study were given prophylactic antibiotics and decision for termination of pregnancy whether by C/S or vaginal deliveries was taken rapidly before such complication developed were i.e. within 24 hrs from the onset of PROM, PPROM). A report done by Swati, who observed that neonatal sepsis was present in 25% of the cases (24).

Conclusions

The fetal weight, maternal age and duration time were more in non vaginal infected pregnant women than vaginal infected pregnant women. C/S and PROM were more in vaginal infected pregnant women than non vaginal infected pregnant women, whereas the NVD and PPROM were more in non vaginal infected pregnant women than vaginal infected pregnant women. The NCOS and Candida were more pathogenic microorganisms associated with the vaginal infections in pregnant women. The Cifixum, Ampicillin, Clotrimazol and Metronid were appropriate antibiotics used for treatment of the vaginal infections, in addition to the ciprofloxacin, gentamicin, chloramphenicol group, taxim and gentamicin but we don’t depend on these antibiotics because them causes of the teratogenicity through pregnancy period.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References


Criminal Responsibility among Murderer Presented to Forensic Committee in Al-Rashad Training Hospital/Forensic Department, 2016-2017

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1Forensic Department, AL-Rashad Training Hospital, Baghdad, Iraq

Abstract

Background: The insanity defense, also known as the mental disorder defense, is a defense by excuse in a criminal case, arguing that the defendant is not responsible for his or her actions due to an episodic or persistent psychiatric disease at the time of the criminal act. This is contrasted with an excuse of provocation, in which defendant is responsible, but the responsibility is lessened due to a temporary mental state. The aim of current study was to find out the results of criminal responsibility assessment for the murderers and related mental illness as well as to study a sociodemographic and personal criterion for murderers. Methods: The study was conducted in AL-Rashad Hospital, forensic department in Baghdad. It was a descriptive retrospective study. Case files of (70) murderers, examined by the forensic committee during the period from 1st January 2016 till 31st December 2017, were studied. Comprehensive data were collected and analyzed by the SPSS version 23. Results: The study showed that murderers were mainly young adults within age group (20-40) years that were married, illiterate/low educational level and had no job or unstable type of jobs. About 30% of victims were 1st degree relative and 30% of them were family in law. The main weapon was used in the murder was gun (45.7%) and knife (40%). According to murderers who presented to the committee, about 38.6% had no mental illness, 17.1% had personality disorder, 32.9% had psychotic disorders and 8.6% had neurotic disorders. The majority of murderers (52.9%) presented to committee were considered responsible, 8.6% had partial responsibility and 28.6% considered irresponsible. Conclusions: This study showed that about more than half of murderers presented had full criminal responsibility and insanity defenses were succeeded only in one third of the cases. The studied murders appeared to be obvious among young adult age groups, with no job or unstable financial resources, and illiterate or had low educational level. The victims were more likely to be from 1st degree relatives or family in-law. The available weapon (gun and knife) was mostly used.

Keywords: murderer, criminal responsibility, mental illness, forensic psychiatric committee.

Introduction

The concept of defense by insanity has existed since ancient Greece and Rome. The first known recognition of insanity as a defense to criminal charges was recorded in a 1581 English legal treatise stating that “If a madman or a natural fool, or a lunatic in the time of his lunacy” kills someone, they cannot be held accountable. The British courts came up with the “wild beast” test in the 18th Century in which defendants were not to be convicted if they understood the crime no better than “an infant, a brute, or a wild beast (1). The M’Naghten test was the product of an English judicial commission created in response to another controversial insanity verdict. It required the defendant to prove that he or she was “laboring under such a defect of reason, from disease of the mind, as not to know the nature and quality of the act he was doing; or, if he did know it, that he did not know he was doing what was wrong.” Notwithstanding criticism of the rule’s exclusive reliance on cognitive incapacity, it was quickly adopted in most American jurisdictions. At least before the latter part of the 20th Century, reform efforts were largely directed at softening the rigid cognitive test, with alternatives such as the Durham product test when
the “unlawful act was the product of mental disease or defect (Durham v.U.S., 1954) and the American Law Institute’s proposal to exculpate a defendant who “lacks substantial capacity to either appreciate the criminality of his conduct or to conform his conduct to requirements of law (2).

The aim of current study was to find out the results of criminal responsibility assessment for the murderers and related mental illness as well as to study a sociodemographic and personal criterion for murderers.

Materials and Method

A descriptive retrospective study was conducted in the forensic psychiatry department at AL-Rashad Hospital in Baghdad, which is the only hospital in Iraq for psychiatric diseases. Clinical data was collected from records of forensic psychiatric committee (which usually joins twice weekly) for the period from 1st January 2016 to 31st December 2017 for all murder cases which were 70 cases.

Comprehensive data was collected about the murderers’ sociodemographic criteria (as age, sex, marital status, education and occupation) and data about crimes (as weapons used and degree of relationship with victim). The murderers were divided into 4 age groups (<20yr, 20-40yr, 40-60yr and >60yr). The victims were divided according to the degree of relationship with murderers into (1st degree, 2nd degree or 3rd degree relatives, or family in-law and foreign) (14).

Clinical data and psychiatric diagnosis by the committee were considered in this study. The courts asked forensic psychiatrists to evaluate the psychiatric disorder, criminal responsibility and if there was a need for mandatory treatment of the murderers. Criminal responsibility decision falls into four categories; ● full responsibility, ● partial responsibility, ● irresponsibility and ● no decision (due to long period between the murder and time of presentation and no clear information of mental state at time of murder).

The assessments by the committee are a duty of a team of three board certified psychiatrists who perform psychiatric diagnostic interviews, mental and physical examinations, psychological assessments, routine laboratory workups and, sometimes, brain-imaging studies. The diagnosis was made according to DSM-5 criteria.

Results

Sociodemographic data

Seventy murderers presented to forensic committee, their age ranged from 14 to 76 years (mean age = 36.39 years, STD=14.095).

Table (1) Socio demographical data of the studied sample

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>63</td>
<td>90.0</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Age/yr</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 (adolescent)</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>20-40 (early adulthood)</td>
<td>39</td>
<td>55.7</td>
</tr>
<tr>
<td>40-60 (middle adulthood)</td>
<td>18</td>
<td>25.7</td>
</tr>
<tr>
<td>&gt;60 (late adulthood)</td>
<td>7</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>21</td>
<td>30.0</td>
</tr>
<tr>
<td>Married</td>
<td>45</td>
<td>64.3</td>
</tr>
<tr>
<td>Divorced</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Employment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idle</td>
<td>17</td>
<td>24.3</td>
</tr>
<tr>
<td>House wife</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>Self-employed</td>
<td>30</td>
<td>42.9</td>
</tr>
<tr>
<td>Military</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>Retired</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Employed</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>24</td>
<td>34.3</td>
</tr>
<tr>
<td>Primary school</td>
<td>26</td>
<td>37.1</td>
</tr>
<tr>
<td>Secondary school</td>
<td>12</td>
<td>17.1</td>
</tr>
<tr>
<td>Diploma</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td>Bachelor degree</td>
<td>5</td>
<td>7.1</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table (1) showed that about 55.7% of murderers were in early adulthood (20-40 years old), 90% were males, 64.3% were married, the majority of them were unemployed or have unstable type of work and majority of them were illiterate or just attended primary school.

**Table (2) Relationship between victims and murderers**

<table>
<thead>
<tr>
<th>Relation of victims to murderers</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st degree relative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>6</td>
<td>8.6</td>
<td>30%</td>
</tr>
<tr>
<td>Mother</td>
<td>2</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Brother</td>
<td>10</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td>2</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Daughter</td>
<td>1</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>2nd degree relative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncle</td>
<td>5</td>
<td>7.1</td>
<td>8.6%</td>
</tr>
<tr>
<td>Nephew</td>
<td>1</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>3rd degree</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cousin</td>
<td>3</td>
<td>4.3</td>
<td>4.3%</td>
</tr>
<tr>
<td>Family in-law</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Husband</td>
<td>3</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Wife</td>
<td>12</td>
<td>17.1</td>
<td>30%</td>
</tr>
<tr>
<td>Ex-wife</td>
<td>1</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Mother in-law</td>
<td>1</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Sister in-law</td>
<td>1</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Brother in-law</td>
<td>3</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Foreign</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foreigner</td>
<td>17</td>
<td>24.3</td>
<td>27.1%</td>
</tr>
<tr>
<td>Neighbor</td>
<td>2</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table (2) showed that about 73% of victims were family relatives with murderer (30% were 1st degree family relatives and 30% were family in-law).

**Table (3) Weapons used in murders**

<table>
<thead>
<tr>
<th>weapons used in murders</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strangulation</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Knife</td>
<td>28</td>
<td>40.0</td>
</tr>
<tr>
<td>Gun</td>
<td>32</td>
<td>45.7</td>
</tr>
<tr>
<td>Cooperate with other</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Hit on head</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td>Drowning</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Burning</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (3) showed that the main weapons used in murders were guns (45.7%) and knives (40%).
### Table (4) Psychiatric illnesses of the murderers diagnosed by forensic committee

<table>
<thead>
<tr>
<th>Psychiatric illnesses</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mental illness</td>
<td>27</td>
<td>38.6%</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>22</td>
<td>31.4%</td>
</tr>
<tr>
<td>Personality disorder</td>
<td>10</td>
<td>14.3%</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>2</td>
<td>2.9%</td>
</tr>
<tr>
<td>Depression</td>
<td>5</td>
<td>7.1%</td>
</tr>
<tr>
<td>Delusional disorder</td>
<td>1</td>
<td>1.4%</td>
</tr>
<tr>
<td>Borderline PD</td>
<td>2</td>
<td>2.9%</td>
</tr>
<tr>
<td>PTSD / Dissociative</td>
<td>1</td>
<td>1.4%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>70</strong></td>
<td><strong>100.0%</strong></td>
</tr>
</tbody>
</table>

Table (4) showed that the majority of murderers had no mental illness (38.6%), while (14.3%) were diagnosed with personality disorders and about (33%) were diagnosed with psychotic disorders (schizophrenia/ delusional disorder).

### Table (5) Relationship between responsibility and sociodemographic state

<table>
<thead>
<tr>
<th>Responsible</th>
<th>Partial responsibility</th>
<th>Irresponsible</th>
<th>No decision</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex Male</td>
<td>31 5 20 7</td>
<td></td>
<td></td>
<td>63</td>
<td>0.063</td>
</tr>
<tr>
<td>Female</td>
<td>6 1 0 0</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Age &lt;20 yr</td>
<td>5 0 1 0</td>
<td></td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>20-40 yr</td>
<td>22 3 10 4</td>
<td></td>
<td></td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>40-60 yr</td>
<td>9 1 6 2</td>
<td></td>
<td></td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>&gt;60 yr</td>
<td>1 2 3 1</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58 9 20 15</strong></td>
<td></td>
<td></td>
<td><strong>92</strong></td>
<td></td>
</tr>
</tbody>
</table>

Age group/yr

For the variable "Age group/yr," the p-value is 0.395.
Table 5 showed that female and younger age murderers were more likely to be responsible for their crimes.

### Table 6  Relationship between responsibility and sociodemographic state

<table>
<thead>
<tr>
<th>Responsible</th>
<th>Responsibility</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Partial responsibility</td>
<td>Irresponsible</td>
<td>No decision</td>
</tr>
<tr>
<td>Education</td>
<td>Illiterate</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54.2%</td>
<td>8.3%</td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>69.2%</td>
<td>7.7%</td>
</tr>
<tr>
<td></td>
<td>Secondary school</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.0%</td>
<td>8.3%</td>
</tr>
<tr>
<td></td>
<td>High education</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37.5%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Occupation</td>
<td>Idle</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41.2%</td>
<td>11.8%</td>
</tr>
<tr>
<td></td>
<td>House-wife</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>83.3%</td>
<td>16.7%</td>
</tr>
<tr>
<td></td>
<td>Self-employed</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>Military</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.0%</td>
<td>16.7%</td>
</tr>
<tr>
<td></td>
<td>Retired</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0%</td>
<td>50.0%</td>
</tr>
<tr>
<td></td>
<td>Employed</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57.1%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Table 6 showed that murderers who had lower level of education and unstable type of job (self-employed) were more likely to be responsible for their crimes.
Discussion

In reviewing the results of this study, we found that murderers had mean age of about 36 years and the young adults were in their 3rd to 4th decade of life (20-40 years old) mostly had committed a murder (55.7%), while adolescents and elderly people were less likely to involve in murders. This result was compatible with the study of (3) who found that the mean age of murderers was 34 years.

Gender had a significant relation with committing murder because most of murderers (90%) were men this is probably related to physical ability difference and weapons accessibility with making attention to the basic difference between males and females in dealing with quarrel due to social and biological aspects. This result was compatible with study done by (4) who found that about 87.7% of murder defendants were males.

Regarding marital status, most of murderers were married (64.3%), this result was not compatible with many national studies and literature which showed that most of murderers had not been involved in long-term relationships or were not married (9,12). This may be related to our society differences when marriage and its continuation do not guarantee stable relationship or stable type of life because our society forces people to get married and continue their relation regardless of how the situation is bad. So, this factor may be misleading when we put in our mind that data collected reported the official type of relation instead of its quality (5).

We noticed that those who were unemployed (24.3%) or had unstable type of jobs (self-employed; 42.9%) were the main part of the sample. That may be related to financial difficulties which lead to unstable type of life with multiple problems and crises which lead to involvement in such criminal acts. This result was compatible with other national studies and literature which showed that most of the murderers were unemployed at time of arrest (1,8).

Regarding educational level, those who were illiterate or had low educational level (34.3% were illiterate and 37.1% had just attended primary school) are more likely to commit murder, that may be related to type of life which make those who have low educational level more difficult to have stable type of job and good financial income so that make them more prone to fall in difficulties and problems. Also, we must put in mind those who can’t precede in education more likely to due to their lower IQ level so that makes them more impulsive and less aware about legal consequences when they drive to do such act. This result was also compatible with study done by (4) who found that most of murder defendants had low educational level. Also, this result was compatible with study from USA (6) who found that schooling significantly reduces the probability of incarceration. In addition, they found that arrest and crime reduction are associated with high school graduation (6).

According to the victims, the majority of them were 1st degree relatives (30%) (the brothers were about half of cases in this group) or family in-law (30%) (The wives were more than half of cases in this group). These findings could be due to high likelihood for murderers to be involved in quarrel with those who have close relationship with them and it is easier for murderers to commit such crime to the persons who live with them in the same house. These results were compatible with literatures and studies which showed that people are more likely to commit crimes against those they know, and the spouses are the principal victims (7).

The main weapons used in murders were guns (45.7%) and knives (40%). That may be due to accessibility of guns and knives especially in Iraqi society. Also, such weapons have more success rate in committing complete murder. This result was compatible with most common methods used to murder in USA which put the firearm as the 1st method followed by cutting/piercing as a 2nd method (8).

Moreover, we found that 38.6% of murderers had no mental illness, 17.1% were diagnosed as having personality disorders, 32.9% were diagnosed as having psychotic disorders and 8.6% were diagnosed with neurotic disorders. This finding was also compatible with (4) who reported that 30% of murder defendant files noted a DSM-IV Axis I disorder and 28% of defendants were reported to have an Axis II disorder, whereas 38% had no psychiatric diagnosis (9-11).

Regarding the sociodemographic state and its relation to criminal responsibility, we found that females are more likely to be considered responsible about their murders, but we put in our mind that female sample is not enough to consider this result ($P$ value = 0.063).

Also, we found that those who had younger age more likely to be considered as responsible for their crimes even this result was not significant statistically ($P$
value = 0.395), but that can be explained by that those who became older in age are less likely to commit such crimes or act in impulsive way without complaint of specific illness.

There was no significant relation between marital status and criminal responsibility ($P$ value = 0.727)

Also, there was no statistically significant relation between educational level and criminal responsibility ($P$ value = 0.478) even results showed that those who were illtreat, or had low educational level, were more likely to be considered responsible.

Conclusions

This study showed that about more than half of murderers presented had full criminal responsibility and insanity defenses were succeeded only in one third of the cases. The studied murders appeared to be obvious among young adult age groups, with no job or unstable financial resources, and illiterate or had low educational level. The victims were more likely to be from 1st degree relatives or family in-law. The available weapon (gun and knife) was mostly used.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

Funding: Self-funding.

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The Effect of Doubled Concentration of Nanoparticles with Gamma-Rays Energy as Simulation Radiotherapy in Lung Cancer

Shihab Ahmed Jasim¹, Nihad A. Saleh²

¹Department of Physics, College of Science/ Kufa University, Iraq

Abstract

Background: Nanoparticles are considered main mediators to improve the efficacy of radiotherapy. By their nano size, they can penetrate the cellular environment and concentrate inside it. Nanoparticles own high cross-section and energy mass attenuation coefficient which means that they have high energy absorption. This study focused on decreasing the irradiation dose required to kill malignant cells by direct injection of NPs inside tumor cells in order to increase the amount of absorbed dose by adding a doubled increased concentration of (zinc and tellurium) NPs in tumor cells with minimum injury to the surrounding healthy tissue and increasing the number of destroyed cancer cells. Method: The theoretical basis for conducting current study was that to increase organ tolerance for radiation dose absorption, the injected particles inside the tumor should have high radiation dose absorption inside tumor. Results: There was increasing apoptosis of cancer cells and decreasing malignant cells survivor in low dose in comparison with high dose without NPs. The number of dead cancerous cells was increasing with doubling increase of NPs which depends on cross-section that is usually depending on high mass energy absorption coefficient and type and amount of energy. Conclusion: Zinc and tellurium nanoparticles can potentiate the effect of radiotherapy on lung tumor cells through increasing number of destroyed malignant cells which depended on atomic number and cross-section.

Keywords: lung cancer, high energy gamma ray, absorbed dose, Zn NPs, Te NPs.

Introduction

According to World Health Organization, the International Agency for Research on Cancer, the number of cancer death is 6.7 million and there are 24.6 million people are still successful with cancer. The highest public cancers are lung (1.35 million) [1].

The radiobiology and radiation injury to the lungs has been described by several authors. The lung is the most radiosensitive organ in the thorax, because lung tissue has a lower density than other soft tissue. A nominal 8Gy corresponds to doses 8-15% higher to lung tissue using cobalt-60 gamma rays. Hence, 8Gy becomes 8.6-9.2 GY (cobalt-60). The earliest signs of radiation injury in the lungs are oedema and changes in blood circulation followed by pneumonitis and pronounced respiratory insufficiency, which appears after a latent period of 1-3 months after doses greater than about 8Gy [2].

The effect of radiation therapy on cancer occurs when applying high energy rays using the technology of nanoparticles [3]. The radiotherapy does not always give the required efficiency to treat tumor cells, because radiation sensitivity of tumor cells is limited, therefore; it appears necessary to increase the concentration of radiation within the ionizing zone and increase the Sensitivity Enhancement Ratio (SER), because of difficult treatment in the area and the limited tolerance dose of the organ [4].

The effective dose of radiation involves use of high energy rays and the concentration of ionizing radiation in the tumor within the dose. Sometimes, this method has limited effect on the treatment process [5].

The Direct injection technique of nanoparticles (NPs), such as ZnNPs and TeNPs in the tumor has some practical advantages such as to focuses the radiation within the desired area and increase the absorbed dose in
the tumor and to avoid surrounding healthy tissue from exposure to unnecessary radiation [6].

Radiotherapy involves the use high Gamma-energy (ionizing radiations) that lead to death or shrinkage of cancer cells, but this interaction causes injury to the enveloping healthy soft tissue. To create greater damage for malignant cells with fewer damage to healthy cells nearby the malignant tumor, we need to increase the organ tolerance for radiation dose absorption inside the tumor by injecting particles that have high radiation dose absorption inside tumor [7]. The choice of such material is for several reasons; it is easy manufactured as nano materials, possesses thermal stability, do not interact with organ tissue and do not have toxic effects [8].

The interaction of electrons and photons within malignant cells creates free radicals that lead to the damage of malignant cells [9,10].

Thus, the greater quantity of absorption of ionizing radiation has better formation of toxic molecules and so increased malignant cells damage. The injection inside cancer cells works on directing specific cellular components within the tumor tissue leading to severe damage at the injection site without affecting nearby health soft tissue [11,12].

The interaction between ionizing radiation with water inside the cell results in formation of free radicals. The accumulation of free radicals leads to formation of toxic molecules. These toxic molecules work cause destruction of cancer cells. Since the production of free radicals within the tumor is larger than in healthy tissue surrounds the tumor, the destruction inside tumor is larger [13].

The biological effect of ionizing radiation depends on absorbed dose, the energy of gamma rays radiation and organs irradiated. When photons enters to the body they lose energy and are finally absorbed, but also they give rise to new photons by multiple scattering. The magnitude of this new effective photon can be estimated by buildup factor which depends on atomic number of absorbing medium, energy of gamma rays and the penetration depth in addition to the shape of the radiation source and the medium [14].

The tables of mass attenuation coefficients and the mass energy absorption coefficients for 40 elements and 45 mixtures and compounds over energy range from 1keV to 20MeV. These tables, although widely used, should now be replaced by the Hubbell and Seltzer tabulation for all elements (Z=1-92) and 48 additional substances for diametric interest. The mass attenuation coefficient, a number of related parameters can be derived, such as mass energy absorption coefficient, total interaction cross-section, molar extinction coefficient, effective atomic number and the effective electron density [15].

The vascularity of malignant tissue is higher than surrounding healthy tissue so that when NPs are injected, they diffuse to the malignant tissue more than healthy tissue so that the absorption of ionizing radiation dose inside tumor become greater due to the presence of NPs.

This study focused on decreasing the irradiation dose required to kill malignant cells by direct injection of NPs inside tumor cells in order to increase the amount of absorbed dose by adding a doubled increased concentration of (zinc and tellurium) NPs in tumor cells with minimum injury to the surrounding healthy tissue and increasing the number of destroyed cancer cells.

**Methodology**

The theoretical basis for conducting current study was that to increase organ tolerance for radiation dose absorption, the injected particles inside the tumor should have high radiation dose absorption inside tumor [16,17].

The ionizing either causes destruction or shrinkage of cancer cells, but it may cause damage to surrounding health tissue, therefore; radiologist needs to find a way to produce maximum destruction in malignant cells with minimum damage to healthy cells surrounding the tumor [18].

Nanoparticles with high energy radiation interaction lead to ensure the production of electron and positron inside tumor which increase the ionization process inside tumor then lead to increase production of free radicals and toxic molecules. As a consequence, accumulation of reaction products leads to cancer cells death [19,20].

The total cross-section for lung with presence of nanoparticles as contrast agent inside lung equals sum of two cross-sections [21]:

\[
\text{Number of survival cells after irradiation} = \text{Number of initial cells before irradiation} \times \text{relative effectiveness}
\]

Where: Number of survival cells after irradiation. : Number of initial cells before irradiation and ( ) relative effectiveness [26].
The total cross-section for lung with presence of nanoparticles as contrast agent inside lung equals sum of two cross-sections $^{[21]}$:

$$\sigma_{total} = \sigma_{lung} + \sigma_{NPs} \quad \text{.................. (1)}$$

Where $\sigma_{total}$, $\sigma_{lung}$ and $\sigma_{NPs}$ equals to lung and nanoparticles cross-sections.

The correlation between cross-section ($\sigma$) and mass energy absorption coefficient ($\mu_{en}/\rho$) can be explained in the following equation $^{[22]}$:

$$\mu_{en}/\rho = \sum_i w_i \left( \frac{N_A \sigma_i}{A} \right) \quad \text{.................. (2)}$$

Where $N_A$: Avogadro's number, $A$: Mass number and $w$: The fraction by weight of the organ with added nanomaterial per volume (μg/ml).

We can modify the equations as follows:

$$\left( \frac{\mu_{en}}{\rho} \right)_{total} = \left( \frac{\mu_{en}}{\rho} \right)_{lung} + \left( \frac{\mu_{en}}{\rho} \right)_{NPs} \quad \text{.................. (3)}$$

Where $\left( \frac{\mu_{en}}{\rho} \right)_{total}$, $\left( \frac{\mu_{en}}{\rho} \right)_{lung}$ and $\left( \frac{\mu_{en}}{\rho} \right)_{NPs}$: Total Lung and Nanoparticles mass energy absorption coefficient.

The exposure rate constant($\Gamma$) relates activity of a point isotropic radiation source to the exposure rate in air at a given distance $^{[23]}$:

$$X = \Gamma \frac{A}{d^2} \quad \text{.................. (4)}$$

Where $A$ is the source activity, $(d)$ is distance from the source.

The equation (7) modification for a lung without and with nanoparticles that adding by direct injection technique with double increase concentrations. Each NP material interacts with extent of radiation falling energy ranging from 1 to 20Mev adding nanoparticles (zinc and tellurium) get from the National Institute of Standards and Technology (NIST2004) $^{[27]}$.

**Results and Discussion**

The irradiation equations applied on lung malignant tumor with usage of Zinc and Tellurium nanoparticles were shown in Figures (1), there was increasing apoptosis of cancer cells and decreasing malignant cells survivor in low dose in comparison with high dose without NPs. The number of dead cancerous cells was increasing with doubling increase of NPs which depends on cross-section that is usually depending on high mass energy absorption coefficient and type and amount of energy.

We noticed that at dose of 5Gy there was an increase in number of living cancer cells because of lowering the cross-section and decrease mass energy absorption coefficient in comparison with the non-injectable cells by NPs. The increasing concentration of NPs gives a possible decrease of irradiation dose with same number of killed malignant cells.

Figures (2) and (3) showed that the number of living cells was less with the use of Tellurium ($Z=52$) which has high cross-section with high atomic number in comparison to Zinc ($Z=30$) at dose of 4Gy. Thus, led to an increase in the number of destroyed cells and decreasing in the number of surviving cancer cells.
Conclusions

Zinc and tellurium nanoparticles can potentiate the effect of radiotherapy on lung tumor cells through increasing number of destroyed malignant cells which depended on atomic number and cross-section.

Therefore, the interaction of Gamma-rays will be concentrated into the tumor without affecting healthy tissue that surrounds the tumor. This means that the absorption of radiation dose will be concentrated into the tumor without the surrounding healthy tissue. High energy Gamma-ray helps increase free radicals that produce interaction with water molecules inside cells.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

Funding: Self-funding.

References


Evaluation the Levels of Thyroid Hormones among Iraqi Pregnant Women

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Abstract

The present study was carried out to evaluate the levels of thyroid hormones among pregnant Iraqi women according to the age. The study group comprised of (140) of full term pregnant women scheduled follow up the alterations of thyroid hormones, the control group included (50) of healthy women volunteers. Serum concentrations levels of T3, T4 and (TSH) were estimated using (ELFA) technique. In the study groups, blood samples were obtained from various ages pregnant women. Range age of the study groups was (20-40) years. In the control group: serum T3 and T4 levels were decreased slightly with increasing age respectively, while TSH levels were increased slightly with increasing age without significant difference P>0.05 and the values were within the normal range. In the hyperthyroidism which included (45) patients women, there were continuously higher in concentration levels of T3, and T4 than that in the control group, but TSH concentration decreased with highly a significant difference P<0.01. While, in hypothyroidism which included (45) patient women, the concentrations of T3 and T4 were lower than that in the control group, but TSH increased with a highly significant difference P<0.05.

Keywords: Thyroid Hormones; Pregnant Women; Enzyme Linked Fluorescent Assay; IRAQ.

Introduction

Female reproduction system is negatively manipulated by both hyper and hypothyroidism, also female infertility is adversely affected thyrotoxicosis. The thyroid gland makes two thyroid hormones thyroxine (T4) and triiodothyronine (T3) which is the active hormone and is made from T4, (1). Thyroid hormones production is regulated by thyroid stimulating hormone (TSH) which is made by pituitary gland in the brain, (1,2). When the thyroid hormones concentrations levels are low in the blood the pituitary releases more TSH and when the levels in the blood are high the pituitary respond by decreases TSH production. The thyroid functions normally, if (TSH) (T3) (T4) are all normal through pregnancy (1,3). The most common causes of maternal hyperthyroidism during pregnancy (80-85 %) is graves’ disease (4). The diagnosis of hyperthyroidism is based on history, physical exam and laboratory testing.

The most common cause of hypothyroidism during pregnancy is the autoimmune disorder known as Hashimoto’s thyroiditis. Untreated or in adequately treated hypothyroidism has been associated with maternal anemia (low red blood cell, count) myopathy (muscle pain, weakness), placental abnormalities, low birth weight infants and bleeding. Because of the important effects of thyroid hormones on the pregnant women and her baby, this project was done (3,5).

This study aimed to determine serum concentrations of thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH), because of their critical role during pregnancy for the health of mother and baby (hypo or hyperthyroidism), so the condition can be recognized and treated immediately before or after birth.

Materials and Method

This study was carried out during the period from June 2013 to October 2013. A group of (ninety) pregnant women with disturbances of thyroid gland hormones (forty five) for each of hypo and hyperthyroidism. (Fifty) pregnant women as a healthy control were included in this study, with age range 20-40 years. They were attending the Specialized center for Endocrinology Hospital women and children in the province of Muthana. The patients and control were grouped depending on their age to four groups (20-24).
Venous blood samples (5 ml) were collected from patients and centrifuged after clotting at 3000 rpm for 10 min for hormone determination. Patients' sera were taken by micropipettes and stored at 2-8°C in disposable tubes for up to 48 hours until assay to obtain more accurate results.

Quantitative Determination of T3 (VIDAS) Test:

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA) was used to determine total triiodothyronine (T3) in human plasma (lithium heparin). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predisposed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. The sample is taken and transferred into the well containing the T3 antigen labeled with alkaline phosphate (conjugate). Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti-T3 antibodies (sheep) coated on the interior of the SPR. Unbound components are eliminated during washing steps. During the final detection steps, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbellifereone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated.

Results are calculated automatically by the instrument in relation to the calibration curve stored in memory (4-parameter logistic model) and are expressed in nmol/l. Samples with a concentration greater than 320 nmol/l, retested after diluted by 1/2 in T4 free human serum (1 volume of sample and 1 volume of T4 free human serum) and retested in the VIDAS T4 assay. If the dilution factor has not been entered when the work list was created, multiply the result by the dilution factor to obtain the sample concentration. Interpretation of test result should be made taking into consideration the patient's history, and in association with thyroid function assessment, including at least a TSH assay.

Quantitative Determination of T4 (VIDAS) Test:

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA) was used to determine total thyroxine (T4) in human plasma (lithium heparin). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predisposed in the sealed reagent strips. All of the assay steps are performed automatically by
the instrument. The reaction medium is cycled in and out of the SPR several times. The sample is taken and transferred into the well containing the AntiTSH antibody labeled with alkaline phosphate (conjugate). The sample/conjugate mixture is cycled in and out of the SPR. The antigen binds to antibodies coated on the SPR and to the conjugate forming a “sandwich”. Unbound components are eliminated during washing steps. During the final detection steps, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically.

The TSH Results are calculated automatically by the instrument Using calibration curve which are stored by the instrument (4-parameter logistic model), the concentrations are expressed in mLU/ml (2nd IRP 80/558). Samples with TSH a concentration greater than 60 mLU/ml, must be realized after dilution in TSH diluted (R1). If the dilution factor has not been entered when the work list was created, multiply the result by the dilution factor to obtain the sample concentration. Interpretation of test result should be made taking into consideration the patient’s history, and the result of any other tests performed.

**Statistical analysis:** Data were summarized, analysed and presented using two software programs; these were the statistical package of social sciences (SPSS version 23) and Microsoft Office Excel 2010. The level of significance was considered significant at \( P < 0.05 \) and highly significant at \( P < 0.01 \).

**Results and Discussion**

Table (1) Shows that the values of T3, T4 decreased slightly with age and TSH decreased slightly with age without significant difference \( P>0.05 \) and the values were within the normal range. The above results agreed with the observation of other studies \((6,7)\). A healthy pregnant woman can adopt herself with this scenario and thyroid in pregnancy produce an extra amount of thyroid hormones on condition of a healthy thyroid gland in combination of enough iodine an essential element in the thyroid hormone structure \((8,9)\).

<table>
<thead>
<tr>
<th>Age group years</th>
<th>No.</th>
<th>T3 level(nmol/l)</th>
<th>T4 level(nmol/l)</th>
<th>TSH level(μIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20-24)</td>
<td>14</td>
<td>2.1871</td>
<td>89.9553</td>
<td>3.4724</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td>±0.08432</td>
<td>±5.67140</td>
<td>±0.53197</td>
</tr>
<tr>
<td>(25-29)</td>
<td>13</td>
<td>1.8542</td>
<td>77.5892</td>
<td>2.4117</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td>±0.06815</td>
<td>±2.90794</td>
<td>±0.24653</td>
</tr>
<tr>
<td>(30-34)</td>
<td>12</td>
<td>1.5592</td>
<td>69.2883</td>
<td>1.5675</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td>±1.1493</td>
<td>±2.23568</td>
<td>±0.20627</td>
</tr>
<tr>
<td>(35-40)</td>
<td>11</td>
<td>1.2356</td>
<td>63.1444</td>
<td>.9689</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td>±1.4063</td>
<td>±1.02542</td>
<td>±0.20925</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>1.7852</td>
<td>77.2014</td>
<td>2.3100</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td>±3.6709</td>
<td>±11.03601</td>
<td>±0.02926</td>
</tr>
</tbody>
</table>

* Normal value:T3=0.4-9nmol/l
In addition, the pregnancy is a physiological period which can be associated with various thyroid malfunctions. The serum concentration of TSH, T3, T4 are the least thyroid parameter which should be evaluated during pregnancy to have a clear picture of a healthy fetus and maternal life. The normal range for thyroid hormone during pregnancy are altered and when thyroid hormone are assessed those specific values should be taken into consideration. Pregnancy itself might have an adverse effect on thyroid function, leaving the pregnant woman with subclinical, overt hypo and hyperthyroidism. It seems that autoimmunity to the thyroid during pregnancy is the major cause for hypothyroidism in particular (10, 11).

Table (2) shows the values of T3, T4 increased with age and TSH decreased with age without significant difference P>0.05 between the first and second group, while there was significant difference P<0.05 between the first and second group with the third group and highly significant difference P<0.01 between the first and second group and the fourth group and significant difference P<0.05 between the third and the fourth group. The protein responsible for the transportation of female sex hormones, defined as Sex Hormone Binding Globulin (SHBG). The protein responsible for the transportation for female sex hormones, defined as and also the serum estrogen level concentration are elevated during hyperthyroidism. Therefore, correlation between SHBG and female sex hormones during periodical cycles and pregnancy should be taken into close consideration. The correlation between SHBG and female sex hormones is a matter under scrutiny (12, 13).

Table (2) Distribution of patients with Hyperthyroidism according to the age range and its relation with T3, T4 and TSH:

<table>
<thead>
<tr>
<th>Age group years</th>
<th>No.</th>
<th>T3 level(nmol/l)</th>
<th>T4 level(nmol/l)</th>
<th>TSH level(μIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20_24)</td>
<td>Mean</td>
<td>13</td>
<td>6.8550</td>
<td>268.7967</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.56013</td>
<td>±26.93433</td>
<td>±.00816</td>
</tr>
<tr>
<td>(25_29)</td>
<td>Mean</td>
<td>12</td>
<td>6.1317</td>
<td>187.9117</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.07111</td>
<td>±8.66305</td>
<td>±.01169</td>
</tr>
<tr>
<td>(30_34)</td>
<td>Mean</td>
<td>11</td>
<td>6.4971</td>
<td>165.0443</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.56988</td>
<td>±6.84556</td>
<td>±.01697</td>
</tr>
<tr>
<td>(35_40)</td>
<td>Mean</td>
<td>9</td>
<td>3.2863</td>
<td>132.3079</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.45297</td>
<td>±7.11519</td>
<td>±.01883</td>
</tr>
<tr>
<td>Total</td>
<td>Mean</td>
<td>45</td>
<td>4.6582</td>
<td>168.1049</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.141758</td>
<td>±46.02076</td>
<td>±.05514</td>
</tr>
</tbody>
</table>

Tables (1) and (2) showed that there was a highly significant difference P<0.01 between the values of T3, T4 and TSH in patients compared with the control group. The above results agreed with the observation of other studies, (14, 15). The most common cause (80%-85%) of maternal hyperthyroidism during pregnancy is Grave’s disease and occurs in one of 1500 pregnant, in addition to other usual causes of hyperthyroidism, very high levels of HCG, seen in severe forms of morning sickness my cause transient hyperthyroidism (16, 17). Many changes occur in thyroid function during the transition phase from the non-pregnant to the pregnant state, changes which stabilize by the end of second trimester or the onset of the third trimester. There is biochemical evidence of functional stimulation of the thyroid, such as in serum thyroglobulin levels, preferential T3 secretion increased T3/T4 ratio and slight increases in basal TSH at delivery (18, 19).

Table (3) showed that the values of T3, T4 decreased with age and TSH decreased with age. There was a highly significant difference P< 0.01 between the
first group and other groups and significant difference \( P < 0.05 \) between the second and third group with highly significant difference \( P < 0.01 \) between the second and fourth group, while there was a significant difference between the third and fourth group \( P < 0.05 \).

**Table (3)** Distribution of patients with Hypothyroidism according to the age range and its relation with T3, T4 and TSH:

<table>
<thead>
<tr>
<th>Age group years</th>
<th>No.</th>
<th>T3 level(nmol/l)</th>
<th>T4 level(nmol/l)</th>
<th>TSH level(( \mu )IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20_24)</td>
<td>Mean 13</td>
<td>.9150</td>
<td>51.6200</td>
<td>58.7650</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.02121</td>
<td>±.38184</td>
<td>±.33234</td>
</tr>
<tr>
<td>(25_29)</td>
<td>Mean 12</td>
<td>.6950</td>
<td>43.3550</td>
<td>52.1550</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.04359</td>
<td>±4.13330</td>
<td>±3.04706</td>
</tr>
<tr>
<td>(30_34)</td>
<td>Mean 11</td>
<td>.4925</td>
<td>31.6150</td>
<td>46.1175</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.09032</td>
<td>±3.65275</td>
<td>±3.35450</td>
</tr>
<tr>
<td>(35-40)</td>
<td>Mean 9</td>
<td>.3320</td>
<td>19.4700</td>
<td>37.3500</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.03347</td>
<td>±5.50463</td>
<td>±3.15824</td>
</tr>
<tr>
<td>Total</td>
<td>Mean 45</td>
<td>.5493</td>
<td>33.3647</td>
<td>46.4913</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>±.21322</td>
<td>±12.69400</td>
<td>±8.23578</td>
</tr>
</tbody>
</table>

Table (1),(3) showed there was a highly significant difference \( P < 0.01 \) between the values of T3, T4 and TSH in patients compared with the control. The above results agreed with the observation of other studies, \(^{20-22}\). The most common cause of hypothyroidism is the autoimmune disorder known as hashimoto’s thyroiditis. Hypothyroidism can occur during pregnancy due to the initial presentation of hashimoto’s thyroiditis, in adequate treatment of a woman already known to have hypothyroidism a variety of causes, or over-treatment of a hyperthyroid woman with antithyroid medications. Approximately, 2.5% of women will have a slightly elevated TSH of greater than 6 and 0.4% will have a TSH greater than 10 during pregnancy, \(^{23-25}\). The incidence of hypothyroidism as normal cause among female is indirectly correlated with age and the prevalence of hypothyroidism in elderly female is about ten times higher compared to younger age in their twenties,\(^{26,27}\).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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Distribution of Abnormal Number of Teeth and Gender Differences: A Sample of Al-Muthanna University Students Aged 18-24 Years Old

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Abstract

Congenital or developmental missing teeth (Hypodontia) is one of important numerical anomalies of teeth. So that many subject field have been carry out in different element of the humans, to find the prevalence of hypodontia. Hyperdontic dentition are teeth that appear in plus to the regular number of teeth.

This review was planned to assess the distribution of abnormal number of teeth decreased (hypodontia), increased (hyperdontia) and gender differences.

This review was applied from 23 /10/ 2016 to 12 /4/ 2019 on 1971 (1065 males, 906 females) students in four colleges in Al-Muthanna University were College of medicine (294), College of dentistry (299), College of pharmacy (185) and college of economics (1193) were clinically examined, only (202) of them had abnormal number of teeth and subjected to orthopanto-mograms to detect the missing teeth and extra teeth. Their age was ranging from 18 to 24 years.

The abnormal number of teeth in (10.2%) students. Hypodontia in (9.18%) students. The hypodontia of maxillary lateral incisor in (34.8%) students followed by hypodontia in mandibular second premolar (30.9%) students. The hyperdontia in maxillary and mandibular teeth (1.06%) students. Mesiodens in (0.7%).

The study displays that about (10.2%) students had abnormal of teeth number. Hypodontia happens more often than hyperdonti. The foremost repeatedly absent tooth was maxillary lateral incisor and the second one was the mandibular second bicuspid. About hyperdontia, the most frequently hyperdontic tooth was Mesiodens. All of them, difference between gender was non-significant.

Keywords: Abnormal number of teeth, Gender differences, AL-Muthanna University.

Introduction

Teeth are classified into kinds according to their specific positions in the dental arch. In mammals, the difference between tooth types depend on the typical contour class, as incisors, canine, bicuspid, and grinder in human race. While difference in shape between tooth types are typical to the teeth in the same family similar each other (¹).

Supernumerary teeth are dentition formed as result of duplicating in the normal full complement as a consequence to excessiveness dental lamina in the dental arch (²).

Congenital missing of all dentition without any associated anomalies is extremely very rare; some studies found that anodontia have suggested to be autosomal recessive hereditary pattern.

Anodontia occurs as an extreme teeth phenotype in ectodermal dysplasia syndrome. In odontology, anodontia, also called anodontia vera, is a rare genetic disorder characterized by the congenital missing of all teeth (permanent or primary dentition. It is associated with other type of dermal and neural syndromes called the ectodermal dysplasia. Anodontia is usually part of a syndrome and rarely to occur as a separated syndrome (³).
Materials and Method

The Sample

Sample distribution was selected from university of Al_Muthanna students. lies south of Capital of Iraq. Age was selected depending on the last birthday giving an age from 18 years 0 months to 24 years 11 months (4).

Number of students presented in Al-Muthanna university were (13366) (Ministry of Higher education and Scientific research, Al-Muthanna University 2016). while the number of population were (842000). The minimum number should be represented of the sample was taken depending on the following equation (5):

\[
\text{The number of sample} = \frac{\text{Total number of students}}{\text{Total number of population}} \times 100000
\]

\[
= \frac{13366}{842000} \times 100000 = 1587
\]

No sort of systemic abnormality, particularly Down’s syndrome and cleft lip & palate because of delayed teeth development in such conditions (6).

Permission for this study was obtained from the Al-Muthanna University.

Methods of Examination

All students were examined clinically under day light using dental mirrors, for all the students that were not surely diagnosed as hypodontic, or hyperdontic, were subjected to orthopanto-mograms (O.P.G). A tooth was detected as congenitally absent when cannot be seen in the dental arch in the radiograph of the region and there was no history or evidence that it was accidentally missed or lost by extraction.

Their radio-graphs are obtained from orthodontic department clinics and radiographic department clinics at the College of Dentistry of Al-Muthanna University.

Statistical Analysis

The processing and analyzing of data done by using (SPSS Inc., version 25 for windows 10 and excel 2018). The most statistical methods were used in order to obtain the results include: inferential statistics, descriptive statistics, Z-test to compare between significant difference of two proportions. The following levels of significance are used:

\[ P > 0.05 \text{ NS Non-significant.} \]
\[ 0.05 \geq P > 0.01 \text{ * Significant.} \]
\[ 0.01 \geq P > 0.001 \text{ ** highly significant.} \]

Results

Distribution of abnormal number of teeth and difference between both gender as shown in (Table,1). The abnormal number of teeth presented in 202 (10.2%) students (104 (9.7%) males and 98 (10.8%) females). Hypodontia in 181 (9.18%) students (96 (9%) males and 85 (9.3%) females). Hyperdontia in 21 (1.06%) students (8 (0.75%) males and 13 (1.4%) females), difference between both gender was non-significant.

Table 1: Distribution in (%) of abnormal number of teeth and gender differences, Hypo.= Hypodontia, Hyper.= Hyperdontia.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>10.2</td>
<td>104</td>
<td>9.7</td>
</tr>
<tr>
<td>Hypo.</td>
<td>181</td>
<td>9.18</td>
<td>96</td>
<td>9</td>
</tr>
<tr>
<td>Hyper.</td>
<td>21</td>
<td>1.06</td>
<td>8</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Distribution of hypodontia in maxillary and mandibular incisor teeth and difference between both gender as shown in (Table, 2): The hypodontia in maxillary central incisor in 4 (2%) students (3 (3.1%) males and 1 (1.1%) females). Unilateral in 3 (1.6%) students (2 (2%) males and 1 (1.1%) females). Bilateral in 1 (0.5%) students (1(1.04%) males and 0(0%) females), The hypodontia in mandibular central incisor in 13 (7.1%) students (6 (6.2%) males and 7 (8.2%) females). Unilateral in 11 (6.07%) students (5 (5.2%) males and 6 (7.05%) females). Bilateral in 2 (1.1%) students (1(1.04%) males and 1(1.17%) females), The hypodontia in maxillary lateral incisor in 63 (34.8%) students (31 (32.3%) males and 32 (37.6%) females). Unilateral in 19 (10.4%) students (8(8.3%) males and 11 (12.9%) females). Bilateral 44 (42.3%) students (23(23.9%) males and 21(24.7%) females), The hypodontia in mandibular lateral incisor in 9 (4.9%) students 5 (5.2%) males and 4 (4.7%) females. Unilateral in 7 (6.07%) students (4 (4.1%) males and 3 (3.5%) females). Bilateral in 2 (1.1%) students (1(1.04%) males and 1(1.17%) females) difference between both gender

<table>
<thead>
<tr>
<th>Central incisor No.</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Max. Cen.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>4</td>
<td>2.2</td>
<td>3</td>
<td>3.1</td>
</tr>
<tr>
<td>Uni.</td>
<td>3</td>
<td>1.6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Bi.</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>T</td>
<td>13</td>
<td>7.1</td>
<td>6</td>
<td>6.2</td>
</tr>
<tr>
<td>Uni.</td>
<td>11</td>
<td>6.07</td>
<td>5</td>
<td>5.2</td>
</tr>
<tr>
<td>Bi.</td>
<td>2</td>
<td>1.1</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>Lateral incisor No.</td>
<td>Total</td>
<td>Males</td>
<td>Females</td>
<td>P sig.</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Max. Lat.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>63</td>
<td>34.8</td>
<td>31</td>
<td>32.3</td>
</tr>
<tr>
<td>Uni.</td>
<td>19</td>
<td>10.4</td>
<td>8</td>
<td>8.3</td>
</tr>
<tr>
<td>Bi.</td>
<td>44</td>
<td>24.3</td>
<td>23</td>
<td>23.9</td>
</tr>
<tr>
<td>Man. Lat.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>9</td>
<td>4.9</td>
<td>5</td>
<td>5.2</td>
</tr>
<tr>
<td>Uni.</td>
<td>7</td>
<td>3.8</td>
<td>4</td>
<td>4.1</td>
</tr>
<tr>
<td>Bi.</td>
<td>2</td>
<td>1.1</td>
<td>1</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Distribution of hypodontia in maxillary and mandibular canine and difference between both gender as shown in (Table, 3): The hypodontia in maxillary canine in 2 (1.1%) students (1 (1.04%) males and 1 (1.17%) females). Unilateral in 1 (0.5%) students (1(1.04%) males and 0 (0%) females). Bilateral in 1 (0.5%) students (0(0%) males and 1(1.17%) females), the hypodontia in mandibular canine in 3 (1.6%) students (2 (2.08%) males and 1 (1.17%) females). Unilateral in 2 (1.1%) students (1 (1.04%) males and 1 (1.17%) females). Bilateral in 1 (0.5%) students (1(1.04%) males and 0(0%) females), difference between both gender was non-significant.
Table 3: Distribution in (%) of hypodontia in maxillary and mandibular canine and gender differences, Max. Can.= maxillary canine, Man. Can.= mandibular canine T=total, Uni.=unilateral, Bi.=bilateral

<table>
<thead>
<tr>
<th>Canine No.</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Max. Can.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>2</td>
<td>1.1</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>Uni.</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>Bi.</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T</td>
<td>3</td>
<td>1.6</td>
<td>2</td>
<td>2.08</td>
</tr>
<tr>
<td>Uni.</td>
<td>2</td>
<td>1.1</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>Bi.</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Distribution of hypodontia in maxillary and mandibular premolar teeth and difference between both gender as shown in (Table 4): The hypodontia in maxillary first premolar in 5 (2.7%) students (2 (2.08%) males and 3 (3.5%) females). Unilateral in 3 (1.65%) students (1 (1.04%) males and 2 (2.3%) females). Bilateral in 2 (1.1%) students (1 (1.04%) males and 1 (1.17%) females). The hypodontia in mandibular first premolar in 3 (1.6%) students (1 (1.04%) males and 2 (2.3%) females). Unilateral in 1 (0.5%) students (0 (0%) males and 1 (1.17%) females). Bilateral in 2 (1.1%) students (1 (1.04%) males and 1 (1.17%) females). The hypodontia in maxillary second premolar in 23 (12.7%) students (11 (11.4%) males and 12 (14.1%) females). Unilateral in 17 (9.3%) students (8 (8.3%) males and 9 (10.5%) females). Bilateral in 6 (3.3%) students (3 (3.1%) males and 3 (3.5%) females). The hypodontia in mandibular second premolar in 56 (30.9%) students (29 (30.2%) males and 27 (31.7%) females). Unilateral in 41 (22.6%) students (21 (21.8%) males and 20 (23.5%) females). Bilateral in 15 (8.2%) students (8 (8.3%) males and 7 (8.2%) females. Difference between both gender was non-significant.

Table 4: Distribution (%) of hypodontia in maxillary and mandibular premolar teeth and gender differences, Max. 1<sup>st</sup> pre.= maxillary first premolar, Man. 1<sup>st</sup> pre.= mandibular first premolar. Max. 2<sup>nd</sup> pre.= maxillary second premolar, Man. 2<sup>nd</sup> pre.= mandibular second premolar. T=total, Uni.=unilateral, Bi.=bilateral

<table>
<thead>
<tr>
<th>First Premolar No.</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Max. 1&lt;sup&gt;st&lt;/sup&gt;pre.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>5</td>
<td>2.7</td>
<td>2</td>
<td>2.08</td>
</tr>
<tr>
<td>Uni.</td>
<td>3</td>
<td>1.65</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>Bi.</td>
<td>2</td>
<td>1.1</td>
<td>1</td>
<td>1.04</td>
</tr>
</tbody>
</table>
Table 4: Distribution (%) of hypodontia in maxillary and mandibular premolar teeth and gender differences. Max. 1st pre.= maxillary first premolar, Man. 1st pre.= mandibular first premolar, Max. 2nd pre.= maxillary second premolar, Man. 2nd pre.= mandibular second premolar. T = total, Uni. = unilateral, Bi. = bilateral.

<table>
<thead>
<tr>
<th>Hyperdontia</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>1.06</td>
<td>8</td>
<td>0.75</td>
</tr>
<tr>
<td>Mesiodens</td>
<td>14</td>
<td>0.7</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>Max. Lateral Incisor</td>
<td>2</td>
<td>0.1</td>
<td>1</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Distribution of hyperdontia in maxillary and mandibular teeth and difference between both gender as shown in (Table 5): The hyperdontia in maxillary and mandibular teeth in 21 (1.06%) students (8 (0.75%) males and 13 (1.4%) females). Mesiodens in 14 (0.7%) students (6 (0.5%) males and 8 (0.8%) females). The hyperdontia in maxillary lateral incisor in 2 (0.1%) students (1 (0.09%) males and 1 (0.11%) females). The hyperdontia in mandibular lateral incisor in 2 (0.1%) students (1 (0.09%) males and 1 (0.11%) females). The hyperdontia in mandibular second premolar in 2 (0.1%) students (0 (0%) males and 2 (0.22%) females). The hyperdontia in maxillary first premolar in 1 (0.05%) students (0 (0%) males and 1 (0.11%) females, difference between both gender was non-significant.

Table 5: Distribution in (%) of hyperdontia in maxillary and mandibular teeth and difference between both gender.

<table>
<thead>
<tr>
<th>Hyperdontia</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>1.06</td>
<td>8</td>
<td>0.75</td>
</tr>
<tr>
<td>Mesiodens</td>
<td>14</td>
<td>0.7</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>Max. Lateral Incisor</td>
<td>2</td>
<td>0.1</td>
<td>1</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Discussion

Prevalence of hypodontia were presented in (9.18%) students, similar with researchers like Sanchez in Argentina (7), Ravn and Nielson in Danmark (8), Mangnusson in Iceland (9), Chung et al in Korea (10) and Dastjerdi et al in Iran (11).

More than other studies including Dolder in Switzerland (12), Werther and Rothenberg in U.S.A (13), Malik in Pakistan (14), Al-Mulla et al in Iraq (15), Silverman and Ackerman in U.S.A (16), Kinan in Iraq (17) and Al-Hamdany et al in Iraq/Mosul (18). This may reflect a persistent problem in different communities with variation in intensity that result due to racial, size of the sample, type of the study, gender and age variations.

Difference between both gender was non-significant (P>0.05), it agreed with many other studies like Dolder in Switzerland (12) and Werther and Rothenberg in U.S.A (13) and in Iran Dastjerdi et al (11).

Maxillary lateral incisors (34.8%) were foremost repeatedly missing which is followed by mandibular second bicuspids (30.9%) and maxillary second bicuspids (12.7%), then mandibular central incisor (7.1%), similar to Kinan (17), Augard and Gayard (19), Al-Hamdany et al in Iraq/Mosul (18), Dastjerdi et al (11), while others as Al Mulla et al (15), Al Judo (20) and Al-Jourany TS in Baghdad/Iraq (21) showed the mandibular second bicuspids were foremost repeatedly missing which is followed by maxillary lateral incisors. This can be due to racial difference and ethnic factor in the populations who were studied.

Difference between both gender was non-significant (P>0.05), it agreed with other studies Werther and Rothenberg in U.S.A (13), in Iran Dastjerdi et al (11) and Pérez et al (22).

Prevalence of hyperdontia presented in (1.06%) students while Gábris. in a research found that the prevalence of hyperdontic teeth were 1.53% (23). Vahid-Dastjerdi et al. examined radiographs of 1751 Iranian population and detected that 0.74% had hyperdontic teeth (24), while Udom et al. detected that the prevalence of 1.8% for hyperdontic teeth. The prevalence might be different in prevalence due to the differences in ethnic factor of population and in the sample size.

Most common hyperdontic tooth was mesiodens which followed by mandibular second bicuspids, maxillary lateral incisor, mandibular lateral incisor and maxillary first bicuspids (23). While Sogra et al, in a research found that most frequently hyperdontic tooth were mandibular bicuspids followed by mesiodens.

Difference between both gender was non-significant (P>0.05), it agreed with Pérez et al (22).

Conclusion

The study displays that about (10.2%) students had abnormal of teeth number. Hypodontia happens more often than hyperdonti. The foremost repeatedly absent tooth was maxillary lateral incisor and the second one was the mandibular second bicuspid. About hyperdontia, the most frequently hyperdontic tooth was Mesiodens. All of them, difference between gender was non-significant.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References

Impact of Residency Area on Dental Caries and Nutritional Status among 10 Years Old Children in Al-Hillah City, Iraq

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¹Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad, Iraq

Abstract

The dental caries are still the most common oral disease affecting children through the world especially in developing countries as in Iraq; however, these are not life threatening human disease.

This study was conducted to assess dental caries experience and the nutritional status among 10 years old primary school children in urban and rural area in Al-Hillah city.

Eight hundred ninety one (891) students, 10 years old, selected randomly from different primary school, in urban and rural area in Al-Hillah city. Oral examination of dental caries was done according to the criteria described by WHO in 1987. Nutritional status was assessed according to body mass index (BMI), then followed the criteria of Centers for Disease Control and Prevention growth chart (CDC).

A high percentage of total samples were affected with dental caries and significantly higher in urban areas than rural areas. The majority of children had normal weight and the lowest percentage had underweight.

This study revealed that a higher prevalence of dental caries experience was recorded, in children of urban than children of rural areas. Therefore, there is need for an improving public and school preventive programs, and encouraged to orient health knowledge in a positive direction.

Keywords: dental caries experience, urban and rural area

Introduction

Dental caries can be defined as a demineralization of the inorganic part of the tooth with the dissolution of the organic substance based on interaction of several factors: diet (fermentable carbohydrate) oral micro flora (acidogenic bacteria), time and host (¹, ²).

A variation in caries prevalence among different countries may be related to several factors, which include: water fluoridation, and dental education, as well as different dietary habit and socioeconomic status, age, gender, race, oral hygiene, and geographical location, all these factors if not available, may lead to higher caries prevalence among developing countries, and the reverse for developed one (³).

Iraq is one of the developing countries that showed as distressing increase in caries prevalence and severity (⁴, ⁵).

There are three mechanisms explained that malnutrition during tooth development can make teeth more susceptible to dental caries. The first is that malnutrition causes defectively formed tooth, the second one is that the eruption of teeth is delayed among malnourished children; the last mechanism is that malnutrition can lead to increased dental caries by affecting salivary gland development and function (⁶, ⁷).

It was thought that provide good nutrition during teeth development was the principle way to prevent dental caries. It was known that the topical effect of diet in the mouth after the teeth have erupted plays a much more important role (⁸).

So, the aim of this study is assessing dental caries and nutritional status in children aged ten years in primary schools in Al-Hillah city.
Materials and Method

Subjects:

Eight hundred ninety one (891) children aged 10 years old selected randomly from different rural and urban primary schools in Al-Hillah city. The cross sectional random sample was calculated for prevalence studies by the formula as $n = \frac{Z^2 P (1-P)}{d^2}$ (9).

$N= \text{Sample size.}$

$Z= \text{Z Statistics for the level of the confidence (at 95% confidence level, } Z=1.96).$

$P= \text{Prevalence of the proportion.}$

$D= \text{Precision (if the precision is 5%, } d=0.05).$

This study was done during the period from December 2018 to February 2019. A pre-study ethical approval was assigned, approval was taken from the General Directorate of Education of Al-Hillah city in order to achieve subject without obligation, also the children’s parent consent form which taken before start the study.

Inclusion criteria: Students with

- No history of medication, (anti- inflammatory or antimicrobial therapy) within previous 3 months.
- No history of orthodontic treatment.
- No history of any systemic disease.

Oral examinations were performed on chairs in a special room, under good illumination by using dental mirror, probe and dental tweezers.

Diagnosis and recording of dental caries were carried out by the criteria of WHO in 1987 (10). Nutritional status was assessed according to body mass index (BMI) indicator by using anthropometric measurement (weight and height) then followed the criteria of Centers for Disease Control and Prevention growth chart (CDC) (11). Children were weight by bathroom scale (12). From the child’s weight and height, BMI index determined according to this formula (12): \[ \text{Body weight / (height)}^2 = \text{BMI Kg/m}^2 \]

The result of that formula was compared with international reference values using CDC growth charts (11).

Results

This study included a total of (891) school children aged (10) years, children living in urban areas constituted (57.6%) of the sample, while the remaining (42.4%) children lived in rural areas, as illustrated in Table (1) illustrated distribution of total sample between the residences of children according to gender.

Table 1: Distribution the residence of the sample by gender

<table>
<thead>
<tr>
<th>Residence</th>
<th>Total No. &amp; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>513 (57.58%)</td>
</tr>
<tr>
<td>Rural</td>
<td>378 (42.42%)</td>
</tr>
<tr>
<td>Total</td>
<td>891 (100%)</td>
</tr>
</tbody>
</table>

Table (2) illustrated distribution of the children by nutritional status between urban and rural residence. Urban residents have shown slightly higher percentage of underweight nutritional status than rural residents (5.26% vs. 4.76%). It had also shown a highly significant relationship, chi-square = 36.06, d.f. = 3, P-value < 0.001.

Table 2: Distribution of nutritional status by residence

<table>
<thead>
<tr>
<th>Residence</th>
<th>Nutritional Status</th>
<th>No. (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Underweight (&lt; 5th percentile)</td>
<td>Normal (5th-85th percentile)</td>
<td>Overweight (85th-95th percentile)</td>
</tr>
<tr>
<td>Urban (n=513)</td>
<td>27 (5.26%)</td>
<td>302 (58.87%)</td>
<td>100 (19.49%)</td>
</tr>
<tr>
<td>Rural (n=378)</td>
<td>18 (4.76%)</td>
<td>292 (77.25%)</td>
<td>34 (8.99%)</td>
</tr>
<tr>
<td>Total (n=891)</td>
<td>45 (5.05%)</td>
<td>594 (66.67%)</td>
<td>134 (15.04%)</td>
</tr>
</tbody>
</table>
Table (3) illustrates the mean value and standard deviation of caries experience by fractions in primary dentition among children in regarding to place of residence. The result revealed that primary missing surfaces (ms) were significantly higher in rural areas, while primary filling surfaces (fs) were significantly higher in urban area.

Table 3: Caries experience of primary teeth with fractions by residency

<table>
<thead>
<tr>
<th>Variable</th>
<th>Residence</th>
<th>Mean ± SD</th>
<th>Z</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>dmft</td>
<td>Urban</td>
<td>3.01 ± 2.40</td>
<td>-0.62</td>
<td>0.539</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>2.89 ± 2.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dmfs</td>
<td>Urban</td>
<td>7.05 ± 6.96</td>
<td>-1.11</td>
<td>0.267</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>7.59 ± 6.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ds</td>
<td>Urban</td>
<td>5.32 ± 5.52</td>
<td>-1.27</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>5.74 ± 5.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ms</td>
<td>Urban</td>
<td>1.52 ± 4.27</td>
<td>-2.68</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>1.95 ± 4.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fs</td>
<td>Urban</td>
<td>0.16 ± 1.08</td>
<td>-3.53</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>0.02 ± 0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (4) illustrates the mean value and standard deviation of caries experience by fractions in primary dentition among children in regarding to nutritional status. It was found that primary teeth (dmft, dmfs, and ds) had a statistically significant relationship with nutritional status. Underweight children had significantly higher dmft, dmfs, and ds than other.

Table 4: Caries experience with fractions in primary dentition by nutritional status

Table (5) demonstrates the mean value and standard deviation of caries experience by fractions in permanent dentition among children in regarding to place of residence. Caries experience was found to be higher in urban area than rural area. DMFT, DMFS, Ds and Fs of permanent teeth were significantly higher in urban areas compared to rural areas while mean value missing surface was higher in rural area than urban.

Table 5: Caries experience with fractions in permanent dentition among children by residency

<table>
<thead>
<tr>
<th>Variable</th>
<th>Residence</th>
<th>Mean ± SD</th>
<th>Z</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMFT</td>
<td>Urban</td>
<td>0.59 ± 0.84</td>
<td>-5.42</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>0.33 ± 0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMFS</td>
<td>Urban</td>
<td>0.87 ± 1.72</td>
<td>-5.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>0.63 ± 1.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>Urban</td>
<td>0.74 ± 1.49</td>
<td>-4.89</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>0.53 ± 1.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>Urban</td>
<td>0.07 ± 0.58</td>
<td>-0.04</td>
<td>0.965</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>0.09 ± 0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FS</td>
<td>Urban</td>
<td>0.05 ± 0.40</td>
<td>-2.15</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>0.01 ± 0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (6) demonstrates the mean value and standard deviation of caries experience by fractions in permanent dentition among children in regarding to nutritional status. Correlations between BMI percentile and each of DMFT and DMFS were assessed using Pearson’s product-moment correlation coefficient; there was no significant correlation between BMI percentile and any of DMFT or DMFS, with correlation coefficient (R) of 0.04 and -0.02 respectively.

Table 6: Caries experience with fractions in permanent dentition among children by nutritional status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nutritional Status</th>
<th>Mean ± SD</th>
<th>Kruskal Wallis H</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMFT</td>
<td>Underweight (&lt; 5th percentile)</td>
<td>0.42 ± 0.66</td>
<td>2.99</td>
<td>0.393</td>
</tr>
<tr>
<td></td>
<td>Normal (5th-85th percentile)</td>
<td>0.47 ± 0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overweight (85th-95th percentile)</td>
<td>0.43 ± 0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obese (&gt; 95th percentile)</td>
<td>0.60 ± 0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMFS</td>
<td>Underweight (&lt; 5th percentile)</td>
<td>0.60 ± 1.14</td>
<td>2.23</td>
<td>0.525</td>
</tr>
<tr>
<td></td>
<td>Normal (5th-85th percentile)</td>
<td>0.82 ± 2.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overweight (85th-95th percentile)</td>
<td>0.60 ± 1.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obese (&gt; 95th percentile)</td>
<td>0.75 ± 1.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>Underweight (&lt; 5th percentile)</td>
<td>0.49 ± 0.92</td>
<td>3.68</td>
<td>0.298</td>
</tr>
<tr>
<td></td>
<td>Normal (5th-85th percentile)</td>
<td>0.69 ± 1.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overweight (85th-95th percentile)</td>
<td>0.51 ± 1.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obese (&gt; 95th percentile)</td>
<td>0.69 ± 1.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>Underweight (&lt; 5th percentile)</td>
<td>0.11 ± 0.75</td>
<td>2.81</td>
<td>0.421</td>
</tr>
<tr>
<td></td>
<td>Normal (5th-85th percentile)</td>
<td>0.10 ± 0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overweight (85th-95th percentile)</td>
<td>0.00 ± 0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obese (&gt; 95th percentile)</td>
<td>0.04 ± 0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FS</td>
<td>Underweight (&lt; 5th percentile)</td>
<td>0.00 ± 0.00</td>
<td>2.88</td>
<td>0.410</td>
</tr>
<tr>
<td></td>
<td>Normal (5th-85th percentile)</td>
<td>0.03 ± 0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overweight (85th-95th percentile)</td>
<td>0.09 ± 0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obese (&gt; 95th percentile)</td>
<td>0.02 ± 0.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The result was shown dmft for primary dentition lower than that seen by Al-Galebi in 2011 matching with age (5). However, this results higher than other previous Iraqi studies by Al-Azawi in 2000 and Radhi in 2009 (13,14). The mean dmfs value of this study was lower than that seen by Diab in 2003 in the central region of Iraq (4), Droosh in 2007 in Sulalmania city (15), and Al-Galebi in 2011 in Al-Nassiyyia city (5).

The mean DMFT value of the present study was lower than that seen by Al-Azawi in 2000 (13), Al-Galebi in 2011 (5), Laith and Al-Rawi in 2016 (16). The result of DMFS was lower than reported by Ali in 2001 (17), Diab in 2003 (4), Al-Galebi in 2011 (5), and Laith and Al-Rawi in 2016 (16).

In present study, the result was shown the dmft/dmfs higher than DMFT/DMFS this result due to the selected age of schoolchildren in this study had been recently transitioned from primary to permanent dentition stage this mean most of permanent teeth were sound that are newly erupted there was no enough time to develop of dental caries (18).

Both dmft/dmfs; DMFT/DMFS were higher in urban area than rural area this result agrees with Al-Sayyab in 1989(19), Al-Azawi in 2000 (13), and finding from this study indicate that, dental caries considered as
a disease of civilization, in addition, rural area was well and natural nutritional status than urban area. This may affect mineralization of teeth and salivary composition, thus decreasing the susceptibility of teeth to dental caries \(^{(20)}\).

This study was shown that the decayed (ds/Ds) was the major component of dmfs /DMFs then ms/Ms and fs/Fs. This may reveal the poor demand for dental treatment for both primary and permanent dentition and even treatment was present it was directed for extraction rather than preserving permanent teeth. These results were in agreement with Baram in 2007\(^{(21)}\) and Al-Galebi in 201 \(^{(5)}\).

There were significantly higher ms in rural area than urban area while fs/Fs were in opposite picture. This indicates poor educational level in rural area towards dental health, this agree with Ali in 2001 \(^{(22)}\) and Al-Sadam in 2013 \(^{(23)}\).

In present study, the result was shown the percentage of dental caries was lower in obese children this agree with Al-Saadi in 2009 \(^{(24)}\). This result could be partially explained that a high amount of fat in the diet binds to various sugars in the diet thus decrease their solubility lead to drop in pH value and weaker acid attacks, and a fatty protective layer over plaque would prevent fermentable sugar substrate from being reduced to acid\(^{(25)}\).

### Conclusion

This study revealed that a higher prevalence of dental caries experience was recorded, in children of urban than children of rural areas. Therefore, there is need for an improving public and school preventive programs, and encouraged to orient health knowledge in a positive direction.

### Ethical Clearance:
The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

### Conflict of Interest:
The authors declare that they have no conflict of interest.

### Funding:
Self-funding

### References
14. Radhi N. Oral health status in relation to nutritional


Molecular Investigation of Plasmid–Mediated Quinolone Resistant Genes among aminoglycoside-resistant uropathogenic *Escherichia coli* Isolates from Babylon Hospitals, Iraq

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¹Faculty of Medicine / Kufa University/Iraq

**Abstract**

**Background:** Uropathogenic *E. coli* is the most frequently causes of UTIs in humans, accountable 75-95% of UTIs. PMQR have been identified in family *Enterobacteriaceae* worldwide. The purpose of this study to examine the dissemination of *aac(6')-Ib-cr* and PMQR genes in isolates of UPEC.

**Method:** A cross sectional study of mid-stream urine of the participate patients their approval for direction usage their specimen. The susceptibility test by disk diffusion for UPEC were isolated from urine and screening of quinolone resistant by multiplex PCR for *qnrA*, *qnrB* and *qnrS* genes and monoplex PCR for *qunD*, *qepA* and *aac(6')-Ib-cr* genes.

**Results:** A total of 1072 mid-stream urine were collected randomly, overall 132 were identified *E. coli*, among these 60 aminoglycoside resistant UPEC isolates were screened to the five quinolone antibiotics. The resistance proportion to nalidixic acid and ciprofloxacin were 75.0%, as well as to lomefloxacin, norfloxacin and levofloxacin were 76.7%, 73.3% and 71.7%, respectively. The susceptibility test for antibiotics showed a high incidence of the resistance to the majority of antibiotics class. It was found that 73.3%, 38.3%, and 20.0% of the isolates harbored *aac(6')-Ib-cr*, *qnrS* and *qnrB* genes, respectively either alone or in combination, while the *qnrA*, *qunD*, *qepA* genes were not detected.

**Conclusion:** All isolates were identified as multiple antibiotic resistances, only one isolate can be considered as possible PDR and high prevalence of *aac(6')-Ib-cr*, *qnrS* and *qnrB* genes among isolates.

**Keywords:** UPEC, *aac(6')-Ib-cr*, *qnrB* and *qnrS*, plasmid mediated quinolone PMQR, multidrug resistance, extended drug resistant, pan drug resistance.

**Introduction**

UTI is the second most common clinical in primary and secondary care suggestion for experiential antimicrobial treatment(1). *E. coli* can cause both complicated and uncomplicated UTIs(2). Fluoroquinolones has represented an alternative therapeutic select for the treatment. But, newly the incidence of fluoroquinolones-resistance between uropathogenic isolates has also been described in different areas(3). Quinolone resistance mechanisms include mutation in target gene, elevated expression of efflux pumps and yielding of modifying enzyme and target protection protein(4). They involve the chromosome encoded and PMQR, three PMQR mechanisms have been identified: target protection by Qnr proteins, drug inactivation by AAC(6)-Ib-cr, and drug efflux by QepA and OqxAB(5). The rapid dissemination of MDR bacteria has become a concern worldwide and complicated the treatment of infections. This phenomenon is a consequence of the ability of bacteria to acquire exogenous genes by mobile elements (e.g. conjugative plasmids, transposons and integrons)(6).

Increased use of ciprofloxacin and other fluoroquinolones antibiotics in Iraq may be cause selection of isolates resistant to aminoglycosides by selecting the isolates that possess AAC(6')-Ib-cr.
The aim of this study to examine the dissemination of \(\text{aac}(6\text{'})\)-\(\text{Ib-cr}\) and PMQR genes in aminoglycosides resistance in UPEC isolates from Babylon hospitals.

**Method**

**Collection of specimens**

This cross section study of the participate patients their approval for direction usage their specimen was conducted in in two main hospitals in Babylon hospitals during the period of six months from March to September 2018 in patients clinically suspected to have UTI. The inclusion criteria for the volunteer patients were attended or admitted in the two hospitals.

**Isolation and Identification of \(E.\ coli\) isolates**

Identification of bacterial pathogens was made based on Gram reactions, culture characters, and routine standard biochemical tests. All \(E.\ coli\) strains were purified on EMB agar plates to confirm that there is no mixed culture. Additionally, \(E.\ coli\) isolates were also confirmatory identified by using commercially available API 20 E kit.

**Antibiotic Susceptibility Test**

Antibiotic susceptibility test was performed by Kirby –Bauer method based to the guideline suggested by the Clinical and Laboratory Standards Institute\(^{(7)}\).

**Examining for Quinolone Resistance and other Antibiotics**

The disk diffusion method was made to identify quinolone resistance in all aminoglycosides resistance UPEC by using Nalidixic acid (30\(\mu\)g), Ciprofloxacin (5\(\mu\)g) Levofloxacin (5\(\mu\)g) Lomefloxacin (10\(\mu\)g), Norfloxacin (10 \(\mu\)g). In addition, all aminoglycosides resistance UPEC were screen for other antibiotics.

**Examining for PMQR Genes**

Multiplex PCR were used to determine the presence \(qnrA, qnrB\) and \(qnrS\) genes and monoplex PCR for \(qunD, qepA\) and \(\text{aac}(6\text{'})\)-\(\text{Ib-cr}\) genes in quinolone resistance UPEC by using T300 (thermo cycle) and Go Taq Green Master Mix (Promega USA). The primer target for each PMQR genes were available elsewhere\(^{(8,9,10,11)}\).

**Results**

Out of 298 isolates of uropathogen, 132 (44.3\%) isolates were recognized as \(E.\ coli\) followed by \(Klebsiella\) spp (22.5\%), \(Proteus\) spp (10.0\%), \(S.\ aureus\) (9.4\%), \(S.\ saprophyticus\) (3.4\%), \(P.\ aeruginosa\) (3.4\%), \(S.\ faecalis\) (2.7\%), \(Enterococcus\) spp (1.7\%) \(Acinetobacter\) spp (1.3\%) and \(Citrobacter\) spp (1.3\%).

**Examining for Quinolone Resistance**

Overall, 78.3\% (47/60) of the aminoglycoside resistant UPEC isolates were non susceptible to at least one of the five quinolone antibiotics by disk diffusion methods. The resistance proportion to nalidixic acid and ciprofloxacin were 75.0\%, as well as to lomefloxacin, norfloxacin and levofloxacin were 76.7\%, 73.3\% and 71.7\%, respectively.

**Co-resistance of \(E.\ coli\) to other Antibiotic**

Antibiotic susceptibility test of all 60 uropathogenic \(E.\ coli\) against 28 antibiotics (belong to twelve classes) displayed high occurrence of resistance to the majority agents of antibiotics. According to the definitions proposed\(^{(12)}\), all of the 60 aminoglycoside resistant UPEC isolates (100\%) were multiple resistance to antibiotics. Based on their MDR, 49 (81.6\%) of the isolates were included, 2 (3.3\%), 1 (1.7\%), 4 (6.7\%), 5 (8.3\%), 18 (30\%) and 19 (31.6\%) were resistant to 3, 5, 6, 7, 8 and 9 antibiotic classes, respectively. While, 10 (16.7\%) isolates was considered as XDR organisms, of which 6 (10\%) were resistant to 10 antibiotic classes and 4 (6.7\%) were resistant to 11 antibiotic classes. PDR could be detected in only one isolate (1.7\%) in this study (Table 1).
Table (1): MDR, XDR and PDR of aminoglycosides resistant UPEC isolate ($n=60$)

<table>
<thead>
<tr>
<th>Type of resistance</th>
<th>No. of isolates</th>
<th>No. of resistance to antibiotic groups ($n=12$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR* ($n=49$, 81.6%)</td>
<td>19  18  5  4  1  2</td>
<td>9  8  7  6  5  3</td>
</tr>
<tr>
<td>XDR* ($n=10$, 16.7%)</td>
<td>6  4  10  11</td>
<td>10</td>
</tr>
<tr>
<td>PDR* ($n=1$, 1.7%)</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

*MDR: multidrug resistance; XDR: extended drug resistance; PDR: pan drug resistance

Examining for PMQR Genes

PMQR genes were identified in 78.3% (47/60) of E. coli, harbor at least only one PMQR gene, and three types of PMQR (qnrS, qnrB and aac(6')-Ib-cr variant) were recognized alone or in combination (Table 2). The aac(6')-Ib-cr variant was present in 73.3% of the isolates, followed by qnrS (38.3%) and qnrB 20.0% of the isolates. Interestingly, qnrA, qnrD, and qepA were not found in any isolates.

Table (2): Distribution of PMQR genes and their combinations among the UPEC isolate ($n=60$)

<table>
<thead>
<tr>
<th>Type of PMQR gene</th>
<th>No. (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>aac(6')-Ib-cr</td>
<td>17 (28.3%)</td>
</tr>
<tr>
<td>qnrS</td>
<td>2 (3.3%)</td>
</tr>
<tr>
<td>qnrB</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>aac(6')-Ib-cr, qnrS</td>
<td>16 (26.7%)</td>
</tr>
<tr>
<td>aac(6')-Ib-cr, qnrB</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>aac(6')-Ib-cr, qnrS,qnrB</td>
<td>5 (8.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>47 (78.3%)</td>
</tr>
</tbody>
</table>

Discussion

Frequency and Resistance of UPEC to Quinolone antibiotic

This study revealed that the UPEC isolates (44.3%) were the major causative agent of significant bacteriuria compared to other uropathogen due to enteric flora, the way of transmission is by fecal contamination and anatomical nearness to the genito–urinary tract in females and the poor hygiene. This result is agreement with other authors have the same findings, which E. coli was the predominant uropathogen.

The resistance rates to nalidixic acid and ciprofloxacin were 75.0%, as well as to lomefloxacin, norfloxacin and levofloxacin were 76.7%, 73.3% and 71.7%, respectively. This may be due to rampant use of quinolones as first line empirical therapy in UTI cases. The data reported in this study is close related with the previous study described that ciprofloxacin resistance rate 79.66% to E coli in Saudi Arabia. In the previous report of the ECDC, the presence of UPEC isolates resistant to fluoroquinolones were existent in low numbers in Sweden (7.9%) and Norway (9.0%), but they were dominant in Italy (40.5%) and Slovakia (41.9%).
Co-resistance of aminoglycosides and quinolones was observed in the UPEC isolates of the present study. The study report in Iraq the identification of a variant of the aminoglycoside modifying enzyme AAC(6′)-Ib, as a common incidence in *Enterobacteriaceae* isolates\(^{(18)}\).

**Antibiotic Resistance**

All UPEC isolates were identified as multiple antibiotic resistances. This result was considered a high level when compared to other report. Frequency of MDR 81.6%, which was relatively high prevalence when compared to rates reported, in Saudi Arabia it was 67.0\(^{\circ}\)%\(^{(19)}\), and in Iran it was 63.0\(^{\circ}\)%\(^{(20)}\). The reason of high MDR may be due to the most cases of UTIs are treated empirically in Iraq, where patients frequently cannot afford to consult a clinicians or have a laboratory tests. Therefore, there may be over cases of bacteria that are not responded to treatment.

XDR, 16.7% this finding are alarming since infections with these XDR UPEC isolates leave physicians with only one or two antibiotic treatment options, leading to increased mortality and morbidity\(^{(20)}\). In present study remarked PDR (1.6\%) of UPEC. No study investigation PDR-producers among UPEC. However, the prevalence of PDR in *Enterobacteriaceae* is rare in Europe and other countries\(^{(21,22)}\). Although infections by PDR *Enterobacteriaceae*, still rare, but have been associated with a high mortality.

**Occurrence of PMQR Genes**

PMQR determinants have been identified in family *Enterobacteriaceae* worldwide, with varying frequency rates\(^{(23)}\). 78.3\% of the isolates harbored at least one PMQR gene (Table 2). In Saudi Arabia, reported that 26.0\% of isolates carried PMQR genes\(^{(24)}\). Previous investigations conducted in Europe described fewer PMQR determinants in ESBL-*E. coli* isolates than found in the current study, 19.0\% in Spain, and 10.0\% in Sweden and Norway\(^{(25,26)}\).

The AAC(6′)-Ib-cr variant, an enzyme encoded by a plasmid-borne *aac(6′)-Ib-cr* gene, that inactivate selected fluoroquinolones (ciprofloxacin and norfloxacin) by acetylation in addition to aminoglycosides\(^{(27)}\). *aac(6′)-Ib* PCR positive products were further digested with BstCI to identify *aac(6′)-Ib-cr* variants. Present study demonstrated that the most prevalent PMQR gene was *aac(6′)-Ib-cr* as 73.3\%. This is also identified in other studies (84.0\%)\(^{(27)}\), (94.0\%)\(^{(28)}\) of the *aac(6′)-Ib* were of the *aac(6′)-Ib-cr* variant. Presence of this variant also increased the incidence of selection of chromosomal mutants (quinolone resistance determine region, QRDR) upon exposure to ciprofloxacin\(^{(29)}\). However, Spread of this variant is undesirable because these fluoroquinolones are used as first choice for treatment of UTIs in Iraq.

*qnrS* was the most prevalent 38.3\%, followed by *qnrB* (20.0\%). Present findings concurred with a previous European survey that *qnrS* was more frequently detected than other *qnr* genes in clinical *Enterobacteriaceae* isolates\(^{(30)}\). In the USA, among ceftazidime-resistant *Enterobacteriaceae* isolates, 23\% were positive for either *qnrA* or *qnrB*, while *qnrS* was absent\(^{(31)}\). In China among ciprofloxacin resistant *E. coli* is 7.5\%. *qnrA*, *qnrB* and *qnrS* were detected in 3.8\%, 4.7\% and 3.8\% of these isolates, respectively\(^{(32)}\). However, variations in distribution of *qnr* genes have been suggested to be attributing to differences in selection criteria or geographic area\(^{(33)}\). None of the *qnrA*, *qnrD* and quinolone specific efflux pumps (*qepA*) were found in present collection. This is not surprising since *qnrD* and *qepA* determinants are rarely found worldwide.

This study was revealed high combination of *aac(6′)-Ib-cr*, *qnrS*, followed by *aac(6′)-Ib-cr*, *qnrB* and *aac(6′)-Ib-cr*, *qnrS*, *qnrB* isolates as shown in Table (2). This could be explained by the coexistence of the *aac(6′)-Ib-cr* and *qnr* genes on the same plasmid\(^{(34)}\). The combination is also observed where 35.1\% and 1.3\% of the *E. coli* isolates carried two and three different PMQR genes, respectively.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


Study of the Effectiveness of Some Raw Plants and Materials in the Treatment of Pediculosis in Najaf province Iraq

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Abstract

Head lice are a public health problem and resistance to over-the-counter medications is a source of concern for many. The current study was conducted to evaluate the activity of three plants as a natural alternative to medical pesticides to treat pediculosis in Najaf province, Iraq. It was noted that the *Lawsonia Inermis* plant (Henna paste) mixed with mercury has scored the highest effectiveness for killing lice in two hours from the first use by 100% followed by the *Allium cepa* juice mixed with *Curcuma longa* plant, where the rate of killing 80% of the second use and finally vinegar mixed with sodium chloride (food salt) by 46.66%. Our suggestion that these blended Materials give better results than if they were alone and are excellent alternatives to medical pesticides.

Key words: pediculosis, raw plant, sodium chloride, mercury, vinegar.

Introduction

Head lice infection was associated with limited morbidity, but it caused anxiety among parents toward school-age. Multiple and excessive treatments to pediculosis have also promoted serious health concerns due to the lack of effective treatment to increase the resistance of lice to synthetic compound, so researchers have begun to looking for new synthetic components of treatment such as compounds obtained from plant source. Although most of the active synthetic pesticides were available in the market and efficient against *pediculus humanus capitis*, but these products were expensive and toxic effect on the human nerves. Hence non-toxic alternative options are needed to treat pediculosis. Natural sources remained less toxic and less expensive; therefore, natural extracts from medicinal plants were main rich source of treatment for various diseases and disorders of the human system.

More than 1000 species of plants had been described in many areas containing chemical components in seeds, stems, roots, leaves and flowers against insect pests but only a few plants had been used to control insects practically on a commercial scale in the past few decades. The efficient treatment of the pesticide to kill head lice and eggs, followed by manual removal of eggs is the most effective procedure for eliminating head lice.

*Lawsonia* is a shrub and dicot plant belong to the Lythraceae family, has only one species (*L. Inermis*) but it has several names such as Henna, Mendi, Shudi, Madurang, Manghati, Madayantika and Goranti. It is widely cultivated throughout Tropical regions as an ornamental plant and although the plant was used in the dye and small number of studies had been recorded in treatment of many diseases like anti-diabetes, immunomodulatory and liver activity.

Mercury is a chemical element previously known as a heavy silver mercury element, liquid in standard conditions of temperature and pressure. It was used in cosmetic recipes such as whiten the skin, treat pimples and freckles in the face, treat sores, warts and skin infections.

For long time, the onion plant (*Allium cepa*) was highly valued for its therapeutic properties, a medicinal plant used, but lately used for its effectiveness of heart vessels, anti-hypertensive and anti-diabetic by a few researchers.

*Curcuma long* belongs to the Zingiberaceae family and is considered to be very cheap, available, effective and acceptable in developing countries, without toxic or harmful reactions. The active ingredients known as curcumin have been shown to be highly therapeutic when used in recommended quantities.
In our study, 6 substances were studied, some of which were extracted from the plants, in addition to other substances, where each extract was mixed with another for the purpose of studying its adverse efficacy of extracts and their use as a human hair pesticide. In the absence of a similar study, the idea of the study came as follows:

1- Study the effectiveness of *Lawsonia* plant mixed with mercury as a lice pesticide

2 – Study the effectiveness of *Allium cepa* juice mixed with *Curcuma longa* plant (Al-Korkom or Curcumin)

3 - Study the effectiveness of vinegar mixture with sodium chloride

**MATERIALS AND METHODS**

A total of 45 randomized samples infected with pediculosis were collected with different ages ranging from 8 - 30 years, Ones were excluded before the examination weren’t eligible for inclusion in our study who used any form of head lice treatment for at least four weeks or any local medicine for 48 hours prior.

The experiment was conducted at room temperature 25°C and a relative humidity of 45%. The blended materials was placed on the hair of the affected women using the brush starting from the scalp with continuous combing the end of the hair and then wrapped in a plastic bag for two hours and then rinsed the hair with empty water with combing to get rid of dead lice And eggs.

Then hair was examined and the experiment was repeated on the second and third day if the lice hadn’t completely eliminated. People were also examined for the side effects of treatment on the skin and eyes, and recorded their impressions regarding smell, itching, etc., during and after treatment. The second test was performed 10 days after the first treatment to determine whether all lice were eliminated in the hair or not. preparation of experimental materials

1- *Lawsonia* paste and mercury

*Lawsonia* leaves and mercury were bought from herbal stores and taken 110 g of ground *Lawsonia* leaves mixed well with 200 ml of water. Then add 5g of mercury to get the henna paste.

2- A mixture of onion juice with ground turmeric:

Red onion (150g) sometimes called purple (*Allium cepa*) peel then squeeze to get onion juice, with a concentration of 100%. Then add 10 g of turmeric (after grinding the turmeric stalks) to the onion juice and mix well.

3- mixture of vinegar and sodium chloride

Concentrated vinegar (250 ml) was taken from the Al-Badawi factory (Iraq) and mixed with salt (25 g). The mixture was added to the vinegar and the salt was well dissolved.

**Result**

45 patients were successfully treated with natural treatments divided into 3 groups and each group contained 15 persons for each combination of experiment materials. The adults and nymphs head lice mortality rate was evaluated after two hours of treatment with Therapeutic materials. Table results show that henna blended with mercury had a high toxic effect in killing lice and eggs by 100% of the first usage, followed by mixture of onion and turmeric plant by 80% of the second usage, while the mixture of vinegar and food salt recorded 46.66% of the third day. The number of lice in the hair has decreased on the first day and the second for the last mixture.

**Table showing effect of tested materials on *pediculus humanus capitis* in vivo.**

<table>
<thead>
<tr>
<th>Tested materials</th>
<th>First try</th>
<th>Second try</th>
<th>Third try</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First day</td>
<td>Second day</td>
<td>Third day</td>
</tr>
<tr>
<td><em>Lawsonia</em> plant with mercury</td>
<td>100%(15)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>onion juice with ground turmeric</td>
<td>80%(12)</td>
<td>20%(3)</td>
<td>-</td>
</tr>
<tr>
<td>vinegar and sodium chloride</td>
<td>46.66%(7)</td>
<td>33.33%(5)</td>
<td>20%(3)</td>
</tr>
</tbody>
</table>
Discussion

The Henna leaf paste is used as a coloring agent for hair. At the same time, the phytochemicals present in the leaves have a toxic effect of head lice. Our results are consistent with a study conducted by (2), that the phytochemical constituents of \textit{L. inermis} by GC-MS) \textit{Gas Chromatography-Mass Spectrometry}, where it revealed the presence of 72 compounds with different molecular weights. In another study, they found to contain naphthoquinone, xanthones, coumarin derivatives, fatty acids, amino acids and other components. It had been shown that the Naphthoquinone which obtained from \textit{L. inermis} leaves had a large immune effect. Quinonic compounds extracted from Henna have also been studied in vitro and have been shown to possess antimicrobial properties. Lawson isolated from \textit{L. inermis} leaves showed a significant antifungal effect (11).

(12) confirmed that it had anti-oxidant, antiviral and parasite effect and reduce larvae activity of some insects, anti-inflammatory, inhibition of enzymes activity, anti-coagulant effect, wounds healing and the activity of inhibition of protein.

Since ancient times, Henna paste has been placed on hands and feet to protect against fungal and to control lice and crust. In a study conducted by (13), 70 phenol compounds were isolated from different parts of the plant, Naphthaconon has been associated with many pharmacological activities, while terpenes and β ionone are responsible for the odor of essential oils isolated from flowers. In addition to other volatile terpenes, some types of non-volatile tryboinoid and two alkaloids and dioxin derivatives have been isolated from the plant. Henna is an important medicinal plant with important biological activities in vitro and in vivo. Although a myriad of pharmacological activities have been documented, the antioxidant and antimicrobial activities are most carefully researched.

(14) found that Henna was used in the treatment of lice mixed with several plant extracts such as wormwood(100%), helba 75% or karkada (50%), Head lice completely disappeared within a week between these patients.

As for mercury, it is known to be a poisonous substance, especially on insects. Thus, mixing it with henna paste gave an excellent result. It is one of the toxins that affects topically by its contact with the body, and affects after absorption on the various organs of the body and some of them called poisonous irritants and is considered a toxin of the nervous system and toxins reproductive system, affecting the activity of the reproductive system in both male and female causing infertility.

In a study conducted on a mummy of Ferdinand II, where samples were taken from the head and pubic hair on the mummy, and then tested for The presence of lice in the hair and mercury concentration, where it tended to accumulate in head hair as well as the presence of mercury in the liquid used for hair washing. This confirms the possibility of applying minerals externally.

Further toxicity tests were conducted to verify the presence of mercury on the skin, body cavities and pubic hair, where the results showed very low values. Therefore, the presence of these elements in the king’s hair could prove exposure to the metal for the purpose of killing the lice and through microscopical and radiographic examination No traces of mercury were shown in cavities of the skull or body in the Ferdinand mummy. Results revealed that parts of the abdomen of adult and seven unfinished had attached to the hair, parts of the lice eggs. The presence of massive mercury in Ferdinand II hair was explained by the use of quicksilver for artificial mummification For the corpse, Considering that there is no trace of the metal in the mummy except in poetry.

While the effects of mercury have been found in all hairs and it seems reasonable to attribute the high value of mercury to the use of solutions and ointments against lice, And the decrease in the percentage of mercury in pubic hair indicates that he has lice as well as the head, and to prove that this region has not received any treatment to control lice (9).

Furthermore, indicators of mercury therapy from other diseases such as ringworm and scabies are described as localized use of mercury on the skin lesion alone (15).

The absence of redness of the skin cells (the crust layer) in human treated with a mixture of henna with mercury is a sign of non-sensitization of this region and is evidence of the lack of absorption of cells of this material and thus non-penetration of the skin, indicating that the effect of this material on the parasite and the possibility of use for external use.
Comparing the results of the current study with a study conducted by(6), where they studied the effectiveness of onion extract in the killing of nymphs and lice in the laboratory and found that the mortality of head lice 10% and 20% after 30 and 60 minutes respectively.

Onion plant has many biological activities including antimicrobials, antioxidants, anti-cancer and anti-inflammatory(16).

(17) conducted that the onion plant as antimicrobial with different concentration of essential oil extracts of three types of green, yellow and red onion tested against two bacteria (staphylococcus aureus and Salmonella enteritidis) and three types of fungus where he was found that the essential oil of green onion led to a decrease in inhibition of bacterial activity when fungal activity was stopped significantly, especially in low concentration.

The onion plant contains two groups of compounds, which form the majority of active substances, sulfur compounds such as Allicin, Allyl propyl disulfide, Flavonoids such as Quercetin. Allicin shows its antimicrobial activity mainly through immediate and total inhibition of DNA and RNA and protein synthesis(18).

Oil products, especially those containing essential oils, may also prevent lice respiration or slow movement, making it easier to remove them from the hair with the soft comb, this is consistent with(19).

It has been suggested that essential oils as an alternative source of insecticide because they are rich sources of biologically active chemicals are commonly used as flavor and flavoring substances for food and beverages(20), because of this emphasis has been placed on plant essential oils or chemicals as potential sources for control of head lice(21). The activity of pesticides from aromatic oils has been well described by(20).

The Turmeric plant was used as a paste for treatment of scabies at the rate of 97% during 3-15 days of treatment and curcumin is the product obtained by dissolving the turmeric extract i.e., the ground rhizomes of Curcuma longa L. (Curcuma domestica Valeton) and purification of the extract by crystallization, it also possesses antioxidant properties(22).

Curcumin was soluble dye in oil and alkalis, stable at high temperatures and in acid, more effective than other spices in its ability to prevent fat peroxidation and its antioxidant effect was eight times stronger than vitamin E(23).

The mixture of vinegar and food salt may be attributed to killing of lice and eggs in the hair of the injured that the acid may work to soften the protective sheath of lice covers and attaches the egg on the hair and this is consistent with(24). The mechanism of action of sodium chloride on lice in laboratory studies is not fully known, but it has been noted that the tear in the digestive system of the lice with the application of food salt and this explains the drought when exposed lice for two hours of sodium chloride and this is consistent with(25).

Conclusions

Use plant raw materials and mix them with more than one substance to get better results in the treatment of pediculosis

Acknowledgment: The authors thank to all patients for their help

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

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Molecular and genotyping for *Cryptosporidium parvum* isolated from children with diarrhea in pediatric hospital of Karbala

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Abstract

The study involved surveilling sampling of children suffered cryptosporidiosis like symptoms. The present study was conducted on 90 children who suffer from abdominal pain and diarrhea. Samples were collected from patients suspected infection by cryptosporidium who attended in pediatric hospital of Karbala Governorate from January-2019 to May 2019. They were (1 month-5 years old age). Each sample was divided into two parts. The first part was used to prepare a moderate thick smears stained with modified zehil nelson stain(m ZN stain) and the second part was preserved at -20 c for molecular detection by nested PCR to identify the positive samples and genotyping by RFLP-PCR that showed the PCR product analysis of HSP70 gene in *Cryptosporidium parvum* from Human stool samples, at (587bp) PCR product. Some positive samples by RFLP-PCR making nucleic acid sequencing. The main purpose of the current study was to explore the prevalence rate of cryptosporidiosis in the mentioned province targeting children at different age categories of both genders. For the gender and infection by *C. parvum*, the current work findings revealed the presence of *C. parvum* in the sampled children distributed under gender categories of male and female and the species was parvum. The results of the AFS showed that 12 (13.33%) out of 90 samples were positive to the presence of cryptosporidiosis. This technique is a good method for detecting *C. parvum* in stool samples of children in both genders, and 27 out of 90 samples was positive by nested PCR and RFLP-PCR respectively. For the age and infection by *C. parvum*, the current work findings revealed the presence of *C. parvum* in the sampled children distributed under three age categories of 1-6 months, 7-12 months, and 13-60 months. Positive results of the age categories were 5 (29.41%) out of 17 samples, 12 (32.43%) out of 37 samples, and 10 (27.78%) out of 36 samples, respectively. The total infection was 27 (30%) out of 90 samples. No significant (*p* > 0.05) differences were recognized for infection rates occurred for the age categories.

**Keywords:** pediatric hospital; *Cryptosporidium parvum*; diarrhea

Introduction

*Cryptosporidium* spp. is a coccidian protozoan parasite that causes waterborne outbreaks worldwide using the fecal-oral route for the transmission of infection. *Cryptosporidium* is one of the leading pathogens which are responsible of the majority of the diarrheal infections (1).

*Cryptosporidium* is considered as a major diarrheal cause of diarrhea in children and immune-compromised people with high death rates for those under 5 years of age (2).

Diagnosis is made by concentration of stools followed by acid-fast staining (AF), a nested PCR assay was developed to detect *C. parvum* DNA directly from stool specimens, after extraction of DNA from formalinized stool (3).

A polymerase chain reaction (PCR) restriction fragment length polymorphism analysis of a 587-bp region of the *Cryptosporidium parvum* 70-kDa heat shock protein (HSP70) gene was developed for the detection and discrimination of the two major genotypes of *C. parvum* (4).

Studies have recognized using modern molecular technologies some subtype families that can induce wide-spread diarrheal conditions (5).

Infection caused by *Cryptosporidium* spp. is a highly
common parasitic diarrhea known in humans and cattle. However, cases that show no symptoms, asymptomatic, are not well recognized for identification as that in the symptomatic case (6).

**Materials and Method**

**study design**

The study was conducted on 90 random samples of faecal smears of children from the laboratories of hospital Paediatric in Karbala City. The age of children ranged from 1 months to 5 years. The study started from January 2019 may 2019. The modified Ziehl-Neelseen stain (acid fast), used for examination of faecal smears to detect oocysts of Cryptosporidium follows. Smears were fixed with methanol alcohol for 5 minutes and allowed to dry at room temperature. Driedsmears were stained for an hour in carbolfucshin prepared by dissolving 15% carbolfucshin in methanol (stock solution). Ten ml of ZiehlFucshin were added to 90 ml of 5% phenol. Smears were rinsed in tap water and differentiated in 2 % H2SO4 solution for 20 seconds while agitating the slide and rinsed in tap water. Smears were counter stained with 5 % malachite green solution for 5 minutes, washed in tap water and left until dried. Stained smears were examined by using 40 X and 100 X oil immersion objectives. Oocysts were obtained from naturally infected children with acute diarrhoea and proved to be infected with Cryptosporidium as confirmed by M.ZN-ST.(acid fast).

**Molecular detection**

The specimens were stored at 4°C for DNA extraction. Faecal specimens from children with diarrhea whose suspected contained Cryptosporidium

Nexted Polymerase chain reaction (nPCR)

The nPCR technique was performed for detection Cryptosporidium parvum based 18S ribosomal rRNA gene from children samples (1 month to 5 years).

Genomic DNA Extraction (stool protocol)

Genomic DNA from feces samples were extracted by using AccuPrep® stool DNA Extraction Kit, Bioneer, Korea, and done according to company instructions.

Primer

The Nested PCR primers for detection Cryptosporidium spp. based 18S rRNA gene and another Nested PCR primers for genotyping Cryptosporidium parvum based hsp70 protein gene were designed according to (4). These primers was provided from Macrogen company, Korea as following table 1.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5′-3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssrDNA gene PCR</td>
<td>F GGGTTGTATTTATAGATAAAGAAC</td>
</tr>
<tr>
<td></td>
<td>R CTITAAGCCTCTAATTTTCTC</td>
</tr>
<tr>
<td>ssrDNA gene Nested PCR</td>
<td>F GACTTTTGGTTTTGAATTGGAATG</td>
</tr>
<tr>
<td></td>
<td>R TAAATTATTACAGAAATCCACTACGAC</td>
</tr>
<tr>
<td>C. parvum HSP70 gene</td>
<td>F AGCAATCTCTGCGGTACAGG</td>
</tr>
<tr>
<td></td>
<td>R AAGACATCCTTGGATCTTCT</td>
</tr>
</tbody>
</table>

1. Direct Isolation of DNA from Patient Stools for Polymerase Chain Reaction Detection of Cryptosporidium parvum.

2. Sensitive genotyping of Cryptosporidium parvum by PCR-RFLP analysis of the 70-kilodalton heat shock protein (HSP70) gene.

**RFLP**

RFLP-PCR technique was performed for genotyping Cryptosporidium parvum based on HSP70 gene. This method was carried out according to described by (4).

RFLP-PCR Technique

RFLP-PCR technique was performed for genotyping
Cryptosporidium parvum based on HSP70 gene. This method was carried out according to described by(4) as following steps:

**PCR master mix preparation**

PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions as following table for each gene:

After that, these PCR master mix component that mentioned in table above placed in standard AccuPower PCR PreMix Kit that contains all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl$_2$, stabilizer, and loading dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (Mygene. Korea).

**DNA sequencing method**

DNA sequencing method was performed for species typing of positive Cryptosporidium sp isolates as following step:

1- The PCR product of 18S ribosomal RNA genes were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system.

2- The DNA sequencing analysis (Phylogenetic tree analysis) was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis based ClustalW alignment analysis and The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

3- The Cryptosporidium species typing analysis was done by phylogenetic tree analysis between local Cryptosporidium sp isolates and NCBI-Blast known Cryptosporidium species.

Finally identified Cryptosporidium species isolates were submitted into of NCBI-GenBank to get Genbank accession number.

**Results and Discussion**

Acid-fast stain age based results microscopic

For the age and infection by *C. parvum*, the current work findings revealed. the. presence of *C. parvum*. in the. sampled children distributed under three age categories of <= 1 years, 1 – 3 years, and > 3yeaes, Positive results of the age categories were 7 (13.0%) out of 54 samples, 3 (10.3%) out of 29 samples, and 2 (28.6%) out of 7 samples, respectively. The total infection was 12 (13.3%) out of 90 samples. No significant (p>0.441) differences were recognized for infection rates occurred for the age categories by microscopic.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive</th>
<th>Negative</th>
<th>Microscopic</th>
<th>Total</th>
<th>Chi-square (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;= 1</td>
<td>Count</td>
<td>7</td>
<td>47</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>13.0%</td>
<td>87.0%</td>
<td>100.0%</td>
<td>1.637 (2)</td>
<td>0.441 NS</td>
</tr>
<tr>
<td>1 – 3</td>
<td>Count</td>
<td>3</td>
<td>26</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>10.3%</td>
<td>89.7%</td>
<td>100.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 3</td>
<td>Count</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>28.6%</td>
<td>71.4%</td>
<td>100.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>12</td>
<td>78</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>13.3%</td>
<td>86.7%</td>
<td>100.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table (2): Relationship between Age groups * Microscopic**

**Age based results nested PCR**
For the age and infection by *C. parvum*, the current work findings revealed the presence of *C. parvum* in the sampled children distributed under three age categories of $\leq 1$ years, $1 – 3$ years, and $> 3$ years. Positive results of the age categories were 16 (29.6%) out of 54 samples, 7 (24.1%) out of 29 samples, and 4 (57.1%) out of 7 samples, respectively. The total infection was 27 (30.0%) out of 90 samples. No significant ($p > 0.231$) differences were recognized for infection rates occurred for the age categories by nested PCR table 3.

**Table (3): Relationship between Age groups * Nested PCR**

<table>
<thead>
<tr>
<th>Variable Negative</th>
<th>Nested PCR</th>
<th>Total</th>
<th>Chi-square (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;= 1</td>
<td>Count 38</td>
<td>16</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 70.4%</td>
<td>29.6%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>1 – 3</td>
<td>Count 22</td>
<td>7</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 75.9%</td>
<td>24.1%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>&gt; 3</td>
<td>Count 3</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 42.9%</td>
<td>57.1%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count 63</td>
<td>27</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 70.0%</td>
<td>30.0%</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

**Age based results genotyping**

For the age and infection by *C. parvum*, the current work findings revealed the presence of *C. parvum* in the sampled children distributed under three age categories of $\leq 1$ years, $1 – 3$ years, and $> 3$ years. Positive results of the age categories were 16 (29.6%) out of 54 samples, 7 (24.1%) out of 29 samples, and 4 (57.1%) out of 7 samples, respectively. The total infection was 27 (30.0%) out of 90 samples. No significant ($p > 0.231$) differences were recognized for infection rates occurred for the age categories by genotyping table 4.

**Table (4): Relationship between Age groups * Genotype**

<table>
<thead>
<tr>
<th>Variable Negative</th>
<th>Genotype</th>
<th>Total</th>
<th>Chi-square (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;= 1</td>
<td>Count 38</td>
<td>16</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 70.4%</td>
<td>29.6%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>1 – 3</td>
<td>Count 22</td>
<td>7</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 75.9%</td>
<td>24.1%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>&gt; 3</td>
<td>Count 3</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 42.9%</td>
<td>57.1%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count 63</td>
<td>27</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 70.0%</td>
<td>30.0%</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>
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Figure 1: Agarose gel electrophoresis image that showed the Nested PCR product analysis of small subunit ribosomal RNA gene in Cryptosporidium parvum from Human stool samples. Where M: marker (3000-100bp) Lanes (1-10) some positive patients samples at (540bp) PCR product.

Figure 2: Agarose gel electrophoresis image that showed the PCR product analysis of HSP70 gene in Cryptosporidium parvum from Human stool samples. Where M: marker (3000-100bp) Lanes (1-10) some positive patients samples at (587bp) PCR product.

For the infection rates and the age of sampled children, it has been shown identified, using RFLP PCR, that 4.8% of infection was detected in children under the age of 5 years old (the only sampled age category); however, and after performing genotypic characterizations, infection was higher due to C. hominis at 75% than that for the case of infection caused by C. parvum at 25.0%. The current work findings revealed the presence of C. parvum in the sampled children under the three age categories under 5 years old (5)(7) Nigeria. METHODS Stool samples were collected from 165 children aged 0-5 years with diarrhea. Cryptosporidium oocysts were examined by wet mount preparation, using formalin ether and a modified acid fast staining method. DNA was extracted from positive samples using QIAamp DNA stool mini kit and PCR-RFLP assay was carried out after quantification. Genotyping and phylogenetic analysis were done to determine the subtype families and their relatedness. RESULTS From the 165 children studied, 8 (4.8%). Our results agree with (8) who revealed that C. parvum infection rates were higher in children under 5 years of old, 3.15%, than those in older age categories especially students of middle schools, 0.82%. These results can be explained as there might have been an immunodeficiency problem facing the sampled children decreasing immune reaction directed toward C. parvum by the immune cells such as CD3+, CD4+ and CD4+/CD8+ and then resulting in immune response reduction (8).

Conclusion

1. Most of examined to the children from 1 month to 5 years whose suffer from abdominal pain and diarrhea was positive in most cases.

2. The detection of the stool-modified-acid-fast stained oocytes is performed using light microscopy; however, this technique is limited to the identification of the genus level only with no vital information regarding epidemiological properties. Currently, Cryptosporidium can be identified by molecular and genotyped using developed molecular techniques such as nested PCR, RFLP-PCR and nucleic acid sequencing in modern laboratories.

3. Nested polymerase chain reaction technique is the best method than others technique used in diagnosis of cryptosporidium by using specific primer for this parasite.

Acknowledgment: I would like to thank all the study subjects who volunteered and cooperated to become a part of this study. They also acknowledge the technical assistance.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

Funding: Self-funding
References


Hyperlipidemia that Induced in Male Rats and Role of Flavonoids Extract of *Quercus infectoria* in Treatment

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¹Medical Laboratory Department/University of Al-Qalam College, IRAQ

Abstract

The aim of the study demonstrates flavonoids of *Quercus infectoria* as anti-hyperlipidemia. 20 adult male rats used and divided to four equal groups (each group consist 5 rats); rats received ad libidium, rats were given normal water containing 0.5% of hydrogen peroxide and 1% of cholesterol in the feed for 60 days for induction of hyperlipidemia. Hyperlipidemia rats treated with (50mg/kg/daily) flavonoids, fourth Hyperlipidemia rats treated with (100mg/kg/daily) flavonoids. The results show non-significant changes (P < 0.05) in levels of HMG-reductase compare with control group. in hyperlipidemia rats, levels of malonedialdehyied (MDA) show significant increase (P < 0.05) in and significant decrease (P < 0.05) in levels of glutathione (GSH) and catalase compare with control group. While, after using flavonoids extract in treatment, the results showed non-significant changes (P < 0.05) in HMG-reductase, MDA, GSH and catalase compare with control group. It was concluded that flavonoids extract of *Q. infectoria* extract has been a protective effect in rats with hyperlipidemia.

**Keywords:** Quercus infectoria; hyperlipidemia; HMG-reductase.

Introduction

The plant *Quercus infectoria* Olivier (Family-Fagaceae) grows as a shrub or small tree, diclinous and monoecious. It is about 2.5 m in height with many spreading branches(1). The various Quercus species originated in Iran, Iraq and Turkey, but are now widespread and particularly common in Asia Minor, Europe and North Africa. Galls are irregular plant growth, which is stimulated by the reaction between plant hormones and powerful growth regulating chemicals produced by insects or mites(2). The Gall of Q. infectoria is described in detail in ethnobotanical and literature to possess various pharmacological actions such as analgesic, antidote, anti-inflammatory, antipyretic, antiseptic, antistomatitis, deodorant, derivative, desiccant, expectorant, germicidal, hypnotic, hypoglycaemic, powerful astringent, sedative, styptic, tonic, tonic to teeth and gum, and wound healing(3,4). Flavonoids and the other phenolic compounds are commonly known as plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl groups. More than 8000 phenolic compounds as naturally occurring substances from plants have been reported(5,6). Its broad spectrum, its biological properties and multiple applications have given rise to interest in investigating their characteristic according their origin. A lot of studies indicate that flavonoids and phenols in propolis can be able to scavenge free radicals in the human body(7-9). So the aim of study is detection of flavonoids role that extracted from *Q. infectoria* against hyperlipidemia in male rats.

Materials and Method

Flavonoid extraction methods

Flavonoid extract was prepared by immersing 100 gm of dried material of *Q. infectoria* in 500 ml ethyl alcohol (100%) for 24 hrs at room temperature using magnetic stirrer(10-12). The mixture was then filtered using Whatman No. 1 filter papers and the process was repeated using the remaining residue with 300 ml ethanol to ensure the complete extraction in each time. The two filtrates were added and treated with 100 ml lead acetate (1%) for 4 hrs for precipitation. The mixture was filtered, then a mixture of 250 ml acetone and 30 ml of concentrated HCl was added to the precipitate, and filtered. The resulting pellet was finally lyophilized (Freeze- dried) at -50o C under vacuum for 12 hrs. The extract was dissolved in ethyl alcohol, the extraction process was repeated for 1 hr, filtered to produce red
filtrate. Finely, the filtrate was placed in a clean and dry Petri dish away from light at room temperature until deep redbrown powdered was obtained.

**Animal model**

Twenty adult male albino rats used in this study, (wt 150-200 gm with age 3-65 month) and obtained from Science college/ Baghdad University, and ensure its normal and there isn’t any infection.

**Experimental design**

Twenty male rats were used and divided as follow (each group consist of five albino male rats):

I. Rats (negative group) were received standard pellet diet only for seven days and then killed to comparison with other groups.

II. Rats (positive group) were given normal water containing 0.5% of hydrogen peroxide (Saudi Hydrogen Peroxide Company (SHPC)) and 1% of cholesterol in the feed for 60 days.

III. Rats with hyperlipidemia and treated with (50mg/kg/daily) flavonoids, and then killed.

IV. Rats with hyperlipidemia and treated with (100mg/kg/daily) flavonoids, and then killed.

**Prepare of blood solution**

The blood collects from rats by cardiac puncher, under anesthesia, and put in test tubes. After clotting, the tubes were centrifugation for 10 min to obtain sera. The serum was taken and stored by deep freezing until used.

**Measurements**

**Determination of HMG - CoA Reductase Concentration**

The concentration of the HMG-CoA Reductase enzyme estimated using My Biosource analysis kit. using Elisa technology.

**Plasma Peroxidation levels (MDA), Glutathione (GSH) and Catalase**

MDA (malonodialdehyied), was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer\(^{(13)}\). GSH level estimated by mixed 2.3 ml buffer with 0.2ml of the sample and then added 0.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture was analyzed by spectrophotometer\(^{(14)}\). Catalase was measured by using the procedure of Biovision-USA kits. All parameters were done on liver extract.

**Statistical analysis**

The Data were analyzed using a statistical Minitab program. A statistical difference between the means of the experimental groups was analyzed using one-way analysis of variance (ANOVA).

**Results & Discussion**

**HMG reductase**

The levels of HMG reductase in positive group show non-significant increase (P < 0.05) compared with control group. The levels of HMG reductase in third and fourth groups show non-significant decrease (P < 0.05) compare with control group as shown in figure (1).

In terms of the enzyme HMG-CoA reductase, the results showed that the concentrations of the enzyme in the group given high cholesterol diet decreased but no significant differences found with the control group. The slight decrease in enzyme concentrations that were not significant with the group could be explained by the fact that the concentration of cholesterol inside the cells is regulated by Internal construction and taking from outside (via diet). Internal construction is done by hepatic HMG-CoA reductase feedback, while the external source is controlled by LDL receptors\(^{(15)}\). About the role of flavonoids to slightly decrees of HMG-reductase may back to its affect lipid metabolism through inhibition of acyl coenzyme A: cholesterol O-acyltransferase and 3-hydroxy- 3-methylglutaryl-coenzyme A (HMG CoA) reductase in rats\(^{(16-17)}\).

**MDA, GSH and catalase**

The levels of MDA (increase), GSH (decrease)
and catalase (decrease) in positive group show high significant changes (P < 0.05) compared with control group. The levels of MDA, GSH and catalase in third and fourth groups show non-significant changes (P < 0.05) compare with control group as shown in figures (2-4).

About the role of hyperlipidemia to increased MDA levels and decreased the levels of GSH and catalase. Anwer et al, referred that the elevated level of LPO is attributed to the enhanced production of ROS\(^{(18)}\). After treatment with flavonoids extract the MDA levels decreased and GSH and catalase levels increased that may back to they protect against oxidative damage by directly neutralizing reactive oxidants\(^{(19)}\). Al-Jumaily et al, referred that the flavonoids pure of Iraqi propolis has led to a reduction of the inhibitory effect as well as for damage to the work of the liver and there by protect members from free radicals and inhibit the liberation of these ROS\(^{(20)}\).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**Reference**

8. Vongsak, B, Kongkiaatpaiboon, S, Jaisamut, S,


Abstract

**Background:** AMI (acute myocardial infarction) is one of the most common cause of death. In this study, the prognostic value of WBC count in patient with AMI was assessed in 24 hrs after admission. STEMI (ST segment elevation myocardial infarction) is frequently associated with leukocytosis, it is that the peripheral leukocyte count have important prognostic implication in AMI.

**Aim of The Study:** This study conducted to evaluate and measure level of WBC count in patient with STEMI and their effect on cardiovascular outcome.

**Patients and Method:** We have 100 patients (male and female) with mean age (40-80) years admitted to the AL-diwaniyha teaching hospital CCU (coronary care unit) department and peripheral blood sampling taken after 24 hrs of admission and another sample after 1 week and we record the main early and delay squally occurred. Patient that admitted to CCU were confirmed with AMI by clinical features, examination and investigations (ECG with ST segment elevation, positive cardiac troponin).

**Results:** The mean WBC count in all patients was 11.260 ± 3.600 X10³/CC. There is no significant difference in mean WBC count among patients with inferior, lateral and posterior wall MI (P > 0.05); however, mean WBC count was significantly highest in patients with extensive anterolateral MI (P<0.001); followed by patients with anterior MI. Early complications were observed in 52 patients (52.0 %), these complications were in the form of arrhythmias such as VF, VT, AF, heart block and bradycardia and acute heart failure. The most common early complication was VF (32.0 %).

Late complications were observed in 28 patients (28.0 %), these complications were in the form of chronic heart failure or unfortunately death of patients. We noted that patient with high WBC after admission have close relation to more damage and necrotic myocyte an liable for early complications like arrhythmia (VT, VF) and acute HF.

**Conclusion:** WBC count remained a significant predictor of complication after admission for patients with STEMI.

**Keywords:** late complications, patient, Leukocytosis, and myocardial infarction

**Introduction**

AMI the most common form where patient presented acutely in previously asymptomatic patient. STEMI occurs when coronary blood flow decrease abruptly, often thrombotic occlusion of coronary arteries previously affected by atherosclerosis, this injury produces or stimulate by risk factors (age, hypertension, DM, smoking, obesity, sedentary life style, alcohol and hypercholesterolemia). The pathogenesis of atherosclerosis is multifactorial. Broadly, endothelial injury and dysfunction result in the adhesion and transmigration of leukocytes from the circulation into the arterial intima as well as the migration of smooth muscle cells from the media into the intima, thus initiating the formation of atheroma or atherosclerosis.
All myocardium that supplied by the offender artery become ischemic. Resulting in chest pain and ECG evidence of transmural (full thickness) ischemia (ST segment elevation) in the leads reflective of that region of the heart.

Subsequently necrosis begins within minutes and progress during several hours in a wave front fashion from the endocardial surface to the epicardia surface. If ischemia persists for several hours, transmural infarction result. In contrast if blood flow is restored during the period of progressive necrosis, the ischemic myocardium is salvaged and size of infarction is reduced.

More than half of patient with AMI died before reaching the hospital.

To confirm the diagnosis of AMI in addition to ECG changes, positive cardiac biomarkers (troponin I, E — CK, MB myoglobin) may be needed.

This study focusing on leukocytosis as dependent predictor for mortality or adverse cardiovascular outcome in patients with STEMI.

In this setting elevated WBC count play a central role in the reparative process that takes place to replace the necrotic tissues.

Traditionally an elevated WBC count is an indicator of systemic inflammation has been acceptable as part of healing response following AMI.

It has frequently been shown to be a predictor of adverse cardiovascular events in addition to part of systemic inflammatory response that mention.

Elevated WBC count to be a proxy for the intensity of the peri-infarction inflammatory response.

The corner stone of this study shown an elevated WBC count measured during acute phase of MI (24 hrs. after admission) associated with adverse out come.

This relation ship strongly associated with extension of infarcted area. Leukocytosis in STEMI affect on seuality through multiple pathogenic mechanisms that mediate inflammation cause proteolytic and oxidative damage to the endothelial cells ,plug the microvasculature ,induce hypercoagulability and promote infarct expansion ,so leukocytosis is a risk factor for early and future cardiovascular events.

There is significant correlations between the ischemic severity and consequence of acute phase response (leukocytosis).

**Aim of the Study:** this study conducted to evaluate level of WBC count in patients with STEMI and record their effect on outcome that occur in patients with leukocytosis.

**Patients and Methods:** This study was done in CCU at Al-diwaniyah teaching hospital from (January 2018 –December 2018) including patients with STEMI, patients with acute coronary syndrome and ST-segment elevation in the ECG more than (1) mm in limbs leads and 2mm in the chest leads or new onset LBBB.

with elevated cardiac biomarkers especially cardiac troponin I, (patient with ACS without ST- segment elevation was excluded). which confirm the diagnosis by supervisor.

A (100) patients with STEMI were included.

**exclusion criteria:**

Patient with any recent infection ,those with hematological disorders , any chronic disease ,drugs, that causes leukocytosis. Those with non STEMI &females during menses were excluded.

Complete history & physical examination was done for every patient. venous blood sample into the plain tube for CBC was taken immediately after admission.

Another sample was taken after 7-8 days .Early complications that occur during the hospitalization time or late complications that occurs any time after discharge: VT, VF, HF, ventricular aneurism, dead , was closely recorded.

**Results**

In this study there is significant increase in WBC count in patient with STEMI The WBC count mean in all patients was 11.260 ± 3.600 X103/ CC, as shown in table 1. There was no significant difference in mean WBC count among patients with inferior, lateral and posterior wall MI (P > 0.05); however, mean WBC count was significantly highest in patients with extensive anterolateral MI (P<0.001); followed by patients with anterior MI, as shown in table 1.
Table 1: WBC count according to location and extent of myocardial infarction

<table>
<thead>
<tr>
<th>P</th>
<th>Mean ± SD</th>
<th>n</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.001 HS</td>
<td>15.903 ±0.936</td>
<td>18</td>
<td>Extensive</td>
</tr>
<tr>
<td></td>
<td>8.257 ±2.204</td>
<td>36</td>
<td>Inferior</td>
</tr>
<tr>
<td></td>
<td>13.053 ±2.058</td>
<td>34</td>
<td>Anterior</td>
</tr>
<tr>
<td></td>
<td>8.266 ±1.691</td>
<td>10</td>
<td>Lateral</td>
</tr>
<tr>
<td></td>
<td>8.010 ±1.010</td>
<td>2</td>
<td>Posterior</td>
</tr>
<tr>
<td></td>
<td>11.260 ±3.600</td>
<td>100</td>
<td>Total</td>
</tr>
</tbody>
</table>

Early complications were observed in 52 patients (52.0 %), these complications were in the form of arrhythmias such as VF, VT, AF, heart block and bradycardia and acute heart failure, as shown in table 2.

Table 2: Prevalence rate of early complications in patients with acute MI

<table>
<thead>
<tr>
<th>%</th>
<th>n</th>
<th>Early</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.0</td>
<td>32</td>
<td>VF</td>
</tr>
<tr>
<td>20.0</td>
<td>20</td>
<td>VT</td>
</tr>
<tr>
<td>4.0</td>
<td>4</td>
<td>AF</td>
</tr>
<tr>
<td>13.0</td>
<td>13</td>
<td>Acute HF</td>
</tr>
<tr>
<td>6.0</td>
<td>6</td>
<td>Heart block</td>
</tr>
<tr>
<td>4.0</td>
<td>4</td>
<td>Bradycardia</td>
</tr>
<tr>
<td>11.0</td>
<td>11</td>
<td>Cardiogenic shock</td>
</tr>
</tbody>
</table>

The most common early complication was VF (32.0 %). Late complications were observed in 28 patients (28.0 %), these complications were in the form of chronic heart failure or unfortunately death of patients, as shown in table 3.

Table 3: Prevalence rate of late complications in patients with acute MI

<table>
<thead>
<tr>
<th>%</th>
<th>n</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>10</td>
<td>Chronic HF</td>
</tr>
<tr>
<td>8.0</td>
<td>8</td>
<td>Death</td>
</tr>
<tr>
<td>1.0</td>
<td>1</td>
<td>Ventricular aneurysm</td>
</tr>
<tr>
<td>10.0</td>
<td>10</td>
<td>Arrhythmia</td>
</tr>
</tbody>
</table>

WBC count Mean were significantly higher in patients with complications, whether early or late. Those with early complications in comparison with patients who were free of early complications, 13.84 ± 1.61 X 10^3 /CC versus 8.47 ± 2.15 X 10^3 /CC, P < 0.001. Moreover, mean WBC count was significantly higher in patients with late complications in comparison with patients who were free of late complications, 13.85 ± 3.600 X 10^3 /CC versus 8.010 ± 1.010 X 10^3 /CC, P < 0.001.
± 3.41 X 10³ /CC versus 10.85 ± 3.41 X 10³ /CC, P < 0.001, as shown in figure 1.

The cutoff value that predicts any type of complication was WBC count of > 10.5 X 10³ /CC with a sensitivity of 88.9 % and specificity of 82.6 %. The cutoff value that predicts early complication was WBC count of > 10.5 X 10³ /CC with a sensitivity of 92.3 % and specificity of 83.3 %. The cutoff value that predicts late complication was WBC count of > 13.5 X 10³ /CC with a sensitivity of 71.4 % and specificity of 83.3 %, as shown in table 4.

**Table 4: Characteristics of ROC curve**

<table>
<thead>
<tr>
<th>Late Complications</th>
<th>Early Complications</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;13.5</td>
<td>&gt;10.5</td>
<td>Cutoff</td>
</tr>
<tr>
<td>0.789</td>
<td>0.929</td>
<td>0.919</td>
</tr>
<tr>
<td>0.696 to 0.864</td>
<td>0.860 to 0.971</td>
<td>0.847 to 0.964</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>95 % CI</td>
</tr>
<tr>
<td>71.4</td>
<td>92.3</td>
<td>88.9</td>
</tr>
<tr>
<td>83.3</td>
<td>83.3</td>
<td>82.6</td>
</tr>
</tbody>
</table>

**Discussion**

In 1974, Friedman et al. (13) first who described the association between WBC count and ACS. They found that an increased WBC count associated with increased risk of developing first AMI. Other studies later on confirmed this observation. Schlant et al. were the first to document an elevation in WBC count as a predictor of morbidity and mortality in patients who survived AMI (14).

Furman et al. (15) examined the association between WBC count and mortality.

Consistent with the findings from the present study.

More recently, Barron et al. (16) examined the association between WBC count and angiographic findings in the thrombolysis state in high TIMI score AMI. They found that patients with a closed infarct-related artery at 60 and 90 minute had a higher WBC count than patients with patent artery. (17)

Many epidemiological studies state that the baseline WBC is associated with increased incidence of AMI and mortality and there is current scientific interest in the prognostic value of the WBC determined during the acute phase of AMI to predict mortality. (18)

These findings are in agreement not only with this study, but also with other studies of other inflammatory markers as CRP and IL-6 elevations of which appear to primarily predict death rather than recurrent ischemic events (19). The basis for this till now unknown, but the early divergence of the cumulative mortality curves suggests that patients with an elevated WBC count have a higher risk of death from the index event (20).

Many explanations have been proposed to state this association: resistance to thrombolytic therapy due to alterations in the microcirculation, hypercoagulable state, a no-reflow phenomenon caused by leukocytes, indirect cardiotoxicity mediated by proinflammatory cytokines, promoters of ischemia-reperfusion injury, and lastly expansion of the AMI. Regarding this final point, it is important to bear in our mind that the leukocyte response that occurs following AMI is a central part of the inflammatory cascade which initiated to replace the necrotic tissue with fibrosis and scarring. This fact may suggest that the greater the amount of necrosis, the larger the leukocyte response, an assertion based on experimental studies that show a direct relationship between the extent of necrosis and the level of both the local and the systemic leukocyte response. (21)

In clinical settings, the extent of AMI is usually estimated using indirect parameters. Thus, various studies have related the WBC to various association with the size of the AMI: the development of heart failure, significant correlations with the peak level of isoenzyme MB of creatine kinase (CK-MB), or with left ventricular ejection fraction. (22)
In this study, WBC acted as an independent predictor of early and late complications. This finding provides indirect evidence in favor of an independent role for WBC in the pathogenesis of post-AMI complications.

Suggest that WBC is a useful and valid biochemical tool for risk prediction of patients with AMI. The neutrophils rule in animal models of ischemia-reperfusion(23)

the findings of this study and finding of Barron et al are consistent with the fact that WBCs may in some way be linked to the cause of the increased. In animal models of ischemia-reperfusion, neutrophils appear to lead to infarct expansion. In a canine model of AMI, neutrophil depletions was associated with a marked reduction in infarct size. The mechanism by which neutrophils cause this damage is unclear. Engler et al. and others state that reperfusion after prolonged ischemia leads to progressive leukocyte capillary plugging and the —no refow phenomenon. This plugging seem to be results in part from neutrophils binding to the ischemic endothelium via the leukocyte integrin CD11b/CD18 (Mac-1) receptor .Three animal studies have demonstrated that treatment with an antibody to the CD18 receptor on neutrophils reduces infarct size (24)

Conclusions

The finding of the current study showed that leukocytosis in patient with STEMI was significantly associated with high rates of mortality in short term follow up also more over the risk of in-hospital death .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References
13. Barron HV, Harr SD, Radford MJ, Wang Y,


Role of Interleukin 33 During Infection with Toxoplasmosis in Rheumatoid Arthritis Patients

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2Biology Department, College of Science, Kirkuk University, Kirkuk, Iraq

Abstract

Toxoplasma gondii is a very common obligate intracellular parasite with highest infection rate among world populations. The Rheumatoid arthritis (RA) considered as an autoimmune disease characterized by loss immunological tolerance to self-antigens. Present study Aims to detection the seroprevalence of toxoplasmosis among RA patients and healthy control and evaluate concentrations of IL-33 to understand their role during infection. Seropositive cases of IgG was 36.7% percentage in RA patients with highest value of IgG 0.395 IU/ml while control group was 100% seronegative. Three cases only were seropositive to IgM with 1.17% and their mean 0.54 IU/ml.

IL-33 levels in RA patients with toxoplasmosis was highest 187.74± 33.24 pg/ml when compared with RA patients 107.88±18.41 pg/ml and control group 55.59±25.75 pg/ml ,there was a significant differences in comparison between studied groups.

Key words:- Toxoplasmosis, Rheumatoid arthritis, IgG, IgM, IL-33.

Introduction

Toxoplasmosis is a common obligate intracellular protozoan parasite zoonotic disease infectious by Toxoplasma gondii, classified as a Coccidia and phylum Apicomplexa (1). All or most marsupials and mammals can serve as intermediate hosts, while several Felidae, enrolled domesticated cats, are the final hosts for T. gondii (2). Human infection generally happened by uptake raw uncooked meat which have cysts (or possibly tachyzoites). animals, including herbivores can be infected by eating vegetables and plants contaminated with oocysts. However, infections can be transmitted through placenta from mother who suffered from infection during pregnancy (3). Infection leading to encephalitis in the fetus, cerebral calcification and mental retardation or psychomotor, T. gondii may be transmitted by transplanted organs or transfused blood (4).

The primary or secondary infections with Toxoplasma gondii can mimicry immune response and make change in the immune response to generate autoimmune disease including autoimmune thyroid diseases, inflammatory bowel disease, systemic sclerosis and rheumatoid arthritis (5,6). Rheumatoid arthritis is an autoimmune disease described by inflammatory polyarthritis of small and big joints, can cause major disability and uneasiness (7). That affects approximately 0.5% to 1% among adults a worldwide (8-9). The pathogenesis of this infection is multifactorial including genetic and ecological factors (10). It is identified by peripheral joints progressive inflammatory synovitis, cartilage damage, corrosions of bone and production of autoantibodies like rheumatoid factor and antibodies of the anti-citrullinated protein (10).

The present study was aimed to evaluate levels of interleukine 33 (IL-33) in patients with rheumatoid arthritis and infected toxoplasmosis infected which diagnoses by anti-Toxoplasma antibodies including IgG and IgM seroprevalence

Material and method

Study subjects

Study was implemented as A case-control study to identify the IL-33 role in the prevalence of T. gondii seropositivity in rheumatoid arthritis patients. samples were collected from patients attending to the department
of Rheumatology in Baghdad and Al-Kindi Teaching Hospitals in Baghdad province, in addition to outpatients clinics according to ethical approval of environmental and health ministry of Iraq. During March to July 2018, samples included 256 of rheumatoid arthritis patients that treated with methotrexate (207 females and 49 males) their age ranged from (15 – 65) years with a mean (40.77± 1.5), and healthy individual consisting of 50 person of healthy people (16 females and 34 males) did not suffer from any pain in the joints and didn’t have a satisfactory history of any autoimmune diseases.

serological testing

About five ml of venous blood were collected from patients and control groups in gel tube, its left at room temperature for 15 minutes then its centrifuged for 5 min at 3000×g . serum were collected in eppendorf tubes and stored at - 20.

The clinical diagnosis of rheumatoid arthritis implemented specialist physician in addition of clinical examination and several laboratory tests including Rheumatoid Factor (RF) (Biosysmeme, Spain) and C- Reactive Protein test (CRP) (Spinract, Spain). The T. gondii antibodies including IgG and IgM were analyzed using ELISA kits (Bioactiva, Germany).

Interleukin level was determined by a commercially available human ELISA kits for IL-33 (Peprotech, USA).

The Statistical Analysis System- SAS (2012) program was used to data analysis. The data was shown as percentage (%) and mean ± standard deviation (SD) and standard error (S.E.). Differences between means were assessed by Least significant difference –LSD test, at the probability level of 0.01 and 0.05 to assess the differences between the study groups.

Results and Discussion

The results of present study were shown that 36.71% were seropositive cases of IgG in 94 patients with RA compared to 162 seronegative cases at 63.2%, while the control group was 100% seronegative. High significant differences recorded between studied groups (p≤0.001) (table 1). The infections of Parasitic may encourage immunomodulatory variable impacts and control of autoimmune disease.

The most interesting association of T. gondii with other diseases is with rheumatoid arthritis. In humans, T. gondii infection may cause a symmetrical polyarthritis of the minor joints of hands, fingers, wrists and knees, as well as T. gondii may be implicated in autoimmune disease through acting as a ligand for toll-like receptors (TLRs).

Table (1): the percentages of T. gondii infection according to Toxo IgG IU/ml ELISA assays in studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rheumatoid Arthritis</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>IgG Antibody</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>94</td>
<td>36.71%</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>162</td>
<td>63.2%</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.0001 **</td>
<td></td>
</tr>
</tbody>
</table>


Table (2) shows that the level of IgM was elevated in group of rheumatoid arthritis patients infected with toxoplasmosis compared with other groups with highly significant differences.

**Table (2): IgM levels estimated by IU/ml for studied groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
<th>LSD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA with toxoplasmosis</td>
<td>3</td>
<td>0.540</td>
<td>0.062</td>
<td>0.029</td>
<td>0.194 **</td>
<td>0.00061</td>
</tr>
<tr>
<td>R.A patients</td>
<td>253</td>
<td>0.0810</td>
<td>0.022</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>0.063</td>
<td>0.018</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the current study, the relation between toxoplasmosis and RA in Iraqi patients was been recognized by the detection of antibodies IgG and IgM in sera of the studied groups by ELISA test. ELISA is an precise serological tool for the diagnosis of toxoplasmosis dependent on Ag - Ab interaction. The reaction can be evaluated objectively by quantization of the color that advanced by an ELISA reader. This technique is economical and also suited for analyzing large number of samples at the same time. The present study is agree with Kuba et al. which found that the seroprevalence of T. gondii IgG and IgM antibody were 33.33% and 20.40% respectively in RA patients treating with methotrexate, and 36.00% and 8.00% in RA patients without treatment, while it was 12.00% and 24.00% in healthy control. Salman and Mohammed found in Kirkuk city that the percentage seroprevalence of toxoplasmosis of IgG and IgM were (47.54% - 6.55%) respectively in RA patients. El-Henawy et al. showed that Anti-Toxoplasma IgG antibodies were positive in (76.7%) of patients with RA versus controls (48.3%), they suggested that higher seroprevalence of anti-Toxoplasma IgG antibodies in RA group refer to correlation between latent Toxoplasma infection and RA. Conversely, other study, show no significant difference of IgG levels which found between RA group and controls and T. gondii infection might be an incidental result.

The elevated seroprevalence of anti-Toxoplasma IgG antibodies in RA group may be expected as a result of diverse cooking methods and oocysts ability to live in different weathers in addition of a close association with cats. An increased risk of T. gondii infection in patients suffered from rheumatic diseases can be share in to changes in innate and adaptive immune responses. The Patients of RA were located to be highly vulnerable to T. gondii infection - particularly throughout periods of immunosuppression that followed treatment with TNF-α inhibitors. The Patients of RA were located to be highly vulnerable to T. gondii infection - particularly throughout periods of immunosuppression that followed treatment with TNF-α inhibitors.

The elevated seroprevalence of anti-Toxoplasma IgG antibodies in RA group may be expected as a result of diverse cooking methods and oocysts ability to live in different weathers in addition of a close association with cats. An increased risk of T. gondii infection in patients suffered from rheumatic diseases can be share in to changes in innate and adaptive immune responses. The Patients of RA were located to be highly vulnerable to T. gondii infection - particularly throughout periods of immunosuppression that followed treatment with TNF-α inhibitors.

Table (3) illustrated that all samples of rheumatoid arthritis have positive results for the test index RF, CRP, except for the control group, which showed the complete passivity of both tests and with highly significant differences p≤0.0001.

**Table (3): index of the Rheumatic Factor and C- Reactive Protein test of the study groups.**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Response</th>
<th>Patients</th>
<th>Rheumatoid Arthritis</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>Ve+</td>
<td>97</td>
<td>100</td>
<td>159</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Ve-</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>CRP</td>
<td>Ve+</td>
<td>97</td>
<td>100</td>
<td>159</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Ve-</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>100</td>
<td>159</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>
of the elevation of rheumatoid factor (RF) titers in serum and synovial fluid is a characteristic feature of RA and perhaps implicated in pathogenesis of this disease (21). Rheumatoid factors are autoantibodies with specificity for the Fc portion of IgG and may be formed in multiple iso-types (22). The results of the test rheumatoid factor were similar with El-Henawy et al. (16) who found the mean of patients of rheumatoid arthritis with toxoplasmosis of 46 patients to be 74.01±8.62 (IU/mL), while the mean of patients without toxoplasmosis was 64.56±3.09 (IU/mL) represented 14 patients.

There is another possible marker for increasing the danger of rheumatoid arthritis, is a C-reactive protein (CRP), which one of the acute phase reactants that increase in inflammation response (23). The results of the current study were agree with Salih (24) which recorded in a study that women in the Kurdistan region suffering from rheumatoid arthritis and all samples were positive for the RF and CRP tests. These autoantibodies are linked with T. gondii especially when they are of high rates (25).

Table (4) shows that the level of IL-33 in the group of RA cases infected with toxoplasmosis was high levels with highly significant differences than other groups.

**Table (4): Levels of IL-33 estimated by pg/ml for study groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Standard errors</th>
<th>P-value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA patients with toxoplasmosis</td>
<td>50</td>
<td>187.74</td>
<td>33.24</td>
<td>5.01</td>
<td>0.0001</td>
<td>39.427 **</td>
</tr>
<tr>
<td>RA Patients</td>
<td>50</td>
<td>107.889</td>
<td>18.41</td>
<td>4.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>55.59</td>
<td>25.75</td>
<td>5.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (5) revealed the comparisons of IL-33 levels between the studied groups.

**Table (5): Multiple comparisons of IL-33 for potential couples between study groups.**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Group 1</th>
<th>Group 2</th>
<th>mean difference</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-33 Concentration (Pg/ml)</td>
<td>RA patients with toxoplasmosis</td>
<td>RA patients</td>
<td>79.851</td>
<td>0.0075</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>RA patients</td>
<td>Control</td>
<td>132.15</td>
<td>0.0002</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>RA patients</td>
<td>Control</td>
<td>52.299</td>
<td>0.0093</td>
<td>HS</td>
</tr>
</tbody>
</table>

The Interleukin-33 (IL-33) is reported as one of cytokine of IL-1 family, which can be induced cytokine syntheses and inflammatory responses mediated through its receptor ST2. It is expressed in several tissues and cells like liver, lung, central nervous system also epithelial, endothelial, smooth muscle, macrophages and fibroblasts cells (26). The pathway of IL-33/ST2 have major role in defense of host and in immune organization in inflammatory and infectious diseases (27). IL-33 have capability to elevated the Th2 immune response and lowering the production of Th1 cytokines (28) also it plays major role in innate and adaptive immunity (29). The gene of IL-33 is located in chromosome 9 (30). Mature IL-33 mediate its biologic effects via T1/ST2 binding by activating NF-xB and MAP kinase (31). The present study is agree with AL-Shamma (32) which showed a highly significant increase IL-33 in concentrations in sera of toxoplasmosis female
of Iraqi patients compared with the healthy control. This finding may indicate that toxoplasmosis might influence cytokine production in these patients (33).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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**References**

Treatment of Induced Lead Toxicity by using DMSA in Puppies

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Abstract

To determine efficacy of DMSA in treatment of sub-chronic lead exposure in dogs, fifteen puppies aged 2-4 months from both sexes were used for experimental induction of lead toxicity. These puppies were adapted for two weeks and divided randomly to three groups, each group contain five puppies; Group 1 which was regarded as negative control; Group 2, this group was drenched 15mg/kg of lead acetate daily for sixty days; Group 3, this group was drenched 15mg/kg of lead acetate daily for sixty days then were treated with DMSA 10 mg/Kg twice daily orally for one week. These animals were clinically examined daily till the end of experiment, then whole blood, bone, liver and brain were collected for estimation the lead concentrations. The results showed presence of several signs after 15 days of exposure which included depression, diarrhea, tremor then these signs subsides after 7 days of treatment with DMSA. Also, the results presented a significant increasing in the concentrations of lead in liver, blood, bone and brain in group 2 as compared with control group, after treatment with DMSA, group 3 showed a significant decrease in lead concentrations in liver, blood, bone, and brain as compared with group 2. In conclusion, DMSA is an effective therapy for clinical cases of lead toxicity.

Key words: DMSA, Puppies, Lead, Toxicity.

Introduction

Lead is a toxic type of heavy metal causes an adverse effects on the GIT, nervous system, also, cardiovascular, urinary and hematological systems, the main mode of action is interrelation of lead affinity with proteins (¹).

Lead poisoning affects thousands to millions of children in many countries which may lead to many biochemical and some neurological dysfunctions (²), and it is commonly occurs via oral route by consumption a contaminated water, leaded paint and ingestion of leaded particles mainly the young dogs that have strange eating habits (³).

The dose of lead that can do the adversarial effects in dogs fluctuates, but 1 mg/kg bw/day of lead acetate can be regarded as a LOEL causing increase in blood pressure after ten days from exposure (⁴).

Rosenman et al. (⁵) stated that colic may combine with other signs such as constipation, vomiting, abdominal pain, cramping, nausea, anorexia, sometime diarrhea and weight loss.

Succimer (DMSA) is one of the most antidotes that used in cases of poisoning by heavy metals especially lead. The main advantage of DMSA is its high effectively when used orally because it is solvable in water (⁶).

Due to importance of lead toxicity in dogs, this study was aimed to induction of lead toxicity in dogs and evaluate the efficacy of DMSA in treating the lead toxicity.

Materials and Method

A Fifteen healthy puppies with same age (2-4 months) and both sexes were adapted for 2 weeks before initiation the experiment. These fifteen dogs were divided into 3 groups, each group have 5 animals:

a. Group 1 (Control): this group regarded as control negative.
b. Group 2 (Pb): this group was drenched orally with lead acetate at 15 mg/ kg per day for sixty days according to Faustman et al. (7).

c. Group 3 (DMSA+ Lead): this group was drenched orally with lead acetate at 15 mg/ kg/ day according to Faustman et al. (7) for 60 days then was treated orally with 10 mg/ Kg DMSA twice daily for one week.

**Clinical examination**

Clinical examination was carried out along the period of experiment for all dogs every day for any changes in appetite, presence of diarrhea or any other symptoms and every 2 weeks the pulse rate, rectal temperature and respiratory rate were examined.

**Lead level measurement:**

Specimens were taken for lead level measurement including whole blood, bone, liver and brain of all animals at the end of experiment (day 68), the tissues preserved by freezing immediately after collection, Lead analysis done by adding 20 ml of nitric acid and one ml of concentrated H$_2$SO$_4$ to digest 1g of tissues then heated at hotplate until foams appeared then 3 ml of nitric acid and 1ml of HClO$_4$ were added without roasting the sample then the volume was completed to 100 ml by using deionized water then the concentration was estimated by Atomic Absorption Spectrophotometer according to (8).

**Statistical Analysis**

All data were examined according to (9).

**Results**

Puppies were monitored daily for development of some clinical signs such as depression, diarrhea, dehydration and other signs. All lead treated puppies showed different degrees of depression appeared after day fifteen till to the end of experiment. Lead treated puppies showed slight decrease in appetite and many lead treated animals showed mild diarrhea and some showed tremor and aggressive behavior after 30 days of lead exposure. After treatment with DMSA, all puppies improved after 7 days of treatment and showed a normal signs. All puppies showed a normal temperature, pulse and respiratory rates before and after lead exposure till the end of experiment.

The results of estimation lead concentrations in different tissues showed that the concentrations of lead in liver, blood, brain and bone were increased significantly in group 2 as compared with control group (G1) , after treatment with DMSA, the group 3 showing a significant decreasing in the concentration of lead in bone, blood, brain and liver as comparing with group 2 (Table 1; Fig. 1,2,3,4).

**Table 1: Lead concentration (ppm) in different tissues of all groups at the end of experiment (Mean ± SE)**

<table>
<thead>
<tr>
<th>Tissues Groups</th>
<th>Blood</th>
<th>Bone</th>
<th>Liver</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Con.</td>
<td>0.1460±0.020C</td>
<td>0.1420±0.018C</td>
<td>0.1100±0.029C</td>
<td>0.1420±0.031C</td>
</tr>
<tr>
<td>G2 Tox.</td>
<td>1.0540±0.099A</td>
<td>13.1040±0.815A</td>
<td>12.0440±0.439A</td>
<td>11.1480±0.321A</td>
</tr>
<tr>
<td>G3 DMSA</td>
<td>0.4600±0.039B</td>
<td>8.3140±0.301B</td>
<td>5.1580±0.403B</td>
<td>6.0500±0.379B</td>
</tr>
</tbody>
</table>

Capital letters mean a significant differences (p<0.05) between groups

![Figure 1: Lead concentration (ppm) in blood of all groups at the end of experiment](image1.png)

![Figure 2: concentration of Lead (ppm) in bone of all groups at the end of experiment](image2.png)
Due to presence of lead sources like leaded gasoline, leaded paint, improper dispose of battery and other sources and lead poisoning have accumulative nature, the risk of lead poisoning of human and animal in Iraq considered high level and its effects ranged from hypertension to death, depends on the dose, rout and body status.

The presented study showed that all lead treated animals exhibit different degrees of depression which appeared after day fifteen and extended to the end of experiment. The presented results were in compatible with results of Fewtrell et al. \(^{(10)}\) who showed that lead interfering with neurological functions in all stages of the life at low levels, and may cause many signs ranged from blindness to encephalopathy that causing dullness, muscular tremor and headache. Puppies showed slight decrease in appetite and eight out of twenty lead - treated animal showed mild diarrhea after 30 days of lead exposure. The current results were similar with results of Rosenman \(et\ al.\) \(^{(5)}\) which showed that colic may combine with other symptoms such as pain of abdomen, constipation, vomiting, cramps, anorexia, nausea, sometime diarrhea and loss of weight. GIT signs happen at blood lead concentration of 100-200 µg/dL, but sometimes associated with level between 40-60 µg/dL.

During lead exposure period, all puppies showed no shafting change in body temperature, pulse rate and respiration. Due to continues low dose of sub chronic exposure of lead there is no shafting change in temperature , pulse rate and respiration and these results are in agreement with those of \(^{(11)}\) which showed that signs occur in association with higher doses or increasing the exposures length. The lead toxicity doesn’t related to the route of exposure and it is depending on blood lead levels.

DMSA is non-toxic, water-soluble, when orally-administered may act as metal chelator, so that it can be used as antidote for heavy metal toxicity \(^{(12)}\).

Ramsey \(et\ al.\) \(^{(13)}\) showed that in dogs that treated with succimer (10 mg/kg B.W., PO, q 8 h) for 10 days, mean blood lead concentrations decreased 53.6, 76.2, and 60.9% from pretreatment value on days 3, 7, and 20, respectively, while, mean urine lead concentrations on days 0, 3, 7, and 20 were 70.0, 485.4, 254.3, and 28.3 micrograms/dl, respectively, they concluded that dogs with lead poisoning, when treated orally with DMSA for ten days it effectively reduced the lead concentrations in blood and absence of lead poisoning signs.

Studies in human with lead poisoning showed that DMSA rapidly increase the urinary excretion of lead, also in persons with low exposure to lead it give a significant improving \(^{(14)}\). An increasing the excretion of lead in urine is due to chelation therapy of DMSA. In a study of Graziano \(et\ al.\) \(^{(15)}\) which done to establish the DMSA therapeutic dose, it showed a 28-fold increase was seen in excretion of urinary lead after the first five doses.

In a study done to estimate blood and brain lead after DMSA treatment, they concluded using of DMSA to reduction of lead concentrations in brain \(^{(16)}\).

In conclusion, DMSA is a good therapeutic agent for treatment lead toxicity in human and animals.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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**References**


Study the Effect of Humidity Variable on the Patient with Jaundice Stay Period inside the Incubator

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Abstract

In this study, a DHT22 sensor was used based on the Arduino platform for the purpose of measuring and monitoring the humidity in the incubator during the period of treatment of new-borns suffering from jaundice. This system was adopted because it has a range of advantages that make it accessible to researchers as it does not need a highly experienced or specialist operator because it is easy to use as well as low-cost. The use of this system provides continuous and real monitoring of the humidity in the incubator to help determine the effect of humidity on the treatment period of the child with jaundice in the hospital. In addition, checking the level in the humidity error and the difference between the required moisture and the actual moisture recorded.

Keywords (Jaundice, Humidity, and sensor)

Introduction

Jaundice which is a disease during which excess bilirubin accumulates in the blood, so the newborns are at raised risk of jaundice. When the newborn with jaundice let without treatment, which will result in neurological impairment and death. Jaundice ensuing from unconjugated bilirubin is well treated with exposure to blue light (1).

Immediately after birth, the newborn moves to a completely new environment that is radically different from the mother’s womb environment, an environment that is not sufficiently protected compared to the womb and is initially unsuitable for a child’s life due to the changing factors surrounding the newborn, especially the moisture factor.

If the relative humidity inside the incubator was increasing, so that will be reduced the loss of water for the skin of the premature new-borns and act as a contributed factor to the maintenance of his corporal temperature (2).

The early days of new-born life are a watershed in his life, because the daily evaporation loss of preterm infants can reach 20% of the body mass, and by increasing air humidity within the incubator, this loss can be reduced. The skin evaporative exchanges between the neonate and the environment are directly proportional to the water vapour partial pressure difference between the new-born’s skin and the air (3).

Thus it should give a healthful hydrothermal environment to decrease the risk of body hypothermia or hyperthermia for newborn infant (4).

The process of measurement the humidity of the therapeutic environment and so understand the perfect conditions for the treatment process is one among the most necessary things that should be of high interest, because it’s an excellent link between the conditions and factors related to the treatment method, and therefore facilitate the study of the impact of humidity alone or combined with different factors in effecting on diagnosing and treatment method.

The Arduino system is considered one among the simplest and most versatile electronic solutions in terms of easy use and speed of learning still as low price of producing components as well as simple to connect and programming by computers, additionally the availability of those electronic components in the local markets, permitting the research worker to work in a very wide
space of ideas to dynamical developing and therefore let to get output a lot of comprehensive solutions and optimality.

The main goal of this study is to manufacture an easy system which will provides a precise perception of measurement the humidity within the incubator and therefore facilitate to calibrate and determine the appropriate degree of treatment.

Theory

Neonatal jaundice is one among the foremost common conditions needing medical attention in newborn babies. About 60% of term and 80% of preterm babies develop jaundice within the 1st week of life, and concerning 10% of breastfed babies are still jaundiced at age one month. Neonatal jaundice is usually harmless, however high concentrations of unconjugated bilirubin could occasionally cause kernicterus (permanent brain damage) (5).

Neonatal jaundice is common, as a result of physiological jaundice or breastfeeding. In some neonates, unconjugated bilirubin concentration, in addition to different risk factors, is sufficient to permit free bilirubin to cross the blood-brain barrier and cause kernicterus. Another subgroup of infants is jaundiced as a result of elevated conjugated bilirubin; a marker for several pathological conditions. Bilirubin measuring should determine those infants in danger (6).

Materials and Method

The hardware and software are the main components of this technique, the hardware consists of two elements that are the AM2301 thermal sensing element and also the Arduino Uno platform.

The incubator was in the hospital which has its heat supply, through this sensing circuit the temperature of incubator is measured during treated the newborn with jaundice.

DHT21

The AM2301 thermal sensing element, that sensing digital humidity which contains the compound that has been calibrated the digital signal output of the humidity sensor. Application of a dedicated digital modules collection technology and also the humidity sensing technology, to make sure that the merchandise has high reliability and wonderful long-term stability. The sensing element includes a capacitive sensing element wet components and a high-precision humidity measuring devices and connected with a high-performance 8-bit microcontroller. The merchandise has wonderful quality, quick response, sturdy anti-jamming capability, and low price. The shape of procedures, the standardization coefficients hold on within the microcontroller, the sensor among the process of the heartbeat to call these calibration coefficients. Standard single-bus interface, system integration fast and simple. Small size, low power consumption, signal transmission distance up to twenty meters, creating it the most effective selection of all types of applications and even the most demanding applications. Product for the 3-lead (single-bus interface) connection convenience. Special packages in keeping with user wants (7). Fig. (1) Illustrates the DHT21 sensor.

Fig. (1) The configuration of DHT21 sensor.

Arduino Uno

The Arduino Uno has shown in fig. (3) utilized in this study, that is a microcontroller board grounded on the ATmega328 (datasheet) was chosen as the microcontroller for this study. This was a perfect choice, as the processor is extraordinarily strong and cost-effective. It contains of 14 digital input/output pins (out of that 6 is utilized as PWM outputs), six analog inputs, a 16 MHz ceramic resonator, facilitation for USB connectivity, a power jack, an ICSP header, and a button. Its styles comprise of help that supports the microcontroller in each possible way. To induce to figure with it one has to merely connect it to a computer with a USB cable or power it with an AC-to-DC adapter or battery (8).
Discussion

The incubator is considered an artificial womb and will have the most vital effect of protecting newborn. One of the most important duties of the incubator is to control the humidity of the newborn to achieve thermal neutrality, which is achieved by control humidity stability in the incubator. However, this study shows that there is a large swing in the humidity from one to another, although the fixed value is the same, and that is due to the difference in efficiency of each incubator parts because of its operational life and its ability to work such as heat source and heat radiator and efficiency of thermal insulation of the incubator walls, In addition to the external effect of room humidity on the humidity of the incubator.

This work opens the way for comprehensive study on the conditions affecting the humidity in the incubator to design an ideal incubator that provides ideal conditions that will help to actually reduce the treatment period for patients with jaundice.

Conclusions

The concluding observations of this study explain the effect of the humidity which is the most popular variable affected on the patient stay period in hospital.

It was clearly found that the performances of the fixed environment factors including temperature, humidity and other factors in the mother’s womb will disappear immediately after birth. This situation places the newborn in a state of sudden environmental change which requires a necessary and quick adaptation. The child who cannot cope with the new environment and if the mother is different in the blood group and RH factor in addition to children who born before 37 weeks (pre-term) the baby will suffer from jaundice disease.

It is clear that the patient stay period of the children with jaundice inside the incubator was closely related to the incubator’s high humidity percentage, for example the humidity of the incubator of the children who had
been cured in a short period varied between 1 and 3 days were at its lowest rates of 43.16% and at its highest rates of 50.6%

It was found that the speed of recovery for children with jaundice which the humidity percentage in its incubator environment is higher than those no closer to the humidity of the mother’s womb. This is due to the thermal regulation of the newborn inside the incubator is closer to the degree of the mother womb.

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References
Prevalence and Classification of Maxillary Canine Impaction among Iraqi patients at An-Najaf City

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Abstract

Canines play an important role in functional occlusion, occlusion protection and in aesthetic smile. So the proper knowledge of permanent canine impaction incidence and its position is essential before orthosurgical procedures. The aim of this study is to highlight the prevalence and difficulty of the treatment of maxillary impaction canine. A 1995 panoramic radiographs were taken, total number of males were 737 (37%) and 1258 (63%) were females, a 79 (3.9%) patients were diagnosed with canine impaction where females were 3.6 times more than males, also most patients diagnosed with unilateral, right-sided and type II impactions.

Keywords: Maxillary canine, impacted canine, classification

Introduction

An impacted tooth is any tooth that fails to erupt into the oral cavity and occupies its proper anatomical position after completion of its root formation. Maxillary canine represents the second most common form of impaction after wisdom teeth(1,2,3). The prevalence of canine impaction ranging from 0.27% in Japanese population(4) to as much as 7.5% among Saudi people(5). females seem to be affected by 2.3-3 times more than males(4,6,7,8). Panoramic radiographs assist in localization of impacted canines in antero-posterior position in relation to adjacent teeth, classification of maxillary canine impaction described according to the long axis angles of canine with occlusal plane(9). So understanding the location and angulation of maxillary impacted canine is important in the treatment plan for both oral surgeons and orthodontics for proper flap design and approach and orthodontic appliances and techniques. The aim of this study was to determine the prevalence of maxillary impacted canine and its classification among sample of Iraqi people.

Materials and Method

This retrospective study collected data from 1995 panoramic radiographs for patients aged 16-37 years old who had attend oral and maxillofacial radiology center in An-Najaf city. All the panoramic radiographs were examined for the presence of impacted maxillary canines that fails to erupt into oral cavity in the future. All the impacted maxillary canines were matched to the seven subtypes of Yamamoto et al (9) classification system according to the long axis angles and occlusal plane [figure 1]. The collected data were analyzed in relation to gender, side either unilateral or bilateral, right or left and classification.

Results

Among 1995 panoramic radiographs there were 737 (37%) males and 1258 (63%) were females, the patients diagnosed with impacted maxillary canines were 79 (3.95%) patients. Females represented the highest proportion of impaction 62 (4.9%) patients compared to males 17 (2.3%) [table 1]. Table 2 described the unilateral impaction (72%) was higher than bilateral impaction (28%) in both males and females. The highest impaction matched type II (53.46%) followed by type IV (27.22%), type I (17.82%), type III (0.99%) and there were no cases for type V,VI and VII in this sample. Also this study reported that the right side of maxilla was highly affected by impaction than the left side [table 3].
Figure 1. A diagram represents the classification system of maxillary canine impaction. Teeth 2, 3 and 4 represent lateral incisor, canine and first premolar, respectively.

Table 1. Number and percentage of male and female patients and canine impaction in each gender and among all the sample.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Criteria</th>
<th>No Impaction</th>
<th>Impaction</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Count</td>
<td>720</td>
<td>17</td>
<td>737</td>
</tr>
<tr>
<td></td>
<td>Percent %</td>
<td>97.69 %</td>
<td>2.3 %</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Total %</td>
<td>36.09 %</td>
<td>0.85 %</td>
<td>36.94 %</td>
</tr>
<tr>
<td>Female</td>
<td>Count</td>
<td>1196</td>
<td>62</td>
<td>1258</td>
</tr>
<tr>
<td></td>
<td>Percent %</td>
<td>95.07 %</td>
<td>4.92 %</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Total %</td>
<td>59.94 %</td>
<td>3.1 %</td>
<td>63.06 %</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>1916</td>
<td>79</td>
<td>1995</td>
</tr>
<tr>
<td></td>
<td>Total %</td>
<td>96.05 %</td>
<td>3.95 %</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2. Number and percentage of canine impaction for both unilateral and bilateral sides of maxilla for each gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Criteria</th>
<th>Canine Impaction</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unilateral</td>
<td>Bilateral</td>
</tr>
<tr>
<td>Male</td>
<td>Count</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Percent %</td>
<td>64.71 %</td>
<td>35.29 %</td>
</tr>
<tr>
<td></td>
<td>Total %</td>
<td>13.92 %</td>
<td>7.59 %</td>
</tr>
<tr>
<td>Female</td>
<td>Count</td>
<td>46</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Percent %</td>
<td>74.19 %</td>
<td>25.81 %</td>
</tr>
<tr>
<td></td>
<td>Total %</td>
<td>56.96 %</td>
<td>21.51 %</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>57</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Percent %</td>
<td>72.15 %</td>
<td>27.85 %</td>
</tr>
</tbody>
</table>
Table 3. Number and percentage of types of maxillary canine impaction for each side for each gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Criteria</th>
<th>Right Canine Impaction</th>
<th>Left Canine Impaction</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Male</td>
<td>Count</td>
<td>2</td>
<td>5</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Percent %</td>
<td>8.69</td>
<td>21.73</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total %</td>
<td>1.98</td>
<td>4.95</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>Count</td>
<td>10</td>
<td>25</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Percent %</td>
<td>12.82</td>
<td>32.05</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total %</td>
<td>9.9</td>
<td>24.75</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>12</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Percent %</td>
<td>11.88</td>
<td>29.7</td>
<td>0</td>
</tr>
</tbody>
</table>

**Discussion**

The current study had shown the prevalence of impacted maxillary canine of 3.9% that lied within the range of other studies that had shown as 0.27% among Japanese (4), 2.1% among Chinese (10), 2.4% among Italian (6), 5.1% among Turkish (11), 5.4% among Hungarian (12) and 7.5% among Saudi population (5). This variation in proportions indicated that the incidence of maxillary canine impaction varied among different ethnic populations. There are many factors contributing to the impaction of maxillary canine as late maxillary canine tooth germ development and its long path of eruption also the delay in its eruption accompanied by increasing bone density and pressure from surrounding bony cavities such as nasal and orbital cavities and maxillary sinus (13). Although the first premolar tooth germ developed later than canine tooth germ, its eruption earlier than canine due to its short path of eruption in comparison to the long path of eruption of canine, so the space between lateral incisor that erupted earlier to them and first premolar must be maintained to facilitate eruption of canine (14). This study reported unilateral impaction (72.15%) was more common than bilateral impaction (27.85%), this result was in agreement with other studies (5,16,17). The current study reported a higher right side impaction than the left side impaction and this finding was confirmed by Altaee ZH study (18). This study reported that type II canine impaction was more predominant followed by type IV, type I and type III respectively and it was in agreement with Hassan AL-Zoubi et al (16) study. Canines play an important role in both occlusal stability and aesthetics so giving a complete picture - for oral surgeons and orthodontics according to population - about their prevalence and type of impaction is essential for proper treatment plan.

**Conclusions**

The prevalence of impacted maxillary canine is 3.9% among sample of Iraqi population. Females are more affected by impaction than males by 3.6 times. Unilateral impaction is more common than bilateral impaction, and the right side is more affected by impaction than the left side. Type II maxillary canine impaction represents the majority of impactions followed by type IV, I and III respectively.
**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


The Efficacy of Chlorhexidine Gel as an Adjunctive Treatment for Patient with Chronic Periodontitis

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Abstract

Background: chronic Periodontitis is considered to be an inflammatory disease and is characterized by the damaging the connective tissue and supporting bone due to secondary infection by periodontal bacteria. Chlorhexidine has been used in the dental practice as an excellent antiplaque agent. Chlorhexidine not only exhibits special property of substantivity, it also possesses a broad antimicrobial spectrum which makes its use in wide variety of oral disorders. Virtually all disciplines of dentistry make use of this material in different formulations like mouth wash, gel, spray, varnish, and restorative material.

Aims of study: The aim of this study was to evaluate the clinical effectiveness of locally delivered Chlorhexidine gel as an adjunctive therapy to scaling and root planing in treatment of chronic periodontitis.

Material and methods: five patients with chronic periodontitis were selected. Pocket depth between 4 and 6 mm was selected and the patient had two sites on his mouth (splitting mouth). A total of 17 control sites were scaled and root planed and 17 test sites were scaled and root planed and Chlorhexidine gel was added. The clinical parameters, probing depth (PD), clinical attachment level (CAL), plaque index (PI) and bleeding on probing (BOP), gingival index were recorded at baseline, 1 week, 2 weeks and 4 weeks.

Results: From baseline to a period of 4 weeks, significant difference was found between test and control group for PD, CAL, PI and BOP.

Conclusions: Subgingival injection of Chlorhexidine gel adjunct with scaling and root planing appeared to cause significant improvement compared with scaling and root planing alone in persons with chronic periodontitis.

Keywords: Chlorhexidine gel, chronic periodontitis.

Introduction

Periodontal disease is an inflammatory condition initiated by a bacterial infection which affects the supporting structures of the teeth. The first stage of the disease is plaque-associated gingivitis (inflammation of the gingivae), which is a reversible inflammatory condition characterized by redness and swelling of the gingivae and a tendency for the gingivae to bleed easily. It is caused by the accumulation and persistence of microbial biofilms (dental plaque) on the teeth. In susceptible individuals, gingivitis may lead to the second stage of periodontal disease which is periodontitis. Periodontitis is an irreversible condition where inflammation of the gingivae is accompanied by connective tissue destruction and loss of the periodontal ligament and alveolar bone supporting the tooth. If untreated, periodontitis may ultimately lead to tooth loss. Chronic periodontitis, formerly known as adult periodontitis or chronic adult periodontitis, is the most prevalent form of periodontitis. It is generally considered to be a slowly progressing disease. However, in the presence of systemic or environmental factors that may modify the host response to plaque accumulation, such as diabetes, smoking, or stress, disease progression may become more aggressive. The use of adjuvant chemical antibacterial agents is considered an important complement to mechanical oral hygiene techniques, both those performed at home, as well as those performed in a professional setting, especially in cases where the patient is unable to maintain an acceptable standard of...
oral hygiene, or when surgical procedures represent a contraindication. (4)

**Chlorhexidine**

It is a bisbiguanide base, is a cationic antiseptic with broad-spectrum antibacterial activity (against Gram-positive and Gram-negative bacteria and certain mycetes). Consequently, it has been extensively studied in medical settings as an antiseptic compound. (5) The bactericidal action is more effective against Gram-positive cocci and weaker in case of Gram-negative ones. There is also a moderate activity against some mycetes, but not against viruses and alcohol-resistant bacilli. (6) The efficacy of CHX is not limited to its antiplaque effect, but extends also to its substantivity, which allows the molecule to remain attached to tissues and have an antibacterial action lasting for 8-12 hours making it possible to reach the minimum effective dose required to inhibit plaque formation, even with very few daily administrations (7). This property comes from CHX’s ability to bind mucins, proteins forming the salivary film covering teeth and oral mucosa surfaces: the CHX cationic portions can bind to the mucin carboxylic segment, which is negatively charged. (7) CHX inhibits bacterial colonization and is slowly released as it is displaced by the calcium ions secreted by the salivary glands. (8)

The antibacterial action of CHX is, therefore, most likely the result of an immediate bactericidal action, followed by a prolonged bacteriostatic action, due to its ability to attach itself to the enamel surfaces. (7)

**Materials and Method**

A total of 34 sites diagnosed as suffering from chronic generalized periodontitis attending the clinic at the Department of periodontics in College of Dentistry/AL-Issra University. All five patients were informed about the purpose of the study and their consents were provided prior to their enrollment into the study. The Exclusion criteria included: any chronic systemic disease, pregnancy, and smoking, use of antibiotics and/or anti-inflammatory drugs within the last 3 months, the Presence of periodontal pocket depth (PPD) sites located on the same side PD between 4 to 6 mm.

**Clinical procedure**

A special preform (stent) was designed for the present study so as to have a systemic and methodical recording of all the observation and information. The sites were divided into two half, one half of the mouth is control sites and the other half is test sites Fig. (1).

![Figure 1: examination kit.](image)

Control sites: 17 sites were treated by scaling and root planing alone.

Test sites: 17 sites were treated with scaling and root planing followed by the placement of the 30ml CHX gel (PRIOKIN) in the periodontal pocket.

The following parameters were recorded at baseline (day 0), 1 week, 2 weeks and 4 weeks using a Michigan “o” probe with marking of 1,2,3,5 by a single examiner:

- Gingival index (GI)
- Sulcus bleeding index
- Plaque index (PI)

The following parameters were recorded at baseline (day 0), and 4 weeks using a Michigan “o” probe with marking of 1,2,3,5 by a single examiner:

- Probing pocket depth - measurement to the nearest millimeter of the distance from the gingival margin to the depth of the pocket
- Relative clinical attachment level (CAL).

After recording all the parameters at the baseline, full mouth scaling and root planing was performed using ultrasonic instruments followed by hand instruments until all supra and subgingival root surfaces felt hard and smooth.

Following debridement, target sites were irrigated gently with cold saline to achieve hemostasis prior to placement of CHX gel (PRIOKIN) and it was applied into the deepest portion of periodontal pocket by means of a thin rounded tip needle.
Results

Probing pocket depth (P.I)

In control sits (table 1), the P.I mean showed a gradual decrease from 1.047± 0.212 at baseline to 0.993±0.595 at 1 week, 0.899±0.899 at 2 weeks and 0.793±0.489 at 4 weeks but however, there was no significant difference (p˃0.05) between the baseline and the 1, 2 weeks but significant difference between the baseline and the 4 weeks. The overall mean total of P.I in control sits was 0.933±0.112.

In CHX sits, the P.I mean showed a gradual decrease from 1.262± 0.439 at baseline to 0.918±0.552 at 1 week, 0.393± 0.552 at 2 weeks and 0.311±0.463 at 4 weeks and there was significant difference between the baseline and 1 week, 2 weeks and 4 weeks. Moreover, there were significant difference between the 1 week with 2 and 4 weeks but not between the 2 and 4 weeks. The overall mean total of P.I in CHX sits was 0.721±0.450. The mean of P.I in CHX treated sits was significant difference (P ≤0.05) when compared to the mean of P.I in control sits fig (1).

Table 1: Clinical parameters assessments (PI, GI and BOP%; Means ±SD) of patient control / treated with Chlorhexidine gel (CHX) examined on 1, 2 and 4 weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Non-CHX (Control)</th>
<th>CHX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1-w</td>
</tr>
<tr>
<td>P.I (M±S.D)</td>
<td>1.047 (±0.212)</td>
<td>0.993 (±0.595)</td>
</tr>
<tr>
<td>G.I (M±S.D)</td>
<td>1.158 (±0.365)</td>
<td>1.109 (±0.772)</td>
</tr>
<tr>
<td>B.O.P (%)</td>
<td>(6/63)</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 2: Overall PI values (mean ±SD) of patient control and treated sits with Chlorhexidine gel (CHX) during the study
Gingival index (G.I)

The G.I mean (table 1) of the control sits decreased from 1.158±0.365 at the baseline to 1.109±0.772 at the 1 week and to 0.523±0.499 at the 2 weeks and to 0.095±0.293 at the 4 weeks, however there was no significant difference between baseline and the 1 week but a significant difference between baseline and 2,4 weeks. The results also showed that there were significant difference between the 1 week with 2 and 4 weeks and between the 2 weeks with 4 weeks. The overall mean total of G.I in control sits was 0.821±0.507.

The G.I mean (table 1) of the CHX treated sits decreased from 1.081±0.274 at the baseline to 0.868±0.664 at 1 week and to 0.180±0.384 at 2 weeks and 0.098±0.297 at 4 weeks. This decrease was significant difference when the baseline compared to 1 week, 2 and 4 weeks. However there was no significant difference between 2 and 4 weeks. The overall mean total of G.I in CHX sits was 0.557±0.491.

The mean of G.I in CHX treated sits was significant difference (P ≤0.05) when compared to the mean of P.I in control sits.

Bleeding index (B.O.P)

The results of the control sits (table 1) in this study showed that B.O.P was 10% and decreased to 8% at 1 week while at 2 and 4 weeks the values were 0%, no significant difference was observed between baseline and 1 week but a significant difference between the baseline and 2,4 weeks.

In the CHX treated sits the data at the baseline was 25% and decreased to 16% at 1 week and to 0% at 2,4 weeks no significant difference was observed between baseline and 1 week but a significant difference between the baseline and 2,4 weeks. On the other hand, both the control and treated sits showed no significant difference was observed between baseline and 1 week but a significant difference (0.05) when the baseline and 1 week compared to 2 and 4 weeks.

Clinical attachment lose CAL

The results (table 2) for the control sits showed a significant decrease from 5.240±1.068 at the baseline to 2.520±0.805 at 4 weeks, while the CHX treated sits also showed a significant decrease from 4.833±0.897 at the baseline to 2.222±0.415 at 4 weeks (table 2).
Table 2: Clinical parameters assessments (CAL and PD; Means± SD) of patient control / treated with Chlorhexidine gel (CHX) examined on baseline and 4weeks

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Non-CHX (Control)</th>
<th>CHX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4-w</td>
</tr>
<tr>
<td>C.A.L (M±S.D)</td>
<td>5.240±1.068</td>
<td>2.520±0.805</td>
</tr>
<tr>
<td>P.D (M±S.D)</td>
<td>5.125±0.619</td>
<td>2.823±0.856</td>
</tr>
</tbody>
</table>

Probing pocket depth (P.D)

Table 2 showed the results of P.D values in control and treated sits, in control sits the value was significant decrease from 5.125±0.619 at the baseline to 2.823±0.856 at 4 weeks. While the P.D values in CHX treated sits showed a significant decrease from 4.529±0.499 at the baseline to 2.176±1.464 at 4 weeks. Table 2 also showed that there were no significant difference between the values of control and CHX treated sits at 4 weeks of the study in both C.A.L and P.D.

Discussion

Chlorhexidine mouth rinsing is ineffective in eliminating a microbiota located beneath the gingival margin. Subgingival irrigation using chlorhexidine solution or even gels turn out to be effective in the treatment of periodontitis presumably due to its ability to retain biologically significant concentration of chlorhexidine for sufficient length of time within the confines of periodontal pocket (9,10). The studies have reported the treatment of periodontal pocket with chlorhexidine irrigation as an adjunct to scaling and root planning, provides a significant improvement in probing depth and reduces the microbial load. (11) The local delivery of CHX gel in periodontal pockets enhances the effect of scaling and root planning, thus CHX gel application shows long lasting (up to 90 days) favorable effects. (12)

In this study the CHX gel is supplied with a special needle having a blunt tip to facilitate the application of the gel without traumatizing or damaging the periodontal tissues. Periodontal probing is one of the most widely used diagnostic tools for the clinical assessment of connective tissue destruction in periodontal disease. Increased PD and loss of clinical attachment are pathognomonic for periodontitis and hence, serve as primary parameters in diagnosis of periodontal disease and evaluation the success of periodontal therapy. The significant reductions observed in plaque and gingival scores from baseline the results of this study are consistent with the results observed by Vinholis (13), when they evaluated the effect of subgingival irritation with a 0.2% CHX collagen gel in periodontal pockets as an adjunct to scaling and root planing. Mizrak observed similar reduction of probing pocket depth with both the group that were treated either by scaling and root planing alone or scaling and root planing with controlled release CHX chip. (14)

Mechanical debridement with subgingival chlorhexidine gel application provide significantly greater improvements in periodontal parameter compared to those obtained by scaling and root planing alone in the treatment of chronic periodontitis. (15) The bleeding upon probing (B.O.P) is a periodontal parameter evaluating the probability of periodontal disease progression. The result of the study are consistent with the findings of Heasman (16) who observed mean reduction of bleeding index score from baseline to the 4 weeks in the site (scaling and root planing with CHX gel) as well as control site (scaling and root planing alone). The result is similar to the results observed in the present study.

This study is considered a non-surgical treatment of chronic periodontitis and the results are similar to those obtained in the study done by Vadiati in 2017 (17) who observed that injection with CHX gel with scaling and leveling of the root surface resulted in a greater improvement in periodontal clinical indices than SRP alone.
A study done by Manthena, et al to compare the effectiveness of CHX varnish and gel and conclude that Subgingival application of highly concentrated CHX varnish and gel following SRP is beneficial in reducing microbial count in moderate to deep periodontal pockets.

**Conclusion**

1. Chlorhexidine gel when applied topically, significantly reduces the clinical parameters (plaque, gingival and bleeding index)

2. Can be highly recommended as an adjunctive treatment for chronic periodontitis.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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**References**


Iron Overload Estimation by Oral Exfoliative Cytology in Beta Thalassemia Major Patients Undergoing Repeated Blood Transfusion

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Abstract

β-Thalassemia major patients require regular blood transfusion therapy lifelong this lead to iron overload in the body tissues, which is a major cause of morbidity and mortality in these patients. Exfoliative cytology, a non-invasive and inexpensive technique based on microscopic evaluation of epithelial cells after a procedure of their fixation and staining. The aims and objectives of this study were: 1. To estimate iron overload by oral exfoliative cytology using Perl’s Prussian blue stain in β-thalassemia major patients. 2. To correlate Perl’s Prussian blue staining positivity with their serum ferritin levels. Smears were obtained from buccal mucosa of 60 β-thalassemia major patients (who had taken ≥10 transfusions) and 30 healthy subjects of the same age group (5-26) year. Smears were stained with Perl’s Prussian blue stain kit. Blood samples were taken from the study and control group at the same time for estimation of serum ferritin levels. Grading criteria were defined for assessing the Prussian blue positivity. Perl’s positivity was observed in 48 out of 60 of thalassemic patients (80%), with a positive correlation to serum ferritin levels. Perl’s Prussian blue staining of exfoliated cells from buccal mucosa can be used to assess iron overload in β-thalassemia major patients, as a screening tool.

Key words: β-thalassemia major, iron overload, exfoliative cytology, perl’s Prussian blue

Introduction

Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis (1). β-Thalassemia major also known as Cooley’s anaemia. The recommended treatment for thalassaemia major involves lifelong regular blood transfusions, usually given every two to five weeks, to maintain the hemoglobin level above 9–10.5 g/dl. Long term transfusion has multiple complications (2). Iron stores in the body exist primarily in the form of ferritin (3). Ferritin is a positive acute phase response protein whereby concentrations increase during inflammation and thereby no longer reflect the size of the iron store (4). Transfusional iron overload develops in patients with chronic anaemia who need to have regular blood transfusions. About 250 mg of iron is the unit of transfused blood, while only about 1 mg of iron that the body excreted per day (3) (5). Hemosiderin deposits in the liver, heart, endocrine glands and lungs of the affected patients (2). Iron deposits have also been found in the gingivae (6) (7). Serum ferritin, Serum iron, iron-binding capacity, Serum non-transferrin bound iron, Bone marrow biopsy (Perl’s stain) for iron within reticuloendothelial stores, Liver biopsy (parenchymal and reticuloendothelial (RE) stores), Liver CT scan or MRI and Cardiac MRI (T2 * or Ferriscan technique) are methods used to monitor iron overload status and effect of iron chelation therapy in these patients (8).

Exfoliative cytology is painless, bloodless, noninvasive, quick and simple procedure (9). it based on microscopic evaluation of epithelial cells after a procedure of their fixation and staining. There are 2 methods in use: the indirect cell collecting method, such as aspiration subjects with Self exfoliated cells, and the direct method, rub cells of mucosal surface (10). This study is an attempt to measure iron overload in those patients by means of exfoliative cytology, an inexpensive technique (11).

Aims and Objectives

1. Assessment of iron overload in beta thalassemia major patients by oral exfoliative cytology using perl’s
Prussian blue stain.

2. Correlation of Perl’s Prussian blue staining with serum ferritin levels.

**Materials and Method**

This cross sectional study was conducted in Ibin Albaladi hospital for gynaecology, obstetric and pediatrics -thassemia center, in Baghdad during the period from January to April 2019. Sampling sessions were limited to the hours between 9:00 and 11:00 AM, data about diagnosis and number of transfusions were taken from patients’ files.

The present study comprised of 90 subjects, with an age range of (5-26) years of both gender, broadly classified into two groups:

1. study group comprised of 60 beta thalassemia major patients
2. Control group: comprised of 30 clinically and hematologically healthy individuals

**Inclusion criteria:**

1. Patients aged (5-26) year, receiving regular blood transfusion ≥10 transfusion.
2. Patient was included based on confirmation by hemoglobin electrophoresis.
3. Control group: clinically and hematologically healthy individuals, in the age range (5-26) years. Subjects selected on the basis of history, clinical examination and blood investigations within normal range.

**Exclusion criteria:** subject with history of any other major illness and newly diagnosed cases yet to receive blood transfusion. Control subjects should have no confirmed acute and chronic liver damage, malignancy, megaloblastic anemia, iron deficiency anemia, and hepatitis.

**Sample collection.**

Patients from the study group and the control group were asked to rinse their mouth with distilled water to remove any debris. Buccal mucosa of the patients was cleaned by guaze and then cells from buccal mucosa were collected by using interdental brush. Scraping were placed on the middle of clean and dry frosted glass slide and spread over a large area to avoid clumping of cells. Slides then placed in coplin jar containing 70% ethanol alcohol for fixation and transferring to laboratory for staining by iron staining kit (abcam 150674).

The smear was first examined at x10 lens followed by examination at x40 lens to study the presence or absence of intra cytoplasmic blue-colored iron granules.

Ten high power fields (objective lens x40) fulfilling the following criteria were chosen for grading purpose:

1. Evenly dispersed cells with minimal overlapping.
2. Minimum 20 epithelial cells and maximum 30 cells per high power field(x40).

The images were captured with a camera attached to the microscope. All the images of the cells were captured with x40 achromatic objective. Captured Images were stored on the computer and analysis was done using the software image J software version 1.52 a. An average of findings in 10 high power fields (x40) were taken and accordingly, appropriate grade assigned.

- Grade 0 - No granules
- Grade I – less than 5 granules/high power field
- Grade II—5–10 granules/high power field
- Grade III—10–20 granules/high power field
- Grade IV—Clumps seen in less than 3 high power field
- Grade V—Coarse granules or clumps seen in 3 or more than 3 high power field.

Venous blood was collected at the same time of taking exfoliative cytology from study and control groups, the sample were centrifuged at 4000 rpm for 20 min to get clear supernatant. then serum ferritin were estimated in the hospital by VIDAS® Ferritin which is an automated quantitative test for the determination of ferritin in human serum or plasma using the Enzyme Linked Fluorescent Assay technique using ferritin kit (bioMérieux).
Figure 1: Perl’s Prussian blue staining of squamous epithelial cells from buccal mucosa showing blue intracytoplasmic iron granules, counter stain used is nuclear fast red stain, positive control tissue used is bone marrow.

Statistical analysis

Data analysis was performed using statistical package (SPSS) ver. (22.0), Chi square test was applied to compare the control and study groups, Analysis of variance (ANOVA) was used and F-test calculated to correlate the buccal smear positivity with serum ferritin levels.

Results

In this study 48 (80%) patients out of 60 in the study group showed positivity for the Perl’s Prussian blue staining in the exfoliated cells of the buccal mucosa. Maximum number of patients, 22 (36.6%) out of 60 had Grade IV positivity, followed by 13 (21.6%) patients having Grade III while 8 (13.3%) patients was Grade II, 3 (5%) patients having grade V, 2 (3.3%) patients having grade I and 12 (20%) patients have grade 0 (negative for perl’s Prussian blue staining). All 30 subjects in the control group (100%) were negative for Perl’s Prussian blue stain. Chi square test was applied to compare the Perl’s staining of the control and study groups. It was found to be statistically highly significant at 1% level of significance (p value > 0.001).

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. &amp; %</th>
<th>Exfoliative cytology</th>
<th>Total</th>
<th>C.S. (*)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neg.</td>
<td>Pos.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>No.</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>71.4%</td>
<td>0.0%</td>
<td>33.3%</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>No.</td>
<td>12</td>
<td>48</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>28.6%</td>
<td>100.0%</td>
<td>66.7%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>No.</td>
<td>42</td>
<td>48</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

(*) Highly Sig. at P<0.01; Testing Based on a Contingency Coefficient.

In order to correlate the buccal smear positivity with serum ferritin levels, ANOVA was used and F-test calculated. The comparison of buccal smear grades of the study group was made with their respective mean serum ferritin levels. This was again found to be significant at 5% level of significance implying that buccal smear grades correlated with the mean serum ferritin levels, as shown in table(2):

<table>
<thead>
<tr>
<th>Marker</th>
<th>Grade</th>
<th>No. of Patients</th>
<th>Mean</th>
<th>Std. E.</th>
<th>Std. Dev.</th>
<th>Min.</th>
<th>Max.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ferritin ng/ml</td>
<td>0</td>
<td>12</td>
<td>7791.0</td>
<td>3179.3</td>
<td>917.8</td>
<td>2501</td>
<td>12000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>2</td>
<td>1862.7</td>
<td>468.3</td>
<td>331.1</td>
<td>1532</td>
<td>2194</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>8</td>
<td>4334.9</td>
<td>2005.3</td>
<td>709.0</td>
<td>1984</td>
<td>7374</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>13</td>
<td>5953.7</td>
<td>3254.1</td>
<td>902.5</td>
<td>2501</td>
<td>12000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>22</td>
<td>5102.9</td>
<td>2357.5</td>
<td>502.6</td>
<td>1515</td>
<td>11725</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>3</td>
<td>8307.2</td>
<td>3428.9</td>
<td>1979.7</td>
<td>5224</td>
<td>12000</td>
<td></td>
</tr>
</tbody>
</table>

(*) S: Sig. at P<0.05; Testing Based on ANOVA.
Result has recorded significant relationship at P<0.05 in light of “Serum Ferritin ng/ml”

Discussion

Blood transfusion therapy being the major cause of iron overload in thalassaemia major. When thalassaemia major patients receive regular blood transfusion, iron overload is inevitable because the human body lacks a mechanism to excrete excess iron \(^{10}\).

In this study, exfoliated cells from the buccal mucosa of 48 of the 60 thalassemic patients (80%) group revealed positivity for Perl’s Prussian blue reaction. Further, it was observed that none of the control subjects showed Perl’s Prussian blue positivity. These results of Perl’s Prussian blue staining positivity were similar to Gururaj and Sundharam et al study (2004) who reported 100% Perl’s positivity in the 10 patients that they examined \(^{14}\), Nandprasad et al study (2010) who observed 65% Perl’s positivity (65 out of 100 patients), Bhat et al study (2013) who reported 71.7% positivity (43 patients positive out of 60), Chittamsetty et al study (2013) who observed 72.5% (29 out of 40 b-thalassemia major patients) and Gupta et al study (2014) who observed 61.6% Perl’s positivity (37 out of 60 cases) \(^{15-18}\). Leekha et al study (2016) who found Values to be positive in 35 (87.5%) out of 40 patients \(^{19}\), And Gajaria et al. study (2017) who observed 98% perl’s positivity (49 out of 50 cases). In the present study, result is higher than of Nandprasad et al. study (2010), Bhat et al. study (2013), Chittamsetty et al. study (2013 and Gupta et al. study (2014), but lower than Gururaj and Sundharam et al. study (2004) and Gajaria et al. study (2017). This variation in positive results can be attributed to the difference in the total sample size of various studies as well as the varying mean serum ferritin levels of the respective patients in the studies \(^{11}\). Also comparison between the study and control group Perl’s Prussian blue staining implied that positivity in the study group was not by chance. Thus, there was a strong association between the thalassaemia patients who were undergoing repeated blood transfusions and suffering from hemosiderosis with their buccal smear iron stain positivity. This proved that iron does accumulate in squamous epithelial cells of buccal mucosa in hemosiderosis and these cells can be scraped to assess iron overload. The controls were healthy and did not suffer from iron overload explaining their negative results. The negative reaction in study group even in spite of high serum ferritin levels in those patients can be explained by either fault in the technique of staining or already shedding of the affected squamous epithelial cells. The excess amount of iron that gets accumulated in various tissues is dependent upon various factors including the formation of iron storage pool. Moreover, amount of ferritin formed in exfoliated buccal cells, may vary, which may invariably affect the Perl’s positivity \(^{11}\). When hemosiderosis occurs, iron accumulates in reticuloendothelial macrophages first and only later spills over into parenchymal cells \(^{20}\). It was observed that the grade of buccal smear positivity correlated well with the serum ferritin levels of these patients and therefore this simple, non-invasive test can be used to monitor iron overload in patients taking regular transfusions.

Nandprasad et al study. And Chittamsetty et al study. Had included the patients with 15 or more number of blood transfusions in their study \(^{15-17}\). In Gajaria et al study (2017), Perl’s Prussian blue positivity was observed even with the number of transfusions being (12), in this study positivity observed with (10) transfusions implying that this test can be used as a screening modality also, besides regular monitoring. Most of these patients were on chelation therapy, yet Perl’s Prussian blue positivity was detected in their buccal smears further validating the usefulness of this test.

The various factors affecting the iron overload in this subset of patients are number of transfusions, age of initiation of iron chelation therapy, whether taking the chelation therapy regularly, socioeconomic status, nutritional deficiencies and other associated comorbidities.

Studies to compare the Exfoliative cytology Perl’s Prussian blue positivity with liver biopsy, T2* MRI of liver/heart, MUGA scan (Multigated acquisition), liver dry weight iron need to be conducted. Also a co-relation of this test with clinical manifestation of iron overload in various organs needs to be done.

Conclusion

Perl’s Prussian blue reaction can be utilized as an objective indicator of iron overload in β-thalassemic patients with high levels of iron overload.

Perl’s Prussian blue staining of exfoliated cells from buccal mucosa can be used to assess iron overload in β-thalassemia major patients, and as a screening modality to detect iron overload in patients yet to start chelation therapy. With the grading system we can even give a
semi quantitative assessment of the same. Since these grades correlated positively with the respective serum ferritin levels, it can be used to monitor iron overload in those Patients.

Acknowledgements: We are grateful to the staff of thalassemia center, blood bank and hematology laboratory in Ibn Albaladi hospital for gynaecology, obstetric and pediatrics in Baghdad and would also like to acknowledge the hard work of our senior and staff of oral pathology laboratory in Baghdad university/college of dentistry.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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References


The Incidence of Contrast Induced Nephropathy-Acute Kidney Injury after cardiac catheterization in Basra Cardiac Catheterization Center. A Prospective Cohort Study

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Abstract

Introduction: Patients who opt for coronary interventions to help them with their IHD problems face several adverse reactions; the most frequently faced is acute kidney injury from contrast media exposure or contrast induced nephropathy. Those patients have higher mortality and morbidity both in the short and longterm period. The incidence of this adverse reaction in Al-Basra Cardia Center is poorly studied so far. We measured the incidence of AKI in this center and tried to correlate the risk with some important covariates identified by previous researches. Method: this was an observational prospective study. It was a part of the double blind single center study (the effect of spironolactone on the incidence of AKI in patients with Stable IHD admitted for coronary intervention, trial registration: ClinicalTrials.gov NCT03329443). Results: The overall incidence of CIN was 20.2% in this cohort sample. Priori defined high risk variables were tested in univariate logistic regression, and if found to be significant they were to be added to a Multi-Logistic regression model analysis. In Regression analysis only GFR (Log Odds ratio) [0.984 (0.971-0.998)] and Mehran Risk score [Mehran >6 (2.456(1.335-4.519), Mehran >11 (3.931) Mehran >16 (12.366) compared to Mehran <5) were positive in the model analysis. Conclusion: there seems to be a high incidence of AKI in this cohort. Important significant factors include low GFR and a good correlation with Mehran risk score.

Key words: AKI, Acute Kidney Injury, CIN, Contrast Induced Nephropathy, Angiography, PCI, Percutaneous Coronary Intervention, IHD, Ischemic Heart Disease, CI-AKI, Mehran risk score

Introduction

Contrast induced nephropathy-Acute Kidney Injury (CIN-AKI) is a well-established complication of coronary angiography or any procedure involving the administration of Intravenous Contrast Agents regardless of the type (1–5)

Affected patients have higher cardiac and non-cardiac adverse events post procedure including and not limited to mortality even in patients without practical clinical manifestations or a need for dialysis or follow up (6–11)

Our Basra Cardiac center is relatively new opened since 2012 and the incidence of CIN-AKI in our center is not rigorously identified as far as we know.

Method

This study was a prospective cohort study at Al-Basra Cardiac Catheterization (Iraq-Basra) between September 2017 and June 2018. The study protocol was reviewed and approved by the institutional review board in the hospital and Baghdad University. All patients gave an informed consent to be included in the study.

Four hundred ninety patients were prospectively followed up that composed the total study group of our study population (the effect of spironolactone on the incidence of AKI in patients admitted for coronary angiography). Patients’ inclusion and exclusion criteria along with detailed trial design and rationale is reported previously (12).

Briefly, Patients’ demographic data were prospectively collected at admission date by researchers
using a prespecified datasheet and is called upon on day 2-3 for further interview and data collection. Coronary angiography/plasty were done by a senior cardiologist according to an established local and global (American College of Cardiology/American Heart Association) standard practice via the femoral approach (13).

The use of CIN prophylaxis was left to the treating physician but whoever received a pre-angio protocol and/or an isotonic fluid after were carefully collected by the research team.

A suitable amount of blood was taken from each patient immediately prior to the procedure and 2-3 days later. Measured parameters included serum creatinine at admission and after 2-3 days, serum Potassium at admission and after 6 hours later. CIN was defined according to K-DIGO guidelines as an increase of 0.3mg/dL or more than 0.25% elevation of Serum creatinine from baseline within 2-3 days of the procedure.

Statistical analysis

All statistical analysis was done using IBM SPSS Statistics 23. Continuous variables were recorded as Mean ± Standard Deviation (SD); categorical variables were recorded as a percentage (%). Normally distributed data were compared using suitable parametric tests. For any deviation from normality, a variable will be subject to a suitable transformation or switch to non-parametric tests. Logistic regression and model analysis was done for analysis of the effect of specifically predefined covariates on the incidence of CIN in the selected group. A p-value of 0.05 was set to be significant.

Result

The overall incidence of CIN was 20.2% in this cohort sample as shown in figure 1. Patients with AKI had a higher Serum Creatinine after 2-3 days Figure 2. They also tend to have a lower GFR [96.54(19.57) vs. 77.24 (21.31)], received more massive amount of Dye [248 (142.32) vs 208 (133.99)], especially more than 150 ml per procedure [71.28% vs. 63.07%], more probably having DM, congestive heart failure, a higher Mehran risk score and an initial Serum Creatinine at presentation, Table 1.
Table 1. Baseline characteristics of patients with and without CIN

<table>
<thead>
<tr>
<th>Negative Mean (SD) or count (%)</th>
<th>AKI KDIGO</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD) or count (%)</td>
<td></td>
</tr>
<tr>
<td>Age (58 (7.96))</td>
<td>58 (7.6)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Gender (Male) (233 (62.8 %))</td>
<td>57 (60.64 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Weight (75.4 (13.86))</td>
<td>78.1 (14.75)</td>
<td>N.S.</td>
</tr>
<tr>
<td>GFR (77.24 (21.31))</td>
<td>69.54 (19.57)</td>
<td>S</td>
</tr>
<tr>
<td>Dye Volume (208 (133.99))</td>
<td>248 (142.32)</td>
<td>S</td>
</tr>
<tr>
<td>Dye Vol &gt; 150mL (219 (59.03 %))</td>
<td>67 (71.28 %)</td>
<td>S</td>
</tr>
<tr>
<td>PCI (234 (63.07 %))</td>
<td>67 (71.28 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Antiplatelets (340 (91.64 %))</td>
<td>86 (91.49 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>ACE Inhibitors (331 (89.22 %))</td>
<td>83 (88.3 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Stent Type DES (252 (67.92 %))</td>
<td>67 (71.28 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Artery none (120 (32.35 %))</td>
<td>27 (28.72 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>RCA (80 (21.56 %))</td>
<td>23 (24.47 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>LAD (19 (5.12 %))</td>
<td>8 (8.51 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>LCX (71 (19.14 %))</td>
<td>17 (18.09 %)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Multiple (81 (21.83 %))</td>
<td>19 (20.21 %)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Interesting to note that the incidence of AKI did not depend on the type of procedure (PCI vs. Angiography), and type of artery involved in the intervention.

Drugs during the periangio period include ACE inhibitors and hydration that were not associated with either an increase or a decrease in AKI incidence, while there was a negative influence in patients taking Metformin on the incidence of AKI in this cohort.

All previously mentioned risk factors in our analysis were added to a Logistic regression model analysis as shown in figure 2. In Multivariate Logistic Regression analysis only GFR (Log Odds ratio) [0.984 (0.971-0.998)] and Mehran Risk score [Mehran >6 (2.456(1.335-4.519), Mehran >11 (3.931) Mehran >16 (12.366) compared to Mehran <5) remained positive in the model analysis.

### Table 1. Baseline characteristics of patients with and without CIN

| Hypertension | 332 (89.49 %) | 84 (89.36 %) | N.S. |
| DM | 122 (32.88 %) | 48 (51.06 %) | S |
| Lasix | 25 (6.74 %) | 7 (7.45 %) | N.S. |
| Metformin | 102 (27.49 %) | 42 (44.68 %) | S |
| Mehran Quartile | | | |
| 1 | 191 (88%) | 25 (12 %) | S |
| 2 | 146 (75 %) | 48 (25 %) | S |
| 3 | 32 (65 %) | 17 (35 %) | S |
| 4 | 2 (33 %) | 4 (67 %) | S |
| Hydration | 27 (7.28 %) | 4 (12.9 %) | N.S. |
| S.Cr1 | 1.08 ( 0.23 ) | 1.14 ( 0.24 ) | S |
| S.NGAL at 0hrs | 14.7 ( 3.81 ) | 15.2 ( 4.33 ) | N.S. |

### Table 2. Univariate and multivariate Logistic regression analyses of predictors of CIN.

<table>
<thead>
<tr>
<th>Univariate Logistic Regression</th>
<th>Multivariate Logistic Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
<td><strong>Odds ratio</strong></td>
</tr>
<tr>
<td>GFR</td>
<td>7.787</td>
</tr>
<tr>
<td>MehranQuartile</td>
<td>23.072</td>
</tr>
<tr>
<td>MehranQuartile(2)</td>
<td>2.05</td>
</tr>
<tr>
<td>MehranQuartile(3)</td>
<td>6.961</td>
</tr>
<tr>
<td>MehranQuartile(4)</td>
<td>7.98</td>
</tr>
<tr>
<td>SCr1</td>
<td>5.899</td>
</tr>
<tr>
<td>Dye Volume</td>
<td>7.757</td>
</tr>
<tr>
<td>Dye&gt; 150</td>
<td>5.599</td>
</tr>
<tr>
<td>DM</td>
<td>8.955</td>
</tr>
<tr>
<td>CHF</td>
<td>13.214</td>
</tr>
<tr>
<td>Metformin</td>
<td>7.785</td>
</tr>
</tbody>
</table>
Discussion

The main finding in our study is that there is an alarmingly high rate of CIN in this trial cohort. Several reports have calculated the risk of CIN in various populations including the higher risk sample (patients admitted for coronary intervention). The risk is usually low and correlate well with Mehran risk score (≈ 3% in low risk groups and up to 60% and more in high risk groups) (4,14,15).

The overall risk in our trial was around 20%, and this is higher than most of the reported incidence in the global centers (2-14%) (14,15). On further analysis patients from Iraqi population might have a similarly high incidence as a similar study in Babil Center has demonstrated a higher risk (40% in a small cohort sample, Mehran score was not reported for an appropriate comparison). Another study was done in Erbil center and reported an incidence of 13% which is also high but come within the high range of some reported incidences in global centers (16,17). Similar high rates (23%-31%) were also have been reported in nearby Iraqi centers (18–20).

Many possibilities could be the basis of this reported outlier. First, this trial cohort has a relatively higher Mehran risk score than the populations usually tested in similar studies reported in the literature. A study by Taher et al. in Egypt has reported an overall incidence of 13%, but the majority of its cohort (84.5%) were in the low risk category. Compared to our cohort which has only 44% in the same risk category. Interesting to note that the incidence of AKI in our low risk group was 12% which is an excellent approximation to the Taher et al. trial (4).

Such high percentages might be due to the lack of using preventive measures at the time of PCI (21–25). The use of hydration before and after the procedure is a well-known prophylactic procedure given liberally to almost all patients at the periPCI time (22–28). Our sample of patients came fasting for at least 8 hours before the procedure. Such fasting may aggravate dehydration and compromise the already defective renal parenchyma during a period of toxicity or ischemia and augment the toxic effects of contrast agents on the nephrons (7,8,28,29). Some of our patients received post procedure I.V. fluid, and they are also given oral fluid after the procedure. Those who received I.V. fluid (mostly because of PCI related issues) had a decreased incidence of AKI but unfortunately not significant because of the low sample size (no. of patients who received post contrast hydration was only 31 patients with an estimated CIN of 13%) incorporated in our analysis.

Mehran risk score was widely accepted as a standard risk score to calculate and manage the risk of AKI in order to apply a suitable preventive measure in selected high risk patients (15,30). This trial tried a similar analysis given the considerable reduction in power (this sample were around 500 patients, and Mehran et al. used more than 5000 patients to develop the model). We analyzed each factor in Mehran score individually and then the complete final score at last (1).

Individual risk factors that were associated with AKI include (DM, HF, S.Cr>1.4 and contrast media over 150 ml/procedure) this cohort did not receive Intraaortic balloon pump counterpulsation (IAPB), nor they had significant hypotension at the procedure. These risk factors were previously described by Mehran et al. as an independent risk factors for the development of CIN (15).

All individually significant risk factors along with Mehran risk score were added to a multivariate logistic regression analysis to define the independent power of each factor alone given the interaction with other factors. In this analysis, only Mehran risk score and GFR remained significant after correction for other covariates in the analysis. This results contrast with a similar study by Shams-Eddin et al who reported that contrast type, volume of contrast above 400 and Age above 65years were the only predictors of CIN. Shams-Eddin et al. did not show any significant effect of Mehran score with CIN, but again the majority of the studies population were in the low range (Mehran <6) score. This study population had a higher sample size (490 compare 200 for Shams-Eddin et al.) and a significant proportion of this cohort were in the mid-High risk range as well (4).

Conclusion

There seems to be a high incidence of CIN-AKI in this trial cohort and the risk correlate well with Mehran risk score.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.
Funding: Self-funding

References
ST-Elevation Myocardial infarction undergoing Primary percutaneous coronary intervention to prevent contrast-induced nephropathy (ATTEMPT): Study design and protocol for the randomized, controlled trial, the ATTEMPT, Am Heart J 2016;172:88-95.


Evaluation of the Correlation Between Vitamin D3 Serum Level, Age, gender and BMI in Rheumatoid arthritis Patients in Al- Kut City/Iraq

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1Middle Technical University/Technical Institute/ Kut/Iraq, 2Al-diwaninya Health Department / Ministry of Health/ Iraq, 3Plant Protection Department- College of Agriculture- University of Misan/Iraq

Abstract

Rheumatoid arthritis (RA) is an auto-immune disease a chronic characterized leading to joint loss (RA) disease affects nearly 1% of the world. This study is aimed to determine the effect of age, gender and vitamin D level on the efficacy of RA. One hundred and twenty patients (twenty male and one hundred female) with RA in this study were divided, into groups according to their age. Vitamin D levels were measured in the serum using the enzyme-linked immunosorbent assay (ELISA) blood test. Assessment of the relationship between disease severity and average level of vitamin D was examined by the scale of scores in different groups with rheumatoid arthritis. At p<0.05, significant levels of the above tests have been detected. The current study indicates there is a significant relationship between overweight high underweight below (18.5) BMI and age rate (26-36) patients with RA with deficiency of vitamin D level in serum. The results showed a highly significant difference between patients’ gender groups in as compared disease severity, female more than male with RA disease.

Keywords: Rheumatoid Arthritis, Vitamin D3, Age, gender, Body Mass Index (BMI)

Introduction

Rheumatoid arthritis (RA) is an immunomodulatory disease that results in erosion, synovial soreness, and damage to the bone, leading to a direct effect on the joints [1]. Premature diagnosis and therapy are pivotal in reducing the damage of this disease, but the cause of this disease and causing rheumatoid arthritis are still unclear. Both genetic and non-genetic elements (like ecological, infectious, and hormonal components) may be the main cause of disease outbreaks [2]. It is one of the most severe diseases affecting about 1% of the world population. This disease is characterized by chronic pathway, unpredictable flares with severe pain that causes disability [3]. Vitamin D is one of the most important ecological risk agents associated with rheumatoid [4]. It is considered as a one of the most significant vitamins in cartilage and bone metabolism. The low concentration of vitamin D in the body has a negative effect on calcium metabolism, bone density, matrix staining, osteoporosis and articular cartilage [5,6]. In all patients with RA, the deficiency of vitamin D is common. Studies have confirmed that the deficiency of vitamin D increases the risk of RA [6]. However, the effect of vitamin D deficiency on pathogenesis of various illness is provocative. Vitamin D that was first recognized in the 1920, a lipid-soluble vitamin [7] is vital for neurodevelopment and neuropsychiatric disorders [8]. It is one of the four vitamins dissolve in fat (A, D, E, and K) stored in body tissues, also called calciferol. Vitamin D is generally used to describe two specific terms (vitamin D2), ergocalciferol (vitamin D3), and cholecalciferol. Vitamin D3 is made in the body when exposed to ultraviolet rays (from the sunlight), is a hormone with steroidal structure, regulates the calcium homeostasis, and bone formation with reabsorption through kidneys, parathyroid glands and bowel while vitamin D2 is a naturally occurring vitamin that is found in plants [9-10]. Vitamin D is the only vitamin that can be manufactured by the human body. About 90% of (vitamin D3) of human requirement reliance on endogenous productions (stimulation by exposure to U.V sunlight) and exogenous sources (principally reinforced foods, diet,) to biological necessities of vitamin D [11].
proliferation the vital role played widely known by vitamin D in them. Another indicator shows that the receptors of vitamin D affect important parts of the brain, such as immune and nerve integrity, and maintain control of proliferation, which acts as a regulator of the proliferation and differentiation of brain cells, which enters the growth of the brain. Vitamin D has various substantial roles in many vital processes such as cellular growth and differentiation, metabolism of calcium, bones, cardiovascular and immunity functions. The main potential risk influences for vitamin D deficiency may comprise age more than 65, in many studies the association among vitamin D deficiency and different diseases both observational and randomized trials have been reported.

**Materials and Method**

One hundred and twenty random cases from patients with RA (Twenty men and one hundred women) ranging in age from 15 to >70 years at Al-Kut Government/Iraq, were divided to many groups according to their age, gender, BMI and Vitamin D concentration. They have been classified into three groups shown below accordant (IOM) as:

1. (> 30 ng/mL) sufficient
2. (20–30 ng/mL) insufficient
3. (< 20 ng/mL) deficient.

They were diagnosed by a rheumatologist. The weight and height were measured in an examination room. Body mass index (BMI) was calculated as (weight (kg)/height (m2)). The level of vitamin D in the serum was measured through by ELIZA after taking a blood sample of 5 cc.

**Statistical Method**

Percentages and Frequencies were equated through the use of three groups of vitamin D with Chi-square analysis. Associations between vitamin D concentrations and disease severity, and BMI in various groups with RA. The considerable level of the mentioned examinations was fixed at p<0.05. The results were studied by using the Statistical Package for Social Sciences (SPSS) version 17.5.

**Results and Discussion**

In this study, we have enrolled 120 patients including (100 women), women comprised 83.3% of the study participants while (20 men) patients comprised 16.7, and a healthy volunteer as controls mean of age (31.1±6.2) years in the RA patients, the mean duration of the disease was of 5 years. There was no significant difference between age and sex groups (p> 0.05). Mean of the 25-OH Vitamin D conc. was 47.65±21.80 nmol/l in RA patients, 93.60±61.82 nmol/l in the healthy volunteer controls (n = 20). We found that the average of the 25-OH D vitamin conc. of the patients (RA) was meaningfully less high than that of controls (p < 0.01). Concerning the (BMI) for patients with RA, in this study, we have recorded a high overweight percentage and the largest proportion in sample was (71.7 %) while underweight percentage was recorded as below (18.5) BMI in patients’ low percentage were composed (5%) as an out total number of the study sample as summarized in Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Rating</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-25</td>
<td></td>
<td>30</td>
<td>25.0</td>
</tr>
<tr>
<td>26-36</td>
<td></td>
<td>34</td>
<td>28.3</td>
</tr>
<tr>
<td>37-47</td>
<td></td>
<td>24</td>
<td>20.0</td>
</tr>
<tr>
<td>48-58</td>
<td></td>
<td>17</td>
<td>14.2</td>
</tr>
<tr>
<td>59-69</td>
<td></td>
<td>12</td>
<td>10.0</td>
</tr>
<tr>
<td>70+</td>
<td></td>
<td>3</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Considering the frequencies and percentage, Table 2 presents the sample characteristics as age, gender, body mass index, and their vitamin D3. Findings depicts that the (28.3%) of study sample are within second age groups (26-36) years old. Regarding gender, most of the study participants were female, it constituted (83.3%) out total number. In regarding with Vitamin D3, most of the study sample (47.5%) were deficiency as (< 20 ng/ml) as well as (26.7% and 25.8%) were sufficient and insufficient respectively.

### Table (2): Statistically association between sample characteristics and their Vitamin D3

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Rating</th>
<th>Vitamin D3</th>
<th>Total</th>
<th>d.f</th>
<th>crit.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Deficiency</td>
<td>Insufficient</td>
<td>Sufficient</td>
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<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-25</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>26-36</td>
<td>13</td>
<td>9</td>
<td>12</td>
<td>34</td>
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</tr>
<tr>
<td>37-47</td>
<td>13</td>
<td>6</td>
<td>5</td>
<td>24</td>
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</tr>
<tr>
<td>48-58</td>
<td>12</td>
<td>3</td>
<td>2</td>
<td>17</td>
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</tr>
<tr>
<td>59-69</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>70+</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>57</td>
<td>31</td>
<td>32</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.048 → S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>49</td>
<td>23</td>
<td>28</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>31</td>
<td>32</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.028 → S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Under weight</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Normal (18.5-24.9)</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Overweight (25.0-29.9)</td>
<td>39</td>
<td>19</td>
<td>28</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Obesity (30.0 and above)</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>57</td>
<td>31</td>
<td>32</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.033 → S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi-square observer,  Chi-square critical, D_f = Degree of freedom,  P-value= Probability value, S= significant, N.S= non significant
The study showed that age rating (26-58) statistically has a significant relationship with vitamin D levels deficiency in patients with RA while the mean age (57-70) was the lowest in vitamin D shortage. The results of the evaluation also showed that women are more predisposed to vitamin deficiency than men.

The study also recorded a significant decrease in the levels of vitamin D for patients with a body mass index BMI (Overweight 25.0-29.9), the mean decreases of vitamin D3 levels in the patients with RA according to their BMI, gender, and age was significant (p<0.05). In addition, the examination of a lack of vitamin D levels and deficiencies in previous studies identified as the level of 25 (OH) -D <20 ng / mL and < less than 30 ng / ml and sufficient (> 30 ng / ml) with Table 2. Vitamin D has a lively role in most physiological roles as well as an important role in bone balance [17]. The main function of vitamin D is to supply besides preserve calcium in addition phosphorus caffeine in the body to ease optimal metabolism. Depressed levels of vitamin D have an important association with extent of diseases and disturbance. It is well known that the incidence of vitamin D and bone diseases such as rickets and osteomalacia are well identified [18]. Findings depicts that the (28.3%) of study sample are within second age groups (26-36) years old, and it is also mean age were (2.63) with standard deviation (1.39). Regarding gender, most of the study participants were female, it constituted (83.3%) out total number. RA is a chronic inflammatory disease of its prominent epithelium (inflammation of the articular membrane) which can usually include wrists, tarsus and each joint. This disease is affected by gender and age, increasing the incidence of this disease with increasing age and hormonal changes in women [19-20].

Concerning body mass index, the overweight were the largest proportion (71.7%). Only small percent were composed (5%) underweight as an out total number of the study sample. Fatness is a medical disorder which means having excess body fat amassed to the range that it may have a negative influence on the health [21]. Healthy life is typically related with adequate concentrations of vitamin D in the serum, which may be described by the external action of men, that is, an increase in duration of revealation to the sun further than women may be due to the nature of life and customs of communities, including Iraq [22]. In regarding with Vitamin D3, most of the study sample (47.5%) were deficiency as (<20 ng/ml) as well as (26.7% and 25.8%) were sufficient and insufficient respectively.

Serum 25OHD below (20 ng/ml), (50 nmol/ liter) defined as insufficiency in vitamin D according of The World Health Organization. However, others have begun in the definition of deficiency of vitamin D serum level 25OHD below (20 ng / ml) and the inadequacy of vitamin D is less (30 ng / ml) (75 nmol / L) [32]. The different lifestyles and physical activity might be the reason behind those results [23, 24]. Exposure to the sunlight is often limited by lifestyle and other options, resulting in insufficient vitamin D intake and insufficient access to the diet; therefore, Patients with a deficiency may need long-term supplements. Moreover, there was a significant correlation between patients in terms of gender, BMI, age, and their vitamin D3 levels (p<0.05). The results of Rajai et al., In 2017, suggested a significant inverse connection between vitamin D serum and rheumatoid arthritis acuteness based on DAS [28] increasing the severity of the disease with low vitamin D serum levels [25].

Conclusions

There were a significant relationship, between levels of vitamin D in the serum and RA severity based on Patients body mass index, age, and , gender have been active affected their deficiency of vitamin D3.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

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Effect of Antimicrobial Agents Extracted from American Cockroach Insect *Periplaneta Americana* L. on Some Species of Microbes

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¹College of Education, University of Samarra/Iraq, ²College of Applied Sciences, University of Samarra/Iraq

Abstract

The current study was conducted for the period of October 2017 until November 2018 to investigate the effect of antimicrobial material of American cockroach *Periplaneta americana* hemolymph against strains of pathogenic bacteria and yeast. The samples of adults American cockroach was collected and divided into three major groups, the first collected their haemolymph without injecting the cockroaches with bacteria (non-immunizator) the second collected their haemolymph after 12 hours of being injected with *E. coli* (immunizator 12h), and the third group after 24 hours of being injected with the same bacteria (immunizator 24h), then divide each totals above to two subgroups one by cooling centrifuge on 4°C and the other by table centrifuge at room temperature (non-cooling). Protein concentration had measured of all transactions and was the highest value is immunizator 12-hour by cooling centrifuges.

Keywords: species of microbes; *Periplaneta americana* L.; Antimicrobial Agents

Introduction

Excessive and non-organizer use of antibiotics contributed increased resistance of microbes to antibiotics and emergence of new strains more resistant than their predecessors, it becomes a great challenge for human health [1], that make various medical institutions looking for new sources instead of antibiotic, one of it the antimicrobial peptides symbolized AMPs for short, scientists and researchers have focused in the past on plants only to find antimicrobial articles, but as the plants produce those articles most insects produce these complex chemicals for various purposes including defense against microbes, mating, communication, and other operations that help bugs survive [2], it has been found that a wide variety of living organisms and especially insects produce AMPs as part of their defensive line, scientists have identified hundreds of these peptides and explained their significance in the innate immune system [3]. This AMPs are self-produces without induction or either after induction by some infectious or inflammatory stimuli like bacteria or bacterial molecules, a hypothesis developed that animals which live in unsanitary and contaminated conditions they evolution of ways to protect themselves from disease causing by microorganisms [4]. Toke [5] stressed that AMPs have a key role in the fight against invading pathogens in insects. The cockroaches are common household insects that live in human environments and feeding at random on a litter therefore various pathogens carried on their body [6], the ability of roaches flourish under such threats and prevent diseases causing because them body are a good source of antimicrobials [7]. So the aim of this study is to investigate f antimicrobial substances in the haemolymph of American cockroaches *Periplaneta americana* on some microbes.

Materials and Method

Types of microbes: In this study used microbes obtained from the microbiology laboratory at the University of Samarra College of Applied Sciences three gram negative bacteria were *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*. and two gram positive bacteria which were: *Staphylococcus aureus* and *Staphylococcus epidermidis*, and one type of yeasts *C. albicans*.

Collection of cockroach samples

90 adult cockroaches were collected from different part of Samarra city from the gardens, houses, and...
sewers manually included male and female, cockroaches diagnosis based on phenotypic characteristics in the natural history museum and research center at the University of Baghdad according to 601 system rankings and Q.S standards, The insects have putted in perforated plastic container to ensure ventilation feeding in laboratory conditions, haemolymph directly extracted [8].

**immunization of Periplaneta americana and extraction**

Immunization is injection a specific type of bacteria to stimulate insect immune system to production antimicrobial materials [9]. E. coli chosen to inject cockroaches as in [10] with dose 100 microliter contain almost CFU/ml had compared with McFarland standard 0.5 [11], insects refrigerated for anesthetized putted on a sterile petri dish on dorsal part, with needle size 1 ml injection 100µ of bacterial suspended solution in phosphate buffer solution between the 4th and 5th abdominal ring slowly to prevent bleeding [12].

**Collection of cockroaches Hemolymph**

All samples were divided to three main groups first was non immunizator did not injected with bacteria their haemolymph had extracted immediately after anesthetized and injected 0.1 ml ringer solution for every insect then hind pair legs were cut with a sterile scissors in coxa membrane [13] the second their haemolymph had extracted after 12 hours of immunization and a third after 24 hours of immunization [14], after the completion of the time required, each major group was split into two subgroups, first extracted with table centrifuge at 1800 rpm and CFU 6000 g for 15 minutes [12] second extracted with cooling centrifuge (Sigma Germany) at temperature 4 ºC with 14000 rpm and accelerate17968 g for 15 min [15], Finally insects had threw extract stored in clean and chilled tubes with information at -20 ºC until used [13], sterilized all of previous extracts using Millipore filters 0.22mµ [7], this crude extract was used in all tests In this study.

**The protein concentration assay**

Determined concentration of proteins for all transactions by Biuret test [16], according to [17]. The total protein concentration was calculated for each sample by equation:

\[
\text{total protein concentration} = \frac{\text{sample absorbency}}{\text{standard absorbency}} \times \text{standard concentration}
\]

**Antimicrobial assay**

Antimicrobial assay was done by Wells Agar diffusion method in Muller Hinton Agar, 20 µl of crud extract putted in every well after spreading 0.1 ml of microbial suspended, incubated plates at 37ºC for 24 hours, measuring Inhibition Zone as in [18].

The toxicity assay on human Polymorphonuclear cell PMNs

PMNs human cells were isolated as in [19] followed method in [20] to measure the viability of PMNs, cells accounted by Haemocytometer under microscope as dyed cells, dead cells that didn’t take the dye are living cells, calculating the percentage by: percentage of cells viability= number of living cells / total x100

**Static analysis**

All the results of the current study were analyzed by analysis of variance test ANOVA of factorial treatments, at implemented P< 0.05 by SPSS software.

**Results**

**The protein concentration of samples**

All value of protein content of haemolymph in table 1, statistical analysis showed high moral differences of protein content of haemolymph immunizator 12-hour cooling centrifuge at P > 0.05 symbol a followed by of non- immunizator cooling centrifuge symbol b followed by the haemolymph immunizator 24-hour cooling centrifuge symbol c, the remaining transactions that symbol typefaces d didn’t show any moral differences at P > 0.05.

<table>
<thead>
<tr>
<th>Type of centrifugation</th>
<th>Cooling g/100 ml</th>
<th>Static symbol</th>
<th>Non-cooling g/100 ml</th>
<th>Static symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Immunizator</td>
<td>1.82</td>
<td>b</td>
<td>0.21</td>
<td>d</td>
</tr>
<tr>
<td>Immunizator 12h</td>
<td>3.182</td>
<td>a</td>
<td>0.225</td>
<td>d</td>
</tr>
<tr>
<td>Immunizator 24h</td>
<td>1.32</td>
<td>c</td>
<td>0.092</td>
<td>d</td>
</tr>
</tbody>
</table>

**Table 1 values of protein content of American cockroach extract**
Antimicrobial activity of American cockroaches extract

All inhibition zone of haemolymph extracted for all transactions are in figure 1. Statistical analysis had moral differences to activity of Immunizator 12-hour cooling centrifuge on *E. coli* with all transactions to level P > 0.05 with 24 mm inhibition diameter symbol **. And moral differences Immunizator 24 hours cooling centrifuge on *E. coli* also inhibit diameter 23 mm compared to the rest of the microbes moral level P > 0.05 symbol * and effect of immunizator 24 hours cooling centrifuge on yeast *C. albicans* with 21.7 mm diameter with the rest of the microbes moral level P > 0.05 symbol *.

**Table 2: Effect of American cockroach haemolymph on Polymorphonuclear leukocytes cell viability**

<table>
<thead>
<tr>
<th>treatment</th>
<th>cells-viability percentage</th>
<th>Static symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>91.3%</td>
<td>a</td>
</tr>
<tr>
<td>Immunizator 24-h cooling centrifuge</td>
<td>80.4%</td>
<td>b</td>
</tr>
<tr>
<td>Immunizator 12-h cooling centrifuge</td>
<td>74.5%</td>
<td>c</td>
</tr>
</tbody>
</table>

Figure 1 Antimicrobial activity of crude haemolymph extracted from *Periplaneta americana*.

Effect of extracted haemolymph on Polymorphonuclear leukocytes cell viability

The extracts Immunizator 12 hour and Immunizator 24 hour both with cooling centrifuge were selected to test their effect on availability of human Polymorphonuclear cell PMNs because their top antimicrobial activity in this study compared to other extract, PMNs cells-viability in table 2. Statistical analysis has shown that control symbol a the highest moral differences that haven’t treated followed by treatment with Immunizator 24-hour cooling centrifuge symbol b decreased PMNs cells-viability because of extract’s toxicity, The latest treatment with Immunizator 12 hours cooling centrifuge moral difference with the first and second transactions moral level P > 0.05 symbol c this means PMNs cells-viability decreases due to the increased of extract toxicity.

Discussion

Cooling centrifugation at temperature 4 °C gave higher levels of protein content compared to the non-cooling for all transactions may reason is the difference in speed between the two devices, the more Speed and power increased the efficiency of the device unplug macromolecules including peptides [21], with terms of immunization, the Immunizator 12-hour extract was the highest protein content due to stimulate their immune system to synthesis peptides after being injected with bacteria, protein content decrease until less than the normal rate after 24 hours of Immunization with bacteria which shows weakness period of insects from bleeding gets, either bugs that didn’t immunized has protein content too because exposure to pathogens, In addition to the natural proteins in their body.

The study found that cockroaches injected with bacteria effective against microbes higher than others did not injected especially on microbe used in immunization *E. Coli*, duration left after injection until extraction was affected too, which was 12 and 24 hours, as well as the centrifuge device used had impact on antimicrobial activity. the haemolymph non-immunizator by cooling centrifuge was affecting on *E. coli*, *C. albicans* and *S. Epidermidis* might because live in environments filled with these microbes [22], cockroaches use similar immune response in vertebrates to face a life-threatening [23]. Seraj et.al. (2003) has an explanation for the effectiveness of the haemolymph non-immunizator by non-cooling centrifuge called him non induced might revert to having a basic AMPs effectively against *E. coli* [14], but the on *S. aureus*, and other types of microbes have been weak because their high resist than the activity of extract. Study did not agree with [24] about
non immunizator haemolymph stating that no affected against both *E. coli* and *S. aureus* may because he used a pathogenic bacteria of nosocomial hospital, there an activity of extract although its protein content indicating that mean presence anther antimicrobial compounds which may fats or proteins associated with lipids or with carbohydrates, like AMP lectins a carbohydrate-binding proteins as in [13]. Some research findings supported the idea of a peptide natively in cockroaches by intestinal and exoskeleton extracts against *S. aureus* and *E. coli* he [2]. The activity of Immunizator 12-hours non-cooling centrifuge decreased for all microbes by changing the centrifuge device to non-cooling despite the stability of the duration of immunization, but had effect on *C. albicans* may target on yeast different from on bacteria. One reason to changes antimicrobial activity is fragmentation haemolymph contents and became useful for bacteria as food or lose activity due to braking of the disulfide bond and Hydrogen bond by heat of centrifuge device which led to change alpha helix or beta sheet shape which help penetrate microbe’s membrane [25], all of the positive charge and hydrophobicity and the length of peptide’s chain were important to activity [26], so may heat of centrifugation had change this properties lowering activity. Our results have agreed with respect to immunization period with [14] that highest effective between 9 to 12 hours after injection then declined after 24 hours. inhibition diameters decreased in the non-cooling centrifuge for most microbial species, Either the immunizator 24 hours cooling centrifuge had decreased antimicrobial ability on most microbes but still highly effective on *E. coli* and was higher effect on *C. albicans* that showed the persistence of antimicrobial activity against microbes even after 24 hours of exposure to microbes. While the antimicrobial activity of immunizator 24-hours non-cooling centrifuge decreased significantly in our study results came in line with [14]. effectiveness of AMPs depend on microbial plasma membrane but some AMPs show broad spectrum activity [27]. differences between study and others due to differences in feeding cockroaches, environmental qualities and humidity factors morph type of species used, and dangerous of bacteria injection [24].

**Conclusion**

Results show that antimicrobial activity of haemolymph extract on microbes with Well diffusion agar was higher inhibiting to immunizator 12 hours by cooling centrifuges on *E. coli* with 24 mm. Also Determined effect of the extract on a viability of polymorphonuclear leukocytes of human.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding.

**References**


Is there Any Correlation between Periodontal Diseases and Sleeping

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²Department of Basic Science, College of Dentistry, University of Baghdad, Iraq.

Abstract

Background: Sleep deficiency has develop a worldwide occurrence, and epidemiologic facts show that little sleep period unfavorably influences human physical health. Essential methods include variation of immune-inflammatory mechanisms. These modifications possibly will donate to potentiation of critical periodontal disease.

Aim of the study: The aim of this study was to assess if there was a relationship between periodontal diseases and sleeping in a sample of patients from the College of Dentistry.

Materials and Method: Forty-Five subjects were sorted into 3 groups (n = 15 each) viz. clinically healthy, gingivitis and periodontitis. Periodontal status of subjects was evaluated by plaque index, gingival index and probing pocket depth. All the study subjects were managed Pittsburgh Sleep Quality Index (PSQI) questionnaire for the judgment of sleep deficiency.

Results: Current study shown that the mean of PSQI was statistically non-significant for all all groups in this study which are: Healthy, Gingivitis and Periodontitis.

Conclusion: This study with initial results is indicative of independent association of sleep deficiency with severity of periodontal disease, but certainly calls on for another studies in the future with larger samples number.

Key words: Periodontal Diseases, Pittsburgh Sleep Quality Index, Sleeping.

Introduction

The gingiva, alveolar mucosa, alveolar bone, cementum and periodontal ligament is the main compositions of periodontium. The periodontium is comprised of these constituents help to reinforce the teeth in their site in the alveolar bone. The color of gingiva is characteristically coral pink in color, but possibly will change because of physiologic pigment with certain races. The age is affected on the texture of gingiva which appear smooth in the period of youth and became stippled at the period of adulthood and then retain to smooth at the period of advancing age. The stippled tissue of gingiva has a consistency like to the peel of an orange and its occurrence does not essentially indicate health(1). The most common oral diseases is gingivitis that concerns humans, it is expressed as an inflammation of the soft tissues around the teeth. As a global health concern, it influences the majority of adolescents and even children (2,3,4 and 5). The term sleep denotes rest or relaxation, it is merely an easy term, but it is a complicated and vital biological activity that is mandatory for all humans on a daily basis irrespective to age, ethnic origin or even sex(6). The role of sleep in systemic physiology and brain function is critical, comprising appetite control, metabolism, and the managing of immune, hormonal, and cardiovascular systems(7). Sleep with two types long and short intervals were related with deprived self-related health. Equally long and short durations of sleep were reported to be predictors, or markers, of type 2 diabetes mellitus, cardiovascular outcomes. Sleep disturbances, including insomnia, independently contribute to the risk of inflammatory disorders and major depressive disorder. Sleep loss induces a functional alteration of the monocyte pro inflammatory cytokine response, resulting in changes of immune cell physiologic characteristics
and several hormones become upregulated \(^{(8,9,10,11,12)}\). The aim of this study was to evaluate if there was an association between periodontal diseases and sleeping in a sample of patients from the College of Dentistry/University of Baghdad.

**Materials and Method**

**Human sample**

The subjects included in this study were drawn from patients attending the periodontics department in the college of dentistry/University of Baghdad from the beginning of February 2018 to the end of April 2018.

The entire study samples composed of 45 patients (17 males and 28 females), classified into three groups, each group composed of 15 patients with age range from 20 to 65 years.

The patients were divided into 3 groups:

- **Group I:** 15 subjects with age range from (20-30) considered as Healthy.
- **Group II:** 15 patients with age range from (20-35) gingivitis patients.
- **Group III:** 15 patients with age range from (30-65). considered as chronic periodontitis patients.

A questionnaire which is self-reported was prepared to be full for each individual who included the PSQI questionnaire.

**Exclusion Criteria**

2. History of Periodontal treatment at the last 3 months.
3. Patients who were smokers.
4. Pregnant females.

**Materials and Instruments**

The following materials and instruments have been used during this study.

1. Disposable mirror.
2. Periodontal probe (Marquis color-coded probe).
3. Mask and gloves.
4. Disposable towel tray.
5. Cotton and Alcohol.

**Clinical Periodontal Parameters**

The clinical examination of periodontal parameters which done by using Marquis color-coded probe include:

**Plaque Index Parameter (PLI)**

The four surfaces of all teeth except 3rd molar were scored and examined according to system of plaque index \(^{(13)}\).

**Gingival Index (GI)**

According to the principles of the gingival index explained in the publication by \(^{(14)}\) each of the four gingival areas of all teeth except 3rd molar (facial, mesial, distal, palatal and lingual) is evaluated for inflammation and given a score from (0-3). Bleeding is assessed by running a periodontal probe along the entire soft tissue wall of the gingival crevice or periodontal pocket.

**Probing pocket depth (PPD)**

Probing pocket depth is determined from the gingival margin to the point at which the probe tip was stopped. It’s generally used to express the effect of inflammation and the causing tissue loss in periodontal disease. The tip of the periodontal probe at all times penetrates into tissue that is below the sulcus or pocket fundus, yet when the advocated probing force of 0.20–0.25 N is applied. \(^{(15)}\). It is imperative to preserve the periodontal probe parallel to the contours of the root of the tooth and to insert the probe down to the base of the pocket. This results in obscuring a section of the periodontal probe’s tip. The first marking visible above the pocket indicates the measurement of the pocket depth. It has been found that the average, healthy pocket depth is around 3 mm. Depths greater than 3 mm can be accompanying with attachment loss of the tooth to the surrounding alveolar bone, which is a distinguishing found in periodontitis \(^{(16)}\).

**Statistical analysis**

Data were analyzed and computed using SPSS (statistical package of social science) software version 24.
Results

The presents of data which was systemically analyzed in tables and the score respond with the aim of the study as follows:

Results of the clinical periodontal parameters analysis

Table (1): Descriptive statistics and groups’ differences for plaque index.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Descriptive Statistics ANOVA test</th>
<th>Groups’ differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  S.D.  S.E.  Min.  Max.</td>
<td>F-test  p-value</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>15  0.95  0.12  0.03  0.62  1.09</td>
<td>10.352  0.000 (HS)</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>15  1.07  0.05  0.01  1.02  1.17</td>
<td>0.029 (S)</td>
</tr>
<tr>
<td>Periostitis</td>
<td>15  1.17  0.20  0.05  0.95  1.63</td>
<td>0.000 (HS)</td>
</tr>
</tbody>
</table>

Table (1) illustration the mean value of plaque index for the (healthy, gingivitis and periodontitis) groups and the values were 0.94, 1.07 and 1.17 respectively.

There was a Highly significance differences in PLI between the three groups were found using ANOVA test. By using Tukey’s HSD test to analyze the any statistically significant difference between each two groups and it was discovered that:

- Highly significant differences were found between healthy group and periodontitis group.
- Non-significant differences were found between gingivitis group and periodontitis group.

Gingival index (GI)

Table (2) show the mean value of gingival index for the (healthy, gingivitis and periodontitis) groups and was 0.94, 1.04 and 1.08 respectively.

Table (2): Descriptive statistics and groups’ differences for gingival index.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Descriptive Statistics ANOVA test</th>
<th>Groups’ differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  S.D.  S.E.  Min.  Max.</td>
<td>F-test  p-value</td>
</tr>
<tr>
<td>Healthy</td>
<td>15  0.94  0.18  0.05  0.58  1.2</td>
<td>0.043 (S)</td>
</tr>
<tr>
<td>Gin.</td>
<td>15  1.04  0.19  0.05  0.67  1.21</td>
<td>0.162 (NS)</td>
</tr>
<tr>
<td>Perio.</td>
<td>15  1.08  0.07  0.02  1     1.21</td>
<td>0.041 (S)</td>
</tr>
</tbody>
</table>
Significance differences in GI between the three groups were found using ANOVA test. By using Tukey’s HSD test to test the any statistically significant difference between each two groups and it was found that:

- Non-significant differences were found between healthy group and gingivitis group.
- Significant differences were found between healthy group and periodontitis group.
- Non-significant differences were found between gingivitis group and periodontitis group.

Probing pocket depth (PPD)

Table (3) show the mean value of periodontal pocket depth which was 1.481. The maximum value was 3.24 and the minimum value was 0.74.

**Table (3): The descriptive statistics of periodontal pocket depth in periodontitis group.**

<table>
<thead>
<tr>
<th>PPD</th>
<th>Descriptive Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>1.48</td>
</tr>
</tbody>
</table>

Pittsburgh sleep quality index (PSQI)

Table (4) show the mean value of the global scores of PSQI index for the (healthy, gingivitis and periodontitis) groups and were 5.47, 6.27 and 5 respectively.

**Table (4): Descriptive statistics and groups’ differences for PSQI index.**

<table>
<thead>
<tr>
<th>PSQI</th>
<th>Groups</th>
<th>Descriptive Statistics ANOVA test</th>
<th>Groups’ differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.D</td>
</tr>
<tr>
<td>Healthy</td>
<td>15</td>
<td>5.47</td>
<td>1.96</td>
</tr>
<tr>
<td>Gin.</td>
<td>15</td>
<td>6.27</td>
<td>3.26</td>
</tr>
<tr>
<td>Perio.</td>
<td>15</td>
<td>5</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Non-Significant differences were found between the three groups is found using ANOVA test.

Table (5) show the percentage and number of good and poor sleeping status in each group. In heathy group (30%) 6 samples had poor sleeping and (36%) 9 samples had good sleeping. In gingivitis group 8 samples (40%) had poor sleeping and (28%) 7 samples had good sleeping. In periodontitis group 6 samples (30%) had poor sleeping and 9 (36%) had good sleeping.

**Table (5): Frequency distributions, percentages according to the sleeping status.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sleeping Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>Healthy</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>
Table (6) display the relation between the scores of PSQI and plaque and gingival indices and non-significant relations were found in all 3 groups. It also show the relation between the scores of PSQI and periodontal pocket depth in periodontitis groups which was also non-significant.

**Table (6): Relation between the PSQI scores and different periodontal indices.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>PLI and PSQI score</th>
<th>GI and PSQI score</th>
<th>PD and PSQI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
<td>R</td>
</tr>
<tr>
<td>Healthy</td>
<td>-0.096</td>
<td>0.73 (NS)</td>
<td>0.09</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>0.134</td>
<td>0.63 (NS)</td>
<td>-0.18</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>0.216</td>
<td>0.44 (NS)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

**Discussion**

This might impair an individual’s capacity to perform adequate oral hygiene practices, thus increasing the risk of periodontal disease\(^{(17)}\). Grover, Malhotra and Kaur made a research in (2015) for Exploring association between sleep deprivation and chronic periodontitis using the Pittsburgh Sleep Quality Index. The results indicated a positive correlation of PSQI with GI and PPD in gingivitis and periodontitis groups. Another study was made by Wiener in 2016\(^{(18)}\) to conclude if there is an relationship of routine insufficient sleep and periodontitis. The data sources for the present study were National Health and Nutrition Examination Surveys of contributors who were of ages 30 years and above who had complete periodontal and sleep data in the NHANES 2009 to 2012 data sets. Contributors were also asked during an interview to report age, sex, race, education level, and smoking status among other questions. The variables were grouped as follows: age (45 years to less than 55 years, 55 years to 69 years); sex (male, female); race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican-American, etc.); education level (high school graduate or less, some college/technical school or above that), and smoking status (current smokers, former smokers, and never smokers). Body mass index was categorized as less than 25, 25 to less than 30, and 30 and above which matched to normal weight, overweight, and obesity. In un corrected analysis, usually sleeping less than 7 hours at night on weekdays or workdays was associated with periodontitis. The association was attenuated and failed to reach significance in adjusted analysis. Han K, Park YM, Park JB made a study in (2018)\(^{(19)}\) to estimate the association between long sleep duration and periodontal disease among men and women using nationally representative data. They found that the propensity of periodontitis increased with longer sleep duration in women. Compared with women who slept 5 hours or less, women with a sleep duration of 6 to 8 hours and 9 hours or more had higher odds of periodontitis. A significant relationship between sleep duration and periodontitis was not found in men. So the connection between long sleep duration and periodontitis was proven by multiple logistic regression analyses after adjusting for confusing factors among Korean women, especially in premenopausal women. Long sleep duration may be considered an independent risk indicator of periodontal disease among Korean women. The Pittsburgh Sleep Quality Index (PSQI) is a self-report questionnaire that assesses sleep quality over a 1-month time interval. The measure consists of 19 separable items, creating 7 components that produce one global score, and takes 5–10 minutes to complete. It was developed by researchers at the University of Pittsburgh (University of Pittsburgh Sleep Medicine Institute, 2016). The questionnaire of PSQI has been used in many settings, including research and clinical activities, and has been used in the diagnosis of sleep disorders.

The PSQI is an useful instrument used to measure the quality and patterns of sleep in the older adult. It is brief, reliable, valid, and standardized self-reported measure of sleep quality. It differentiates “poor” from “good” sleep by measuring seven domains: Subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, and sleep disturbances, use of sleep medication, and daytime dysfunction over the last month\(^{(20)}\).
The (PSQI) suffers from the same problems as other self-report inventories in that scores can be easily overstated or diminished by the person completing them. Like all questionnaires, the way the instrument is administered can have an effect on the final score. The PSQI is a quite new measure and as a result has not received enough investigation to determine the totality of the psychometric measures (21).

Conclusion

Non-significant differences in PSQI were discovered between the (healthy, gingivitis and periodontitis group) (i.e. the sleeping quality and quantity didn’t show an obvious effect on the periodontal status). Non-significant relation was found between PSQI and PLI, GI and PPD in all groups. Gingivitis group shows the lowliest sleeping states among the 3 three groups. The sample size which may be the cause for these results.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

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Establishing the Ordinary Narration of Men with Originally Negative Prostate Biopsies

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Abstract

The prostate is an organ of reproduction in the man, resembling chestnut or walnut in shape and size, located directly under the bladder and in front of the anus surrounding the first part of the urethra. The aim of study is determine the health results of Basra Governorate, Iraqi males with history of a single negative transrectal prostate biopsy TRUS-Bx, from January 1, 2010 to December 31, 2018. The researcher used billing claims from Basra Educational Hospital database (BEH) to determine the patients whom have understand about TRUS – BX with using billing claim of PNB (Prostate Needle Biopsy) and was separated for concurrent ultrasound pelvic. The 94% from database was practicing in Basra as billing claim which meaning that who doesn’t accept billing will compensate accordingly. The drugs tests were implantation for all patients as Basra Educational Hospital ethical. Compassion, specialty, high quality predictive rate and negative prophetic value had been used to decide the analytical rate of PCa diagnosis of TRUS-Bx. The records were then analyzed by the use of SPSS version 14. The ethical was asked patients to own an eleven minutes rest before measure of their pressure level of blood and whole experiment. Young people (under the age of 45) are considered low levels (the risk of prostate cancer is few), when they are less than 2.5 Nano g/ml. For older men (older than 45 years), the value is low if it is less than 4 Nano grams/ml. In spite of the foregoing, it is known that about half of the cases of Prostate-Specific Antigen PSA in proportions that do not exceed these limits. For this reason, it is important to monitor the PSA values every year. Large-scale prostate biopsy identifies large prostate cancer in many men and of whom the previous sextant was a benign biopsy. This procedure should be considered when the suspicious results are for the morrow, despite the previous negative TRUS-BX.

Keywords: Prostate Biopsy, Radical Prostatectomy, Prostate Cancer

Introduction

The prostate is an organ of reproduction in the man, resembling chestnut or walnut in shape and size, located directly under the bladder and in front of the anus surrounding the first part of the urethra (1). The constant growth of the prostate leads to its enlargement, or the so-called Benign Prostatic Hyperplasia (BPH), which means the increase of its cells, which increases the amount of PSA protein secreted, a portion of it appears in the blood is measured by the type of prostate disease, whether benign hypertrophy, bacterial infection, Chronic inflammation or cancer.

Only men have a prostate, a small gland located below the bladder near the rectum. The prostate gland surrounds the urethra, which is the corridor inside the penis through which urine and men pass (2). The prostate is often described as the size of a walnut and naturally grows as men grow older. This may sometimes pose problems, such as difficulty urinating. These problems are common in older men and are not always symptoms or signs of cancer (3).

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In the early stages there may not be any symptoms. In later stages, however, some prostate cancer symptoms may include:

- Frequent or sudden feeling of need to urinate
- Having difficulty urinating (e.g. difficulty in starting urination or being unable to urinate despite feeling the need for it or slow flow of urine)
- Some pain when you pee.
- The presence of blood in the urine or semen
- Pain in the lower back or upper thighs or hips

The factors most relevant to the development of prostate cancer are:

- **Age:** Prostate cancer is an age-dependent disease, which means that the likelihood of infection increases as age progresses. The risk of prostate cancer by the age of seventy five is 1 in 7 men. This risk is increased by the age of 83 to 1 in 5 men.

- **Family history:** If you have a first-class relative with prostate cancer, you are more likely to be infected than men who don’t have family histories like this.

- **Heredity:** Genes are present in each cell of the body. They control the way cells grow in the body and behave.

- **Diet:** There is some evidence that eating too much processed meat or fat-rich foods may increase the risk of developing prostate cancer.

- **Lifestyle:** There is evidence to show that the environment and lifestyle may affect the risk of prostate cancer.

**Prostate-Specific Antigen Test**

PSA is a diabetic protein (Glycoprotein-protein with leftover sugars) produced by prostate cells. It is actually a protease, a protein that is capable of dismantling other proteins. It is present at a high rate in the tissues of the prostate and semen.

The significant increase in the PSA level, which exceeds 0.75 Nano-g/ml within a year, increases the likelihood of prostate cancer and is a reason for taking a prostate sample. However, despite the limited nature of PSA screening, it remains the best predictor of prostate cancer or to follow patients who have received treatment for prostate cancer. Prostate cancer rarely returns, after treatment, without a high level of PSA.

Also, you should tell your doctor if you have a urine catheter (catheter) or a prostate biopsy or if your prostate inflammation is diagnosed because these cases may increase the PSA rate.

**Radical Prostatectomy (RP)**

The traditional surgical procedure for the resection of the prostate is to open a wound of approximately 15 – 20 cm in the abdomen beneath the navel. As the complications of this process are manifold, the surgeons thought of an alternative, where the patient’s suffering is less and the duration of recovery is shorter, the process known as Minimal Invasive Surgery (MIS), which can be translated by the smallest surgery aggression or an incursion into the body of the patient which was
introduced almost two decades ago\(^{(11)}\).

The laparoscopic Radical prostatectomy or (LRP) device or telescope flattens the natural depth of the position on which the operation is conducted but faces various obstacles, including hardening or non-movement of the wrist/wrist in this device\(^{(12)}\).

In Canada, Dr. Stephen Butler - from the St. Joseph’s Health Centre in London - is successfully using it between 10 – 12 times with the robot – so we can be more precise than normal open surgery. Also, the robot avoids the important nerves and muscles of the nerve and urinary incontinence\(^{(13)}\).

**Aim**

The aim of study is determine the health results of Basra Governorate, Iraqi males with history of a single negative transrectal prostate biopsy TRUS-Bx, from January 1, 2010 to December 31, 2018.

**Methodology**

The experiment was described as cohort study using related data from Basra Educational Hospital in Iraq which consist from males only who have history of single negative transrectal prostate biopsy between periods January 1, 2010 to December 31, 2018.

**Sample**

The researcher used billing claims from Basra Educational Hospital database (BEH) to determine the patients whom have understand about TRUS – BX with using billing claim of PNB (Prostate Needle Biopsy) and was separated for concurrent ultrasound pelvic. The 94% from database was practicing in Basra as billing claim which meaning that who doesn’t accept billing will compensate accordingly. The drugs tests were implantation for all patients as Basra Educational Hospital ethical.

**Inclusion criteria**

1. Age older than legal consent > 35 years.
2. Prostate needle biopsy.
3. Ultrasound Pelvic with 3 days of (PNB) prostate needle biopsy

**Exclusion criteria**

1. Woman gender
2. Males age less than 35 years.
3. Death prior biopsy.
4. Before January 1, 2010
5. After December 31, 2018
6. Implantation of hormone pellets.
7. Censored in the first 60 days from index biopsy.
8. Any evidence of infection.
9. Patient’s refusal to participate in the study.

**Statistical Process**

The average, mean and standard deviation were present in variables established; whilst frequency and ratio have been decided on to represent qualitative variables. Compassion, specialty, high quality predictive rate and negative prophetic value had been used to decide the analytical rate of PCa diagnosis of TRUS-Bx. The arrangement stage among single negative transrectal prostate biopsy was determined using RP rates. The records were then analyzed by the use of SPSS version 14.

The researcher used sensitivity analysis for comparing the results of patients that have negative TRUS – BX and patients have any type of prostate biopsy. Additional, the researcher was used following tests for all patients beside drugs test:

1. PCa diagnosis rates
2. Frequency distribution of repeat biopsies
3. RP rates

**Ethics**

The ethical was asked patients to own an eleven minutes rest before measure of their pressure level of blood and whole experiment. Ethics Committee of Basra Educational Hospital in Basra government (Iraq) and AHAPs permitted the analysis method, and printed consent were attained from all of the members before beginning of the study.

**Results**

**Demographic**

As mention in methodology section, we haven’t
sample size, we used database of Basra Educational Hospital database as principle objectives.

The databases of Basra Educational Hospital have 395650 males’ patients, amount of 293908 patients (74.3 %) were excluded because of many reasons such as have not first prostate biopsies, prior in PCa diagnosis that called prostate cancer. The amounts of 101742 patients have negative prostate biopsy with ratio of (25.7 %). The 101742 was total sample of our study, 65412 patients have TRUS-Bx with ratio (64.3 %) and 36330 patients haven’t TRUS-Bx with ratio (35.7 %).

The final sample was 65412 male patients have TRUS-Bx, as shown in Table 1.

**Table 1 Sample Demographic Distribution**

<table>
<thead>
<tr>
<th>Parameter Number</th>
<th>Number and percentage of Women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 – 44</td>
<td>14758</td>
<td>22.56 %</td>
</tr>
<tr>
<td>45 – 54</td>
<td>16241</td>
<td>24.83 %</td>
</tr>
<tr>
<td>55 – 64</td>
<td>15421</td>
<td>23.58 %</td>
</tr>
<tr>
<td>65 – 74</td>
<td>10214</td>
<td>15.61 %</td>
</tr>
<tr>
<td>&gt; 75</td>
<td>8778</td>
<td>13.42 %</td>
</tr>
<tr>
<td>Total</td>
<td>65412</td>
<td>100.00 %</td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>35912</td>
<td>54.90 %</td>
</tr>
<tr>
<td>Urban</td>
<td>29500</td>
<td>45.10 %</td>
</tr>
<tr>
<td>Total</td>
<td>65412</td>
<td>100 %</td>
</tr>
</tbody>
</table>

(NS= No statistical significance)

The numbers of males with age between (35 – 44 years) were 14,758 patients (22.56 %), age between (45 – 54 years) were 16241 (24.83 %) patents, age between (55 – 64 years) were 15421 (23.58 %) patients, age between (65 – 74 years) were 10214 (15.61 %) patients, and greater than 75 years old were 8778 (13.42 %) patients. P value for all above results was more than (0.05), which was statistically not significant.

**Prostate diagnosis**

The sample size was 65412 male patients between periods from January 1, 2010 to December 31, 2018 to determine the single negative transrectal prostate biopsy TRUS-Bx.

We found that the patients have prostate cancer diagnosis for 5 years with age 35 – 44 was (7.50 %), 45 -54 (11.85 %), 55 – 64 (12.02 %), 65 – 74 (15.81 %) and greater than 75 was (16.10 %). The PCa diagnosis for 10 years with age 35 – 44 was (11.15 %), 45 -54 (17.70 %), 55 – 64 (20.87 %), 65 – 74 (20.98 %) and greater than 75 was (20.27 %). The PCa diagnosis for 15 years with age 35 – 44 was (14.60 %), 45 -54 (21.07 %), 55 – 64 (24.21 %), 65 – 74 (23.19 %) and greater than 75 was (20.78 %).
Table 2 PCa diagnosis

<table>
<thead>
<tr>
<th>Age</th>
<th>5 Years</th>
<th>10 Years</th>
<th>15 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 – 44</td>
<td>7.50</td>
<td>11.15</td>
<td>14.60</td>
</tr>
<tr>
<td>45 – 54</td>
<td>11.85</td>
<td>17.70</td>
<td>21.07</td>
</tr>
<tr>
<td>55 – 64</td>
<td>12.02</td>
<td>20.87</td>
<td>24.21</td>
</tr>
<tr>
<td>65 – 74</td>
<td>15.81</td>
<td>20.98</td>
<td>23.19</td>
</tr>
<tr>
<td>&gt; 75</td>
<td>16.10</td>
<td>20.27</td>
<td>20.78</td>
</tr>
</tbody>
</table>

Figure 3 PCa diagnosis as taken single negative TRUS-BX

Young people (under the age of 45) are considered low levels (the risk of prostate cancer is few), when they are less than 2.5 Nano g/ml. For older men (older than 45 years), the value is low if it is less than 4 Nano grams/ml. In spite of the foregoing, it is known that about half of the cases of Prostate-Specific Antigen PSA in proportions that do not exceed these limits. For this reason, it is important to monitor the PSA values every year.

When diagnosing prostate cancer that has not spread out of the prostate, it is usual to have a radical resection of prostaglandins or prostate biopsies. After the surgical procedure, the level of PSA in the blood should be reduced to 0. If the PSA level is higher than 0, the order indicates (in this case) the presence of residue from the prostate tissue, or the spread of the tumor elsewhere. The same applies if the prostate is frozen (Cryotherapy).

Sometimes a decision is taken to conduct radiotherapy. If the PSA level continues to rise three times in a row, it is considered a failure of treatment.

Additional, we made frequency distribution of repeat prostate biopsies in sample size for negative prostate biopsies in Iraq men in Basra, all results shown in frequency distribution of repeat prostate biopsies was identified in 19624 of 65412 men (30 %). The biopsy revealed a Gleeson degree of 6-8 (Median 6.4). In 26709 out of 65412 patients (41 %) and of whom prostate cancer was identified was only 100 positive core biopsy. Although the PSA biopsies was higher and had a total of free PSA less in those with cancer, it was only a statistical indicator of positive PSA speed biopsy (P < 0.001). Prostate cancer was observed in 64% of men with PSA. The 19079 patients of total sample (65412 patients) undergoing prostate resection patients were identified as having a great disease in all. Extensive biopsy complications included urinary retention in 15320 patients and rectal bleeding.

The results show that 45% of the radical prostate (RP) was not suffering from the single negative transrectal prostate biopsy TRUS-Bx, while 30% of patients were died due to the radical prostatectomy and suffered from prostate cancer.

The sensitivity analyses incontestable that once this restriction wasn’t applied, and every one man with a negative prostate diagnostic test were enclosed, the PCa diagnosing rates weren’t clinically considerably completely different. The patients UN agency underwent biopsies at completely different time points within the study amount had variations within the accumulative incidences of the assorted study outcomes, which may well be a mirrored image of the impact of changes in variety of cores sampled on malady outcomes. Similarly, temporal changes within the relative frequencies of finger-guided versus TRUS-BX over the study amount may are assessed to additional demonstrate changes in diagnostic testing technique over the years and to judge the temporal changes in patient exclusion thanks to having undergone a finger-guided biopsy.

Conclusion

The aim of study is determine the health results of Basra Governorate, Iraqi males with history of a single negative transrectal prostate biopsy TRUS-Bx, from
Young people (under the age of 45) are considered low levels of prostate cancer risks. Large-scale prostate biopsy identifies large prostate cancer in many men and of whom the previous sextant was a benign biopsy. This procedure should be considered when the suspicious results are for the morrow, despite the previous negative TRUS-BX. The method of radical prostatectomy (RP) is not treated as a panacea for prostate cancer in several cases, and depends on the degree of cancer prevalence in this area.

For future work, we will suggest that knowledge transfer occurs by sharing the results of this study with the rest of the medical community. We will present our findings at various international conferences such as the annual American Urological Association Meeting. We also plan on publishing our results in a peer-reviewed journal, ensuring that our results become available to as wide an audience as possible.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**

Mutans Streptococci and Removable Orthodontics

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Abstract

Numerous studies have investigated the influence of orthodontic therapy and appliances on the oral microbial flora. Little is known about the effect of removable orthodontic appliances on oral colonisation by mutans streptococci. The original aim of the present investigation was to assess the mean and the statistical mean difference of colony values of S. Mutans in a removable orthodontic appliance fabricated from two types of acrylic resin for an Iraqi sample. According to certain predetermined sample criteria; the present study consist of eleven consecutive young patients scheduled for orthodontic treatment with removable orthodontic appliances at the department of orthodontics at the college of dentistry/ University of Babylon as well as dental student seeking orthodontic treatment. Each subject included in the present study had to wear an upper (hot cured) and lower (cold cured) well adaptive removable orthodontic appliance. A swab was taken from the lingual/palatal gingival and inoculated within the brain heart agar then cultured within Mitis- Salivarius Bacitracin (MSB) agar. The colonies were calculated for each plate, afterwards the colony forming unit (CFU) will be calculated. of the present study showing that all bacterial colony values were higher in the lower than upper arch. However, a statistical non significant difference was registered between the colony mean values of both arches. The present study signify both types of acrylic resins (heat/ cold) can be used as an orthodontic acrylic base in removable appliances within the orthodontic practice with a careful monitoring of patients treated orthodontically for risk of caries development.

Keywords: mutans streptococci, heat cure acrylic, cold cure acrylic.

Introduction

Acrylic resins are mostly used as denture and orthodontic base material in dental practice. These are available in different forms according to the polymerization reaction as heat cure acrylic resin, rapid cure auto polymerizing acrylic resin, light cure resin and specialized form resins used for microwave. Usually, heat-cured (polymethyl methacrylate) PMMA is used as the so-called “gum-work” for removable full dentures or removable partial dentures;¹⁰ the cold-cured PMMA is used for denture repair, reline and orthodontic removable appliances involved in thumb deterrent, tipping teeth, block movements, overbite reduction, space maintenance and retention.²,³

Nowadays orthodontic treatment is adopted by wide section of society not only for the correction of malocclusion but also improves mastication, speech and appearance, as well as overall health, comfort, and self-esteem.⁴ Although the orthodontic appliances has many known benefits, these appliances are also associated with a number of damages and disorders of oral cavity.⁵

Oral cavity is a complex environment supporting a many distinct bacterial species or phylotypes, of which over 50% are yet to be cultivated, residing specifically in diverse niches in the oral cavity and executing different roles.⁶,⁷ Presence of ortho-dontic appliances in oral cavity alters the balanced ecosystem of oral microbiome; as it provides an additional retentive site for food, different physio-chemical environment and surfaces for adhesion and attachments of normal oral microflora.⁸ Regarding the long term existence of baseplates of orthodontic appliances (BOA) in mouth and their surface porosities may have a negative impact on oral microbiota, promote the biofilm formation and may contribute to dental caries, gingival inflammation and periodontal disease.⁹,¹⁰ Streptococcus mutans (SM) is considered one of the main organisms in plaque that contributes to the initiation of caries. Despite being ubiquitous in the oral cavity, SM prevalence often indicates caries susceptibility and poor oral hygiene.¹¹
Numerous studies have investigated the influence of orthodontic therapy and appliances on the oral microbial flora. [12,13] Little is known about the effect of removable orthodontic appliances on oral colonisation by mutans streptococci. [14] The original aim of the present investigation was to assess the mean and the statistical mean difference of colony values of S. Mutans in a removable orthodontic appliance fabricated from two types of acrylic resin for an Iraqi young sample.

**Materials and Method**

**Sample enrollment:**

According to certain predetermined sample criteria; the present cross sectional study consist of eleven out of seventeen (7 males and 4 females) consecutive young patients scheduled for orthodontic treatment with removable orthodontic appliances at the department of orthodontics with age ranging from 18 to 25 years were enrolled to be included in this study during the period from December 2018 to July 2019. The study sample were requited from patients attending the teaching hospital at the college of dentistry/ babylon University as well as dental student seeking orthodontic treatment. As each patient was receiving upper and lower orthodontic appliance; so the study sample were further subdivided into eleven upper as well as lower arch that receiving appliances.

**Inclusion sample Criteria:**

1) Good oral hygiene  2) Cooperative compliant patient  3) Clinically healthy gingiva 4) Well fabricated upper and lower removable orthodontic appliance  5) Patients’ malocclusion is within the scope of removable appliance.

**Exclusion sample criteria:**

1) History of systemic diseases 2) medicament such as antibiotics, steroids, or nonsteroidal anti-inflammatory drugs at least 3 months prior to appliance insertion 3) Presence of fixed bridges/crowns or partial dentures 4) Smoking habit.

**Materials**

1) Transport media 2) Petri dish, test tube, flask, rick 3) Loop and glass Spreader. 4) Micro and electro-pipette. 5) sterile saline and distilled water. 6) Mitis salivarius agar, brain heart agar, agar agar. 7) Bacitracin. 8) Cold and Hot Cure Acrylic. 9) Incubator, Dry oven, Autoclave. 10) Refrigerator. 11) Millipore filter(0.4μm) 12) Gram stain.

**Methodology (bacterial isolation):**

Each subject included in the present study had to wear an upper and lower well adaptive removable orthodontic appliance. The upper and lower appliances were fabricated from heat and cold cure acrylic resin, respectively following the manufacturer’s instructions ( Surrey, U.K., England). At the time of 1st appliance activation after 2 weeks of their insertion; a swab was taken from the lingual/palatal gingival area were the acrylic base plates were extended using a selective transport media (fig. 1) . After 24 hour of their initial anaerobic incubation at 37º C; the swab was inoculated within the brain heart agar as a broth media that facilitating over growth of bacteria and incubated again for 24 hour. Afterwards and before cultivation, serial dilution was done to be ready for cultivation (0.1 ml of sample is plated) within Mitis- Salivarius Bacitracin (MSB) agar using a sterile spreading glass. After 24 hour of incubated bacterial cultured plates at 37º C, a growth of bacterial colonies (a cluster of clones in bluish distinctive colour) were seen clearly and distributed on the surface of MSB agar (fig. 2 left ).

The colonies were calculated for each plate using colony counter device, afterwards the colony forming unit (CFU) will be calculated and the data were submitted and stored in an excel program within a computer for satistics.

**S. Mutans identification:**

The identification of S.mutans is based on (according to the information in Bergeys Manual of Determinative Bacteriology 9th ed.,1994) [15] distinctive colonial morphology on selective and nonselective agar, Gram staining, distinctive cell shape on light microscopy. Morphologically, a gram-stained isolated Mutans Streptococci were viewed under a light microscope at an objective magnification of 40x. The size of the observed colonies ranged from 0.5 to 1 μm. The mutants bacterial cells resembled chains of cocci long as beads in their spatial arrangement. (Fig.2 right).
Figure 1: A transport media with plates

Figure 2: The pictures shows the macro- and micromorphology of Streptococcus Mutans isolate from study sample. (left) S. mutans colonies with a characteristic rounded bluish color grown on the MSB selective agar; (right) The isolated colony of S. mutans is gram stained and viewed under light microscope, the coccus cells arranged in chains.

Colony forming unit

A colony-forming unit (CFU) is a unit used to estimate the number of viable bacteria or fungal cells in a sample. Not all bacterial cells produce colonies; For this reason results are reported as colony forming units (CFU)/ml of bacterial culture. Ideally only plates with 25-250 colonies are used. In order to make the calculation of the number of cells/ml in the original samples less formidable, dilutions are designed to be easy to handle mathematically. The number of colonies present in a particular test sample was determined using the formula

\[ CFU/ml = \frac{\text{Number of colony} \times 1}{\text{dilution} \times \text{volume of sample plated}}. \]

\[ CFU/ml = (X) \times 10^6 \times 10. \]

\[ CFU/ml = 0.0X \times 10^9. \] In present study for a given no. of colonies; a 0.1 ml from a $10^{-6}$ dilution of the original sample was plated.

**Mitis- Salivarius Bacitracin agar (MSB agar):**

Following the method of Geigy [18]; Al-Mudallal et al [19]; Naji [20] in this agar media preparation:

MSB agar is a selective media were used for cultivation of S. mutans. It was prepared by addition of selective agents: bacitracin antibiotic and sucrose, at the optimal levels determined to the Mitis –Salivarius Agar (MSA).

**Mitis- Salivarius Agar (MSA)**

Is the main components of the prepared media that suppress the growth of most microorganisms but allows the growth of Streptococcus Spp. This media prepared according to the manufacturer’s instruction by dissolving 90 g of MSA in 1000 ml distilled water. Afterwards sterilized by autoclave at 121°C 1.5 bars per square inch for 15 minutes, and left to cool until 45 °C. To inhibit bacteria other than Mutans Streptococci; bacitracin and sucrose were added to the Mitis agar medium; since a relative resistance of S. mutans to high concentration of both bacitracin and sucrose had been reported.

**Bacitracin antibiotic**

Under aseptic condition, bacitracin antibiotic solution was added to the agar media. A bacitracin stock solution was prepared by dissolving 0.2661g of bacitracin powder in 100 ml of de-ionized water. This will supply concentration of 200 IU /L (1 unit of bacitracin =0. 0133 mg). [18] Millipore filter (0.4μm) was used to sterilize bacitracin solution. A new fresh solution was prepared every 2-3 weeks and stored in a refrigerator.

**Sucrose**

After the sterilization of the Mitis agar medium till cooling, sucrose was added to provide concentration of 200 g/L. Sucrose solution was sterilized by Millipore filter(0.4μm). 200 g/L of sucrose can inhibit the growth of S. sobrinus and S.cricetus and enhance the growth of S.mutans.[19] Moreover, The inclusion of sucrose leads to the formation of glucans and the appearance of colony formation to aid identification.

After the addition of these two components to the Mitis agar, a Mitis -Salivarius Bacitracin (MSB) agar will be form. The latter formed media then poured into
plates while solidify and stored in a refrigerator (after overnight incubation at 37 °C) till use thereafter.

**Results**

The results of the present study showing that all bacterial colony values were higher in the lower than upper arch. However, a statistical non significant difference was registered between the colony mean values of both arches.

**Table (1): Reveals the mean, maximum, minimum, standard deviation, error values for the upper and lower Streptococcus Mutans colonies.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>meanU/L</th>
<th>mean difference</th>
<th>t.test</th>
<th>p.value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>USC</td>
<td>127</td>
<td>-21.09</td>
<td>-.65</td>
<td>.995</td>
<td>20</td>
</tr>
<tr>
<td>LSC</td>
<td>148</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Each colony value is multiplied by 10^7.

**Table (2): Reveals the mean difference between upper and lower Streptococcal colonies and independent t-test. A non significant difference was found between both colony values of upper and lower arches.**

<table>
<thead>
<tr>
<th>variable</th>
<th>no.</th>
<th>Mean/cfu*</th>
<th>Max</th>
<th>Min</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>USC</td>
<td>(11)</td>
<td>127</td>
<td>263</td>
<td>50</td>
<td>56</td>
<td>23</td>
</tr>
<tr>
<td>LSC</td>
<td>(11)</td>
<td>148</td>
<td>285</td>
<td>72</td>
<td>43</td>
<td>22</td>
</tr>
</tbody>
</table>

*P-value ≤ 0.05 was significance

**Discussion**

Plaque accumulation followed by enamel demineralization and gingivitis is a well-known complication in orthodontic therapy when fixed or removable appliances are used. When use the orthodontic appliances, the acrylic plates are placed in contact with teeth, and thus the resulting plaque accumulation commonly is due to food retentive configuration of the acrylic materials. Regarding the long term existence of base plate of orthodontic appliances in mouth and their surface porosities may have a negative impact on oral microbiota, promote the biofilm formation and may contribute to dental caries, gingival inflammation and periodontal disease.

Streptococcus mutans (SM) is considered one of the main organisms in plaque that contributes to the initiation of caries. For this reason it is necessary to investigate the degree of adhesion as well as virulence of S. Mutans using the two types of orthodontic acrylic resins in isolated study by CFU calculation.

Regarding the present study result; the colony forming unit for the lower S.colonies are higher than the upper colonies in all values (mean, minimum, maximum) as the mean lower S. colonies is (148 × 10^7 cfu/ml) while (127 × 10^7 cfu/ml). Such higher lower colony units of S. mutans in spite of smaller mandibular surface area suggesting that cold cured acrylic resins of removable orthodontic appliance is more favourable environment for mutans S. colonization than hot cured resins. However, a non statistical mean difference was found between the S. colony units for both acrylic resins ( P. value = 0.9) which can be attributed to sample size methodological factors. It was found that the use of removable appliances may lead to the creation of new retentive areas and surfaces, which favour the local adherence and growth of Mutans Streptococci.

In cold-cured acrylic resins the problem is even more prominent, because they display more surface porosities than the heat-cured ones. These porosities hamper the complete removal of dental plaques, such that mechanical cleaning often turns out to be inadequate. Surface roughness may contribute to the positively correlated rate of microbial colonization and plaque maturation on
surfaces. Lewis also showed that heat cured PMMA, often the kind used for making a Hawley retainer, showed the most bacterial adherence, specifically by S. mutans. Importantly, S. mutans adhered more, or to the same extent to acrylic, as it did to enamel. A study demonstrated that the subgingival flora did not change, implying that the periodontal condition of the patient was not affected. Moreover; although bacterial levels may increase with orthodontics, patients with removable retainers are similar to healthy nonappliance wearers demonstrating no increased gingivitis or periodontitis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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References


The Impact of Prenatal Nutritional Status on Occlusion of Primary Teeth among Kindergarten Children in Al-Kut City/Wassit

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²College of Dentistry, University of Baghdad/Iraq

Abstract

Background: When malnutrition begins early in life it affects adversely various aspects of growth including dental arch and increase the severity of oral problems in later life. The aim of present study was to assess the effect of prenatal malnutrition on the occlusion of primary teeth.

Material and method: This survey was conducted among urban kindergarten children in Al-Kut city of Wasit governorate. The sample 959 children aged 4 and 5 years were randomly selected from different areas of Al-kut city. The assessment of nutritional status was performed using prenatal anthropometric measurements, and primary dentition terminus was used for assessing dental arch relation.

Result: According to prenatal anthropometric indices preterm children constitute 23.46%, whereas low birth weight children constitute 13.66%. Flush terminus plane was the more prevalent type of occlusion for the total sample 47.86% while among preterm and low birth weighted children the mesial step terminus was more prevalent type of occlusion.

Conclusion: The prenatal malnutrition had some effect on the arch relation among kindergarten children.

Keywords: Wassit; primary teeth; prenatal Nutritional Status

Introduction

Nutritional status is a state that is resulting from the balance between the nutrients intake and the nutrients expenditure 1. Nutritional anthropometry remains the most practical and useful means for assessment of the nutritional status of population, particularly among infants and young children. Nutritional anthropometry is able to detect an imbalance of energy and nutrients in relation to need. Anthropometric indices are indices of post natal malnutrition 2 and prenatal malnutrition which include gestational age and birth weight 3.

Gestational age defined as the number of weeks from the first day of the last normal menstrual period to the date of delivery. It is one of the varieties of anthropometric indices of prenatal malnutrition 4. Full term infants are those that born between 37 to 42 weeks of gestation, post term infants are those born after 42 weeks of gestation and preterm infants are those of less than 37 weeks gestation 5. Birth weight is another anthropometric index of prenatal malnutrition 3. WHO (1972) and Mayes et al (1997) define any infant weighting less than 2500 gram at birth should be called low birth weight child, this definition is useful to identify a group of newborn at high risk of postnatal health complication and death 6. Weight at birth is a good indicator not only for the mother’s health and nutritional status, but also the newborn’s chances for survival, growth, long term health and psychosocial development; low birth weight increased risk of dying during their early months and years 7.

The science of occlusion is described by Foster (1982) that the occlusion of the teeth is any position in which the upper and lower teeth come together and the articulation of teeth is the functional movement of the lower dentition in contact with upper dentition 8. The term normal occlusion encompasses minor deviations from the ideal, which do not constitute the esthetic
or functional problems. It is not possible to specify precisely the limits of normal occlusion and so there can be disagreement even between experienced clinicians about categorization of border line cases. On the other hand Jones et al (2000) defined the normal occlusion as that occlusion which classifies the requirements of function and aesthetic but in which there are minor irregularities of individual teeth. Malocclusion is defined by Jones et al (2000) as an occlusion in which there is a mal relation between the arches in any of the planes of space or in which there are anomalies in tooth position beyond the limit of normal. Malocclusion refers to any degree of irregular contact of the teeth of the upper jaw with the teeth of the lower jaw. This would include overbites, under bites and crossbites. There is no specific system of deciding how much misalignment is too much. WHO (1997) including malocclusion under the heading of handicapping dentofacial anomaly, because it can cause problems with the child’s bite, gum tissue, speech development and appearance. As far there is no previous Iraqi study concerning the effect of prenatal nutritional status on the dental arch relation this study was conducted in order to assess the effect of gestational age and birth weight on the occlusion of primary teeth.

**Materials and Method**

After taking permission from Ministry of education, eight kindergartens had been selected randomly from 23 governmental one in AL-Kut city. A cluster sampling is done by carrying out a complete enumeration of each of selected kindergarten. Any children with systemic disease and uncooperative children were excluded. The age of the child in this study was calculated according to the criteria mentioned by World Health Organization (1987) that was according to the last birthday. Prenatal nutritional status was assessed by using anthropometric measurements including Birth weight according to the criteria of Roberton in 1993 that include When child’s birth weight is ≥ 2500 gm is consider as normal birth weight while when child’s birth weight is < 2500 gm is consider as low birth weight; other anthropometric measurement include gestational age that assessed according to Steer (1995) that include description of full term infants are those born between 37-42 weeks of gestation, while post term infants are those born after 42 weeks of gestation however the preterm infants are those of less than 37 weeks gestation. Concerning occlusion of primary teeth primary terminal plane was used in this study; this terminal plane can be classified into one of the three categories:

1) Flush terminal plane (flush terminus): means that the anterior- posterior positions of the distal surfaces of opposing primary second molars are in same vertical plane.

2) Mesial step terminus: is defined as a lower second primary molar terminal plane that is mesial to the maxillary primary terminus.

3) Distal step terminus: is descriptive to the situation in which the mandibular second primary molar terminus is distal to the upper second primary molar terminus.

Statistical analysis: data description, analysis presentation were performed by using Statistical package for social sciences (SPSS). Descriptive analysis: frequency, percentage used for nominal variables. Level of significance: not significant at P>0.05, significant at P≤0.05, and highly significant at P≤0.01.

**Result**

In the present study the total sample involved was 959, whereby 398 children aged 4 years (41.50%) and 561 (58.50%) children aged 5 years. The distribution of the sample according to gender illustrates that the male constitute 49.74% while female constitute 50.26% of the total sample. However in the present study the prevalence of preterm children was 23.46% while the prevalence of low birth weight in the present study was 13.66%.

The distribution of children according to relationship of primary terminal plane by age and gender is shown in Table 1. Data analysis of primary terminal status in this study showed that for the total sample the percentage of children with flush terminus (47.86%) was higher than percentage of mesial step terminus (46.72%) and distal step terminus (5.42%). This figure is true among children aged 5 years as well as among total male. While the opposite picture was found among total female as well as 4 year old children that the highest percentage was mesial step terminus and the lowest percentage was distal step terminus.

Table 2 demonstrates the distribution of children according to relationship of primary terminal plane by gestational age and birth weight. Data analysis shows that among full term children teeth with flush terminus
was the more prevalent, while among preterm the prevalent type was mesial step terminus. Concerning birth weight; children with normal birth weight had the higher percentage of flush terminus, on the other hand teeth with mesial step terminus was the more prevalent among low birth weight children.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The distribution of children according to relationship of primary terminal plane by age and gender.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>Gender</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Both</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Both</td>
</tr>
<tr>
<td>Total</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Both</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The distribution of children according to relationship of primary terminal plane by gestational age and birth weight.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary terminal plane</td>
<td>Gestational age</td>
</tr>
<tr>
<td></td>
<td>Full term</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Mesial step</td>
<td>343</td>
</tr>
<tr>
<td>Distal step</td>
<td>36</td>
</tr>
<tr>
<td>Flush terminus</td>
<td>355</td>
</tr>
</tbody>
</table>

**Discussion**

There are no previous available Iraqi studies dealing with prenatal nutritional measurement (gestational age, birth weight) in relation to oral health conditions that can be used to compare the results of this study with. However in the present study the prevalence of preterm children was 23.46%, this percentage was higher than that found by Offenbacher (1999) in Turkey 17 and Blanc (2005) in United States 18. On the other hand the prevalence of low birth weight in the present study was 13.66% that was lower than that found by Allen.
These differences may attributed to the difference in the whole environments of the pregnant women that include nutritional status of the pregnant women, their general health, their social class, ethnic group, and their oral health 20, 21, 22, 23.

There was no available Iraqi study concerning occlusion of primary teeth in preschool children to compare our result with. However the percentage of flush terminus plane was the more prevalent type for the total sample, the flush terminus plane indicate or predict the normal occlusion later on in permanent teeth 16, in addition the most prevalent type of primary terminal plane among preterm and low birth weight was mesial step in the present study, while among full term and normal birth weight was the flush terminus. This may attributed to the fact that poor nutrition in pre and post natal period highly affect the development of mandible and position of teeth 24.

**Conclusion**

Flush terminus was the most prevalent type of occlusion (47.86%). Among preterm and low birth weight children the mesial step terminus was the most prevalent type of occlusion.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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The Association between Mn-SOD Gene Polymorphism and Peripheral Neuropathy in Type2 Diabetic Patients of Babylon Province-Iraq

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Abstract

Background: Oxidative stress has been known to be implicated in the onset and development of impaired insulin secretion and insulin resistance and both are involved in diabetes. The mechanisms involved in oxidative stress-induced diabetic peripheral neuropathy include the generation of reactive oxygen species ROS, excesses reactive nitrogen species RNS, lipid peroxidation, DNA damage, and reduction in cellular antioxidants. Polymorphisms in genes responsible for encoding these antioxidant enzymes causes the development of diabetic peripheral neuropathy (DPN). Aim: this study was aimed to indicated the role of genes encoding manganese (Mn-SOD) superoxide dismutase in the pathogenesis of DPN in a type2 diabetic patients of Babylon province. Ala(-9)Val polymorphism of Mn-SOD gene polymorphism were studied in type2 diabetic patients with (n=30) and without DPN (n=30).

Results: Polymerase chain reaction (PCR) technique were used for detection Mn-SOD polymorphisms. This technique included the use of PCR primers (Forward and Reverse) to produce a restriction site in the amplified Mn-SOD gene product just with the polymorphic base. Then, the product of (PCR) was digested with Bsh T1 restriction enzyme to detect Ala(-9) polymorphic position. The results of Ala(-9)Val polymorphism showed that the frequency of Ala/Ala, Ala/Val, and Val/Val were 63.3%, 20%, and 13.3% in healthy control subject and 36.6%, 33.3%, and 30% in diabetic without neuropathy countered by 23.3%, 20%, and 56.6% in diabetic with neuropathy. This proposed that the Ala(-9)Val polymorphism in the Mn-SOD gene is significantly associated with a risk for progression of diabetic peripheral neuropathy.

Conclusions: Homozygote pattern Ala/Ala were more frequent in control groups compared with homozygote pattern Val/Val were significantly more frequent in diabetic peripheral neuropathy patients.

Keywords: Oxidative stress, diabetic neuropathy, SOD, Mn-SOD polymorphism.

Introduction

The exposure to high levels of circulating glucose and fatty acids especially in non-insulin sensitive tissues like eye, kidney, and nervous system results in oxidative and nitrosative stress(1) leading to imbalance between oxidants and antioxidants in favour of the oxidants and consequently diabetic complications(2).

Oxidative stress has been known to be implicated in the onset and development of impaired insulin secretion and insulin resistance and both are involved in diabetes(3). The mechanisms involved in oxidative stress-induced diabetic peripheral neuropathy include the generation of reactive oxygen species ROS, excesses reactive nitrogen species RNS, lipid peroxidation(4), DNA damage, and reduction in cellular antioxidants triggering the disruption of lipids of the myelinated structure of nerves leading to loss of axons and damaging the microvasculature of peripheral nervous system(5) causes hyperexcitability in the afferent nociceptors and central neurons resulting in generation of spontaneous impulses within the axons and dorsal root ganglions of the nerves forming the pain that are associated with diabetic peripheral neuropathy(6).

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SOD catalyze the diversion of superoxide anion into hydrogen peroxide and oxygen:

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

SOD activity was primarily reported by McCord and Fridovich in 1969\(^7\) they consequently demonstrated that SOD is the key antioxidant enzyme implicated in the detoxication of superoxide radicals and it plays an essential protective roles against cellular and histological disruption that are causes by ROS\(^8\) and also has an important role in inhibiting inflammatory response which is closely correlated with minimizing hyperalgesia, several forms of SOD have been found in all mammalian tissues with different locations within the cell, these are metalloproteins each containing a distinct metal ion in its center: intracellular Cu-Zn-SOD or SOD 1, Mn-SOD or SOD 2, and extracellular EC-SOD or SOD 3, and each of which is produced by a special gene\(^9\).

Mitochondrial SOD (Mn-SOD) is present in the mitochondrial matrix in two separating forms, dimeric Mn-SOD and tetrameric Mn-SOD and each has a subunit contains one Mn (III) ion\(^1\). Mn-SOD is generated in a constitutive manner, but can also be triggered by cytokines such as IL-1 and TNF, endotoxin, and by numerous oxygen metabolites in particular cell types having important role in occurrence of tissue damage in the case of oxidative stress also has been believed that transcriptional regulation of Mn-SOD is mediated by the activation of nuclear transcription factor \(\text{NF-kB}\) propped by oxidants\(^1\) ).

Mn-SOD or SOD2 gene is the only known antioxidant enzyme present in matrix of mitochondria suggesting that is the first line of defense against free radicals production, and it located on chromosome 6q25.3 consisting of five exons interrupted by four introns and the promoter which control the gene expression, structural and/or functional SNP of the Mn-SOD encoding gene, and have an important role in maintenance of cellular ROS levels\(^1\)

In human there are at least 190 SNPs have been reported, the best known functional SNP that is associated with diabetic peripheral neuropathy is Ala-9Val SNP is a Restriction Fragment length Polymorphism (RFLP) with chromosomal position in the codon 56 (2 exon at position 9) of Mn-SOD gene, the substitution of C to T (GCT to GTT) that is alanine to Valine results in structural changes of the mitochondrial targeting sequence of the enzyme leading to less efficient transport of Mn-SOD into the mitochondrial matrix and can compromise the capacity to neutralize superoxide radicals in the cell\(^1\)

Materials and Method

Study subjects

The study samples were collected in Marjan teaching hospital in AL-Hilla City/Babylon province-Iraq. Subjects in this study comprised from (30) patients suffer from type 2 diabetes with peripheral neuropathy, (30) patients without peripheral neuropathy as a positive control group with duration of disease (1-5, >5-10, >10 years) and with average age between (35-65 year). In contrast, the study included (30) apparently healthy people aged between (35-65) as control matched with disease group. The presence of type 2 diabetic peripheral neuropathy or not were diagnosed for all patients by a specialized doctor.

Venous blood samples were collected from fasting patients and control subjects after a period of fasting 8-10 hours by vein puncture using 5ml disposable syringes, 2 ml was placed into EDTA tubes mixed gently for 3 minutes and then being divided into two parts: the first part used in hematological tests and especially for HbA\(_1c\) assay and the second part was stored in – 20 ºC for using later for genetic analysis.

Table (1): Clinical features of the study groups.

<table>
<thead>
<tr>
<th>Group Indicates</th>
<th>Healthy Control group (n = 30)</th>
<th>Type2 patients without DPN group (n = 30)</th>
<th>Type2 patients with DPN group (n = 30)</th>
<th>(P) value of group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (Years)</td>
<td>(\ldots\ldots)</td>
<td>(7.76 \pm 1.95)</td>
<td>(12.33 \pm 7.64)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>(49.06 \pm 10.06)</td>
<td>(50.76 \pm 9.64)</td>
<td>(51.73 \pm 8.04)</td>
<td>0.53</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>(27.58 \pm 2.22)</td>
<td>(27.48 \pm 0.69)</td>
<td>(28.44 \pm 2.57)</td>
<td>0.99</td>
</tr>
<tr>
<td>HbA(_1c)</td>
<td>(9.92\pm0.96)</td>
<td>(8.45\pm0.03)</td>
<td>(4.89\pm0.26)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

(Mean ± SD): Mean ± Standard Deviation; \(n\): number of samples; *Significant at \(P \leq 0.01\)
DNA extraction and genotyping of Mn-SOD

The manufacturer protocol (Geneaid/Korea) was followed for extraction the DNA from frozen blood samples by using some components of the extraction kit. The purity of extracted DNA were determined by using Nano-droop apparatus. Sequences of primers used for PCR amplification of Mn-SOD include the:

Forward strands F: ‘5-CCAGCAGGCGAGCTGGCCG-3’ and

Revers strands R’5-TCCAGGGCGCCGTAGCGT-3’, the band size of about (91 bp).

Polymorphic sites were amplified by using polymerase chain reaction technique (PCR). The PCR reaction mixture considered of < 250ng/μl template DNA, 400μM of each dNTP, 12.5 μl buffer of 1U Go Taq DNA polymerase (Promega), 10 μM of each primer and 3 mM MgCl$_2$ in 25 μl of total reaction volume.

Amplification reactions were carried out by using GTC Series thermocycler (Cleaver Scientific/UK) apparatus. The following program was set in the thermocycler after determination of the optimum annealing temperature to amplify Mn-SOD gene (55°C for 30 seconds + 37 cycle). PCR-RFLP technique included the addition of restriction enzyme BshTI of about (0.5 μl to 10 μl PCR product), incubated at 37°C overnight.

Statistical analysis

SPSS version 23 was used for analysis the clinical variables whereas, Chi-square test used for comparison genetic frequencies of Mn-SOD between patients and control group. Odds ratios (ORs) with confidence intervals (95% CL) and their associated P-Values were used to calculate the results. A P-Value of ≤ 0.05 considered statistically significance.

Results

Mn-SOD genotyping

The results of Mn-SOD gene genotyping show that the PCR product had one band about (91bp) for both type2 diabetic patients and control group as shown in figure (1).

![Figure 1: The electrophoresis pattern of PCR product for Mn-SOD gene, 1% agarose, 75V, 20mA for 1h.](image)

RFLP-PCR for Mn-SOD gene

The results of PCR-RFLP of Mn-SOD for type2 patients with and without DPN and control group by using Bsh TI restriction enzyme show that the homozygous AA pattern has one band about (91bp), the homozygous VV pattern has two bands about (17 and 74 bp) and the heterozygous AV patterns has three bands (17, 74, and 91 bp) as shown in figure (2).

![Figure 2: Electrophoresis pattern of RFLP-PCR for PCR product (91bp) with restriction enzyme Bsh TI, 3% agarose, 75V, 20mA for 2h.](image)
The genotype and allele frequency of Mn-SOD gene polymorphism in type2 patients with and without DPN and control subjects

The homozygote pattern (AA) were more frequent in control group (63.3%) than type2 patients with DPN (23.3%) with odd ratio (0.1762), while the homozygote (VV) were more frequent in type2 patients with DPN (56.6%) than control group (13.3%) with odd ratio (8.5000), as shown in table (2).

Table (2): The genotype distribution of Mn-SOD gene polymorphism in type2 patients with and control subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype Frequency %</th>
<th>Type2 diabetic patients with DPN</th>
<th>ODD Ratio</th>
<th>CI 95%</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>63.3</td>
<td>23.3</td>
<td>0.1762</td>
<td>0.0572-0.5431</td>
<td>0.002**</td>
</tr>
<tr>
<td>AV</td>
<td>20</td>
<td>20</td>
<td>0.8214</td>
<td>0.2398-2.8140</td>
<td>0.75</td>
</tr>
<tr>
<td>VV</td>
<td>13.3</td>
<td>56.6</td>
<td>8.5000</td>
<td>2.3714-30.466</td>
<td>0.001**</td>
</tr>
<tr>
<td>Allele Frequency%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A(0.63)</td>
<td>A (0.23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V(0.37)</td>
<td>V (0.77)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**P≤0.01,   DPN: Diabetic Peripheral Neuropathy

On the other hand, the homozygote pattern (AA) were more frequent in type2 patients without DPN (36.6%) than type2 patients with DPN (23.3%) with odd ratio (0.52), whereas the homozygote pattern (VV) were more frequent in type2 patients with DPN (56.6%) than type2 patients without DPN (30%) with odd ratio (3.05) as shown in table (3).

Table (3): The genotype distribution of Mn-SOD gene polymorphism in type2 patients with and without DPN.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype Frequency %</th>
<th>Type2 diabetic patients without DPN</th>
<th>Type2 diabetic patients with DPN</th>
<th>ODD Ratio</th>
<th>CI 95%</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>36.6</td>
<td>23.3</td>
<td>0.52</td>
<td>0.1705-1.6204</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>AV</td>
<td>33.3</td>
<td>20</td>
<td>0.50</td>
<td>0.1547-1.6163</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>VV</td>
<td>30</td>
<td>56.6</td>
<td>3.05</td>
<td>1.0533-8.8390</td>
<td>0.03*</td>
<td></td>
</tr>
<tr>
<td>Allele Frequency%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (0.36)</td>
<td>A (0.23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V (0.64)</td>
<td>V (0.77)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P≤0.05,   DPN: Diabetic Peripheral Neuropathy

Discussion

Antioxidant enzymes represents one of the crucial cellular protective mechanisms against oxidative stress in the human body so that the polymorphism of the antioxidant genes can lead to change the enzyme activity\(^{(14)}\). Mn-SOD is a mitochondrial enzyme which is responsible for the formation of \(\text{H}_2\text{O}_2\) from...
superoxide radicals, the variant allele of Mn-SOD has been associated with increased oxidative stress which is induced by diabetes mellitus leading to the development of diabetic neuropathy(15). Oxidative stress can trigger the damage of neurons by nerve lipid peroxidation, the impairment of mitochondrial DNA, the respiratory chain inhibition, and the cross-linking of the neurofilament protein(16). The oxidative disorders also cause rapid changes in glia cells, suffering, severe pain, disability, cardiac death and silent myocardial ischemia, all these disorders are some of the most important consequences of diabetic peripheral neuropathy(17).

Shimoda-Matsubayashi et al.(18) indicated that the (Ala) allele has an alpha-helical structure which represents a common conformation for mitochondrial leader signals, while the (Val) allele may alter its conformation from alpha-helix to beta-sheet starting from the position (16) because of amino acid substitution, this meaning that the (Val) allele is lesser transported into the mitochondria than the (Ala) allele of the enzyme. In the inner mitochondrial membrane, the poor recognition of signal sequence by membrane receptor may results in mistargeting and impairment splitting of a particular sequences leading to reduction of enzyme activity of an imported protein like Mn-SOD within the mitochondrial compartment. Processing studies have been suggested that the basal level of the Mn-SOD activity may be most increased for (AA), followed by (AV), and then (VV)(19). The (AV) dimorphic site is located in Mn-SOD gene within exon 2(20), found that Knock-out mice lacking exon (1) and (2) display a progressive motor disorders because of neuronal degeneration.

In mitochondria, the (V) allele of the Mn-SOD may be present in a lower concentration so that the homozygous (VV) should have lower resistance to oxidative stress than patients with other Mn-SOD alleles which is a common feature of diabetes mellitus with different aging and neurological impairment, the ineffective targeting of Mn-SOD may drop out the mitochondria with low defended against superoxide radicals results in protein oxidation, mutations of mitochondrial DNA and damage which are common in the development and progression of diabetic neuropathy, and neurodegenerative disorders like Alzheimer and parkinson’s disease(21).

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding**: Self-funding

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Lemon Crude Extract Modulates Ito Cells Activation in High Cholesterol Diet-Induced Liver Steatosis in Male Mice

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¹Faculty of Dentistry/ University of Babylon, Iraq

Abstract

Background: Different types of polyphenols exist in many sources such as fruits, vegetables and herbs. For example, lemon has mainly flavonoids (such as Hesperidine, Eryoketrine, Narignine, Hesperidine, routine and chlorogenic acid). Research conducted in vivo and in vitro showed that lemons have different health benefits, such as anti-cancer effect, antimicrobial, antihyperlidemia and protective effect against liver disease. In addition, lemons are used to treat liver disorders. Therefore, current research was aimed to investigate the protective effect of the prolonged use of Lemon Crude Extract.

Methods: Sixty male albino mice (4 weeks old, weighing between 20-25g) were used. They were divided into 3 groups (n= 20) for each group during the period from January 2018 to May 2018. Group I was used as control, Group II induction group was fed with (HCD), Group III was fed a (HCD) and treated with 1:1m/v of 100% (LCE) for 12 weeks.

Results: Histological variations were identified in Group II by disturbed hepatic architecture, congestion in blood sinusoids and portal veins and infiltration of lymphocytes. Also, there was peri portal steatosis that was observed in HCD-fed mice. In addition, there was significant increase in Ito cells that was identified by desmin antibody. Moreover, these variations were less noticeable in Group III.

Conclusion: Lemon has a protective effect against activation of Ito cells in fatty liver.

Key words: Steatosis, LCE, Ito cells, HFD, fatty liver.

Introduction

Modern lifestyle has had an impact on most food habits in humans leading to eating high-calorie food filled with high carbohydrate and fat, which are known as (fast food). This leads to increased risk of increasing weight, obesity and chronic non-transmissible diseases, such as liver disease (1). Several studies dealt with the importance of active compounds that constitute foods which have maintaining and prevention of various diseases. For example, polyphenols have an effective role in the healing and treatment of many diseases (2). They contain an enzyme capable of modifying the immune function (3). Different types of polyphenols exist in many sources such as fruits, vegetables and herbs. For example, lemon has mainly flavonoids (such as Hesperidine, Eryoketrine, Narignine, Hesperidine, routine, and chlorogenic acid) (4). Research conducted in vivo and in vitro showed that lemons have different health benefits, such as anti-cancer effect, antimicrobial, antihyperlidemia and protective effect against liver disease (5). In addition, lemons are used to treat liver disorders (6).

Ito cells, also called hepatic stellate cells, fat-storing cells, lipocytes, peri-sinusoidal cells, vitamin A-storing cells, are located in the space of Disse between hepatocytes and sinusoidal endothelial cells (7). These cells constitute about 5% of the total number of liver cells, they help in reducing the turnover of parenchymal cells and normalize liver regeneration (8). Ito cells are transformed to myofibroblasts which cause fibrosis then cirrhosis. Myofibroblasts secrete a large amount of extracellular matrix proteins like collagens type I and III, proteoglycan, glycoproteins cytokines and chemokines (9). They stimulate hepatic fibrogenesis, together with fibroblasts and parenchymal cells, into mesenchymal cells (10).

Materials and Method

Lemon Crude extract

Fresh mature (lemon citrus) fruits are used. The taxonomic identity of the plant was made by the Biology Department, Faculty of Science/ University of Babylon. Extract preparation was modified from (11). The fruits
with their peel about (500g washed well with deionized water then cut to small pieces, after that their seeds were removed then blended and squeezing using a commercial blender and was filtered to remove the residues by filter with Whitman filter paper (2l).

**Preparation of high fatty diet**

Fatty diet was prepared by adding 1% cholesterol to the standard diet. Cholesterol was purchased from Sigma Company. The high-fat diet was prepared every 2 days, kept at 4°C until used and left at the room temperature for 1h before use (12).

**Histological and Immunohistochemistry**

**Light microscopic observations**

The livers were collected then as soon as fixed with 10% buffered formalin and embedded in paraffin. Sections (5μm) were prepared and then stained with hematoxylin-eosin dye for photo microscopic observations.

**Immunohistochemical Technique**

The following markers were used in this study (Dako Cytomation Denmark):

- Monoclonal Mouse Anti-Human Desmin, Clone: D33, Code Number: M 0760. After DE waxing, dehydration, washing (with distilled water) and then pre-treatment of tissues with heat-induced epitope retrieval in Micro Wave Oven was done prior to staining (using Labeled Streptavidin Biotin LSAB™+/HRP kit, code number K0697 detection system). The staining procedure followed dakocytomation technique (13).

**Results**

**Histological findings**

The liver sections obtained from Group I showed hepatocytes with one cell thickness arranged in cords and can identify the central vein at its center and Ito cells lined the space of Disse (Figure 1). The liver sections obtained from Group II showed many variations in the form of narrowing and congestion in sinusoids, most hepatocytes showed cytoplasmic vacuolation appeared with ballooned hepatocytes and cellular infiltration in addition to multiple small microvesicular steatosis (Figure 2). The liver sections obtained from Group III showed that most of the hepatocytes showed renewal of their cytoplasm (Figure 3).

**Immunohistochemical findings**

Immunohistochemistry was used to demonstrate the presence of Ito cells. Examination of anti-desmin immunohistochemical-stained sections of both Group I and group III revealed anti-desmin positive cells in between hepatocytes (Figures 1 and 3). Examination of anti-desmin immunohistochemical-stained sections obtained from group II revealed an apparent increase in number of anti-desmin-positive cells (Figure 2).

**Discussion**

Lemon is one of the main citrus, it is extensively cultivated in the middle area. It has antimicrobial, anti-parasitic, antiviral and anticancer effects. It was proved that it lowers blood sugar, inhibits low-density lipoprotein oxidation (14,15). The current study dealt with the protective role of Lemon Crude Extract (LCE) against fatty liver induced in male albino mice. In this study, Livers obtained from group II showed disturbed hepatic architecture which was explained as increased oxidative damage in hepatocellular proteins or necrotic changes in hepatocytes that lead to abnormality in the orientation of the hepatocyte plates and disturbing hepatic architecture, dilatation of central veins, blood sinusoids and portal veins were attributed to inflammatory changes or ischemia and hypoxia following high-fat diet (15). In addition, cellular infiltration was observed, this result is considered diagnostic of steatohepatitis. The adipocytes in steatosis secrete many immune modulator elements in the form of pro-inflammatory cytokines (IL-6, TNF-α and ROS). All these factors contribute to chronic inflammatory condition and to hepatocytes injury (16,17).

Microvesicular steatosis is an abnormality in metabolism, synthesis and export of lipids which is associated with defective beta-oxidation of fatty acids (18). Furthermore, cytoplasmic vacuolation was attributed to lipid peroxidation because of oxidative stress that damages cell membrane as well as membranes of cell organelles leading to an increase in their permeability and disturbance of the ions concentrations in the cytoplasm and cellular organelles (19).

The present study showed an increase in the numbers of Ito cells. These findings may be attributed to fibrosis and altered phenotype called capillarization that lose the ability to prevent Ito cells activation and inactivate activated hepatic stellate cells (20). In the present work, we observed that daily administration of LCE with HCD diet ameliorates previous changes. A previous
study revealed that daily administration of lemon juice has hepato-protective role against steatosis in alcohol-induced liver injury in Mice\(^{(21)}\). Lemon contains many compositions including phenolic compounds, vitamins, carotenoids, essential oils, minerals and dietary fiber\(^{(22)}\). The hepato-protective effect of lemon may be attributable to preventing oxidative damage including lipid peroxidation\(^{(23)}\). Some studies concluded that vitamin C alone could reduce oxidative stress induced by ethanol and the hepato-protective effect of vitamin C treatment was more effective than silymarin, quercetin and thiamine\(^{(24, 25)}\). Flavonoids interact with hydroxyl radicals, and then inhibit oxidases\(^{(26)}\). In a previous study\(^{(27)}\), lemon Flavonoids were shown to possess hepato-protective effects on liver damage induced by carbon tetrachloride, the mechanism of the protective effect was related to the antioxidant activity\(^{(27)}\).

Figure 1 Liver sections for control group showed central vein (C.V), hepatocytes (H) and Ito cells (I) (H&E A 200X).

Figure 2 Liver sections for GII showing cellular infiltration (CI), Ito cells (I) ballooning degeneration (B) with microvesicular steatosis (MI) (H&E 200X).

Figure 3 Liver sections for GIII showing normal structure of liver (H&E 200X).
**Conclusion**

Lemon has a protective effect against activation of Ito cells in fatty liver.

**Ethical Clearance**: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

**Funding**: Self-funding.

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Assessment of Serum Advanced Glycation End-Product Level and Its Effect on Periodontal Health Status in Type 2 Diabetic Patients with Chronic Periodontitis

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Abstract

Background: The bidirectional relationship between diabetes mellitus and periodontitis was obvious as both of them are considered chronic diseases. The risk of developing periodontitis was reported to be higher in diabetic patients specially with poorly control diabetes mellitus, which in turn can negatively impact glycemic control. Advanced glycation end-products have intertwined relationship with oxidative product; increased in advanced glycation end-products could lead to oxidative stress and vice versa. The aim of current study was to investigate the possibility of using serum levels of (AGEs) for identification of the periodontal pathological condition in periodontitis patients with and without diabetes. Method: Twenty healthy individuals (control group), 30 patients with severe chronic periodontitis and 30 poorly controlled diabetic patients with severe chronic periodontitis were included. Full mouth (plaque index PlI, gingival index GI, bleeding on probing BOP, probing pocket depth PPD, clinical attachment loss CAL) were recorded by periodontal probing and serum advanced glycation end-products concentration were assayed using enzyme linked immunosorbent assays. Results: A non-significant difference was reported in plaque index PlI, gingival index GI, bleeding on probing between diabetic and non-diabetic patients with chronic periodontitis, while probing pocket depth, clinical attachment loss revealed a significant difference between diabetic and non-diabetic patients. Advanced glycation end-products presented with highest level in diabetic group (26.92) followed by chronic periodontitis group (15.91) then the control group (6.60), however, the correlation was non-significant with periodontal parameters. Conclusions: It is possible to use serum advanced glycation end-products level in the early diagnosis of chronic periodontitis in patients with and without diabetes.

Keywords: chronic periodontitis, advanced glycation end-products, plaque index, gingival index, bleeding on probing.

Introduction

Diabetes mellitus has a series of metabolic disorders recognized by disorders in insulin action, secretion or both causing hyperglycemic state (¹), diabetes complications occurs with long periods of poor glycemic control (²).

Periodontal diseases are inflammatory diseases caused by infection of the supporting tissues with bacteria (³). In spite of the essential role of bacteria in dental plaque, alone it’s not enough for the initiation or advancement of periodontium breakdown. So, host response activation causes irreversible tissue destruction, then inflammation and disease resulted from interaction of these microbiota with immune defenses (⁴).

The gingivitis and periodontitis considered as the most popular forms of periodontal diseases. Gingivitis, a stable form, it is considered a reversible inflammation of the gingiva with no involvement of the attachment apparatus, while periodontitis includes the deeper structure of periodontium leading to loss of attachment with the destruction of periodontal structure (⁵).

Chronic periodontitis (CP) is considered as the most popular type of periodontitis it mostly affects adults between 40 - 50 years old and it is reported as the essential cause of tooth loss. It progresses slowly, but may be subjected to periods of exacerbation (⁶).

The relationship between diabetes mellitus and periodontitis was reported as a bidirectional cyclical relationship (⁷). Moreover, in several studies the presence
of diabetes was associated with higher incidence, prevalence and severity of chronic periodontitis (4,8). Thus, CP was counted as the sixth complication of diabetes (7), and diabetes considered as a risk factor for boosting periodontal disease (9). The risk of developing periodontitis was reported to be three-fold higher in people with poorly-controlled diabetes, which in turn can negatively impact glycemic control (10).

Advanced glycation end products (AGEs) are proteins or lipids that turn into glycated following exposure to sugars. AGEs represent a heterogeneous complex that is produced continuously under physiologic conditions and their production is greatly increased in case of atherosclerosis, hyperglycemia as well as inflammation and oxidative stress (11). A study conducted in 2018 confirmed the results of previous studies about the intertwined relationship between AGEs and oxidative product (OP) (12). Furthermore, recent studies confirmed the past evident on the involvement of AGEs in periodontal disease (13) and diabetes complications (14). All of that motivated us to perform current work in order to study the possibility of using serum levels of (AGEs) for identification of the periodontal pathological condition in periodontitis patients with and without diabetes.

Materials and Method

Study participants

Human samples consisted of eighty males; age range was (35-55) years. Information taken from each participant about the name, age, full dental and medical history, if he was taken any medications, smoking or drinking alcohol, level of HbA1c for diabetic patients and the diabetic period.

The study was approved by the ethical committee of college of dentistry/ university of Baghdad. Each subject was informed about the aims of the investigation according to the informed consent written in simple English and Arabic words, and they were free to choose whether to participate in this study or not. Participants were categorized into three groups: A. Poorly-controlled type 2 diabetes mellitus with sever Chronic periodontitis (CP+pT2DM); composed of 30 males with mean clinical attachment loss ≥ 5mm, HbA1c >9% and on oral hypoglycemic medication. B. Sever chronic periodontitis (CP) without any systemic disease; composed of 30 males with mean clinical attachment loss ≥ 5mm. C. Systemically healthy with clinically healthy periodontium (control group); composed of 20 males seemingly healthy without any systemic diseases with apparently healthy periodontium.

Inclusion and exclusion criteria

The inclusion criteria were 1. patients with generalized severe chronic periodontitis CP must have CAL ≥3mm at >30 % of the sites with mean of CAL ≥5mm (15), 2. At least 16 teeth present, 3. Patients with T2DM for ≥5 years and on oral treatment only, 4. All participants within normal range of body mass index (18.5-24.9kg/m²). The exclusion criteria were: 1. Females were excluded from the study, 2. Presence of systemic disorders other than T2DM, 3. Patients who were receiving periodontal treatment within three months before the study, 4. Patients with T1DM and T2DM receiving insulin as a treatment, 5. Having a course of antibiotic in three months prior to the study, 6. Medications that may affect periodontal tissue and 7. Drinking alcohol or smoking.

Clinical periodontal parameters

All participants were subjected to full mouth periodontal examination excluding 3rd molar by Michigan O periodontal probe for (plaque index PlI, gingival index GI, bleeding on probing BOP, probing pocket depth PPD, clinical attachment loss CAL) on four surfaces (mesial, buccal/ labial, distal and lingual/ palatal).

HbA1c measurement

A volume of 5ml venous blood was gathered from each individual, for diabetic group 1ml placed into Ethylene diamine tetra acetic acid (EDTA) tube in order to measuring HbA1c and 4ml gathered into a serum separating tube (gel tube) and then centrifuged at 3000rpm for 15 minutes. Serum was divided into Eppendorf tubes then labelled and stored at -50°C till used for analysis.

The measurement of HbA1c for patients with T2DM was done by using Standard A1c Care Test kit analyzer. The procedure was executed according to manufacturer’s instructions.

Biochemical analysis of advanced glycation end products (AGEs)

Assessment of AGEs level was performed using ELISA kit (96 wells) for quantitative measurements
of AGEs in serum (CUSABIO AGEs ELISA kit Catalog Number.CSB-E09412h, china) according to manufacturer’s instructions.

**Statistical Analysis**

Data were processed and analyzed using the Statistics Package for Social Sciences (SPSS; version 22) with both descriptive and inferential statistics. Means were used to express all values. LSD test and t-test were used in data analysis to compare mean values among the groups. Correlation between AGEs and clinical periodontal parameters was tested by Person’s Correlation Coefficients (r) test. Moreover, in the statistical evaluation, differences were considered significant when probability value \( P<0.05 \) and \( P>0.01 \).

**Results**

Thirty (CP+pT2DM) patients, thirty CP patients and twenty healthy control individuals were studied. PLI and GI of control group revealed significant differences from (CP+pT2M) and CP groups (Table 1). Moreover, BOP presented non-significant difference, while CAL and PPD showed significant difference from (CP+pT2M) and CP groups (Table2).

**Table 1 Intergroup comparisons of the mean values of PLI and GI parameters between all pairs of study and control groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLI</td>
<td>CP+pT2DM</td>
<td>Control</td>
<td>1.99100*</td>
<td>0.06711</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>CP+pT2DM</td>
<td>CP</td>
<td>0.03433</td>
<td>0.06002</td>
<td>0.569</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>Control</td>
<td>1.95667*</td>
<td>0.06711</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

**GI**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP+pT2M</td>
<td>Control</td>
<td>CP+pT2M</td>
<td>1.94100*</td>
<td>0.03893</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>Control</td>
<td>0.01367</td>
<td>0.03482</td>
<td>0.696</td>
</tr>
</tbody>
</table>

**Table 2**: Intergroup comparisons of mean percentages of score 1BOP, PPB and CAL parameters between study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>No.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOP</td>
<td>CP</td>
<td>30</td>
<td>70.40</td>
<td>9.27</td>
<td>-0.827</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>CP+pT2DM</td>
<td>30</td>
<td>73.01</td>
<td>14.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPD</td>
<td>CP</td>
<td>30</td>
<td>5.24</td>
<td>0.64</td>
<td>-2.349</td>
<td>0.022*</td>
</tr>
<tr>
<td></td>
<td>CP+pT2DM</td>
<td>30</td>
<td>5.58</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAL</td>
<td>CP</td>
<td>30</td>
<td>5.14</td>
<td>0.41</td>
<td>-3.428</td>
<td>0.001**</td>
</tr>
<tr>
<td></td>
<td>CP+pT2DM</td>
<td>30</td>
<td>5.57</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at \( P>0.05 \). **: Significant at \( P>0.01 \).
Regarding AGEs, the highest mean value was noticed in diabetic group followed by CP group then the control group (Figure 1). As shown in (Table 3) the AGEs reported significant difference between (CP+pT2M) and CP and control groups as well as between CP and control groups. Furthermore, serum AGEs had a non-significant correlation with all the clinical periodontal parameters (Table 4).

![Figure 1](image_url) mean serum concentrations of AGEs (ng/ml) for study and control groups.

**Table 3**: Intergroup comparisons of mean serum concentrations (ng/ml) of AGEs between all pairs of study and control groups

<table>
<thead>
<tr>
<th>parameter</th>
<th>groups</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGEs</td>
<td>CP+pT2DM</td>
<td>Control</td>
<td>20.319767</td>
<td>4.473531</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>11.002300</td>
<td>4.001248</td>
<td>0.007**</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>Control</td>
<td>9.317467</td>
<td>4.473531</td>
</tr>
</tbody>
</table>

*: Significant at $P > 0.05$. **: Significant at $P > 0.01$.

**Table 4 Correlations between levels of AGEs with the clinical parameters at each study and control groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Statistical analysis</th>
<th>CP+pT2DM</th>
<th>CP</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLI</td>
<td>r</td>
<td>-0.132</td>
<td>-0.202</td>
<td>-0.113-</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.486</td>
<td>0.285</td>
<td>0.635</td>
</tr>
<tr>
<td>GI</td>
<td>r</td>
<td>-0.084</td>
<td>-0.331</td>
<td>-0.214-</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.658</td>
<td>0.074</td>
<td>0.364</td>
</tr>
<tr>
<td>BOP</td>
<td>r</td>
<td>-0.114</td>
<td>-0.219</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.549</td>
<td>0.246</td>
<td></td>
</tr>
<tr>
<td>PPD</td>
<td>r</td>
<td>-0.229</td>
<td>0.192</td>
<td></td>
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<tr>
<td></td>
<td>P-value</td>
<td>0.223</td>
<td>0.308</td>
<td></td>
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<tr>
<td>CAL</td>
<td>r</td>
<td>-0.138</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.466</td>
<td>0.900</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

A significant difference in PI and GI between control group and both of diabetic and non-diabetic groups with CP, while a non-significant difference was reported between (CP+pT2M) and CP groups. This could be attributed to the effect of microbial dental plaque since it is considered as the essential factor in the pathogenesis of periodontal disorders (5,16), and its causes gingival inflammation due to its accumulation on teeth with sign and symptom of inflammation (erythema, edema, bleeding, tenderness and enlargement) (17). Since both groups had been chosen with strict criteria with same degree of severity, this may explain the non-significant difference between study groups, (CP+pT2M) and CP. Moreover, BOP reported a non-significant difference between study groups. This could be attributed to similarity in subgingival microbiota between diabetic and non-diabetic patients (18).

On the other hand, the CAL and PPD revealed significant differences between study groups. This could be attributed to the effect of DM on the production of proinflammatory cytokines, like IL-6, by human gingival fibroblasts which cause an increase in their production when compared to non-diabetic (19). Also, patients with hyperglycemia and periodontal disease reported an increase in the expression of TLRs in periodontal tissues which cause higher inflammatory response in those patients (20). Thus, the incidence, prevalence and severity of periodontitis will be higher in diabetic than non-diabetic individuals (21).

The biochemical analysis of serum AGEs demonstrated the highest level in diabetic group followed by CP group and then control group with significant differences between them. This could be explained by the significant association of AGEs with oxidative stress and inflammation (22). Levels of ROS were reported to be higher in periodontitis patients than healthy controls (23).

On the other hand, poor glycemic control leads to an increase in AGEs accumulation (24,25) and their formation rate was highly increased in case of hyperglycemia and oxidative stress (26). So, in case of diabetes together with condition of chronic high oxidative stress will lead to further acceleration in the process of formation of AGEs (22).

Regarding the correlation between AGEs and clinical periodontal parameters, although the correlation was non-significant, there was an elevated level of this marker in chronic periodontitis groups compared to healthy control group suggesting its association with periodontitis and this could be explained by the association of AGEs with progression of periodontal disease (26).

Accumulation of advanced glycation end-products (AGEs) in oral tissues including periodontal fibroblasts and gingival tissues and its interactions with their receptors (RAGE) reported to be associated with impaired fibroblastic growth in periodontal tissues (25) and causes an increase in formation and release of ROS from cells of periodontal ligament with subsequent induction of metalloproteinase (MMP) and proinflammatory cytokines. All that will lead to degradation of connective tissue, osteoclast activation and bone loss (27).

Conclusions

The increased level of serum AGEs in CP groups suggested their potential role in initiation or progression of periodontitis in patients with and without diabetes. Also, the higher level of AGEs in CP group compared with control group may raise the potential involvement of AGEs in the etiology and progression of periodontal diseases. Furthermore, it is possible to use serum AGEs level in the early diagnosis of CP in patients with and without diabetes.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of interest: The authors declare that they have no conflict of interest.

Funding: Self-funding.

References


Serum Fatty Acid Synthase Level in Patients with Prostate Cancer and Benign Prostatic Hyperplasia

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Abstract

Background: Human Fatty Acid Synthase is highly expressed in many human cancers. Previous studies have shown that this enzyme is expressed at very high levels in prostate cancer and that the growth of prostate cancer cell line can be inhibited by pharmacological inhibitors that target this enzyme. Additionally, some studies have reported that this enzyme is overexpressed not only in tissue, but also in serum of patients with various cancers. The aim of this study was to evaluate serum levels of this enzyme in patients with prostate cancer and in patients with benign prostatic hyperplasia as well as to investigate whether it can be used as a biomarker for detection of prostate cancer and benign prostatic hyperplasia. Methods: By using an FASN ELISA kit, we measured serum levels of Human Fatty Acid Synthase in 35 patients with prostate cancer and 35 patients with benign prostatic hyperplasia. We also measured serum FASN levels of 35 healthy volunteers enlisted as normal controls. Results: Serum FASN levels in prostate cancer patients were significantly higher than in healthy control subjects, but FASN levels in patients with benign prostatic hyperplasia were not significantly higher than in healthy control subjects. Conclusions: Serum FASN levels are expressed at significantly high levels in human prostate cancer. Serum FASN levels were not expressed at significantly high levels in human benign prostatic hyperplasia. FASN serum levels may be additional biomarker for prostate cancer detection.

Keywords: Fatty acid synthase, prostate cancer, benign prostatic hyperplasia, ELISA.

Introduction

Prostate cancer (PC) and benign prostatic hyperplasia (BPH) are common prostate tumors. Prostate cancer is the most widely recognized non-cutaneous malignancy tumor in men [1]. Benign prostatic hyperplasia is a non-cancerous increase in size of prostate gland. It is the most common benign tumor found in men [2].

Although transrectal ultrasound-guided prostate biopsy is gold standard in the diagnosis of prostate cancer, it can be hurtful with possible side effects of biopsies including pain, serious infections and bleeding [3].

Additionally, serum prostate-specific antigen (PSA) levels do not have a direct correlation with increasing grade and stage of prostate cancer [4]. Thus, a novel biomarkers that have a stronger association with prostate cancer and have direct correlation with increasing grade of prostate cancer and have less side effects are needed.

A marker that is considered in this study is human fatty acid synthase (FASN), a metabolic enzyme that catalyzes the biosynthesis of longchain fatty acids [5]. FASN was first identified as oncogenic antigen 519 in patients with a poor prognosis for breast cancer [6]. In normal human tissue de novo fatty acid synthesis is suppressed and the low levels of lipogenic enzymes expression are maintained. Normal cells preferentially depend on dietary lipids to satisfy their metabolic needs. In contrast, increased lipogenesis is a major hallmark for tumor progression with cancer cells change to dependence on de novo fatty acid synthesis to support rapid cell growth [7]. The main enzyme responsible for the synthesis of fatty acids in the cell is fatty acid synthase (FASN) [8]. FASN has been found to be common sense overexpressed in about every type of cancer and is associated with their progression and development [9]. This enzyme is overexpressed not only in tissues, but also increased enzyme concentration in serum of patients with various cancers such as colorectal...
cancer, gastric carcinoma and esophageal neoplasia [10-12]. Serum FASN levels are elevating in patients with prostate cancer compared with healthy controls [13]. In addition, the levels of serum fatty acid synthase are associated with stage of disease in patients with colorectal cancer [14].

The aim of this study was to evaluate serum levels of this enzyme in patients with prostate cancer and in patients with benign prostatic hyperplasia as well as to investigate whether it can be used as a biomarker for detection of prostate cancer and benign prostatic hyperplasia.

Materials and Method

Study subjects

The present study included 35 patients with prostate cancer (15 patients underwent trans urethral resection or prostate biopsy surgery and 20 patients from oncology hospital. In addition, 35 patients with benign prostatic hyperplasia who were diagnosed and had blood samples collected between September 2018 and April 2019. Patients were divided into three groups; control, BPH and PC. Fasting serum samples were collected from patients preoperative or prior to treatment in addition to serum samples from 35 healthy individuals (controls).

FASN ELISA

A total of 100μl serum was analyzed using a commercially available ELISA kit, FASN ELISA (cusabio biotech), according to manufacturer’s recommendations. Using a pipette, a volume of 100μl of standard and sample was added per well. Then covered with the adhesive strip provided, incubated for 2 hours at 37°C, then the liquid of each well was removed by inverting plate and rapidly flicking the liquid away from the plate. Using a pipette, a volume of 100μl of Biotin-antibody (1x) was added to each well and covered with a new adhesive strip. Incubated for 1 hour at 37°C, then each well was aspirated and washed, and the process was repeated two times for a total of three washes. It was washed by filling each well with wash buffer (200μl) using an auto-washer and it was left to stand for 2 minutes. After the last wash, any remaining wash Buffer was removed by decanting. The plate was inverted and blot it against clean paper towels. Then by pipette (100μl) of HRP-avidin (1x) was added to each well. The micro titer plate was covered with a new adhesive strip and incubated for 1 hour at 37°C. The aspiration/wash process was repeated for five times as in step 6, then (90μl) of TMB substrate was added to each well and incubated for 15-30 minutes at 37°C in the dark. The a volume of 50μl of stop solution was added to each well using a pipette, the plate was gently tapped to ensure thorough mixing. The optical density was determined of each well within 5 minutes, using a micro plate reader set to 450nm.

Statistical Analysis

Statistical analysis was carried out using Microsoft excel 2013 and SPSS version 20. The numerical data expressed as mean ±SD. Furthermore, comparisons between mean serum concentrations of FASN in control and study (PC and BPH) groups were performed. All P values were two sided and statistical significance was set at P≤0.05. Receiver Operating Characteristics (ROC) curve was calculated to estimate the sensitivity and specificity of the used FASN as biomarker and discriminatory ability.

Results

In this study, ELIZA method was used for estimation of the concentration of FASN in sera of patients with prostate cancer, benign prostatic hyperplasia and healthy controls (Figure 1). FASN concentration in sera of patients with prostate cancer ranged from (0.87ng/ml) to (24.19ng/ml) with a mean±SD of 5.8±4.9ng/ml, and showed a significant elevation in comparison with control group (P=0.001; Table 1). On the other hand, the mean±SD FASN concentration in sera of benign prostatic hyperplasia patients was (3.3±1.8) and showed no significant elevation in comparison with control group (P= 0.17; Table 2).

Table (1) Comparison between serum FASN concentrations in PC and control groups

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC group</td>
<td>5.8±4.9</td>
<td>2.7±2</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Table (2) Comparison between serum FASN concentrations in BPH and control groups

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH group</td>
<td>3.3 ±1.8</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>2.7±2</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Figure 1 Concentration FASN in PC, BPH and control groups.

The Receiver Operator Characteristic (ROC) curve showed a significant discriminatory ability of increased serum FASN levels for PC (Figure 2).

Figure 2 ROC curve

The cut-off value of the serum FASN concentration in patients with prostate cancer was (3.31ng/ml) with sensitivity of 71%, specificity of 23% and an area under curve of 0.77; Table 3).

Table 3 Sensitivity and Specificity of FASN ROC curve in patients with prostate cancer

<table>
<thead>
<tr>
<th>Cut-off value</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Area under curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.31ng/ml</td>
<td>23%</td>
<td>71%</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Discussion

A novel biomarker that has stronger association with prostate cancer and has direct correlation with increasing grade of prostate cancer is needed. Identification of serum protein markers of prostate cancer and benign prostatic hyperplasia together with other markers already known could help provide such a non-invasive diagnostic and prognostic screening tool. FASN is a major component of the de novo fatty acid synthesis pathway that catalyzes the oxidation NADPH-dependent formation of large free fatty acids from two carbon donors. A strong association was found between increased FASN levels in malignant tissue and the presence of other unfavorable prognostic indicators in primary prostate cancer, as determined by immunohistochemistry methods [15]. Patients with prostate cancer rich in FASN display significantly poorer clinical prognosis than those with prostate tumors containing lesser amounts of FASN enzyme.

In this study, FASN concentrations were determined in sera of patients. The findings indicated that the concentrations of FASN in sera of patients with prostate cancer were higher than in sera of healthy subjects. We have detected high FASN concentration in 71% of participant patients with prostate cancers depending on FASN cut-off value and the mean±SD was (5.8±4.9ng/ml).

This finding was in agreement with study of [13], however, the mean and standard deviation of serum FASN in that study was (0.79±0.76 units/I) which was different from the mean and standard deviation in our study, because the (ng/ml) unit was used in our study to measure sera FASN concentration.

This was the first study that serum concentration of FASN in patients with benign prostatic hyperplasia was determined. The results shown that no significant different between BPH and healthy subjects groups (P=0.17; Table 2). This approved that FASN has a role in development of prostate cancer and indicating that serum FASN concentration could aid as a diagnostic marker for prostate cancer, because most men with an elevated PSA level turns out not to have prostate cancer; just about 25% of men who have a prostate biopsy due to an elevated PSA level actually are found to have prostate cancer when a biopsy is done [16] and novel biomarkers that have stronger association with prostate cancer are needed.
A receiver operatory characteristic (ROC) curve is a graphical plot used to show the diagnostic ability of binary classifiers. It was first used in signal detection theory, but now it is used in many other areas as medicine. ROC curve is instituted by plotting the sensitivity against the (1-Specificity).

Sensitivity is the ratio of observations that were correctly predicted to be positive out of all positive observation. It equals:

\[
\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}}.
\]

In contrast, specificity is the ratio of observations that are correctly predicted to be negative out of all negative observations. (1 – Specificity) equals:

\[
\text{Specificity} = \frac{\text{False positive}}{\text{True negative} + \text{False positive}}.
\]

In this study discriminatory ability of increased serum FASN levels for PC was tested by ROC curve (Figure 2). The area under curve was 77% and this indicated how well serum FASN can distinguish between patients with prostate cancer and healthy subjects.

The sensitivity was 71%. This meant that the positive detection rate was 71%. This is higher than positive detection rate in study of [13] which was 53%. The latter study had only 29 samples with prostate cancer and may be this the cause that made their results vulnerable to bias, additionally the incubation time and temperature of the first incubation ELISA method in that study were overnight at 4°C and this is different from current study where the incubation time was 2 hour at 37°C.

**Conclusion**

This study suggested that serum FASN levels were expressed at significantly high levels in human prostate cancer. Thus, FASN serum level may be an additional biomarker for prostate cancer detection with a sensitivity and specificity of 71% and 23%, respectively. The study concluded that there was no significant different between serum FASN of patients with BPH and healthy subjects. In addition, serum FASN concentration is not useful biomarker for diagnosis of PBH. This approved that FASN has a role in development of prostate cancer. Subsequent studies should investigate how serum FASN levels relate to clinical responses to prostate cancer therapies. Subsequent studies to investigate relationship between transcription factors of FASN and Gleason score. Determine the cut-off value of serum FASN concentration for each class of Gleason score in patients with prostate cancer. Also, studying correlation between serum concentration of FASN with stage of prostate cancer is highly recommended.

**Ethical Clearance:** The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

**Funding:** Self-funding.

**References**


Antibacterial Activity of Crude Extracts of *Spirulina Platensis* Against Some Pathogenic Bacteria and Fungi Isolated from Different Sites on Human Body

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¹Faculty of Basic Education, University of Diyala, Iraq

Abstract

*Spirulina platensis* are filamentous, undifferentiated, non-toxigenic cyanobacteria that have been used as food since ancient times. There have been numerous studies on its antioxidant and antimicrobial actions. *Spirulina* as many other cyanobacteria species have the potential to produce a large number of antimicrobial substances, so they are considered as suitable organisms for exploitation as biocontrol agents of plant pathogenic bacteria and fungi. In the present study, antimicrobial activity of *Spirulina platensis* solvent extracts in serial dilution was investigated against pathogenic bacteria and fungi. The antimicrobial activity of *Spirulina platensis* was determined against pathogenic bacterial and fungal isolates. The methanol extract of *Spirulina platensis* showed maximum zone of inhibition against all the bacterial and fungal isolates.

Keywords: *Spirulina platensis*, Inhibition zone

Introduction

Early interest in *Spirulina* was focused mainly on its potential as a source of protein, vitamins, especially vitamin B12 and provitamin A (β-carotene), and essential fatty acids like γ-linolenic acid (GLA). Recently more attention has been given to study its therapeutic effects, which include reduction of cholesterol and nephrotoxicity by heavy metals, anticancer properties, protection against radiation, and enhancement of the immune system⁽¹⁾. *Spirulina* also possesses other biological functions such as antiviral, antibacterial, antifungal, and antiparasite activities⁽²⁾.

Microalgae, such as *Ochromonas* sp., *Prymnesium parvum*, a number of blue green algae produce toxins that may have potential pharmaceutical application. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, of which many are based on their uses in traditional medicine⁽³⁾. *S. platensis* produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Pathogen resistance to synthetic drugs and antibiotics that are already in use makes search for plants with antimicrobial activity more important, as they can substitute for synthetic antibiotics and drugs⁽⁴⁾.

Characteristics of plants that inhibit microorganisms have been investigated in laboratories since 1926. The past decade has witnessed a significant increase in the prevalence of resistance to antibacterial and antifungal agents. Resistance to antimicrobial agents has important implications for morbidity, mortality and health care costs in U.S. hospitals, as well as in the community. These developments and the associated increase in bacterial infections intensified the search for new, safer, and more efficacious agents to combat serious bacterial infections⁽⁵⁾.

Materials and Method

Preparation of biomass and harvesting

A prepared flask containing 100 ml of Bg-11 culture media and transfer 25 ml of isolated algae then incubated for 14 days; transfer this culture growth to 500 ml of culture media and incubate again for 14 days, then transfer this culture growth to 1000 ml of Finally, the culture of growth is transferred to glass basins of 4L dimensions (50 cm long, 40 cm wide and 30 cm high) for biomass culture⁽⁶⁾. These pools were covered with a piece of gauze and the air was supplied with rubber. With bubble stone⁽⁷⁾.
The 20-day biomass culture was harvested by centrifugation at 4000 rpm for 10 minutes. The samples were washed with sterile water and dried in the oven with 38-40 °C. These samples were then weighed and stored in the refrigerator.

Preparation of organic extract of the spirulina platensis

According to with some modifications, were followed to prepare the crude extracts of algae as follows: One gram of spirulina platensis powder was extracted with 250 ml of 97% ethanol using a Soxhlet extraction device at 76 C for 3-4 hours until the solvent becomes insoluble the color. The raw extract was dried by rotary evaporator at 40 °C.

C. The residues (raw extracts) were collected and stored at -20 °C until use again. The extract was weighed and the percentage of the extraction yield was calculated in terms of the primary algae material used in extraction.

The method of posting agar well:

Antimicrobial and fungal activities from the crude extracts of Spirulina platensis were tested using the Agar method for good propagation. Four different concentrations were prepared (10-20, 30, 40 µg/mL). Nutrient agar plates were fortified with 100 ml in a 24-hour broth culture of tested bacteria or 100 ml of Dextrose Sabouraud’s culture soup 5 days of tested fungus. Four wells (6 mm) were manufactured and filled with 100 ml extract. The dishes were incubated for 24 hours at 37 °C for bacteria or for 3 days at 30 °C for fungus. The diameter of the region was measured to discourage recorded results. In addition, antimicrobial activity was compared with the standard.

Analysis by Gas Chromatography – Mass Spectrometry (GC-MS)

For GC-MS analysis, a high-temperature column (Inert cap 1MS; 30 m × 0.25 mm id × 0.25 µm film thickness) was purchased from Agilent Technologies (SHIMADZU—Japan), by employing a high-temperature column. Derivatization of each sample was eliminated. The injector and detector temperatures were set at 280 °C while the initial column temperature was set at 100 °C. A 5 µL sample volume was injected into the column and ran using split (1:10) mode After 1 min, and the oven temperature was raised to 225 °C at a ramp rate of 12.5 °C/min (hold time 4 min). The oven temperature was then raised to 300 °C at a ramp rate of 7.5 °C/min (hold time 5 min). The helium carrier gas was programmed to maintain a constant flow rate of 17.5 mL/min and the mass spectra were acquired and processed using both Agilent GC-Mass. Solution (SHIMADZU—Japan) and postrun software. The compounds were identified by comparison of their mass with NIST library search and authentic standards.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in Inhibition Zone Diameter. Least Significant Difference-LSD test was used to significant compare between means in this study.

Result

The present study included bioactivity of crude extracts of Spirulina platensis on some pathogenic organisms isolated from different site as presented in the table (1).

The result showed the different sensitivity to the series of extraction on inhibition zone to an microorganism under study, table (2).

The zone of inhibition of Spirulina platensis extracts against bacteria was ranged between (0-13 mm at 10 mg/l and 8mm - 15mm, 8- 13mm and 7mm-15mm at 20mg/l, 40mg/l and 50mg/l respectively.

The zone of inhibition of Spirulina platensis extracts against fungi was ranged between (0-9 mm at 10 mg/l and 7mm - 8mm, 0-7mm and 9mm-13mm at 20mg/l, 40mg/l and 50mg/l respectively.
Table (1): pathogenic isolation site

<table>
<thead>
<tr>
<th>No</th>
<th>Gram Positive Bacteria ( + ve)</th>
<th>Source of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus epidermidis</td>
<td>Skin</td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td>Sputum (Chest infection)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No</th>
<th>Gram Negative Bacteria ( - ve)</th>
<th>Source of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Klebsiellasp</td>
<td>Urine (UTI)</td>
</tr>
<tr>
<td>2</td>
<td>E.Coli</td>
<td>Urine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No</th>
<th>Fungi</th>
<th>Source of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida albicans</td>
<td>skin ulcers</td>
</tr>
<tr>
<td>2</td>
<td>Aspergillusniger</td>
<td>Ear</td>
</tr>
</tbody>
</table>

Table (2) Antimicrobial Activity of crude extracts of Spirulina platensis as presented by inhibition zone diameter (mm)

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Control</th>
<th>10 mg/l</th>
<th>20mg/l</th>
<th>30mg/l</th>
<th>40mg/l</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0</td>
<td>6</td>
<td>10</td>
<td>8</td>
<td>7</td>
<td>2.07 *</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>2.88 *</td>
</tr>
<tr>
<td>St. epidermis</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>11</td>
<td>8</td>
<td>3.19 *</td>
</tr>
<tr>
<td>St aureus</td>
<td>0</td>
<td>13</td>
<td>15</td>
<td>13</td>
<td>15</td>
<td>3.64 *</td>
</tr>
<tr>
<td>c. albicans</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>13</td>
<td>2.72 *</td>
</tr>
<tr>
<td>Asperniger</td>
<td>0</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>9</td>
<td>2.16 *</td>
</tr>
<tr>
<td>LSD value</td>
<td>-</td>
<td>2.69 *</td>
<td>3.44 *</td>
<td>2.89 *</td>
<td>2.96 *</td>
<td>---</td>
</tr>
</tbody>
</table>

* (P<0.05).

The St aureus bacteria show more sensitivity and inhibition zone which reach a significant level (P<0.05) in all dilution were compare with other microorganisms, while Asperniger was less sensitive in compare with other organisms as shown figure (1) as well as the result show a significant differences in any dilution between all pathogenic organisms inhibition zones, furthermore there are found a significant difference between serial dilution against the same pathogen under probability levels (P<0.05).

Discussion

The antibacterial activity of algal compounds extracted from algae depends upon the type of solvent used for extraction.

Spirulina has been studied because of its therapeutic properties and the presence of bioactive compounds\(^{(13)}\). The occurrence of antimicrobial compounds in plants was well documented and these compounds are known to possess antimicrobial activity in biological systems. But, the antioxidant characteristics of algae and cyanobacteria are less well documented, although decreased cholesterol levels have been reported in hypercholesteremic patients fed Spirulina and the antimicrobial activity of phycobiliproteins extracted from Spirulina platensis has also been demonstrated.

Many investigations mentioned that the methanol extracts of Nostoc muscorum revealed antibacterial activity on Sclerotinia sclerotiorum by\(^{(14)}\). Also the methanolic extract of a blue green alga has been investigated by\(^{(15)}\) for in vitro antimicrobial activity against Proteus vulgaris, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus and Rhizopus nigricans using agar cup diffusion method. The antimicrobial activity of methanolic extract of S. platensis was also explained.
by\textsuperscript{(16)} due to the presence of \textit{γ}-Linolenic acid and compound was also present in the methanol extract in the present study as observed by GC-MS analysis. Previous publications reported that the compounds such as 1-Octadecene, 1-Heptadecane were found in both algae and plants show anticancer, antioxidant and antimicrobial activity\textsuperscript{(17,18)}. Antimicrobially active lipids and active fatty acids are present in a high concentration in this alga. It was hypothesized by \textsuperscript{(19)} that lipids kill microorganisms by leading to disruption of the cellular membrane as well as bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. Present investigations is contradictory with the results of other studies\textsuperscript{(20,21)} may be due to the production of bioactive compounds related to the seasons, method, organic solvents used for extraction of bioactive compounds.

### Conclusion

It is concluded from the study that extracts of algal strain used in the present investigation showed better antibacterial activity against the pathogens used, but further researches should be made to identify and purify natural product against antibacterial and antifungal. The enhanced antibacterial activity expressed in sequential extraction might be due to the fact that both hydrophobic and hydrophilic bioactive compounds were extracted. An improved knowledge of the composition, analysis, and properties of \textit{S. platensis} with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application of this cyanobacteria.

### Reference


The Problem of Multidrug Resistance Bacterial Strains in Daily Clinical Practice in Dealing with Typhoid Fever in Mid-Euphrates Region of Iraq: A Cross Sectional Study

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Abstract

Background: in our community, typhoid fever is common infectious disease based on daily clinical practice and the emergence of multidrug resistant strains a evidenced by worldwide reports and our daily clinical observation necessitate optimizing antibiotic approach to treat such common infectious disease.

Aim of the study: we aimed in this study to highlight the problem of multi-drug resistance in daily clinical practice when treating typhoid fever.

Patients and methods: The present cross sectional study included 95 cases with clinical and laboratory evidences of typhoid fever. Venous blood sample was obtained from each patient and sent to the lab for purpose of culture and sensitivity. The study was carried out at the department of Medicine / Al-Diwaniyah teaching hospital, Al-Diwaniyah province, Mid-Euphrates region of Iraq. The study started on January 2018 and extended through October 2018.

Results: Regarding amoxicillin, azithromycin and cefotaxime, isolates obtained from 60 patients (63.2 %) were resistant whereas isolates from 35 patients were sensitive. Ciprofloxacin and ceftriaxone were totally ineffective since resistance was recorded in all enrolled isolates (100.0 %). On the other hand, chloramphenicol, trimethoprim-sulphamethoxazole and imipenem produced the best results since all isolates were sensitive to these antibiotics. The resistant strains of Salmonella enterica serotype typhi were more associated with urban areas than with rural regions, 75 % versus 58.2 %; however, the difference did not reach statistical significance (P > 0.05).

Conclusion: antibiotic approach to typhoid fever is a dynamic rather than a static phenomenon that needs revision regularly and that drug individualization according to culture and sensitivity rule should be adopted by all health workers and institutes when dealing with outbreaks of Salmonella enterica serotype typhi.

Key words: multidrug resistance, typhoid fever, Iraq

Introduction

Typhoid fever, a term first used by Pierre Louis in 1829 to describe an infectious disease which was proved later to be caused by Salmonella enterica serotype typhi (¹). This gram negative rod shaped baceterium has only one reservoir which is the human being (²). The disease is mainly encountered in geographic regions with low socioeconomic status in which poor sanitation is common and it is mainly seen in young adults and children (³). The incidence of typhoid fever in 2000 was estimated to reach 21.7 million cases worldwide with 216,000 cases died of the disease. In 2010 the disease was estimated to affect 11.9 million patients and to kill 129,000 individuals in low to intermediate income regions of the world (⁴, ⁵). Indeed, these figures may not represent the gloomier picture and be an underestimate to the truth as a large number of cases are treated on outpatient basis. The biggest proportion of the 200 to 300 cases diagnosed annually in the United States is attributed to travelling to
endemic areas (6). In Iraq, the reported annual incidence of the disease in Sulaimania was estimated to be 21 cases per 10000 per year (7). Unfortunately, despite thorough search in the available published articles in the internet, we did not get an article dealing with the true incidence of the disease in the Mid-Euphrates region of Iraq during the last 10 years; however, in clinical practice the disease is fairly common. The introduction of antibiotics has dramatically reduced the rate of mortality caused by typhoid fever from approximately 15 % in the pre-antibiotic era to less than 1% following antibiotics administration (8).

Chloramphenicol was the first antibiotic to be indicated to treat Salmonella enterica serotype typhi infections (9). Resistant forms of the bacterium were identified in the population with just 2 years of administrating the drug (8). Nowadays, ofloxacin or ciprofloxacin have been considered as the mainstay of treating Salmonella enterica serotype typhi infections (10). In spite of the risks accompanying quinolone treatment in children, they are justified for severe infection or when other modes of therapy are lacking (11). A wide spectrum cephalosporin (e.g. ceftriaxone) or azithromycin may be indicated. When resistance to a quinolone is discovered. Combination therapy of the macrolides, cephalosporins and fluoroquinolones have been justified in patients when above measures fail to control the disease (5, 8).

The detection of enteric fever becomes difficult in a lot of countries around the world due the development of multidrug resistance (MDR) by Salmonella enterica serotype typhi as well the alteration in the presentation mode of the disease (11). The term MDR was used to describe a condition in which all three first line medications; chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole fail to control the disease (13). Dated back to 1972, the first report of cases resistant to chloramphenicol was registered, following which reports around the world have been recorded about the emergence of Salmonella enterica serotype typhi that are resistance to alternative forms of monotherapy until we reached the era of multi-drug resistance (12, 15). The isolation of the first MDR strain was done in Southeast Asia about 30 years ago following which the bulk of data about MDR has been grown (15). Therefore the use of quinolone or 3rd generation cephalosporins has been justified in the treatment of typhoid fever instead of the traditional approach of chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (16). The prevalence rate of MDR in five Asian countries was estimated to range from 7 up to 65 % (17).

Because of the relatively high incidence rate of typhoid fever in Iraq and the difficult control of the disease following the published guideline, we aimed in this study to highlight the problem of multi-drug resistance in daily clinical practice when treating typhoid fever.

Patients and methods

The present cross sectional study included 95 cases with clinical and laboratory evidences of typhoid fever. The mean age of enrolled patients was 43.14 ±5.21 years with an age range of 11-58 years. Venous blood sample was obtained from each patient and sent to the central laboratory of Al-Diwaniyah teaching hospital for purpose of culture and sensitivity. The study was carried out at the department of Medicine / Al-Diwaniyah teaching hospital, Al-Diwaniyah province, Mid-Euphrates region of Iraq. The study started on January 2018 and extended through October 2018.

The study was approved by the Ethical Approval Committee of College of Medicine / University of Al-Qadisiyah. Statistical analysis was done using statistical package for social sciences (SPSS, version 23) and Microsoft Office Excel 2010. Data were expressed as mean standard deviation, number and percentage. Chi-square test was used to study association between categorical variables. The level of significance was considered at P ≤ 0.05.

Results

In the present study, 95 patients with clinical features suggestive of typhoid fever were enrolled and the diagnosis was confirmed based on blood culture results. The study included 45 male and 50 female patients with an age range of 11 to 58 years and a mean age of 43.14 ±5.21 years, as shown in table 1. According to residency, patients were classified into 67 patients from urban regions and 28 patients from rural areas accounting for 70.5 % and 29.5 %, respectively, table 1. The antibiotic sensitivity results are demonstrated in table 2. Regarding amoxicillin, azithromycin and cefotaxime, isolates obtained from 60 patients (63.2 %) were resistant whereas isolates from 35 patients were sensitive. Ciprofloxacin and ceftriaxone were totally ineffective since resistance was recorded in all enrolled isolates (100.0 %). On the other hand, chloramphenicol,
trimethoprim-sulphamethoxazole and imipenem produced the best results since all isolates were sensitive to these antibiotics, as shown in table 2. The resistant strains of *Salmonella enterica* serotype typhi were more associated with urban areas than with rural regions, 75% versus 58.2%; however, the difference did not reach statistical significance (P > 0.05), table 3. The distribution of patients according to 10 years age intervals is shown in figure 1 together with corresponding proportions of sensitive and resistant strains. It is obvious that resistant strains are more frequent among patients younger than 40 years of age; nonetheless, we were not able to assess the significance level because chi-square was not valid.

**Table 1: Demographic data of the study sample**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>95</td>
</tr>
<tr>
<td>Age range</td>
<td>11 - 58 years</td>
</tr>
<tr>
<td>Mean age ±SD</td>
<td>43.14 ±5.21 years</td>
</tr>
<tr>
<td>Male : Female</td>
<td>45:50:00</td>
</tr>
<tr>
<td>Residency</td>
<td>Urban, n (%)</td>
</tr>
<tr>
<td></td>
<td>Rural, n (%)</td>
</tr>
</tbody>
</table>

**Table 2: Results of blood culture and sensitivity**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>35</td>
<td>36.8</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>35</td>
<td>36.8</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>35</td>
<td>36.8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>95</td>
<td>100.0</td>
</tr>
<tr>
<td>Trimethoprim-</td>
<td>Sulphamethoxazole</td>
<td>95</td>
</tr>
<tr>
<td>Imipenem</td>
<td>95</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Table 3: Association between residency of patients and drug resistance (amoxicillin, azithromycin and cefotaxime)**

<table>
<thead>
<tr>
<th>Results of sensitivity test</th>
<th>Total</th>
<th>Rural</th>
<th>Urban</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 95</td>
<td>n = 67</td>
<td>n = 28</td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>60 (63.2%)</td>
<td>39 (58.2%)</td>
<td>21 (75.0%)</td>
<td>0.122*</td>
</tr>
<tr>
<td>Sensitive</td>
<td>35 (36.8%)</td>
<td>28 (41.8%)</td>
<td>7 (25.0%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

n: number of cases; *: Chi-square test; NS: not significant at P ≤ 0.05

**Figure 1: The distribution of patients according to age showing the proportion of multi-drug resistance**

**Discussion**

In this study it was obvious that the oldest drug chloramphenicol was the best drug to treat nowadays strains of *Salmonella enterica* serotype typhi that are common in our community in addition to Trimethoprim-Sulphamethoxazole. Of the newly introduced antibiotics, imipenem was also 100% effective in fighting these *Salmonella enterica* serotype typhi strains. The striking finding in this study, was that the first line drug ciprofloxacin was totally ineffective despite being recommended by the well known medical textbooks to control typhoid fever. Ceftriaxone also showed failure to eradicate these resistant strains. Multi-drug resistance was encountered in 63.2% of isolates enrolled in the current study, which is indeed a horrible figure that require prompt awareness to adopt new strategies in fighting *Salmonella enterica* serotype typhi that are common in our community.

In the near past, ciprofloxacin was recommended as the first-line drug to treat *S. typhi* infections as
majority of the conventional antibiotics became of no benefit leading to excessive use of this drug, namely ciprofloxacin, for the control of typhoid fever. Nevertheless, resistance to ciprofloxacin was recorded in the late 80s and early 90’s of the past century, and the number of reports of treatment failure following this antibiotic became increasingly higher toward the beginning of this century. In our study we found supporting evidence that ciprofloxacin is no more effective in controlling the disease and recommendation should be shifted to other effective drugs such as the “old fashion” chloramphenicol or other effective antibiotics and that sensitivity results must direct the proper choice of antibiotic therapy in an individual manner. Mutation on genetic level of the S. typhi strains may explain the emergence of such resistance as shown by some authors. When resistance to ciprofloxacin was observed other drugs such as ceftriaxone and azithromycin gained wide acceptance as effective regiments in controlling the disease, however, several later reports documented the failure of these antibiotics in treating significant proportion of patients suffering from typhoid fever around the world in addition to our finding in the current study that ceftriaxone showed total failure and that significant proportion of patients included in our study were infected with bacterial strains that are resistant to azithromycin. We believe that the irrational use of antibiotics in our community is one of the major reasons for the emergence of resistant bacterial strains and that the ministry of health should take measures in cooperation with other governmental institutes that insure rational and well controlled prescription of antibiotics.

**Conclusion**

it is obvious that antibiotic approach to typhoid fever is a dynamic rather than a static phenomenon that needs revision regularly and that drug individualization according to culture and sensitivity rule should be adopted by all health workers and institutes when dealing with outbreaks of *Salmonella enterica* serotype typhi.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


Analysis of CTLA-4 (+49A/G) Gene Polymorphism and the Risk of Pulmonary Tuberculosis in Babylon province of Iraq

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¹College of Medicine, University of Babylon, Hilla, Iraq

Abstract

Background: Pulmonary tuberculosis (pTB) is an infectious disease caused by the bacillus Mycobacterium tuberculosis (M. tuberculosis). It typically affects the lungs, but can also affect other sites (extra-pulmonary TB). The disease is spread when sick individuals expel bacteria into the air, for example by coughing.

Aim of Study: To highlight the effect of CTLA-4 (+49A/G) gene polymorphism on the risk of pulmonary Tuberculosis (pTB).

Patients and Methods: This case-controlled study used single specific primer-polymerase chain reaction (SSP-PCR) to analyze the CTLA-4 (+49A/G) gene polymorphism in 60 patients with pTB who were referred to consultant clinic for respiratory diseases in Hilla – Babylon province/ Iraq during the period from December 2017 to July 2018, and 60 healthy persons’ control. Blood samples were collected from both groups according to the standard methods.

Results: Data analysis revealed that the frequencies of AA, AG and GG genotypes in patients were 73.33%, 23.33%, and 3.34% respectively. In controls, this frequency was 51.67%, 35%, and 13.33% respectively. Logistic regression test detected a significant difference in the frequency of the (GG genotype) mutant homozygous of this polymorphism between patients and controls (3.34% versus 13.33%), The GG genotype of CTLA-4(+49A/G) showed a significantly decreased risk of pulmonary tuberculosis disease (OR= 0.18, 95% CI= 0.04 – 0.88, P value = 0.035).

Conclusion: The GG genotype of CTLA-4 may decrease the risk of pTB.

Keywords: Pulmonary Tuberculosis; Mycobacterium; CTLA-4.

Introduction

Tuberculosis (TB) has existed for millennia and remains a major global health problem. It causes ill-health for approximately 10 million people each year and is one of the top ten causes of death worldwide. It has been the leading cause of death from a single infectious agent(1). The major risk factors that increase early death of TB patients are being positive for human immunodeficiency virus (HIV), being of old age, being underweight or undergoing retreatment(2). About one-third of the world’s population are estimated to be infected with M. tuberculosis, albeit mostly without clinical symptoms. These silent carriers bear a life time risk of developing active disease, with more than 95% of cases and deaths occurring in the developing world(3). Population genetic studies have made significant contributions to reveal the role of human genetic variation in susceptibility to TB infection(4). Macrophages and neutrophils play a decisive role in host responses to intracellular bacteria including the agent of tuberculosis, the role of both reactive nitrogen intermediate (RNI) and reactive oxygen intermediate (ROI) as central mediators of innate immune defense in human M. tuberculosis infection is well established(5).

The entry of M. tuberculosis into the body induces activation of cellular immune mechanisms which play important roles in the mechanism of T cells. TH1 cells induce macrophage activation and phagocytosis reactions. Eradication of these pathogens requires secondary administrative cellular immune mechanisms such as cytotoxic T lymphocytes (CTL)(6).
CTL-associated antigen 4 (CTLA-4) is a CD28 receptor that inhibits T cell proliferation through combination with B7 molecules. The human CTLA-4 gene is located on chromosome 2q33. The CTLA-4 (rs231775) polymorphism is located in the first exon of CTLA-4 +49A/G base substitution can cause a change from threonine to alanine amino acid in the coding region of CTLA-4 (7), (8), revealed a significant relationship between the GG genotype of CTLA-4 and the increased risk of TB.

The present study aimed to investigate a possible correlation between CTLA-4+49A/G gene polymorphism and the risk of pTB in a sample of the Iraqi population.

Materials and Method

Study Groups:

This study designed into two groups. The first group involved 60 pTB patients. Those patients were referred to consultant clinic for respiratory diseases in Hilla – Iraq during the period from December 2017 to July 2018. All patients were subjected to full clinical and radiological examinations prior to laboratory examination. The subjects with pTB were selected from those who had a confirmed diagnosis by a healthcare professional and who presented clinical symptoms, radiological evidence and positive sputum acid-fast bacillus (AFB) smears. The second group involved 60 apparently healthy individuals were considered as control group. This control subjects had no previous history of pTB, autoimmune disease, diabetes, and chronic disease.

Collection of Blood Samples

The blood was collected by venous procedure. The site of venipuncture is sterilized by 2% iodine. Five milliliters of venous blood were collected from each participant and kept in EDTA tube. A total of 120 blood samples were collected (60 subjects with pTB patients and 60 healthy controls). DNA extraction was accomplished using (Favorgen/ Taiwan) kit according to the manufacturer’s instructions. After estimation of the quality of DNA by Nanodrop, the Specific Sequence Primer (SSP-PCR) method was used to analyze the genotyping of CTLA4 (+49A/G) with the primers given in (Table -2). The PCR conditions for the amplification of CTLA-4 were as follows: initial denaturation phase comprised 5 minutes at 94°C; next 30 cycles (1 minutes at 94°C, 1 minutes at 57°C, and 1 minutes at 72°C); then, seven minutes at 72°C. The samples were stored at 4°C until electrophoresis as in (figure -1).

Statistical Analysis

The Statistical Package for the Social sciences (SPSS, version 20) was used for statistical analysis. Risk association between the genotype and TB susceptibility was estimated by the calculation of adjusted odd ratio and 95% confidence intervals using multivariate logistic regression. For this analysis, subjects who were homozygous for the wild type genotype were considered as reference, and polymorphisms as dependent variables. Chi- square was used for testing the deviation from Hardy- Weinberg equilibrium, and to compare between patients and control. A $P$-value < 0.05 was considered statistically significant.

Results

The demographic characteristics of study population for pTB patients and controls groups were involved the following factors as in (Table -1).

Table-1: Demographics characteristic of study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients group</th>
<th>Control group</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
<td></td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-30</td>
<td>29 (48%)</td>
<td>23 (38%)</td>
<td>0.269</td>
</tr>
<tr>
<td>31-50</td>
<td>18 (31%)</td>
<td>22 (36%)</td>
<td>0.439</td>
</tr>
<tr>
<td>51-70</td>
<td>13 (21%)</td>
<td>15 (26%)</td>
<td>0.666</td>
</tr>
</tbody>
</table>
Cont.. Table- 1: Demographics characteristic of study population

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35 (58%)</td>
<td>41 (68%)</td>
<td>0.256</td>
</tr>
<tr>
<td>Female</td>
<td>25 (42%)</td>
<td>19 (32%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Rural</th>
<th>Urban</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>42 (70%)</td>
<td>25 (42%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Urban</td>
<td>18 (30%)</td>
<td>35 (58%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Smoker</th>
<th>Non-smoker</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>24 (40%)</td>
<td>20 (30%)</td>
<td>0.444</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>36 (60%)</td>
<td>40 (70%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Vaccinated</th>
<th>Non vaccinated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>33 (55%)</td>
<td>43 (72%)</td>
<td>0.058</td>
</tr>
<tr>
<td>Non vaccinated</td>
<td>27 (45%)</td>
<td>17 (28%)</td>
<td></td>
</tr>
</tbody>
</table>

|                  |              |               |         |
| BMI              |              |               |         |
| <25 kg/m²        | 44 (73.33%)  | 16 (26.67%)   | 0.001   |
| ≥25 kg/m²        | 16 (26.67%)  | 44 (73.33%)   |         |

The AA genotype was found in 44 (73.33%) subjects with pTB as well as in 31 (51.67%) controls subjects. The AG genotype was observed in 14 (23.33%) subjects with pTB and 21 (35%) control subjects. The GG genotype was observed in 2 (3.34%) patients with pTB and 8 (13.33%) control subject. The A allele frequency was 102 (85%) in subjects with pTB and 83 (69.17%) in control subjects. Allele frequency for the G allele was 18 (15%) in subjects with pTB and 37 (30.83%) in control subjects as in (Table -3).

Table – 2: The Primers of CTLA-4 gene and their corresponding genes used in the present study

<table>
<thead>
<tr>
<th>Types of primer</th>
<th>Primer sequence (5’-3’)</th>
<th>Product Size(bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>GTGGGTCAAAACACATTTCAGTGTCAGG</td>
<td></td>
<td>(9)which is developed by the obligate intracellular Mycobacterium leprae (ML</td>
</tr>
<tr>
<td>Reverse</td>
<td>TCCATGTCTGCTTCAAAAGTCCTCACTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>ACAGGAGAGTGCAGGCGGCAAGTCTAGT</td>
<td>AA: 120bp AG: 229bp GG: 162bp</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>GCACCAAGCTCAGCTGAACCTTGATG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table- 3: Genotypes and Alleles Frequency of \textit{CTLA4} Gene +49A/G Polymorphism in Patients with pTB and the Control Group

<table>
<thead>
<tr>
<th>\textbf{CTLA-4 rs 231775}</th>
<th>\textbf{pTB Cases (60)}</th>
<th>\textbf{Controls(60)}</th>
<th>\textbf{P-value}</th>
<th>\textbf{OR(95%CI)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>44(73.33%)</td>
<td>31(51.67%)</td>
<td>0.036</td>
<td>0.47(0.21 -1.06)</td>
</tr>
<tr>
<td>AG</td>
<td>14(23.33%)</td>
<td>21(35%)</td>
<td>0.07</td>
<td>0.18(0.04-0.88)</td>
</tr>
<tr>
<td>GG</td>
<td>2 (3.34%)</td>
<td>8(13.3%)</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>0.51</td>
<td>0.165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele A</td>
<td>102(85%)</td>
<td>83(69.17%)</td>
<td>0.005</td>
<td>0.4 (0.21-0.75)</td>
</tr>
<tr>
<td>Allele G</td>
<td>18(15%)</td>
<td>37(30.83%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

T-cell activation is a complex process that requires >1 stimulatory signal. TCR binding to MHC provides specificity to T-cell activation, but further costimulatory signals are required. Binding of B7-1 (CD80) or B7-2 (CD86) molecules on the APC with CD28 molecules on the T cell leads to signaling within the T cell. Sufficient levels of CD28:B7-1/2 binding lead to proliferation of T cells, increased T-cell survival, and differentiation through the production of growth cytokines such as interleukin-2 (IL-2), increased energy metabolism, and upregulation of cell survival genes.

\textit{CTLA-4} +49 A/G binding to B7 may actually produce inhibitory signals that counteract the stimulatory signals from CD28:B7 and TCR: MHC binding\textsuperscript{(10)}. Proposed mechanisms for such inhibitory signals include direct inhibition at the TCR immune synapse, inhibition of CD28 or its signaling pathway, or increased mobility of T cells leading to decreased ability to interact with APCs\textsuperscript{(11)}. The polymorphism in \textit{CTLA-4} +49 A/G gene may reduce the risk of being infected with pTB, and can down-regulate the inhibition of cellular immune response\textsuperscript{(12)+6230G>A (rs3087243}. The \textit{CTLA-4} +49 A/G (G) GG genotype are more frequent in healthy subjects than patients and may associate with a protection role against intracellular infection\textsuperscript{(13)}.

(Figure-1: Genotypic patterns of cytotoxic T-lymphocyte associated antigen-4 +49A/G polymorphism using SSP-PCR visualized under UV transluminator. M: DNA marker, lanes 1,5 and 9: AG genotype, lanes 3,4,6,7and 8: AA genotype, lane 2 and 10: GG genotype.)
**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**

Screening Motives among Attendants of Breast Cancer-Early Detection Clinics, in Iraq

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Abstract

Background and Objectives: The early detection model for breast cancer in Iraq is a combined program of an early diagnosis for symptomatic attendants and an opportunistic unsystematic breast clinical examination screening that mainly depend on attendants’ request. With an aim to initiate breast cancer screening program, the current study’s objective is to illustrate the prevalence and motives of screening behavior among attendants.

Method: A cross sectional study was conducted for a period of three months, interviewing 500 randomly selected attendants of two breast cancer early detection clinics in Baghdad city. Data presented in frequency and percentages and analyzed by binary logistic regression, P ≤ 0.05 reflected a significant association.

Results: The results showed that only 42 (8.4%) attended the breast clinics with screening intention. Being employed (OR= 3.198; 95% C.I.= 1.284 – 7.964; P=0.013) or a student (OR=6.605; 95% C.I.= 1.125 – 38.759; P=0.037), of high socioeconomic status (OR=2.695; 95% C.I.= 1.036 –7.012; P =0.042) and having a positive family history of breast cancer (OR= 5.17; 95% C.I.= 2.466 –10.855; P <0.001) were significant reasons for breast cancer screening.

Conclusions: Reforming the health system and tackling access barriers to screening should be endorsed through applying multiple components interventions to increase women participation in the service with a special focus on the less empowered under-served ones.

Keywords: Breast cancer; early detection; Screening; Screening intention; Screening motives.

Introduction

Breast cancer; the most frequent cancer among women, impacting 2.1 million new cases each year, contributing about 11.6% of the total cancer incidence burden worldwide. In 2018, it is estimated that 627,000 women died from breast cancer—that is approximately 15% of all cancer deaths among women. ¹ In Iraq, it stands as the second cause of cancer mortality (12.01%) and the commonest cancer among Iraqi women (34.27%) with 4922 newly diagnosed cases in 2016, of them 828 (16.8%) women were below 40 years of age. In Baghdad, the capital city of Iraq, breast cancer incidence reaches to 41.65 per 100,000 women. ²

Early detection practices are the most important ways to catch breast cancer early and increase survival rates, hence it is the second pillar of the national cancer control program (NCCP) with the principles of providing national educational campaigns about early warning signs of breast cancer via mass media involvement and school curricula, in addition to strengthening the efficacy of breast early detection program and encouraging researches in the area. ³

The early detection model of breast cancer in Iraq is a combined program of an early diagnosis for symptomatic attendants presenting with breast pain, mass and nipple discharge etc. and an opportunistic unsystematic breast clinical examination screening. ⁴,⁵ That might be followed by mammograms (for 40 years and older women) or ultrasound (for women younger than 40 years of age), these are provided on a woman’s request or by her physician’s recommendation.
Therefore, screening is not regulated, there are no centrally prearranged invitations nor a follow-up system for screening. \[6\]

According to the triple assessment protocol trailed in the breast early detection clinics, patients with positive radiological evaluation (mammography and/or ultrasound) are sent for tissue sampling through fine needle aspiration or excisional biopsy. \[5\] Due to lack of awareness and screening opportunities, breast cancer is usually detected at a later stage. According to the national Iraqi cancer registry in 2016, more than 80% of cases presented with infiltrative ductal carcinoma as their main morphology. \[2,7\]

**Justification of the issue:**

It had been reported that making the decision to implement screening as part of breast cancer control strategy should be preceded by promoting education not only to public but also to health care professionals. \[5\] Yet resource limitations dictate the national priorities. Regularly monitoring cancer control activities to ensure they are effective and remain effective is essential. \[8\]

As a result, providing information on the prevalence of screening among breast clinics attendants would reproduce the public willingness to screen plus it will provide a feedback results on the awareness campaigns that had been ongoing since establishment of the NCCP program from one hand, and identifying the demography of screeners would serve as a contrast to find out those who need to be targeted by the awareness campaigns from the other hand.

**Research questions:**

1. Do people visit the early detection clinics for screening?
2. What is the prevalence of screen-attendants?
3. What motivate attendants to screen for breast cancer?

**Aim**

Initiating breast cancer screening program.

**Objectives:**

2. Demographic characteristics of the screeners.

**Patients & Methods**

A cross sectional study was conducted from the first of November 2018 to the end of January 2019, in two major hospitals in Baghdad- Iraq (Al Yarmook Teaching Hospital and Al Eliwiya Maternity Hospital). Each hospital had an active, functioning and fully equipped breast cancer early detection clinic (i.e. with an ultrasound, mammography and fine needle aspiration units attached to the breast clinic). These clinics are installed in the outpatient wards of the hospitals. Using the sample size equation \[9\]: 

\[ n = \frac{z^2 \cdot p(1-p)}{E^2} \]

where \( n \) is the sample size, \( Z \) is the critical value in a two-tailed test and equals to 1.96, \( E \) is the sampling error equals to 5%, \( P \) is the estimated prevalence, here it is considered 0.50.

Through systematic random sampling, 500 randomly selected attendants were approached, verbal consents were obtained after explaining the aim and objectives of the study. Interviews were completed within facility premises using a structured questionnaire that was developed based on literature review, meeting with focus group and supervisors’ experiences. The requested information was age of respondent, educational achievement, employment, husbands’ education, area of residency, reasons for attending the clinic, source of referral to the breast clinic, family history of breast cancer and a set of questions regarding socioeconomic status (SES) which was determined using a newly proposed Iraqi scale. \[10\]

The questionnaire was tested and piloted on twenty-five clinic-attendants and changes were made accordingly. The questionnaires which were distributed during the pilot phase were not used in the final analysis. Data were coded and analyzed using **statistical package for the social sciences** version 18. Binary logistic regression was used to identify the demographic predictors for screening among attendants. P value equal or less than 0.05 was considered significant.

**Results**

 Majority of women who attended the breast early detection clinics were symptomatic. Figure (1) illustrates the reasons for attending the breast clinics among the studied sample. The mean age for symptomatic attendants (complaining) was 42±11.8 years ranging from 16 up to 66 years. While the average age for the
asymptomatic group (screening) was 44.5 ±10.4 years, within a range of 22 to 63 years.

Figure (1): Distribution of the sample according to reasons of attendance the breast clinics (n=500)

The main complaint was having breast pain (245; 49%), followed by feeling a lump (150; 30%), nipple discharges (45; 10.8%) and other symptoms (9; 1.8%). Only 42 (8.4%) attended the breast clinics with screening intention. Eleven (26.2%) women were employed and three participants were university students at the time of the interview. As for the source of referral to the breast clinic, 18 (42.9%) women were self-referred, 13 (31%) had a friend or a family member registered in the same breast clinic and 11 (26.2%) were recommended to screen their breasts regularly by their private physicians. Table (1) demonstrates the distribution of the studied sample by the sociodemographic characteristics according to reason for visiting the breast clinic.

Logistic regression analysis was employed to predict the probability that a participant would attend breast clinic for screening. The predictor variables were age, residency, employment status, family history of breast cancer, socioeconomic status, participants’ and their husbands’ education levels. A test of the full model versus a model with intercept only was statistically significant, \(X^2(15, N = 500) = 58.035\), \(p < 0.001\). Table 2 shows the logistic regression coefficient, Wald test, and odds ratio for each of the predictors.

Having a positive family history of breast cancer is 5.173 times more likely to attend breast clinic for screening than those with no such history. High socioeconomic status women were 2.695 times more likely to screen for breast cancer. Being employed or student were 3.198 and 6.605 times more likely to request breast cancer screening respectively.

### Table (1) the distribution of the sample by demographic characteristics according to the reasons of attendance (n=500)

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Asymptomatic (Screening)</th>
<th>Reasons for attendance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below 40</td>
<td>12 (28.6%)</td>
<td>186 (40.6%)</td>
</tr>
<tr>
<td>40 and above</td>
<td>30 (71.4%)</td>
<td>272 (59.4%)</td>
</tr>
<tr>
<td>Participants’ Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>4 (9.5%)</td>
<td>70 (15.3%)</td>
</tr>
<tr>
<td>Primary</td>
<td>9 (21.4%)</td>
<td>114 (24.9%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>10 (23.8%)</td>
<td>100 (21.8%)</td>
</tr>
<tr>
<td>High school</td>
<td>7 (16.6%)</td>
<td>77(16.8%)</td>
</tr>
<tr>
<td>Institutions and universities</td>
<td>12 (28.6%)</td>
<td>97 (21.2%)</td>
</tr>
<tr>
<td>Husbands’ Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>1 (2.4%)</td>
<td>46 (10.0%)</td>
</tr>
<tr>
<td>Primary</td>
<td>1 (2.4%)</td>
<td>62 (13.5%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>5 (11.9%)</td>
<td>90 (19.7%)</td>
</tr>
<tr>
<td>High school</td>
<td>13 (31.0%)</td>
<td>101(22.1%)</td>
</tr>
<tr>
<td>Institutions and universities</td>
<td>22 (52.4%)</td>
<td>159 (34.7%)</td>
</tr>
<tr>
<td>Employment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>28(66.7%)</td>
<td>385(84.1%)</td>
</tr>
<tr>
<td>Employed</td>
<td>11 (26.2%)</td>
<td>61 (13.3%)</td>
</tr>
<tr>
<td>Students</td>
<td>3 (7.1%)</td>
<td>12 (2.6%)</td>
</tr>
<tr>
<td>Residency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>36(85.7%)</td>
<td>381(83.2%)</td>
</tr>
<tr>
<td>Rural</td>
<td>6 (14.3%)</td>
<td>77(16.8%)</td>
</tr>
<tr>
<td>SES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>12(28.6%)</td>
<td>237(51.7%)</td>
</tr>
<tr>
<td>Middle</td>
<td>8(19%)</td>
<td>118 (25.8%)</td>
</tr>
<tr>
<td>High</td>
<td>22(52.4%)</td>
<td>103 (22.5%)</td>
</tr>
<tr>
<td>Family History of Breast Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17(40.5%)</td>
<td>370(80.8%)</td>
</tr>
<tr>
<td>Yes</td>
<td>25(59.5%)</td>
<td>88 (19.2%)</td>
</tr>
</tbody>
</table>
Table 2: Binary logistic regression analysis of screening motives among attendants (n=500)

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Wald X²</th>
<th>P value</th>
<th>OR</th>
<th>95% C.I. for OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Age ≥ 40 years</td>
<td>0.468</td>
<td>0.849</td>
<td>0.357</td>
<td>1.597</td>
<td>0.590 - 4.324</td>
</tr>
<tr>
<td>Participants’ Education level</td>
<td>2.037</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>0.068</td>
<td>0.009</td>
<td>0.924</td>
<td>1.071</td>
<td>0.261 - 4.396</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.171</td>
<td>0.057</td>
<td>0.812</td>
<td>1.186</td>
<td>0.291 - 4.835</td>
</tr>
<tr>
<td>High</td>
<td>-0.360</td>
<td>0.204</td>
<td>0.651</td>
<td>0.698</td>
<td>0.147 - 3.320</td>
</tr>
<tr>
<td>Institutional and higher</td>
<td>-0.590</td>
<td>0.537</td>
<td>0.464</td>
<td>0.554</td>
<td>0.114 - 2.687</td>
</tr>
<tr>
<td>Rural residency</td>
<td>0.868</td>
<td>1.989</td>
<td>0.158</td>
<td>2.382</td>
<td>0.713 - 7.956</td>
</tr>
<tr>
<td>Husbands’ education level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>0.204</td>
<td>0.020</td>
<td>0.889</td>
<td>1.227</td>
<td>0.070 - 21.596</td>
</tr>
<tr>
<td>Secondary</td>
<td>1.036</td>
<td>0.784</td>
<td>0.376</td>
<td>2.817</td>
<td>0.284 - 27.898</td>
</tr>
<tr>
<td>High</td>
<td>2.127</td>
<td>3.362</td>
<td>0.067</td>
<td>8.392</td>
<td>0.864 - 81.556</td>
</tr>
<tr>
<td>Institutional and higher</td>
<td>1.872</td>
<td>2.537</td>
<td>0.111</td>
<td>6.503</td>
<td>0.649 - 65.125</td>
</tr>
<tr>
<td>Positive family history of Breast Cancer</td>
<td>1.644</td>
<td>18.893</td>
<td>0.000</td>
<td>5.173</td>
<td>2.466 - 10.855</td>
</tr>
<tr>
<td>Socioeconomic Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>-0.048</td>
<td>0.007</td>
<td>0.932</td>
<td>0.953</td>
<td>0.312 - 2.906</td>
</tr>
<tr>
<td>High</td>
<td>0.991</td>
<td>4.129</td>
<td>0.042</td>
<td>2.695</td>
<td>1.036 - 7.012</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>1.162</td>
<td>6.236</td>
<td>0.013</td>
<td>3.198</td>
<td>1.284 - 7.964</td>
</tr>
<tr>
<td>Students</td>
<td>1.888</td>
<td>4.372</td>
<td>0.037</td>
<td>6.605</td>
<td>1.125 - 38.759</td>
</tr>
<tr>
<td>Constant</td>
<td>-5.571</td>
<td>20.119</td>
<td>0.000</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Nearly one out of twelve clinic-attendants, was there for screening purposes, this low (8.4%) percent is consistent with another Iraqi study where only 9.5% of women visited the breast center for routine screening. [11] While in Jordan the percent rises to 25.4% (16.8% had clinical breast examination and 8.6% had periodic mammography screening). [12] This might be explained by the neglect of screening education and promotion, which is probably related to Iraq’s unique situation of conflicts (wars and civil wars) that in turn modified priorities. Breast cancer screening and even clinical services on breast cancer have been neglected particularly due to lack of available equipment, funds, organizational structure and quality criteria. [7] Having breast pain was the main complaint for visiting the breast clinic followed by feeling a lump, which goes in line with a previous study. [11] Lack of women knowledge about breast cancer screening would result in missed screening opportunity. [13] When public screening-demands are low, doctors’ words remain a proven influence for pursuing screening particularly when clients don’t fully realize the idea of visiting the health facility without having symptoms. [6, 14-16] It had been reported that the most frequent reasons for not going to breast cancer screening exams regularly were absence of breast problems. [17] Henceforth increasing population demand for screening together with healthcare providers’ delivery interventions [18] through engaging community and family physicians in screening services might be useful to increase service beneficiaries; since these physicians are the health
promoters, educators and the preventive force for the health system. [19, 20]

Similar to reported findings in literature, employed ladies are more empowered, financially capable of seeking screening and most likely will self-refer to the breast clinic. Being a student means more exposure to educational campaigns whether via media or school curriculum that in turn influence a positive attitude toward screening. Having a relative diagnosed with breast cancer might rise the perceived risk of breast cancer and eventually motivates screening behavior. [12,14, 21,22]

Therefore; in the absence of a breast cancer screening program, it appears clearly that the most empowered women are the ones who are motivated for requesting screening whether it might be due to their knowledge or ability to pay for services, or due to perceived risks of breast cancer. This sequentially indicates a need to reform awareness campaigns and extend its audience further to the less privileged. Initiating a breast cancer screening though is still a goal, yet increasing women participation in the early detection service and mainly among the screening group might be the first step to attain that goal.

Conclusion and Recommendations

There is an urgent need to increase both public and healthcare providers’ awareness about screening, reforming the health system and tackling access barriers to screening should be endorsed through applying multiple components interventions to increase women participation in the service with a special focus on the less empowered under-served ones.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

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Study the Toxicity and Anticancer activity of Some New Amic Acid and Their Derivatives of Mefenamic acid

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Abstract

A series of amic acid derivatives of mefenamic acid were synthesized with the aim of inhibiting topical gastrointestinal toxicity of mefenamic acid. The key intermediate amic acid (III) was prepared from the reaction of acid hydrazid of mefenamic acid(II) with phthalic anhydrides in dry actone. The new type of imide compound (IV) was dehydrated the amic acid(III) with acetic anhydrous and sodium acetate. The esterification of hydroxyl groups of amic acid (III) produce corresponding ester(V), which was condensed with hydrazine hydrate to give acid hydrazide (VI), then the later compound reacted with syringaldehyde in dry benzene to yield new schiff base (VII). The new derivatives containing heterocyclic unit (VIII)-(X), four and five, member ring were successfully formed such as azetidin-2-one, thiazolidin-4-one, and,imidazolidin-4-one. The structures of the synthesized compounds were confirmed using FTIR, ¹HNMRI, Mass and CHN-S. The antibacterial activities of some synthesized compounds were screened and showed a highest or low inhibition against Staph.aureus (G+), Bacillus subtilisa (G+), Klebsiella pneumoniae (G-), and E.coli (G-). Also, The cytotoxic effect of different concentrations of some the synthesized compounds was tested against MCF-7 cell line (human breast carcinoma cells) and positive results were obtained for some of them, which encouraged us to study the toxicity using living organisms (mice) to evaluate its acute toxicity and proved the resules of non-toxicity of the derivatives.

Keywords: Mefenamic acid, imidazolidin-4-one, antibacterial activities, MCF-7 cell line, acute toxicity study.

Introduction

Mefenamic acid is one of the anthranilic acid derivatives class of non-steroidal anti-inflammatory drugs (NSAID)) is thought to be due to their interference with prostaglandin biosynthesis, and is used to treat strong analgesic and antiinflammatory agents in the treatment of degeneration of joint cartilage, rheumatoid arthritis and musculoskeletal disease [¹].

The general view in the literature shows that mefenamic acid undergoes a number of reactions, this has been used in the preparation of many compounds containing heterocyclic moiety of therapeutic molecules for different applications, including the presence of effective anti-cancer drugs, antimicrobial, inflammatory and cytotoxic activities[²].

Amic acid and its derivatives have been extremely used in drug industry, advanced materials and biologically active heterocycles. In fact, amic acid carrying suitable position of carboxylic group are prime precursors for synthesis of heterocyclic derivatives[³].

A large number of heterocyclic compounds have been explored for developing pharmaceutically molecules. Among this the derivatives: azetidin-2-one (β-lactam), thiazolidin-4-one, and imidazolidin-4-one, have been playing an important function in the pharmaceutical chemistry [⁴]. This study is aimed at synthesis new active molecules with fewer side effects that can be successfully used as drugs, the cytotoxic effect and non-toxicity proved the resules of this derivatives.

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Experimental

Materials and instruments:

All solvents and chemicals were purchased from Sigma- Aldrich, and GCC Chemicals company. Melting points were recorded using electrothermal melting point apparatus and are uncorrected. The FTIR spectrums were registered using KBr discs on Shimadzo (Ir prestige-21) Fourier Transform Infrared Spectrometer. $^1$HNMR spectra were recorded on Bruker, Ultra Shield (300)MHz, Switzerland and using DMSO-$d_6$, and CDCl$_3$ with reference to TMS as internal reference. Elemental micro-analyses were carried out using an EuroEA Analyzer. Mass spectra of compounds were measured by Electron Impact (EI) 70eVmass using a MS Model: 5973 spectrometer.

Synthetic Procedures

The routes for synthesis new derivatives as depending on to the Scheme (1):

\begin{align*}
\text{O} \quad \text{HN} & \quad \text{O} \quad \text{HN} \\
\begin{array}{c}
\text{Ar} \quad \text{C} \quad \text{Cl} \\
\text{HN} \quad \text{O} \quad \text{N} \\
\text{O} \quad \text{H} \quad \text{N} \\
\text{O} \quad \text{H} \quad \text{N} \\
\end{array} & \quad \text{O} \quad \text{N} \quad \text{H} \\
\begin{array}{c}
\text{ClCH}_2\text{COCl} \\
\text{HSCH}_2\text{COOH} \\
\text{glycine Et}_3\text{N} \\
\end{array} & \quad \text{O} \quad \text{HN} \quad \text{N} \\
\begin{array}{c}
\text{Ar} \quad \text{C} \quad \text{H} \\
\text{HN} \quad \text{O} \quad \text{N} \\
\text{O} \quad \text{H} \quad \text{N} \\
\text{O} \quad \text{H} \quad \text{N} \\
\end{array} & \quad \text{O} \quad \text{HN} \quad \text{N} \\
\begin{array}{c}
\text{Ar} \quad \text{C} \quad \text{H} \\
\text{HN} \quad \text{O} \quad \text{N} \\
\text{O} \quad \text{H} \quad \text{N} \\
\text{O} \quad \text{H} \quad \text{N} \\
\end{array} & \quad \text{O} \quad \text{HN} \quad \text{N} \\
\begin{array}{c}
\text{Ar} \quad \text{C} \quad \text{H} \\
\text{HN} \quad \text{O} \quad \text{N} \\
\text{O} \quad \text{H} \quad \text{N} \\
\text{O} \quad \text{H} \quad \text{N} \\
\end{array}
\end{align*}

\(\text{Ar} = 4-\text{OH,3,5-OC}_3\text{CH}_{12}\)
Preparation of methyl 2-(2,3-dimethylanilino) benzoate (I)

This compound (I) was prepared following the procedure described by Lit. [5] yield 80%; m.p(96-98)°C.

Preparation of 2-(2,3-dimethylphenylamino) benzohydrazide (II)

This compound (II) was prepared following the procedure described by Lit. [5], yield 78%; m.p (118-120)°C.

Synthesis of 2-(2-(2-(2,3-dimethylphenylamino) benzoyl)hydrazine-1-carbonyl) benzoic acid (III)

In a flask with magnetic stirrer, a solution of (2.55g, 0.01 mol) of acid hydrazide (II) in 20 mL of acetic anhydride was dripped into a solution of (1.48g, 0.01 mol) phthalic anhydride in 20 mL of acetic anhydride for 30 min at room temperature. Stirring was continued for 5 hrs. The brown solid precipitate was filtered, washed, dried and recrystallized from ethanol to give a new amic acid (III), yield 74%, m.p (167-169)°C; FTIR (ν ,cm⁻¹): 3392-2571(OH), 3340-3309 (NH), 3012 (C-H arom.), 2939-2922(C-H aliph.), 1712, 1653 (C=O), and 1255 (C-O); ¹H NMR (δ ppm): 6.66-8.09 (m,11H,Ar-H), 9.45, 7.91, and 7.58 for (s,1H,NH); mass spectra (relative intensity%): m/z=403(5), 223 (75), 178(70), 123 (70), 69(100), 104(68), 51(25):Elemental analysis: Calcd. for C₂₉H₂₉NO₄: C, 71.68; H, 4.93; N, 10.90; Found: C, 71.98; H, 5.11; N, 11.03.

Synthesis of methyl 12-(2-(2-(2,3-dimethylphenyl) amino) benzoyl) hydrazine-1-carbonyl) benzoate (V):

A mixture of compound (III) (4.03 g, 0.01mol), absolute methanol 30mL and H₂SO₄ 5.4 mL was refluxed 6 hrs. After cooling washed the mixture with NaHCO₃ solution, the resulting of a white crystals solid was filtered off, washed with water, dried and recrystallized by ethanol to give compound (V), yield 71%; mp (137-139) °C; FTIR (ν ,cm⁻¹): 3330-3309 (NH), 2987 (C-H arom.), 2939-2922(C-H aliph.), 1732, 1685, 1657 (C=O), and 1255 (C-O); ¹H NMR (δ ppm): 2.08 (s, 3H, CH₃), 2.27 (s, 3H, CH₃), 3.86(s,3H,OCH₃), 6.66-8.09 (m,11H,Ar-H), 9.66,and 9.18 for (s,1H,NH); Elemental analysis for this compound : Calcd. for C₂₉H₂₃N₃O₄; C, 69.06; H, 5.51; N, 10.07; Found: C, 69.45; H, 5.80; N, 10.30.

Synthesis of N′-(2-(2,3-dimethylphenyl) amino) benzoyl) phthalohydrazide (VI):

A mixture of ester compound (V) (4.17gm,0.01mol), was dissolved in absolute ethanol 3mL. Hydrazine hydrate(0.5gm,0.01 mol) was added slowly and the mixture was refluxed for 6hrs. The mixture was cooled and the pale yellow solid was filtered, then used ethanol to recrystallized, yield 70%, m.p (158-160)°C, FTIR (ν ,cm⁻¹): 3305-3145(NH₂,NH), 3001(C-H arom.), 2941-2822(C-H aliph.), 1660,1639(C=O amide); ¹H NMR (δ ppm): 2.11 (s, 3H, CH₃), 2.28 (s, 3H, CH₃), 6.56-8.05 (m,11H,Ar-H), 9.13, and 8.18 for (s,1H,NH); Elemental analysis: Calcd. for C₂₉H₂₃N₃O₅; C, 66.18; H, 5.51; N, 16.78; Found: C, 66.70; H, 5.78; N, 17.01.

Synthesis of (Z)-N′1-(2-((2,3-dimethylphenyl) amino) benzoyl) -N′2-(4-hydroxy -3,5-dimethoxybenzylidene) phthalohydrazide (VII):

A mixture of new compound (VI)(4.17g, 0.01 mol) , Syringaldehyde (1.82g,0.01mol) with EtOH 15 mL and 6hrs, cooled after that filtered the product and crystallized from ethanol,dark yellow powder, yield 70% , m.p (150-152) °C, FTIR (ν ,cm⁻¹): 3548(OH), 3469-3344(NH), 3016 (C-H arom.), 2968-2935(C-H aliph.), 1651(C=O amide), 1626(C=N); ¹H NMR(δ,ppm,CDCl₃): 2.10 (s, 3H, CH₃), 2.29 (s, 3H, CH₃), 2.51(s, 1H,OH ), 3.84 (s,6H, OCH₃),6.68-8.58 (m,13H,Ar-H),7.32(s,1H(CH-N)
A number of new mefenamic acid derivatives were synthesized by the reaction between the carboxylic acid group in mefenamic acid and methanol in acidic medium (I), which was converted to acid hydrazide (II), then the later reacted with phthalic anhydride with heating in dry acetone to give compound (III) in good yield. FT-IR spectrum for (III) show the new absorption stretching bands due to O-H of COOH moiety in the region (3392-2571)cm⁻¹, a stretching band to C=O for COOH appeared at 1712 cm⁻¹, a stretching band asymmetry and symmetry of N-H group appeared at 3340-3309 cm⁻¹. Imide compound (IV) was synthesized by cyclization of amic acid (III) using acetic anhydride and anhydrous sodium acetate. FT-IR spectrum for (IV) show characteristic absorption bands at 3344 cm⁻¹ due to N-H imide, and absorption bands at (1747, 1645) cm⁻¹ to carbonyl imide groups.[6]

The esterification of hydroxyl groups of amic acid (III) produce corresponding ester(V), the FTIR spectrum for (V) a display absence absorption stretching bands O-H and C=O groups together presents of new bands at 1732 cm⁻¹and 1255 cm⁻¹assigned to carbonyl and C-O groups of ester moiety, respectively. Ester(V) was condensed with NaOH to give acid hydrazide (VI), the FTIR spectrum display stretching vibration asymmetry and symmetry of NH and NH groups in the region(3305-3145)cm⁻¹ as well as stretching absorption at 1651cm⁻¹for C=O amide. When the reacted compound(VI) reacted with thionyl chloride, new Schiff base (VII) was formation. FTIR spectrum for

\[
\delta(C)\text{ (ppm): 2.10 (s, 3H, CH₃), 2.29 (s, 3H, CH₃), 2.51 (s, 1H,OH),3.33 (s, 6H, OCH₃); 3.87 (s,2H, CH₂-S); 6.68-7.87 (m,13H,Ar-H), 7.37 (1H, CH-N), 12.98, 9.45, 9.18, for (s,1H,NH); CHN-S: Calcd. for C₃₄H₃₂Cl₅N₅O₂: S, 62.28; H, 5.07; N, 10.68; S,4.89; Found: C62.57; H, 5.38; N, 10.91; S,4.99.
\]

\[
\text{Synthesis of } S\text{-2-(2-(2-(4-hydroxy-3,5-dimethoxyphenyl) (2R,3S)-3-chloro-2-(4-hydroxy-3,5-dimethoxyphenyl) -4-oxothiazolidin-3-yl)-2-(2-(2,3-dimethylphenyl) amino) benzoyl) hydrazine-1-carbonyl benzamide (X)}
\]

The Schiff base (VII) (5.81gm, 0.01 mol), glycine (0.75gm, 0.01mol) and triethylamine 1mL in ethanol 15mL was refluxed9hrs. The mixture was neutralized with diluted Hydrochloric Acid and then poured into ice- water. The yellow crystals were filtered off, washed with water and crystallized from ethanol, yield : 60% , m.p (198-200 °C); FTIR (ν,v,cmm⁻¹): 3550 (OH), 3410-3380(NH), 3064 (C-H arom.), 2962-2933(C-H aliph.), 1736,1610(C=O); ¹H NMR (δ ,ppm): 2.09 (s, 3H, CH₃), 2.29 (s, 3H, CH₃), 1.85(s, 1H,OH), 3.75 (s, 6H, OCH₃); 3.87 (s,2H, CH₂-N); 6.41-7.91 (m,13H,Ar-H), 11.12(s,1H,NH), 9.18 (s,1H,NH), 8.13 (s,1H,NH); Elemental analysis: Calcd. for C₃₄H₃₂N₅ClO₇: C, 63.94; H, 5.37; N, 13.16; O, 17.54; Found: C63.57; H, 5.83; N, 13.82.

Results and Discussion

A number of new mefenamic acid derivatives were synthesized by the reaction between the carboxylic acid group in mefenamic acid and methanol in acidic medium (I), which was converted to acid hydrazide (II), then the later reacted with phthalic anhydride with heating in dry acetone to give compound (III) in good yield. FT-IR spectrum for (III) show the new absorption stretching bands due to O-H of COOH moiety in the region (3392-2571)cm⁻¹, a stretching band to C=O for COOH appeared at 1712 cm⁻¹, a stretching band asymmetry and symmetry of N-H group appeared at3340-3309 cm⁻¹. Imide compound (IV) was synthesized by cyclization of amic acid (III) using acetic anhydride and anhydrous sodium acetate. FT-IR spectrum for (IV) show characteristic absorption bands at 3344 cm⁻¹ due to N-H imide, and absorption bands at (1747, 1645) to carbonyl imide groups[6].

The esterification of hydroxyl groups of amic acid (III) produce corresponding ester(V), the FTIR spectrum for (V) a display absence absorption stretching bands O-H and C=O groups together presents of new bands at 1732 cm⁻¹and 1255 cm⁻¹assigned to carbonyl and C-O groups of ester moiety, respectively. Ester(V) was condensed with NaOH to give acid hydrazide (VI), the FTIR spectrum display stretching vibration asymmetry and symmetry of NH and NH groups in the region(3305-3145)cm⁻¹ as well as stretching absorption at 1651cm⁻¹for C=O amide. When the reacted compound(VI) reacted with thionyl chloride, new Schiff base (VII) was formation. FTIR spectrum for
compound(VII) display absence of bands of NH$_2$, NH groups with a new stretching band C=N group at 1626 cm$^{-1}$. Finally, three derivatives containing heterocyclic unit (VIII-X) four, and five member ring synthesized from reacted the shiff base(VII) with chloroacetylchloride, thioglycolic acid, and glycine in triethylamine, respectively. All compounds are identified by physical properties, and by spectral methods FT-IR, $^1$HNMR and elemental analysis.

**Biological Activity**

Mefenamic acid has analgesic action three times more than that of aspirin. However, like all classical NSAIDs are associated with an increased risk of gastrointestinal ulcers. This study involves synthesis, evaluate antibacterial activity , cytotoxic effect, and toxicity in vivo of new amic acid and their derivatives of mefenamic acid, which are expected to have fewer side effects than the original compound.

**Antibacterial activity**

The antibacterial activity of the compounds were examined (in vitro) against *Staph. aureus* (G+), *Bacillus subtilisa* (G+), *Klebsiella pneumoniae* (G-), and *E.coli* (G-) using the agar diffusion method[7]. Most of the derivatives display high or low biological activity versus bacteria, the data are listed in table(1) . Compound (VIII) showed good inhibition against *Staphyllococcus aureus*, this could be related to the presence of azetidin-2-one(β-lactam), while compound (X) showed slightly active against *Klebsiella pneumonia* and *E.coli*.

**Anticancer screening**

The cytotoxicity of new amic acid and their derivatives of mefenamic acid (III, IV, VII, VIII, IX) against a human breast cancer cell line[8] for 48h. MCF-7 cells were seeded in (96-well)culture plates at 200 µl/ of cell suspension was filled to each well and the plates covered by plate and sealed at Para film and put in an incubator, then incubated for 24 h in humidified chamber at 37°C with 5% Carbon dioxide gas and medium fill up with 10% bovine serum and 1% of penicillin / streptomycin mixture until the cells reached confluence. The plate was checked out for contamination, and cultured at different concentrations 10 - 500 µg/ML, while 200µl of maintenance medium were added to each well of monitoring group, then plates were tight with Parra film and regressed to the incubator. Evaluation of cytotoxicity was carried out after 48 , the supernatant was removed, 150 µL of DMSO was added to the solution then shaking, and absorbance values reeds at $\lambda = 450$ nm to calculated the rate of inhibition of cell growth. Cytotoxicity assay for derivatives (III, VII, and VIII) caused good inhibitory effect on the growth of cell line except compounds (IV, and IX), table (2). compound (III)showed more than 50% inhibition for MCF-7and compound (VII and IX) less than 50% inhibition for MCF-7cell line.

**Acute Toxicity Test**

In this research, three groups of 45 albino mice (each consisting of 15 mice) were used to evaluate the acute toxicity of some synthesized compounds (III, IV, VII, VIII, IX), using the Lorke-written method[9]. Mice were fasted for 18 h with free arrival to water before experiment. The compounds were dissolved in distilled water and treated via the oral route (5 and 10 g/kg). The mice are fed, weighed and observation of general signs of toxicity symptoms, behavior, and mortality for 14 days. The study exhibited : no mortality with doses 5 and 10 g/kg body weight, no contrast in the weight of the mice (weighted every day) between the group control and the treated groups, no change in mice behaviors was in the weight of the mice daily measured between the group control and the treated groups, no modification in mice behaviors was recorded, and no toxicity symptoms were announced next 14 days. Moreover, some mice were sacrificed by cervical dislocation and kidneys, liver, and heart were weighed. Finally, visual estimate of organs of mice showed normal cases.

**Conclusion**

Amic acid represent a major class of organic compounds and used some of these derivatives in therapeutic purposes, therefore modification structure of the amic acid derived from mefenamic acid have allowed using multistep processes to the synthesized of new derivatives may be having a broad spectrum of biological activity. Some amic acid and their derivatives of mefenamic acid were screened for their antibacterial, anticancer activity and acute toxicity test . We need to further examination to know mechanism by which the heterocyclic compounds act to give a potent cytotoxic effect that might get the mefenamic derivatives being blight anticancer product.

**Acknowledgement** : This work was supported by the Bio Technology Research Center in Al-Nahrain University.
**Table (1): antibacterial activity of some compounds**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Zone of inhibition in millimeter</th>
<th>Escherichis coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>IV</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>VIII</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>IX</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>X</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

**Table (2): The inhibition of cells growth of some compounds µl/well**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>inhibition of cells growth for MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>53.1%</td>
</tr>
<tr>
<td>IV</td>
<td>0%</td>
</tr>
<tr>
<td>VII</td>
<td>31.1%</td>
</tr>
<tr>
<td>VIII</td>
<td>10.1%</td>
</tr>
<tr>
<td>IX</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


8- Qingqing H, Tiange C, Qianwen L, Yinghong

Investigating Effects of *Salvia Officinalis* (Sage) on Development of Mice Embryos Kidney and Some Hormonal Effect of Treated Mothers

Ban Thabit Al- Ani1, Rana R Al Saadi2, Raad Ghazi Reshan3

1High Institute of Infertility Diagnosis and ART’s, Al Nahrain University, Baghdad, Iraq

**Abstract**

**Background** *Salvia officinalis* (sage) is commonly employed in both medicinal and culinary preparations, *Salvia* plants are antioxidant and can improve ‘head and brain’ function.

**Aim of study**: To examine effects of aqueous plant extract of sage on development of mouse embryos kidney and hormonal effect of treated mothers.

**Material and Method**: Thirty adult albino Swiss female mice were used in this experiments study, their weight was 23-27 gm, three groups with 10 mice per group were categorized as:

G(A) orally treated with sage aqueous extract (167.8) mg/kg, G(B) administration orally with sage aqueous extract (83.9) mg/kg, G(C) orally treated with distilled water (control group) for 6 weeks. For histological study, the pregnant female mice have been sacrificed at day 17 of gestation of each group and 30 mice embryos were fixed in Bouin’s fixative, paraffin infiltration. Specimen stains with H&E. After 24 hours from last orally dose of treatment period, 3 animals from each groups were sacrificed under ether anesthesia, 3 ml blood sample was collected from each animals, serum reproductive hormonal assay of FSH, LH, E2 and P4 were performed depends on kit assay procedure of ELISA kit.

**Result**: Histological examination of embryonic kidney after maternally oral administrated of *sage* reveals, different histopathological changes of embryonic renal tissue, also results revealed an increase that is highly significant (*P*< 0.01) in the numbers of fetuses belongs to G(A) and G(B) mothers at day 17 in comparison with G(C). Statistical analysis revealed in contrast with G(C) a highly significant (*P*< 0.01) rise in weights of fetuses (males and females) belonged to mothers of G(A) and G(B), while, serum hormones level show highly significant (*p*≤0.01) rise in E2 and P4 level in G(B) when compared with G(A), while significant decrease (*P*<0.05) in FSH level in G(A) and G(B), and there was a significant rise (*P*<0.05) in LH hormone level in G(A) and G(B).

**Keywords**: *Salvia officinalis* (sage), aqueous extract, mice embryo.

**Introduction**

In the past years, *Salvia officinalis* (sage) Medicinal plants were regarded as the only form of healthcare accessible to the mainstream of human population (1). Likewise, numerous plants was empolyed as sex stimulants in classical medicine (2). It contains steroid and flavonoid such as genistein, daidzein and coumestrol (3) that are recognized as phytoestrogens. The metabolites of phytoestrogens act an estrogenic effect on central nervous system which stimulates cell division and growth of the genital tract of female animals (4). *Sage* is similarly employed internally in the treatment of night sweats, excessive lactation, profuse perspiration (as in tuberculosis), excessive salivation (as in Parkinson’s disease), depression, anxiety, menopausal problems and female sterility (5) (Fig.1), *Sage* likes being a panacea; it was employed as sexual debility; to treat mental and nervous conditions for menstrual and menopausal problems (6). There are numerous bioactive compounds existing in this plant, like terpenes and phenolic compounds (7)and (8), that are believed to be the key for...
various therapeutic properties accredited to sage\(^{(9)}\). 

\textit{Sage} have antioxidant activities may be therapeutically helpful and it was revealed that the antioxidant potential of plants due to their phenolic components \(^{(10)}\). Nevertheless, their impact on preimplantation embryos does not have reasonable amount of studies to examine such impact \(^{(11)}\). The estrogenic activity of \textit{sage} was demonstrated experimentally, the herb has strong effects in cases of oligomenorrhea and amenorrhea \(^{(12)}\). Therefore, it can be used as premenstrual tension, cysts, pelvic congestion fibroids, irregular (or heavy menses), and endometriosis for hormone imbalances, and it is not permitted to use in pregnancy \(^{(13)}\).

\textbf{Figure (1): Sage (\textit{S. officinalis})} \(^{(5)}\)

\section*{Materials and Method}

\subsection*{Preparation of Plant Extracts}

Plant extracts were done according to \(^{(11)}\). Leaves were air-dried and powdered using coffee grinder. Using soxhlet apparatus and water bath, 50 gm. of the leaf powder were removed for 3 H in 250 ml of the solvent (distilled water) at 45°C. Via a rotary evaporator, the leaf extract solution was evaporated at 45°C and the resulting crude cutting was held at -20°C.

\subsection*{Sage Doses and Concentrations:}

Aqueous plant extract was assessed in a mammalian model (albino mouse). Two doses of sage extract were used (83.9 or 167.8 mg/kg). These doses were correspondent to 10 and 20%, respectively of the dose in mice.

\subsection*{Laboratory Animals:}

Albino Swiss female mice were used in the experiments. Their age and weight at the beginning of experiments was 6-8 weeks, and 23-27 gm, respectively. Mice were grouped, each of which was kept in a distinct plastic cage. The mice were preserved at a temperature of 23 – 25°C, and had free access to food (standard pellets) and water.

\subsection*{Animals Groups:}

Effects of two doses (83.9 and 167.8) mg/kg of sage extract (aqueous) with control were investigated. Therefore, animals were grouped into three groups according to treatment procedure:

\textbf{GA}: Orally with the aqueous extract of sage (83.9) mg/kg (10 animals) for 6 weeks.

\textbf{GB}: Orally with the aqueous extract of sage (167.8) mg/kg (10 animals) for 6 weeks.

\textbf{GC}: With distilled water (control = 10 animals) for 6 weeks.

\subsection*{Blood sample collection and hormonal assay:}

After 24 hours from last orally dose of treatment period, 3 animal from each group G (A), G (B), G (C) sacrificed under ether anesthesia, blood was collected from the animals in each group by cardiac puncture used disposable syringe of 5ml, blood samples were collected in a centrifuge tubes for maximum coagulation and separation of serum, after centrifuged at 3000 rpm/10minutes, serum was isolated, frozen at (-20°C) and then processed for hormonal assays. The serum reproductive hormonal assay of E2 and p4 were performed depends on kit assay procedure of ELISA kit (Germany).

In the metestrus phase, females remained with mature healthful males for mating (1 male/ 2 female). Vaginal plug incidence regarded as the initial day of pregnancy \(^{(14)}\) the following days were consecutively counted. The pregnant female (PF) was extracted into separate cages.

When PF in G(A), G(B) and G(C) reach day 17 of gestation, animals undergo abdominal incision, plenty of died and alive fetuses in each horn were reported. Each fetus was washed and weighted for all groups. The embryos were stable in Bouin fixative and paraffin and implanted sections were stained with eosin and hematoxylin, two pathologists unaware of the plant and the dose individually read and reviewed the specimens.
Results

Daily administration of either 83.9 mg/kg b wt. as G(A) and 167.8 mg/kg B wt. as G(B) of Salvia officinalis (sage) to female mice for 6 week until day 17 of gestation cause some changes including:

A- Weights changes: significant rise \( (P < 0.01) \) in fetuses weights (males and females at day 17 of gestation) in G(A) and G(B) in comparison with G(C) as showed in (Table 1). Differences recorded between body weight of males and females fetuses at right and left uterine horns in G(A) and G(B) administered mothers. These results showed that female’s weights were higher than the males. These differences lacked significance (Table 1).

B - Numbers of fetuses: important rise \( (P < 0.01) \) in the numbers s of fetuses belongs to G(A) and G(B) mothers at day 17 in comparison with G(C) (Table 2).

C- Level of serum hormones: The result of hormonal level show a highly significant \( (p \leq 0.01) \) increase in E2 level in G(B) in contrast with G(A) as show in (Fig.2), Similar results seen in P4 level in G(B) in contrast with G(A) (Fig.3).

D - Histological Observations including:

1- Control group (fetal kidney)

The embryonic kidney of the control group displays the histological features of the normal renal tissue (Fig.4).

2- Treated group (Histopathological Changes of kidney fetus)

Fetus kidney shows that, glomeruli appeared shrunken, swollen with wide, narrow capsular spaces (CS) respectively (Fig.5). The convoluted tubules showed some abnormal changes in its histological organization; Nuclei of its epithelial cells were pyknotic (P) with condensation in chromatin material. Also, an evidence of degeneration in glomeruli (G) of Bowman’s capsule (BC) was recorded (Fig.5).

Table (1): Changes in fetal body weight at day 17 of gestation belongs to G(A) and G(B) treated with Salvia officinalis (sage) as compared with GC (Mean± S.E.).

<table>
<thead>
<tr>
<th>Fetus at Day 17</th>
<th>Doses of Salvia officinalis (sage) (Mg/kg b. w.)</th>
<th>Weight of fetuses(g) Mean± S.E.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Among groups</td>
</tr>
<tr>
<td>Weigh of fetuses</td>
<td>control</td>
<td>0.471±0.038</td>
<td>( \leq 0.001** )</td>
</tr>
<tr>
<td></td>
<td>G(A)</td>
<td>0.41±0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G(B)</td>
<td>0.34±0.009</td>
<td></td>
</tr>
</tbody>
</table>

NS= no statistical significant difference.

* =Statistically significant difference \( (P<0.05) \).

** =Highly statistically significant difference \( (P< 0.01) \).
Table (2): Changes in numbers of male and female fetuses in left and right horns of uterus belongs to pregnant mice of G(A) and G(B) at day 17 of gestation as compared with control G(C) (Mean± S.E.).

<table>
<thead>
<tr>
<th></th>
<th>G(A)</th>
<th>G(B)</th>
<th>Control G(C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of males</td>
<td>No. of Females</td>
<td>No. of males</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
</tr>
<tr>
<td>Left</td>
<td>16</td>
<td>33.33%</td>
<td>19</td>
</tr>
<tr>
<td>Right</td>
<td>32</td>
<td>66.66%</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>100.00%</td>
<td>57</td>
</tr>
<tr>
<td>Total male &amp; Female fetuses</td>
<td>105</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>P value</td>
<td>0.833</td>
<td></td>
<td>0.771</td>
</tr>
</tbody>
</table>

Figure (2): Effect of *Salvia officinalis* (sage) on Estrogen level (E4) in female mice serum.

Figure (3): Effect of *Salvia officinalis* (sage) on Progestrone (P4) level in female mice serum.

Figure (4): This slide show fetus Kidney of control group G(C) , cortical region containing, a glomeruli (G) within Bowman’s capsule (BC) and both proximal (PT) and distal (DT) tubule.

Figure (5): This slide shows fetus treated kidney at day 17 of pregnancy , histopathological changes of glomeruli tuft , decrease and increase in capsular space of Bowman’s capsule due to swollen and shrinkage in glomeruli respectively (G) , nacrosis (asters) in the proximal (PT) and distal tubule (DT) capsular space (CS), Bowman’s capsule (BC). (H&E, A400X, B1000X, C200X).

Discussion

The daily administration of aqueous extract of *salvia officinalis* as 83.9 mg/kg b wt. as G(A) and 167.8 mg/kg B wt. as G(B) orally to pregnant female mice showed a significant reduction in fetal weight, these result agreed with that found by Evans *et al* (15) who proved that infants born to mothers with prenatal exposure to steroids had an increased risk of low birth weight. This reduction because *Salvia officinalis* contain steroid, isoflavones, and isoflavonoids (13) The mechanism of decreased fetal growth because progesterone possess both membrane and cytoplasmic receptors in a numerous reproductive and non-reproductive tissues including the brain and is a powerful inhibitor of GnRH for that exogenous progesterone may disturb the normal hormone work and interfere with normal genital development in fetal reproductive tract as demonstrated by Mari S (16).

In experimental groups G(A) and G(B), there were a highly significant decrease in fetuses number at day 17 of gestation and at birth time, this result explained on bases that progesterone make it harder for fertilized ova to be linked to the walls of the uterus, resulting in a lower probability of pregnancy as shown by Nagel (17).
Moreover, there are some differences in numbers of female fetuses which was higher in comparison with the numbers of male fetuses in G(A) and G(B), these results are due to that sex hormones powerfully impact the functional and anatomical organization of the sex organs, muscular and skeletal structure, and central nervous system (CNS), therefore is a major contributor to sexual discrepancy (18).

After 6 weeks of administration of *silva officinalis*, preovulatory secretion of P4, even though restricted, can apply positive feedback on the estrogen-primed pituitary to enlarge LH release (19). This phytoestrogen that is operating at high or low concentration disturbs diverse target tissues and performs as agonist or antagonist to E2. Phytoestrogens have ability to affect rudimentary cell biology to their impact on cellular enzymes, in addition to their aptitude to impact growth factors, instance, cytokines, and to control genes apply antioxidant actions even at a low level as shown by (20). The Corpus Luteum secretes E2, P4, and inhibin, as documented by Knobil (21). The estrogens use negative feedback at both low and high concentrations. On the other hand, progestins are operational just at high concentration (22).

Kidneys at second and third trimesters typically have the same configuration as in postnatal life, their appearance depend on the gestational age at normal configuration help in early diagnosis and optimize fetal safety then reduce the high prenatal morbidity and mortality (23).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


Association among Vitamin D Deficiency with some Inflammatory Marker in Iraqi Patients with Autoimmune Thyroiditis

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1College of Nursing University of Baghdad/ Baghdad, Iraq, 2College of biotechnology/Al-Nahrain University / Baghdad, Iraq, 3College of Nursing /University of Baghdad/ Baghdad, Iraq

Abstract

Background: Hashimoto thyroiditis (HT) is a common chronic autoimmune disease of the thyroid gland, characterized by painless goiter and elevated thyroid antibodies. Hypovitaminosis D (vitamin D insufficiency and deficiency) is common among patients with HT.

Method: The study include (60) subjects compared with 30 apparently healthy control group were visiting nursing home hospital in Baghdad medical city, Levels of serum thyroid-stimulating hormone (TSH), free-triiodothyronine (FT3), free thyroxine (FT4), TgAbs, and TPOAbs were determined with automated immuno chemiluminescent assay (ICMA) kits (Abbott Laboratories, IL, USA). Levels of serum 25-hydroxyvitamin D 25(OH) D) were determined using a competitive protein-binding assay (Roche Diagnostics, Mannheim, Germany). The inter-assay variation coefficient for 25(OH)D measurement was 8.5%.

Results: The level of the study parameters in autoimmune thyroiditis which show significant correlation in age ,BMI, vitamin D, anti TPO antibody,IL-17, TNF-α and thyroid function (p<0.05) (p<0.01) test but calcium show non significant correlation between patients and control group.

Conclusion: The present work shows a significant association between circulating 25(OH)D and HT, also IL-17.

Keywords: Vitamin D; autoimmune thyroiditis; Iraqi patients

Introduction

Autoimmune thyroid disease (ATD) is the most prevalent endocrinopathy (1). The prevalence of ATD was as high as 60% of patients, 40% of whom additionally suffered from thyroid disorders including overt hypothyroidism (24%), subclinical hypothyroidism (8%), and hyperthyroidism (8%) (2). According to Krzewska et al. the ATDs Hashimoto’s thyroiditis and Graves’ disease, are the most prevalent autoimmune diseases in children and adolescents. The autoimmune pathology in clinical practice seems a difficult task for endocrinologists, with many unknowns. Modern laboratory diagnosis with high sensitivity allows the detection of autoantibodies in autoimmune thyroiditis (AIT) and the monitoring of a wide spectrum of markers of the immune status of patients for diagnostic research and treatment (3).

Hashimoto thyroiditis (HT) is the most common chronic autoimmune thyroid disease characterized by painless goiter and elevated serum thyroid antibodies. HT may emerge with stimulation of environmental factors in genetically susceptible individuals. Predisposing genes are human leukocyte antigen (HLA), cytotoxic T lymphocyte antigen-4 (CTLA-4), protein thyrosin phosphatase non-receptor type 22 (PTPN22) and thyroglobulin (Tg) genes (4). In recent years, vitamin D deficiency is reported to cause autoimmune diseases. Vitamin D receptors (VDR) were shown to be present in intestinal epithelium cells, osteoblasts, renal cells and most importantly immune system cells (T lymphocytes, monocytes, dendritic cells and also B lymphocytes). Vitamin D inhibits T lymphocyte proliferation, particularly T helper 1 (Th1) lymphocytes. It may increase T helper 2 (Th2) lymphocyte formations. Role of vitamin D in pathogenesis of HT is reduction...
of anti-inflammatory Th lymphocytes and elevation of inflammatory Th1 cells. T helper 17 (Th17) cells were also found to be associated with HT pathogenesis (5).

25-hydroxyvitamin D [25(OH)D] is involved in the regulation of many physiological processes in the body and beneficial effects related to treatment with cholecalciferol were noted in immunodeficiency, cardiovascular disorders, anemia, diabetes, various pathologies of the liver, and gastrointestinal tract disorders, as well as for tuberculosis and malignant tumors of the breast and intestine (6,7). Both genetic predisposition and environmental factors may contribute to the development of autoimmune diseases. Increased levels of immune inflammation markers may affect the course of immuno-endocrine pathology. A gradual development of the autoimmune inflammatory process, when combined with hypothyroidism and DM, may significantly contribute to the development of endothelial dysfunction and the consequent development of vascular complications (8).

Recently, Th17 cells and their hallmark cytokine (interleukin, IL)-17 have been recognized as crucial contributors to the pathogenesis of thyroid autoimmunity (9). Production of IL-17A and IL-17F is characteristically attributed to Th17 cells. These ILs can act on a broad range of cells, including epithelial cells, fibroblasts, and macrophages inducing the release of proinflammatory tissue mediators such as interleukins IL-1β, IL-6, and IL-8, growth and hematopoiesis stimulating factors – tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor, and granulocyte colony-stimulating factor (G-CSF), and tissue components degrading enzymes metalloproteinases (10). Early studies demonstrated that IL-17 stimulates production of other cytokines such as IL-6, IL-8, and G-CSF in nonimmune cells such as connective tissue fibroblasts and epithelial cells via activation of the nuclear factor-κB (NF-κB) transcription factor (10).

In addition, IL-17 demonstrates strong synergic action being combined with other cytokines, such as IL-1β and TNFα. Previous studies have shown that patients suffering from AITD have enhanced levels of IL-17A and Th17 lymphocytes revealed in blood and colonizing thyroid tissue as well as a marked in vitro differentiation of Th17 cells (10).

Methods and material:

The study included (60) subjects compared with 30 apparently healthy control group were visiting nursing home hospital in Baghdad medical city between October to April 2019. The diagnosis of Hashimoto thyroiditis was made on the basis on clinical examination, and Anti TPO antibody in all of patients.

The Demographic and clinical variables were obtained from participant report and electronic medical records. Demographic include age, sex, and body mass index (BMI). BMI was calculated as weight (kg)/squared height (m²). A fasting morning venous blood sample was obtained from each participant. Levels of serum thyrodstimulating hormone (TSH), free-triiodothyronine (FT3), free thyroxine (FT4), TgAbs, and TPOAbs were determined with automated immuno chemiluminescent assay (ICMA) kits (Abbott Laboratories, IL, USA). Levels of serum 25-hydroxyvitamin D (25(OH)D) were determined using a competitive protein-binding assay (Roche Diagnostics, Mannheim, Germany). The inter-assay variation coefficient for 25(OH)D measurement was 8.5%.

Inclusion Criteria

The age range samples were taken between 18–45 years for two groups and without any chronic condition other than thyroid are included in this study.

Exclusion Criteria

The criteria were excluded from this study including: bone and muscle cardiac disease, pancreatic, hepatobili, diabetes, hypertension, malignancy, oral contraceptive pills (OCP), and pregnancy.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. The standard deviation was used to significant compare between means. The correlation coefficient between difference parameters in this study was estimated.

Results

Table(1) show the level of the study parameters in autoimmune thyroiditis which show significant correlation in age, BMI, vitamin D, anti TPO antibody, IL-17, TNF-α and thyroid function (p<0.05) (p<0.01) test but calcium show non-significant correlation between patients and control group.
Table 1 shows the level of the study parameters in autoimmune thyroiditis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD panties</th>
<th>Mean ± SD control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(year)</td>
<td>31.5±8.2</td>
<td>39.4±10.2</td>
<td>0.00096*</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>29.9±2.2</td>
<td>27.03±2.7</td>
<td>0.00023*</td>
</tr>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>11.15±4.35</td>
<td>35.16±4.2</td>
<td>0.00001**</td>
</tr>
<tr>
<td>Anti TPO antibody (IU/ml)</td>
<td>173.58±19.43</td>
<td>13.69±1.5</td>
<td>0.00001**</td>
</tr>
<tr>
<td>IL-17 (pg/ml)</td>
<td>2.23±0.7</td>
<td>1.48±0.44</td>
<td>0.00001**</td>
</tr>
<tr>
<td>TNF-α(pg/ml)</td>
<td>10.81±1.69</td>
<td>6.5±1.6</td>
<td>0.00001**</td>
</tr>
<tr>
<td>TSHmIU/mL</td>
<td>3.5±1.17</td>
<td>1.42±0.12</td>
<td>0.00001**</td>
</tr>
<tr>
<td>FT3 ng/L</td>
<td>0.9±0.3</td>
<td>7.0±1.5</td>
<td>0.00001**</td>
</tr>
<tr>
<td>FT4 ng/L</td>
<td>4.75±2.3</td>
<td>9.8±1.5</td>
<td>0.00001**</td>
</tr>
<tr>
<td>PTH(pg/ml)</td>
<td>45.17±5.7</td>
<td>40.89±2.1</td>
<td>0.000195*</td>
</tr>
<tr>
<td>Calcium mg/dl</td>
<td>8.7±1.5</td>
<td>9.07±0.46</td>
<td>0.168664NS</td>
</tr>
</tbody>
</table>

* (P<0.05), ** (P<0.01), NS: Non-significant.

Table 2. Correlation coefficient between vitamin D, anti-TPO, TNF-α, IL-17 and other parameters

<table>
<thead>
<tr>
<th>The group</th>
<th>Correlation coefficient(r)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vit. D Anti TPO TNF-α IL-17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.0141 -0.1416 0.1268 -0.0547</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.0437 0.0873 -0.2315 -0.3268</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH</td>
<td>-0.3024 -0.2948 -0.426 0.2143</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>0.0712 0.1379 0.0185 -0.049</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>-0.1353 0.079 0.1797 -0.0246</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>-0.0606 0.1245 0.1797 0.2484</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>-0.374 0.198 -0.1773 0.0659</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit. D</td>
<td>----- 0.1094 0.1848 -0.2689</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti TPO</td>
<td>0.1094 ----- -0.1321 -0.0988</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.1848 -0.1321 ----- 0.1883</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-17</td>
<td>-0.2689 -0.0988 0.1883 -----</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Hashimoto’s thyroiditis (HT) is the most prevalent autoimmune disorder characterized by the destruction of thyroid cells caused by leukocytes and antibody-mediated immune processes accompanied by hypothyroidism. In recent years, evidence has emerged pointing to various roles for vitamin D, including, proliferation and differentiation of normal and cancer cells, cardiovascular function, and immunomodulation. Vitamin D deficiency has been especially demonstrated in HT patients (11).

Our results show the level of the study parameters in autoimmune thyroiditis which show significant correlation in age, BMI, vitamin D, anti TPO antibody, IL-17, TNF-α and thyroid function (p<0.05) (p<0.01) test but calcium show non-significant correlation between patients and control group.

In recent study The results of this study showed a significant reduction of anti-Tg Ab (anti-thyroglobulin antibodies and) TSH hormone in the Vitamin D group compared to the start of the study; however, there was a no significant reduction of anti-TPO Ab in the Vitamin D group compared to the placebo group (p=0.08). No significant changes were observed in the serum levels of T3 and T4 hormones. Therefore, vitamin D supplementation can be helpful for alleviation of the disease activity in HT patients (12).

Recently, the receptors of 1,25-dihydroxyvitamin D have been found on many cells, including immune cells(13).The expression of 1α hydroxylase (cy27B1) activity in many of these cells supports(14-16) the idea that the Vitamin D has immunomodulating effects. Several genetic studies have shown an association between gene polymorphism of Vitamin D receptor and of 1α hydroxylase (cy27B1) (17-20) with autoimmune thyroid diseases (AITDs).

However, the relationship between Vitamin D and AITDs is still a controversial issue (21). Many studies have already pointed out a relation between low concentration of Vitamin D and AITDs; (22-25) however, the cause and effect relationship is not known. The findings of one prospective case-control study were performed in Amsterdam(26) and did not support the association between low Vitamin D level and early stages of thyroid autoimmunity.

Kivity et al. in a cross-sectional study compared the level of Vitamin D in patients with AITDs, non-AITDs, and healthy people. They showed that the prevalence of Vitamin D deficiency was significantly higher in patients with AITDs than healthy persons. The rate of Vitamin D deficiency was also higher in patients with non-AITDs than healthy population.(27)

Shin et al. in Korea studied the association between Vitamin D and TPO-Ab in patients with and without AITDs. They demonstrated that there was a significant negative correlation between Vitamin D level and TPO-Ab in patients with AITDs. (23)

In a study in Turkey, Bozkurt et al. studied the relation between Vitamin D deficiency and Hashimoto’s thyroiditis. They compared the level of Vitamin D and severity of Vitamin D deficiency between euthyroid patients with Hashimoto’s thyroiditis and a healthy control group. Their results pointed out that there was correlation between severity of Vitamin D deficiency and thyroid volume, level of thyroid autoantibodies as well as duration of Hashimoto’s thyroiditis. They concluded that Vitamin D may have potential role both in development and progression of Hashimoto’s thyroiditis to hypothyroidism. (24)

In a study in Tehran, Iran, Mansournia et al. reached to the similar conclusions. Furthermore, they showed that by 5 ng/mL increases in the level of Vitamin D, the risk of occurrence of Hashimoto’s thyroiditis would be decreased by 19%.(28).

On the other hand, some studies did not support the association between Vitamin D and thyroid autoimmunity (29-32). Effraimidis et al. have conducted two case–control researches in the framework of Amsterdam AITD cohort study to determine the association between Vitamin D and early stages of thyroid autoimmunity. In one cross-sectional study, they compared the concentration of serum Vitamin D between euthyroid participants with genetic susceptibility for AITDs and negative thyroid antibodies and controls who were healthy women without family history of AITDs.

Although Th1-driven autoimmune response has long been considered as dominant for HT development, recent studies are suggesting an evident participation of Th17 cells in AITD, particularly HT pathogenesis.
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Funding: Self-funding

References


A Statistical Study on Microorganisms that Cause Tooth Decay and Prevention and Treatment Methods

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Abstract

The current research includes knowledge of the effect of microbes on dental caries, by providing the environment suitable for the growth of bacteria and fungi that cause this decomposition, fermentation and decay and thus erode the texture of the tooth and then full tooth decay. These are the macrophages that lead to tooth decay (streptococcus species, Staphylococcus aureus, Candida, Lactobacillus, Actinomycosis, Micrococcus, Antibacterials, Bacillus, Actinobacillus) in different percentages: 33.3%, 21.3%, 14.7%, 12.1%, 8.2%, 4.5%, 3.4%, 1.3%, 1.2%), respectively. There are many other factors that help tooth decay occur: the host (tooth and saliva), microorganisms in the form of tooth plaque, substrate (diet).

Keyword: microbes , tooth decay, Prevention and Treatment.

Introduction

Dental caries is an infectious microbiological disease of the teeth that results in localized dissolution and destruction of the calcified tissues. It is the second most common cause of tooth loss and is found universally, irrespective of age, sex, caste, creed or geographic location. Saliva has a cleansing effect on the teeth. Normally, 700–800 ml of saliva is secreted per day. Caries activity increases as the viscosity of the saliva increases. Eating fibrous food and chewing vigorously increases salivation, which helps in digestion as well as improves cleansing of the teeth. The quantity as well as composition, pH, viscosity and buffering capacity of the saliva plays a role in dental caries (¹, ²). Dental caries is one of the most common preventable diseases which is recognized as the primary cause of oral pain and tooth loss. It is a major public health oral disease which hinders the achievement and maintenance of oral health in all age groups (³).

Dental caries refers to the localised destruction of susceptible dental hard tissues by acidic by-products from the bacterial fermentation of dietary carbohydrates.

It is a chronic disease that progresses slowly in most of the people (⁴), which results from an ecological imbalance in the equilibrium between tooth minerals and oral biofilms (plaque) (⁵). Caries is an etiologically complex disease process. It is likely that numerous microbial, genetic, immunological, behavioral, and environmental contributors to risk are at play in determining the occurrence and severity of clinical disease. Assessment tools based on a single risk indicator are therefore unlikely to accurately discriminate between those at high and low risk. Multiple indicators, combined on an appropriate scale and accounting for possible interactions, will certainly be required.

Caries may be characterized by the experience of pain, problem with eating, chewing, smiling and communication due to missing, discolored or damaged teeth. The microbial community of caries is diverse and contains many facultatively and obligately-anaerobic bacteria belonging to the genera Actinomyces, Bifidobacterium, Eubacterium, Lactobacillus, Parvimonas and Rothia (⁶). It can also be caused by other bacteria, including members of the mitis, anginosus and salivarius groups of streptococci, Propionibacterium, Enterococcus faecalis, Scardovi, Prevotella, Selenomonas, Dialister, Fusobacterium, Pseudorambacter, Veillonella, Atopobium, Granulicatella, Leptotrichia and Thiomonas (⁷-¹⁰).

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Bacteroides, Prevotella, and Porphyromonas species are prevalent on mucosal surfaces and reach very high concentrations in dental plaque, gingival crevices and tonsillar crypts (7).

Traditional approaches to the treatment of dental caries have focused on repairing the consequences of the disease (cavities) rather than the disease itself. From our perspective, person-centered approaches, such as individual risk assessment, active surveillance, oral health literacy, and preventive interventions/therapies, supplemented, when necessary, by surgical care (drilling, filling, extraction) are the essential evidence-based approaches for the effective management of this disease. We see that factors such as fear, total and reimbursed costs, provider availability, transportation, and even parent or caregiver characteristics, including financial distress, depressive symptoms, and limited social networks, can be barriers to care (11). Recently, nanotechnology has emerged in the treatment and protection of teeth, such as nanopowder technology and nanotechnology for metals, which has become a major concern for scientists and researchers.

Materials and methods of work

First\ Materials:-

All diagnostic and general plant media, used reagents and solutions are manufactured by a company (Mast and Oxoid).

Second\ methods of work:-

1. Collection of samples

A total of 40 mouth and teeth swabs were collected for patients that have dental caries in the Dental Unit at Manpuole Hospital in Andrapradesh city, Hyderabad State, India, during (April and May of 2014).

2. Isolation and diagnosis

Samples were growth directly on pre-prepared culture media and laboratory tests were performed to diagnose the bacteria based on the methods of (smith, 2002) and (colle and et al, 1969)(12), according to the following diagnostics:-

A) Cellular diagnosis:

This was done by examining the pigmented dye of gram to determine the shape and assembly of bacterial cells and the nature of their interaction with the dye.

B) culture diagnosis:

It ensures the diagnosis of the shape and size of bacterial colonies and their effect on the general and specific agro-culture and diagnostic communities.

C) Biochemical tests:

Diagnosis of bacteria is based on their ability to produce enzymes and their biochemical effectiveness.

3. Statistical analysis:

The results of isolation and diagnosis were analyzed statistically using the Kay square test (Daniel, 1978)(13).

Results and Discussion

The percentages of germs isolated from oral infections showed that streptococcus species represented (33.3%) , followed by Staphylococcus aureus species (21.3%) , while the Bacillus and Actinobacillus species represented (1.4% ,and 1.5%) respectively. While the rest of the isolates recorded different ratios of germs as shown in the table (1)

Table (1): Proportion of germs isolated from oral infections

<table>
<thead>
<tr>
<th>No.</th>
<th>Types of germs</th>
<th>Percentages of germs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>streptococcus species</td>
<td>33.3%</td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td>21.3%</td>
</tr>
<tr>
<td>3</td>
<td>Candida species</td>
<td>14.7%</td>
</tr>
<tr>
<td>4</td>
<td>Lactobacillus species</td>
<td>12.1%</td>
</tr>
<tr>
<td>5</td>
<td>Actinomyces species</td>
<td>8.2%</td>
</tr>
<tr>
<td>6</td>
<td>Micrococcus species</td>
<td>4.5%</td>
</tr>
<tr>
<td>7</td>
<td>Antrobacteriacea species</td>
<td>3.4%</td>
</tr>
<tr>
<td>8</td>
<td>Bacillus species</td>
<td>1.3%</td>
</tr>
<tr>
<td>9</td>
<td>Actinobacillus species</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

In general, the results of this study were consistent with previous , (Saini, 1999) , (Socransky, 1970) such as (Gibbons ,et al. 1946) (Carlsson, 1967) (14-16). The prevalence and spread of germs of various kinds in the oral cavity, which leads to tooth decay, especially in the
case of negligence and non-cleaning of the teeth and lack of care, which provides a catalyst that leads to loss of age and seek compensation instead of because the presence causes the mouth to disease including tissue damage and even cancer, which finds the right environment to occur easily. Tooth decay is so widespread that many people do not treat it with the proper seriousness. It is common, for example, lack of interest in the injury of children tooth decay in milk teeth. However, dental caries can lead to complications and complications of serious and far-reaching, even in children whose teeth have not yet developed. Among these complications. Abscesses, Abscess in the teeth, tooth loss, broken teeth, chewing problems, Acute infections. In addition, when dental caries reaches a stage where the aches are very severe, this may interfere with normal daily life, to the extent that it prevents the student from going to school or to work. If the aches are severe and hinder the process of eating or chewing, they may lead to malnutrition and weight loss. If decay leads to tooth decay, this may negatively affect self-confidence. In some very rare cases, abscess caused by dental caries may lead to severe contamination that may endanger the patient’s life if not properly treated. The percentage of pathogens that cause oral diseases varies depending on the type of the isolation and method of infection.(17).

Dental plaque is a thin, tenacious microbial film that forms on the tooth surfaces. Microorganisms in the dental plaque ferment carbohydrate foodstuffs, especially the disaccharide sucrose, to produce acids that cause demineralization of inorganic substances and furnish various proteolytic enzymes to cause disintegration of the organic substances of the teeth, the processes involved in the initiation and progression of dental caries. The dental plaque holds the acids produced in close contact with the tooth surfaces and prevents them from contact with the cleansing effect of saliva.(18, 19).

A Biological Factors of dental caries:-

There is interaction and cooperation between a group of factors that are active and help to prevent tooth caries, and these factors are the conditions that provide suitable and controlled in the incidence of this disease, We can epitomize it up with the following:-

1-Host (teeth and saliva):(20, 21)

A\ Tooth:

Composition:- Deficiency in fluorine, zinc, lead and iron content of the enamel is associated with increased risk caries. Morphological characteristics:- Deep, narrow occlusal fissures, and lingual and buccal pits tend to trap food rubbish and bacteria, which can cause caries. As teeth get worn (attrition), caries declines.

   Position:- The interdental areas are more oversensitive to dental caries. Malalignment of the teeth such as crowding, abnormal spacing, etc., can increase the susceptibility to caries.

B\ Saliva: (22, 23)

Saliva has a cleansing effect on the teeth. Generally, 700– 800 ml of saliva is secreted daily. Caries activity increases as the viscosity of the saliva increases. Eating fibrous food and chewing vigorously all them increases salivation, which helps in digestion as well as lead to cleansing of the teeth. The quantity as well as composition, pH, viscosity and buffering capacity of the saliva plays an important role in dental caries.

   • Quantity: Reduction of salivary secretion as found in xerostomia and salivary gland aplasia gives rise to increased caries activity.

   • Composition: Inorganic fluoride, chloride, sodium, magnesium, potassium, iron, calcium and phosphorus are inversely related to caries. Organic ammonia retards plaque make up and neutralizes the acid.

   • pH: A neutral or alkaline pH can neutralize acids created by the action of microorganisms on carbohydrate food substances.

   • Antibacterial factors: Saliva keep enzymes such as lactoperoxidase, lysozyme, lactoferrin and immunoglobulin (Ig)A, which can inhibit plaque bacteria.

2-Microorganisms in the form of dental plaque: (24, 25)

Dental plaque is a thin, tenacious microbial film that forms on the enamel layer. Microorganisms in the dental plaque ferment carbohydrate foodstuffs, especially the disaccharide sucrose, to produce acids that cause demineralization of inorganic substances and furnish various proteolytic enzymes to cause disintegration of the organic substances of the teeth, the processes implicated in the initiation and progression of dental
caries. The dental plaque conclude the acids produced in close contact with the tooth surfaces and prevents them from contact with the cleansing action of saliva.

3-Substrate (diet): (26, 27)

The role of reiterated carbohydrates, especially the disaccharide sucrose, in the aetiology of dental caries is well established. The total amount consumed as well as the physical form, its oral clearance rate and frequency of consumption are important factors in the aetiology. Vitamins A, D, K, B complex (B6), calcium, phosphorus, fluorine, amino acids such as lysine and fats have an inhibitory effect on dental caries. So, caries requires a susceptible host, cariogenic oral flora and a suitable substrate, which must be present for a sufficient length of time.

Prevention and control of dental caries:

Increase the resistance of the teeth.(20, 21)

Systemic use of fluoride:
1- Fluoridation of water, milk and salt.
2- fluoride supplementation in the form of tablets and lozenges.
3- consuming a fluoride-rich diet such as tea, fish, etc.

Topical:
1-Use of fluoridated toothpaste and mouth wash.
2-use of fluoride varnishes (in-office application, longer duration of action, high fluoride content).
3-use of casein phosphopeptide–amorphous calcium phosphate (CPP–ACP), which is available as tooth mousse, helps to remineralize the soft initial carious, demineralized areas of the teeth.

Treatment of dental caries

Treatment comprises removal of decay by operative procedures and restoration with appropriate materials such as silver fillings, gold inlays, composite resin, glass ionomer cement, full metal or porcelain crowns, etc. In advanced cases, where the pulp of the tooth is involved, endodontic treatment may be required. Where there is extensive destruction of the tooth structure or when endodontic treatment is not feasible, extraction of the tooth and replacement by an artificial prosthesis may be required.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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References


In vitro Antifungal efficacy of *Saccharomyces Cerevisiae* Mediated Nanoparticle Against Resistant *Candida Albicans* Isolates

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Abstract

Dry yeast *Saccharomyces. cerevisiae* was obtained from markets in Kirkuk and used to study their ability to prepare silver nanoparticles and shows that the silver nitrate solution at 1Mm changes its color to brown and is an initial indication of the formation of nanoparticle particles and X-ray diffraction peaks were measured at (111), (202) and (220) respectively. Particles also appeared in clear minutes in the scanning electron microscopy images at a size of 44 nanometers. A total of 103 vaginal swabs were taken from women who suffered from vaginitis from the Kirkuk General Hospital. The results revealed that Candida types were responsible for 67% of the cases. *Candida albicans, C.glabrata, C.tropicalis, C.krusei* and *C.parapsilosis* were isolated. 30.8%, 22.8%, 20%, 15.8% and 11.5%, respectively. In addition, the sensitivity of the drug in the drilling method against the isolates of Candida albicans showed high resistance to fluconazole and gresiofulvin antagonists, with 100% of gresiofulvin (85%) of fluconazole. For nystatin, Resistance ratios (14%) The solution of the nano-silver particles recorded from *Saccharomyces Cerevisiae* yeast was also shown. had a significant inhibitory effect on Candida albicans, a multi-resistance yeast antimicrobial resistance (21,21,22mm) at 100% and (10,13,10mm)at 50% and inhibition(5, 4, 5 mm) has a 35% dilution and a 25% concentration did not receive any inhibition.

**Keyword:** *Saccharomyces cerevisiae*, silver nanoparticles, *Candida albicans*

Introduction

Nano science is the science that is interested in studying and characterizing materials ranging from 100 to 100 nanometers apart, as well as assigning their chemical, physical and mechanical properties to study related phenomena arising from their small size. Nano science is also known as nanoscale science because it studies materials in the atomic or molecular ladder, which differ significantly from the properties of the same materials in a larger scale. Biological and chemical nanoparticles can be obtained from bacteria and fungi that have the ability to produce nanoparticles with antimicrobial properties. Yeast microbes are the oldest beneficial microorganisms used by humans for the purpose of fermentation and bread making since ancient times. Archaeologists in Egypt, during their excavation in archaeological areas, found grinding stones, bread fermentation chambers, as well as drawings of bakeries and ferrets dating back 4000 years. The first to be observed through the microscope was the scientist Anthony van Levenhoek (1680), but at that time he did not describe it as a living organism, but rather the first German form of Fleishman, the first to isolate yeast (*Saccharomyces. cerevisiae*). Are widely used industrially in fermentation production of alcohol and calcioir. Saccharomyces means saccharine. This type of yeast has two types: *S.cerevisiae* and *S.boulardii*, which are used in the manufacture of bioreactors. These two types of non-pathogenic species were thought to be the first type of species, but it was later identified as a new species.

Vaginitis is one of the most common problems in women of fertile age. There are more than one cause of this type of inflammation such as bacteria, viruses, parasites, chemical and other chemical factors, but the most common causes are yeast *Candida genus*. The other normal microflora in the mucous membranes play a role in limiting their growth and formation, such as the
Lactobacilli in the vagina that inhibits the adhesion of candida to the vaginal epithelial cells(7).

Material and Method

Collect sample

103 samples were collected from Kirkuk General Hospital (vaginal swabs) By a sterile speculum as it was inserted into the vagina and the sample was taken from the cervix by a sterile swab and sent directly to the laboratory.

Direct test

The samples were examined directly by making two slices, the first one was Smear, as it was sprayed on the glass slide and gram-stain to observe the positive yeast cells of this dye The second slice was placed with a drop of the swab of the swab solution and tested under the force (x40) after placing the lid of the slide to observe the white blood cells WBC and epithelial cells .

Laboratory culture

After the vaginal swabs were transferred to a laboratory directly planted on the center of the malt extract agar, three replicates of the sample were incubated under 37 ° C for 24 hours. Yeast growth was examined and secondary cultivar of the dishes showed positive result. The dishes which showed no growth Considered negative (6).

Diagnosis of candida spp

To examine the growth of the yeast cell well by preparing a slice of the colony and pigment chromium with the observation of the virtual shape of the colony and then conducted the following tests(8).

- Potassium nitrate reductase test
- Urea by drolysis test
- Germ tube production test
- Test the production of Chalmyspores
- Carbohydrate fermentation test
- sensitivity of fungal antibiotics test

A sensitivity test was occurred by spread method in the solid medium and according to (9) the use of 6 types of antifungal processed by Oxide.

Preparing a yeast culture and producing nanoparticles

Saccharomyces cerevisiae yeast was obtained dry from the market and then prepared a pure culture by preparing a culturing medium consisting of (1% Yeast extract, 2% Peptone and 2% of Dextrose) YPD broth in flask 100 ml and heated at 35° in 48 hours and then centrifuge at 10,000 cycles in one minut For 10 minutes, Then use supernatant solution in silver nanoparticles synthesis. .

We take the supernatant and put it in a 1000-ml flask and then add g17 silver nitrate to 100 ml of supernatant yeast And then i was put in completely dark conditions and after the discoloration the nanoparticles formation were examined by X-RD , ( Shimatzu) in physics department collage of science Baghdad university and SEM (TESCAN.VEGA) in university of technology . Iraq (Nanotechnology and Advanced Materials Research Center)to detect the size and the shape of the particles.

Preparing for soluation of nanoparticles

Use the solution of silver particles after filtering from the yeast using the sterile filter (filter paper No 1) and sterilize by the exact filtration unit 0.22mm and attended the following stacks:

First concentration: Contains 1mM of AgNPs solution and is 100% concentrated

The second concentration was attended by adding 5 ml of the initial solution in 5 ml of sterile distilled water to become the concentration to 50%.

The third concentration was attended by the addition of 3.5ml of the initial solution and diluted in 6.5ml of distilled water to a concentration of 35%.

The fourth concentration was attended by 2.5 ml of the initial solution and diluted in 7.5ml of distilled water to a concentration of 25%

Results and Discussion

Isolation and Diagnosis

The current study has shown that 70 isolates to candida spp yeasts by studying some of the apperance and microscopic characteristics and biochemical tests as follows:
Characteristics of fungal colony

The colonies appeared smooth, convex, creamy white, and characteristic yeast, and the cells were spherical or oval and the presence of spores and pseudohyphae\(^{(10)}\).

Potassium nitrate reduction test and analysis of urea

The result of these negative tests of *Candida albicans* as shown in table (2) testing the fermenting and the representation of carbohydrates (sugars)

The test results showed a fermentation gas in a tube for positive and also change color from violet to yellow centered. Table (2) representation of carbohydrates the show of growth around the hole topic by sugar and change color from violet to yellow is a positive result.

Growth properties test on liquid medium

Use this test to diagnose types of *candida spp* through surface growth characteristic table 2

Test production Germ tube

This test is a rapid diagnosis of *Candida albicans* yeast which showed a combination filament arose from yeast cell.

Chlamydospores Production test

This test was used to disperse the yeast *Candida albicans* from other types of *candida*, which showed spherical cells with a thick wall.

<table>
<thead>
<tr>
<th>Type of Candida spp</th>
<th>reduce KNO3</th>
<th>Type of Candida spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu Gala Lac Suc Glu Lac Suc Tre Raff ss</td>
<td>growth Surface production Germ tube analysis urea</td>
<td></td>
</tr>
<tr>
<td>- v - - + - + + - +</td>
<td>+ - + - -</td>
<td>C.albicans</td>
</tr>
<tr>
<td>- + - - + + - -</td>
<td>- - - - -</td>
<td>C.glabrata</td>
</tr>
<tr>
<td>- - + + + - -</td>
<td>- + - - -</td>
<td>C.krusei</td>
</tr>
<tr>
<td>- + - + + - -</td>
<td>- - - - -</td>
<td>C.parapsilosis</td>
</tr>
<tr>
<td>- + - + + - -</td>
<td>- + - - -</td>
<td>C.tropicalis</td>
</tr>
</tbody>
</table>

According to the tests mentioned above, *Candida albicans* yeast was 21%, followed by *C.glabrata* with 16 isolates, 22.85%, and *C.krusei*, 14 isolates, 20%, *C.parapsilosis*, 11 isolates, 15.71% and *C. tropicis*, 8 isolates. Or 11.42%.

Formation of silver nanoparticles AgNPs

The formation of nanoparticles was observed through indicators as follows:-

Visual observation

The results of this study showed a silver nano particles are AgNPs using yeast *S.cerevisiae* (image 1) through show color change resulting from the reduction of silver nitrate AgNO3 this color shift caused by raising surface of alblasmon (Foundation of this vibration is electron delivery group) Silver nano particles\(^{(11)}\).

X-Ray Diffraction

(Fig. 1) shows the X-ray diffraction spectra of the nanoparticles (AgNPs) prepared using the yeast of *S.cerevisiae*. We show the peaks of diffraction (111), (202) and (220) at angles (38.20, 48.34 and 63.43) The angles are adjacent to the angles mentioned with the card of the International Center for Diffraction Data (JCPDS) and are consistent with\(^{(12,13)}\).
Scanning electron microscopy

Figure 2 shows the scanning electron microscope images of the nanoparticle particles formed by the S. cerevisiae yeast, which appear in clusters that confirm the formation of nano-silver particles.

Antifungal susceptibility test

The antifungal sensitivity of Candida albicans isolates was studied using 5 types of fungal antifungal solutions, and the results were determined by measuring Zone of Inhibition as shown in Table (3) based on. The results showed that there was a difference resistance of isolates under study to antifungal used isolates showed high resistance to fluconazole and gresiofulvin with 100% resistance and 85% to fluconazole. Clotrimazole resistance was 71%, Ketoconazole (23%). In contrast, nystatin was the best treatment option with 14% resistance.

The results indicated relative resistance to most of the yeast isolates under study for most of the antibiotics used. Nystatin was one of the most potent antifungal with the highest inhibitory due to the fact that nystatin inhibits the important Ergosterol synthesis in the cell membrane of the yeast. Inhibition between (25-17) mm This result is consistent with the outcome of the which found that nystatin was one of the best inhibitor in the growth of candida. Ketoconazole antifungle ranged from 22 to 16 mm in diameter with a clear effect, with 77% of isolates yeast. Clotrimazole was the third in terms of the ability to inhibit the growth of candidiasis, and explained that this antibody is effective in inhibiting the growth of Candida.

Fluconazole was the fourth most important inhibitor of the growth of the types of Candida under study. The rate of inhibition was 18-11%. This result may be due to the large number of antibiotic use, as well as the type of resistance these isolates possess against most of the antibiotics used.

Grisofulvin was the last to inhibit the growth of the types of candida under study. Inhibition of 100% was observed. Grisofulvin had no effect on Candida yeast. There was no inhibiting growth in the concentrations used.

Table (2) represents the resistance and sensitivity of Candida albicans to the antibiotics used

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Chloramphenicol</th>
<th>Ceftriaxone</th>
<th>Clindamycin</th>
<th>Ciprofloxacin</th>
<th>Linezolid</th>
</tr>
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</table>

Table (2) represents the resistance and sensitivity of Candida albicans to the antibiotics used
Effect of AgNPs on Candida albicans Resistance:

Antimicrobial activity of AgNPs has been verified against antimicrobial resistance by diffusion method. The diameter of the inhibition zone in millimeters around each hole was determined at the level of different concentrations of NPs. The study showed that the silver nanoparticles produced from S. cerevisiae yeast had high efficacy on both isolates (3), (11) and candida albicans respectively resistant to antifungal agents as shown in Table (3).

In the 100% concentration, the inhibition diameter (22, 21.21 mm) against the three isolates with multiple resistance to antifungal resistance and 50% dilution (10,10,13 mm) While he has 35% loss reached diameter inhibition (5, 4, 5). The concentration of 25% did not get any discouragement and that the small size of the nanoparticles and the large surface area where the smaller size collected in larger numbers on the surface of the cells leading to increased toxicity of microorganisms through its effect on the permeability of the plasma membrane and thus cell death (19, 20).

The mechanism in which nanoparticles interact with yeast cells is that microorganisms carry negative charges while nanosynthetic oxides carry a positive charge, creating an electromagnetic attraction between the yeast and the surface of the minutes. The nanoparticles release the ions that interact with the thiol- Which increases the permeability of the membrane and thus cell death.

<table>
<thead>
<tr>
<th>Resistance Candida albicans isolates</th>
<th>Concentration of nanoparticles produced from Saccharomyces cerevisiae %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of the inhibition zone for isolation(19mm)</td>
<td>Diameter of the inhibition zone for isolation(11mm)</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
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<tr>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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**Reference**

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Application of Chemical Techniques in the Manufacture of Some Industrial Dental Materials

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Abstract

This method of manufacturing Separating Medium was used in the field of chemical polymerization, in which a semi-gelatinous liquid was placed on the dental kits to prevent the adhesion of the gypsum material to the dermis made of hot processed polymer. After the experiments on the plant seeds, specifically the flax plant, the final extraction of the substance has been reached. It has given all the desired good specifications that are used in the field of dentistry. It is known that this material is a polar compound was diluted with distilled water after preparation. It is worth mentioning that 10% of the industrial pigments have been added to give them the red or pink color as these colors are desirable and common in the commercial markets as well as It is recommended for the dental products manufacturers, as well as 10% of the original alcohol and 1% of formalin to prevent the growth of bacteria and other types of microorganisms that cause rotting and decomposition of the manufactured material.

Keyword: Superating Medium, Chemical Techniques, Extractions.

Introduction

It is important to know the importance of polymers in our daily lives and the extent of their applications in all areas in the individual, as well as their importance in many fields of industrial and medical. Doctors are not familiar with a variety of polymer materials. The use of polymers in the field of dentistry in many of the scientific experiments conducted by the researchers, by selecting the choice of dental adhesive and other materials of the other teeth, including the manufacture and production of the insulating material between the layers of plaster and acrylic work stage dental mold synthesis. It is possible to make this choice, more specifically, if we can clarify the mechanical, characteristics of various dental adhesives. The main objective is to establish strong bonds for remaining age and for composite white fillings, which are usually some other polymers. They resemble self-etch adhesives in composition as they contain acidic monomers(¹), and are characterized by chemical interaction with dentin hydroxyapatite(²,³).

Linum usitatissimum belongs to the Linaceae family, an important flowering plant grown all over the world. Although this species consists of more than 100 species, L. usitatissimum has long been considered a valuable agricultural plant due to the production of oils And other synthetic and medical products . Traditionally, flax products and compounds have been prescribed to treat many chronic human diseases such as diabetes, high cholesterol, cardiovascular disease and other related conditions .There are two types of flax (flax fiber and flax seed) which are widely used nowadays. A significant trend has been observed in reducing the area of flaxseed in favor of flaxseed in some countries in recent years and because of the ever-increasing importance of flax as a plant used in many chemical, pharmaceutical, food and animal feed industries. Oilseed crops (⁴). Flaxseeds are used as a diet and a preventive enhancer, and have been used for a long time in both gastrointestinal diseases (constipation, inflammation of the bowel, colon, gastritis) and respiratory diseases (cough and dyspnea). This type of plant has also been used in the industry, because its seeds contain a high percentage of organic fibers which made it used in the manufacture of paper, ink, cloth and fishing nets, as well as in the manufacture of soap and oils that Working on hair (⁵). A separate medium is defined as representing

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a coating layer formed on a surface that prevents the second surface from sticking to the first surface (6). This layer is a medium that rises above the surface of the material such as the plaster, metal or wax to prevent adhesion between the denture And other materials that are in contact with him during preparation (7). And also used to seal all the pores in the stone model without adding any additional materials (8). Separating media are materials that used for filling any porous surface in order to effect easy separation of other materials which are later poured against them. Therefore the acrylic resin must be carefully protected during processing from the gypsum surface in the mould spaces for two reasons: 1-Any water incorporate in to the resin from the gypsum during processing will defined affect the polymerization rate and the cold of the resin: the denture procedure will craze readily of water after the processing: particularly if the resin is not cross-linked. 2-Disolved polymer and free monomer must be prevented from soaking in to the investing medium, portions of gypsum material will be joined to the denture after polymerization: with result that it will be virtually impossible to separate the investing material from the resin (9).

Application

Materials and Method

The amount of 100 grams of flax seed is taken and the quantity is boiled in 5 liters of distilled water and 100 degrees Celsius. The boiling process lasts from half an hour to an hour. The longer the boiling period, the more the solution becomes somewhat thick or semi-gelatin. The mixture is heated through sieves for a liquid that is seed free and free from impurities, and then leaves the solution to cool down. After that, the amount of formalin and 1% of the solution is added to the solution and 10% of the ethyl alcohol is added to prevent the growth of microorganisms from bacteria, fungus and others. Then we put (0.001) of the red industrial pigments and mix the solution well in order to homogenize to obtain a liquid substance that is similar in specifications, shape and work to the insulating material. And add varying proportions of polymer solution suitable for use as a mixture to give a liquid or gelatin to meet the required specifications that lead to the purpose we are looking for. After the manufacture of the material, a sample of hot acrylic was prepared. Using the usual plastic technique, 3ml of the insulating material, using a small brush, was placed on the plaster containing the gypsum material (prepared by the dental kit). Then the acrylic sample was placed and pressed with 30 pascal. (15-60 °C). After the cooling process, the mold was opened and it was observed that the acrylic material was not attached to the stone material. Their success and efficiency It is known that flaxseeds are available in the market, cheap and easy to prepare, and the possibility of storage for a long time without decomposition, used in this research is a natural plant material gives good results in the separation of gypsum material from the kit. The teeth are made of acrylic, and have no negative effects on the mechanism of the work of dental molds. Through the preparation of the mold, and the traditional technique follows its steps, the bottom of the tooth flask is filled with a dental stone according to the manufacturer’s instructions: W / P is 25ml / 100g; a layer of stone mixture is placed on the metal mass to avoid air retention. Insert the metal block into the stone mixture after coating with separate media. It is known that after the stone was laid, both stone and metal patterns were coated with separate media. The top half of the flask is placed at the top of the bottom part and the stone is packed, and with continuous stirring, to get rid of the trapped air. The stone was allowed to harden for 60 minutes before opening the flask. Then the bottle and the metal are opened. The patterns are removed from the mold carefully and carefully. The material is then wiped with a soft brush.

Results and Discussion

Polymers are materials made from long, repetitive chains of molecules. These materials have unique properties, depending on the type and how they interconnect. The purpose of the extraction phase is to obtain the desired component and leave the unwanted components aside, which are the residues of the seeds from which the material was extracted. This extraction should be done well and sufficient to obtain a material of desirable specifications and highly efficient in the formation of the crust necessary to prevent the adhesion of gypsum material to the dentures. This extraction process is done by heating the seeds used (flax seed) with distilled water to 100 degrees Celsius. The boil is then extracted from the seeds and the boiling continues from half an hour to an hour. This response to the extraction was obtained by the presence of water as a polar compound and the fact that flaxseed is a plant component, which is considered a complex carbohydrate, so it decomposes with water and by the temperature as a catalyst for the period mentioned above because these seeds are surrounded by a protective layer cannot access water easily. The normal situation. (10%, 15%,
The optimum concentration of the polymer mixed with the extract was 20%, giving the desired result in the separation between the acrylic and stone. If the polymer was used alone in this separation process, it did not give the result to be obtained, as well as the extract if we used it alone. The result is not the same as after mixing. Therefore, the extract and the polymer used should be mixed with this ratio because it gives the physical and The chemical that qualifies it to be usable as a buffer separating the gypsum and acrylic layers. The measurements taken are as shown in Table (1). It is not surprising that we get liquid synthetic material such as the insulation of plant seeds such as flaxseeds. Purifying the desired component of the “desired material” after its removal from the unwanted component (seed) is very important because it ultimately gives us a transparent liquid slightly tilted to a homogeneous, semi-gelatinous, polarized color that can be diluted by ordinary water. Gives the red or pink color desired when the dental laboratory owners any color of the dental kit. Pigment additives, dyes or colored materials are the basic material where the product acquires attractive colors or may act as light or oxidative protective materials and other physical and chemical properties (9). After obtaining the required liquid “flaxseed extract” mixed with the polymer, the molded gypsum material is coated with a soft brush and left for at least five minutes, then the acrylic material prepared to form the tooth kit is then applied to special equipment and subjected to temperature ranging from (60 - 80) for half an hour and harden in the end. Here, after the opening of the molds, we saw that the gypsum material was not attached to the mold. This is the secret to success in the experience of manufacturing this material, which is of great benefit. However, if the insulating material is not applied, this gypsum material will stick to the denture, making it difficult to remove it, distorting the kit and not properly and accurately imprinting it in the patient’s mouth, as well as transferring water molecules from the gypsum material in the mold to the dental kit during the process of sclerosis. Poor specifications and not good research The water molecules in the kit appear in clear bubbles and this is evidence of its lack of validity. The selection of these flaxseed seeds in our current research is not random, but is done through scientific research and laboratory experiments. This is why chemical industries start with simple raw materials obtained from natural sources such as mines, forests, agricultural fields, , In addition to the most abundant material in nature which is the water that cannot be established or the duration of any vital effectiveness without it and it is agreed that one of the most important goals of science, scientists, scientific researchers and industry workers is to harness their science creatively to develop new technology A new material “utilized in the industrial and medical fields, and the use of raw materials improved and in most cases in developed countries find new technology protection it by registered Kpraouat invention and discoveries as the discoverers true for these materials and save their rights periods specified time by a special (10). In this research, a comparison was also made between the insulating material extracted from the flaxseed and other good insulation materials available in the market. The Indian “ISOLIATE” model was selected in comparison. The two models were used to show that the proportion of gypsum materials adhering to the dental prosthesis is very small compared to The original article. Some characteristics of both materials were measured for comparison purposes and as shown in Table (1):

The table (1): shows the comparison of the chemical properties of the insulating material (manufactured by extraction and polymers) and the Indian product (ISOLATE).

<table>
<thead>
<tr>
<th>Chemical Properties</th>
<th>ISOLATE) Type)</th>
<th>Extraction Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.D.S</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Conductivity</td>
<td>2.62</td>
<td>3.17</td>
</tr>
<tr>
<td>PH</td>
<td>8</td>
<td>6.55</td>
</tr>
</tbody>
</table>

However, when the table is observed, soluble salts are found to be non-existent in both substances. However, the conductivity and pH function of the Isolate insulation material is slightly higher than that of flaxseed. This little difference is not affected by the work of the insulating material in the formation of the cortex that separates the two electrodes when used. This discrepancy is shown in Figure (1):
Figure (1): Shows the comparison between the Indian and the extract in terms of turbidity, conductivity and acid function, respectively.

It is known that the manufacture of separating medium, which is used in the manufacture of teeth from flax seeds, works to produce a polar compound diluted with normal and distilled water. This material creates a layer of thin crust that prevents the adhesion of gypsum material to the dentures made of hot acrylic treated. It is possible to have a separating medium with a transparent yellowish color if a distinctive dye is added to it from an industrial, not a pharmaceutical, substance.

**Conclusion**

The main objective of this study is to obtain local products that enrich the markets with manufactured materials inside the country and not to import foreign materials as much as possible, in order to promote the economy of the country. Therefore, the country is satisfied with this material which is reached in scientific and industrial ways and applied practically in all Laboratories and dental clinics. It is a realistic study in which plants invest, specifically flax plants, which are mixed in calculated proportions with polymer solutions. Therefore, a high quality industrial material is produced with high efficiency.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**

Isolation and Identification of *Pantoea agglomerans* from Open Heart Operations Unit of Marjan Hospital in Hilla City

Enas R . Al-Kidsawey¹, Kawther H. Mhdi¹, Mohammed A. Al kaif³

¹Hilla university college/Ministry of higher Education and Scientific research/Iraq

Abstract

The nosocomial infection of *Pantoea agglomerans* has become an important cause of many systemic infections in humans, and considered of present study was to isolate and identify this pathogen from open heart operation unit of Marjan hospitals in Hilla city during a period from April to September 2018, (19) isolates of *Pantoea agglomerans*. Was isolated from (150) swaps which were collected from the environmental of open heart operation unit. 8/30 isolates from system, 3/35 from bed, 3/25 from earth, 3/35 from door and 2/25 from nurse hands isolated identification of microbes were done according on the morphological, cultural, microscopically characterization and list of biochemical test. To emphasize the identification of *Pantoea agglomerans*. We used the API20 technique biochemical strips (biomerieux SA/marc y-1 Etoile France).

The antimicrobial susceptibility pattern of the isolates was studied using different types of antibiotics. The microbes showed resistance to the routinely used antibiotics.

**Key Words:** *Pantoea agglomerans*, characteristic, Isolation, nosocomial, hospital.

Introduction

*Pantoea agglomerans* is a Facultative anaerobic gram negative bacteria, previously named *Erwinia herbicola* (¹) or *Enterobacter agglomerans*, motile with peritrichous Flagella non spore forming, commonly found in environment such as water, soil, dust, air, sewage, seeds of vegetables and food. As well as reported as both commercial and opportunistic pathogens of animal and humans (²). *Pantoea agglomerans* is an opportunistic microbes can be isolated from different sites of hospitals such as wall, door, bed, floor, clothes of nurse, and also could be isolated from clinical specimens including blood, wounds, urine, throat, and stool(³). *Pantoea agglomerans* is worldwide bacterium commonly isolated from plant surfaces, and many research has shown that *Pantoea agglomerans* can serve as a plant pathogen (⁴). It was identified as a nosocomial pathogen to human in mid-1960s. It could be a cause of opportunistic human infections, mostly by wound infection with plant material or by hospital-acquired infection especially in human with immune compromised (⁵). The survey for infective agent is very important to reduce the occurrence of infection with different types of microbes, therefore to prevent spreading opportunistic microbes and spreading drug resistant bacteria between the patients the CDC recommends use of contact isolation, precaution, enhanced environmental cleaning, dedicated patient care equipment and reduce using of antibiotics. This study mainly aims to isolation and identification and determination the prevalence of *Pantoea agglomerans* from the hospital environment of open heart operation in Marjan hospital and to recognize the antimicrobial susceptibility.

Materials and Method

One hundred fifty (150) swaps were collected from April to September 2018 from environmental locations of open heart operation of Marjan hospital Hilla city. The Swaps which was taken from system, bed, doors, earth and nurse hand, was moistened by normal saline (2 ml) of sample was placed in sterile tubes containing brain heart infusion broth (BHT), transporting by ice box to...
the laboratory (6), each sample was cultured in Luria Bertani (LB) agar by spreading methods, the plate were incubated at (37°C) for 3 days, the colonies which appeared after that were transferred to slants of the same medium, then the colonies were collected according to the morphological, characteristics appearance, agar surface and purified by culturing on nutrient agar plates, and identified with gram stain, and the main characters of colonies on solid medium such as shape, texture, and pigmentation for characterization the biochemical and physiological test, were used the isolates were maintained at (4°C) and cultured in LB medium. The biochemical tests used for identification depending on macroscopic motility tests: oxidase, catalase, indol, H₂S production, vogas proskanter (7; 8), for further identification various carbon sources used such as: glucose, xylose, mannose, Rhamnose, salicin, sucrose, Trehalose, maltose (9). The identification was emphasized by using API20E (Biomerieux SA/ marcy_1 Etoile France). The test done according to the manufactures direction, the systems were incubated at (35 °C) and the results were read after (20-24) hrs. Stored for long time by the identified isolates were culturing in tryptic soy broth (TSB) with 20% glycerol at -20°C (Merk co.).

Antibiotic Susceptibility

The antibiotic susceptibility test was done according Kirby-Baner (10) disc diffusion method by using Muller Hinton agar plate. the antibiotic used: ampicillin (10 Mg), carbenicillin (100 Mg), ceftazidiume (30 Mg), cefotaxime (30 Mg), imipenem (10Mg), streptomycine (10Mg), amikacin (30 Mg), gentamycine (10 Mg), tobramycine (10 Mg), pipracillin (100Mg), colistin (25 Mg), tetracycline (30Mg).

Results

The results in Table (1) are summarized the total of (150) environmental sample, the highest number of of Pantoea agglomerans was isolated from system 8/30 (26.6%) followed by earth 3/25 (12%), then bed samples 3/35 (8.57%), door 3/35 (8.57%) and 2/25 (8%) for nurse hand sample. p

Table (1): The number and percentage of Pantoea agglomerans isolated from different locations of open heart operation unit of Marjan hospital.

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of Sample</th>
<th>No. of Pantoea agglomerans</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>System</td>
<td>30</td>
<td>8</td>
<td>26.6</td>
</tr>
<tr>
<td>Earth</td>
<td>25</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Bed</td>
<td>35</td>
<td>3</td>
<td>8.57</td>
</tr>
<tr>
<td>Door</td>
<td>35</td>
<td>3</td>
<td>8.57</td>
</tr>
<tr>
<td>Nurse hands</td>
<td>25</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

The results showed that (19) isolates was isolated from different environmental locations of open heart operation unit of Marjan hospital were positive for catalase, simmon citrate, motile, negative indol, H₂S production and the other test.

The API20E test strips was used which emphasized that identification of (19) isolates have the same profile with systems indicate that the isolates are aerobic, ferment, galactose, lactose, glucose, arabinose, mannitol, inositol, rhamnose, maltose, melibiose and vogas proskauer was positive, indol negative, citrate positive.

The results of antimicrobial susceptibility was summarized in Table (2).

All 19 isolates of of Pantoea agglomerans showed high resistance toward most antibiotics which mean that the isolates have multidrug resistance (MDR) and they can adapted to antimicrobial agents, this process enable these pathogens to oppose the action of certain antibiotics remanding the antibiotics ineffective.

Table (2) Antibiotic susceptibility of Pantoea agglomerans. N= 19

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance %</th>
<th>Intermediate%</th>
<th>Sensitive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>16(84.21)</td>
<td>3(15.78)</td>
<td>-</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>15(78.94)</td>
<td>3(15.78)</td>
<td>1(5.2)</td>
</tr>
<tr>
<td>Ceftazidium</td>
<td>7(36.8)</td>
<td>2(10.5)</td>
<td>10(52.63)</td>
</tr>
</tbody>
</table>
**Cont.. Table (2) Antibiotic susceptibility of *Pantoea agglomerans*. \( N=19 \)**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotax</td>
<td>10(52.63)</td>
<td>4(21)</td>
<td>5(26.31)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>3(15.78)</td>
<td>3(15.78)</td>
<td>13(68.42)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>11(57.89)</td>
<td>3(15.78)</td>
<td>5(26.31)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>13(68.42)</td>
<td>2(10.5)</td>
<td>4(21)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>15(78.94)</td>
<td>4(21)</td>
<td>-</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>10(52.63)</td>
<td>2(10.5)</td>
<td>7(36.85)</td>
</tr>
<tr>
<td>Pipracillin</td>
<td>6(31.5)</td>
<td>5(26.31)</td>
<td>8(42.10)</td>
</tr>
<tr>
<td>Colistin</td>
<td>-</td>
<td>1(5.26)</td>
<td>18(94.73)</td>
</tr>
<tr>
<td>Tetracydine</td>
<td>-</td>
<td>2(10.5)</td>
<td>17(89.47)</td>
</tr>
</tbody>
</table>

**Discussion**

The results of our study showed that, the isolation of (19) isolates of *Pantoea agglomerans* on pure culture, All the isolated colonies of yellow pigmented with slightly concave centers (5mm) in diameter, The isolates are a Gram negative rod and partially hemolytic sheep red blood cells.

The phenotypic characteristic of the isolates agree with the characteristic of *Pantoea agglomerans* Which described in the bergeys mannal of determinative bacteriology (9,11).

The identification of isolated pathogen was done by using several biochemical tests which summarized in Table (2) which showed that the characteristic of *Pantoea agglomerans* (12).

The results of identification ensured by API 20 system technique.

Recently the microbes of *Pantoea agglomerans* Regarded as opportunist pathogen which cause severe infection when introduced in to system of humans or animals (13).

The infection caused by this pathogen often involve patient that are already by other origin disease (14).

There are a little information about *Pantoea* spp. Especially in Iraqi hospitals. The result of this study showed that the occurrence of *Pantoea agglomerans*. In the open heart operation in Marjan hospital in Hilla city. This results are very important to clarify the possible role of *Pantoea agglomerans*. As a nosocomial pathogen in common parts of hospital infection and regarded as a first report of this pathogen in such part of hospital, survey for infective agent is very important to reduce the infection with different type of pathogen. In recent years there is an increased in nosocomial infection especially in neonate intensive care unit (NICN) also in oncology department, these unusual microorganisms including *Pantoea agglomerans*. So the aim of present study was tried to isolate and determine the occurrence of *Pantoea agglomerans*. From hospital environmental of open heart operation in marjan hospital *Pantoea agglomerans*. Which isolated in this study showed multi resistant to many antibiotics used (15).

The antimicrobial resistance of *Pantoea agglomerans* are of great concern both in human and veterinary medicine wide world. Antibiotic resistance is a serious problem in the treatment of human and animal with microbial diseases (13). The antibacterial susceptibility test of *Pantoea agglomerans* Is done by disc diffusion method using the Kirby and Bauer technique (10) Table (2)

*Pantoea agglomerans* is an opportunistic pathogen and when in to human and animals organs caused severe diseases especially in fatal infection, the most serious are individuals with underlying diseases such as young Persons (16). Infection caused by *Pantoea agglomerans* is difficult to diagnosis. To our knowledge there is no previous report on the isolation and identification of *Pantoea agglomerans* from environmental of open heart operations of Marjan hospital. The results of our study showed that contamination of the environment unit with *Pantoea agglomerans*. These results is very important especially the isolated pathogens showed multdrug resistance to many antibiotics used in this study and have inherent capability to remain viable.
and grow well at room temperature because of this the contamination occurred in these parts of hospital.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


Immunological and Molecular Study of Human Cytomegalovirus contribution to Anemia in patients with Chronic Kidney Disease

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Abstract

Background: Renal failure is a condition in which the kidneys fail to remove metabolic end-products from the blood and regulate fluid, electrolytes and pH balance of extracellular fluids. The aim of current study was to investigate the role of human cytomegalovirus in renal failure and its contribution to anemia among patients with renal failure. Methods: This study was carried out at AL-Sadder Medical City Hospital during the period from December 2018 to February 2019. The study involved a total of 50 patients (32 males and 18 females) with kidney disease with age range between (20-70) years and 20 healthy individuals considered as controls. The human cytomegalovirus antibody were detected by cassette (rapid test) and compared the results of cytomegalovirus diagnosis (by rapid test) with molecular technique (Real time-Polymerase chain reaction ) results. Erythropoietin hormone concentration was detected by ELISA technique. Results: Data obtained from rapid test showed that positive patients for human cytomegalovirus were 32(64%) for all cases, while patients negative for human cytomegalovirus were 18(36%) compared with those of controls. Real time-Polymerase chain reaction amplification for presence of human cytomegalovirus DNA in serum samples revealed that human cytomegalovirus genome was detected in 10(20%) of the 50 serum samples. Erythropoietin hormone showed lower concentration in patients than controls. Erythropoietin showed significant decrease (P<0.05) in all groups of patients compared to those of control group. Conclusion: Human cytomegalovirus seemed to have relationship with chronic and acute renal failure and can affect patient’s immune status. Also, the decrease of erythropoietin hormone is the mean cause of anemia in renal failure patients.

Keywords: renal failure, Human Cytomegalovirus virus, Erythropoietin hormone, Real time-Polymerase chain reaction technique.

Introduction

Renal failure is a systemic disease and usually turns into a route cause for several kidney and urinary tract diseases. Renal failure induces a slow and progressive decline of kidney function enhanced by various factors including infections, diabetes, auto immune diseases, endocrine disorders, cancer and toxic chemicals [1]. The immunity of patients with hemodialysis becomes weak and this causes viral infections such as Human Cytomegalovirus (HCMV) [2]. It is commonly a result of complications arising from other serious medical conditions. Unlike acute renal failure, which happens speedily and suddenly, chronic renal failure occurs gradually (over a period of weeks, months or years) as the kidneys slowly stops working leading to an end-stage renal disease (ESRD) [3,4].

Anemia is a frequent complication during the later stages of chronic kidney disease. When present, it may cause symptoms such as fatigue and shortness of breath. The pathogenesis of anemia in chronic kidney disease is complex, but a central feature is a relative deficit of erythropoietin (EPO) [5]. The latter is a glycoprotein produced in the kidney under hypoxic conditions. It functions as the principal regulator of red blood cell production by controlling the proliferation, survival

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and differentiation of immature erythroid progenitors into mature red cells [6].

On the other hand, HCMV is one of the causes of inflammation in the kidney in the developed world; 40–60% of individuals are infected by time they reach adulthood, with seroprevalence approaching 100% in some populations [7]. Although initial HCMV infection is often asymptomatic in healthy individuals, it can cause severe and sometimes fatal disease in immune compromised individuals and neonates [8].

HCMV is one of the most common causes of birth defects resulting from an infectious agent, with 20% of congenitally infected infants exhibiting permanent neurological squeals including blindness, deafness and/or mental disability [9]. HCMV can also cause severe diseases in organ transplant recipients and AIDS patients after either primary infection or reactivation of a latent infection [10]. HCMV establishes a persistent infection, remaining silent in the host and undergoing productive reactivation cycles that contribute to its efficient transmission. HCMV infects and replicates in a wide variety of cells including epithelial cells of gland and mucosal tissues, smooth muscle cells, fibroblasts, macrophages, dendritic cells, hepatocytes and vascular endothelial cells [11,12]. The aim of current study was to investigate the role of human cytomegalovirus in renal failure and its contribution to anemia among patients with renal failure.

**Patients and Method**

This study was carried out at AL-Sadder Medical City Hospital during the period from December 2018 to February 2019. A total of 50 patients (32 males and 18 females) with kidney disease, with age range between (20-70) years, and 20 healthy individuals considered as controls were included. A 5-ml blood sample was collected from each participant. The blood samples were obtained by vein puncture from all patient after cleaning the skin with 70% alcohol, then blood left for about 30 minutes at room temperature, then they were centrifuged for 5 minutes at 3000rpm to separate serum and then transferred into other tubes. Serum samples were kept in capped plastic tubes and frozen at -20°C until being used. Each sample was labeled and given a serial number together with patients’ name.

**Immunological tests**

**CMV cassette rapid test:** The sample and test components were prepared to room temperature if they were refrigerated or frozen. The sample was mixed thoroughly before the test was performed, after which the cassette was placed on a clean surface. The test strip was numbered with the patient’s sample number. The pipette was filled with sample and the droplet retained. One drop (about 10µl) of the test sample was placed in each sample hole. The 2 drops (80µl) from the sample diluents were added directly to each specimen well. The results were read within 15 minutes.

**Molecular tests:** Sample extraction: (G- spin DNA extraction kit).

A 150-µl sample was added into microcentrifuge tube and a 570-µl volume of VNE Buffer was added into the sample and mixed well by vortexing and then incubated for 10 minutes at room temperature. A 570-µl volume of ethanol was added to the sample mixture and mixed well by plus-votexing. VNA Column was combined with collection tube and transferred up to 700ml of sample mixture to the VNE Column and then centrifuged at 8,000×g for 1min then discarded the flow-through and then combined with the VNA Column with used collection tube. The rest of sample mixture was transferred to the VNA Column and centrifuged at 8,000×g for 1min. A 500-µl volume of wash Buffer1 was added to the VNA Column and centrifuged at 8,000×g for 1min. A 750-µl volume of wash Buffer2 was added to the VNA Column and then centrifuged at 8,000×g for 1min. A 750-µl volume of wash Buffer2 was added to the VNA Column and then centrifuged at 8,000×g for 1min. Then centrifuge at full speed (18,000×g) for an additional 3min to dry the VNA Column and then discarded the flow-through and the Collection tube. The VNA Column was combined with elution tube and added 50µl of preheated RNase-free water to the membrane center of the VNE Column and then the VNE Column left to stand for 2min, centrifuged for 2min to elude the nucleic acid. Nucleic acids were stored at -70°C.

**Erythropoietin hormone assay procedure**

A 100-µL volume of dilutions of standard, blank and samples were added into the appropriate wells. Wells were covered with the sealing plate and incubated for 1 hour at 37°C. The time was started after the last sample addition. The liquid of each well was removed without wash. A 100-µL volume of detection reagent A was added to each well and they were covered with plate sealer and incubated for 1 hour at 37°C.
solution was aspirated and washed with 350μL of 1× wash solution to each well using a squirt bottle, and let it sit for 1~2 minutes. Then the remaining liquid was removed from all wells completely by snapping the plate onto absorbent paper. Totally washed 3 times and after the last wash, any remaining wash Buffer was removed by aspiration. The plate was inverted and blotted against absorbent paper. A 100-μL volume of detection reagent B was added to each well and the wells were covered with plate sealer and incubated for 30 minutes at 37°C. The aspiration/wash process was repeated for 5 times as conducted. A 90-μL volume of substrate solution was added to each well. Wells were covered with a new plate sealer, incubated for 10-20 minutes at 37°C and protected from light. The liquid will turn blue by the addition of substrate solution. Fifty-μL volume of stop solution was added to each well. The liquid will turn yellow by the addition of stop solution. The liquid was mixed by tapping the side of the plate. Any drop of water and fingerprint on the bottom of the plate were removed and confirmed there was no bubble on the surface of the liquid. Then, the microplate reader was run and conducted measurements at 450nm immediately.

**Statistical analysis**

All values were expressed as means ± SE. The data were analyzed using of SPSS (T test) version 23 and Microsoft Excel computerized programs. P value less than 0.05 was taken as the lowest limit of significance.

**Results**

Fifty patients with kidney failure, who referred to Central laboratory at Al-Sadder Teaching Hospital in AL-Najaf Governorate with age range of (20 to 70) years, were distributed according to gender 32(64%) males and 18(36%) females (Table 1).

**Table (1) Distribution of patients with renal failure according to gender**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Controls No.(%)</th>
<th>Patients No.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>10(50)</td>
<td>32(64)</td>
</tr>
<tr>
<td>Female</td>
<td>10(50)</td>
<td>18(36)</td>
</tr>
<tr>
<td>Total</td>
<td>20(100)</td>
<td>50(100)</td>
</tr>
</tbody>
</table>

**Age groups and percentages of the study groups**

In the current study, renal failure patients’ age ranged between (20-70) years. The current study showed that patients within the age group (20-30) years represented 18% of the study sample, while the age group (>50) years represented the highest percentage of 32%; (Table 2). In addition, result showed that 20% and 30% were within age groups (31-40) and (41-50) years, respectively, while the age of control group ranged between (20-60) years (Table 2).

**Table (2) Distribution of patients according to age groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age/yr</th>
<th>Controls No.(%)</th>
<th>Patients No.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>20-30</td>
<td>6(30)</td>
<td>9(18)</td>
</tr>
<tr>
<td>A2</td>
<td>31-40</td>
<td>6(30)</td>
<td>10(20)</td>
</tr>
<tr>
<td>A3</td>
<td>41-50</td>
<td>4(20)</td>
<td>15(30)</td>
</tr>
<tr>
<td>A4</td>
<td>&gt;50</td>
<td>4(20)</td>
<td>16(32)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20(100)</td>
<td>50(100)</td>
</tr>
</tbody>
</table>

**Detection of HCMV by rapid test**

Out of the 50 patients, only 32(64%) were positive for HCMV, whereas 18(36%) showed negative results for HCMV by rapid test (Figure 1).

**Figure 1 Serology of HCMV among renal failure patients.**
Detection of HCMV nucleic acid (DNA) by RT-PCR

The results of RT-PCR amplification for presence of HCMV DNA in serum samples (Figure 2) showed that HCMV genome was detected in 10(20%) of the 50 serum samples tested from patients suffering from renal failure with viral loads ranged from $(25 \times 10^2$ to $281 \times 10^4$) IU/ml (Table 3).

Table (3) Percentage of HCMV in serum samples by RT-PCR

<table>
<thead>
<tr>
<th>Results</th>
<th>Number of samples</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Negative</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure (2) Real-time PCR amplification.

Erythropoietin hormone detection with participants' gender

In the present study, there was a decrease in the level of erythropoietin (EPO) hormone concentration for patients with renal failure compared with control groups. The concentration of erythropoietin hormone in males (positive and negative for HCMV) decreased to (3.8905 pg/ml and 9.0727 pg/ml), respectively, compared with male controls (14.71 pg/ml), while the concentration of EPO in females (positive and negative for HCMV) decreased to (3.6909 pg/ml and 8.7286 pg/ml), respectively, compared with female controls (14.62 pg/ml) with significant difference ($P<0.05$; Figure 3).

Figure (3) Erythropoietin hormone concentrations according to participants' gender.

Discussion

The present study showed that the percentage of males (64%) with kidney failure was more than females (36%). There was no reason to give a response to another category in terms of infection, but the weakness of immune system may be the main cause of the disease. This result was agreed with the results of [13, 14, 15] who shown that males with renal diseases are more susceptible to infections than females, due to daily efforts of males as compared to females in addition to the increase in muscle mass in males than females leading to high proportion of creatinine in males and kidney damage. In this study, one of the common immunological techniques, cassette technique, was used. After the examination of samples of patients with renal failure and control samples, current results showed that out of 50 patients, only 32 (64%) were positive for HCMV, whereas 18(36%) showed negative results for HCMV by rapid test. The results were consistent with the results obtained by [16] who found the presence HCMV antibody in patients with kidney failure (83%) compared to control group. The proportion of positive serological test differed for this virus in patients with hemodialysis and that the cause of this variation was linked to the number of patients screened, the geographical distribution and sensitivity of the immunological techniques used to determine the Anti-CMV antibodies [17].

The results of current study were positive for human cytomegalovirus among the group of patients with renal dysfunction for males and females using one of the sensitive molecular techniques, RT-PCR. The results in this study reported that there was about 10(20%), out of 50, samples gave positive viral DNA existence and these results were in agreement with [18] who found that about 32(32.7%) of the samples tested gave positive results for viral genome and the difference in such subject areas may be ascribable to the sample type or in some case
disease type and stage\textsuperscript{[19]}. RT-PCR has been developed to detect HCMV because of its time-saving feature and high sensitivity and specificity\textsuperscript{[20]}. Current study showed a significant decrease in erythropoietin hormone, for both genders (negative for HCMV), responsible for the production and development of red blood cells and these lead to cause anemia in these patients, this result was in agreement with \textsuperscript{[21]}. The results showed that the level of EPO in renal failure patients (positive for HCMV) decreased more than in patients negative for HCMV, and this study was in agreement with \textsuperscript{[22]}. HCMV targets renal glomerular, vascular, epithelial, interstitial and tubular cells, including fibroblast-type cells in the renal cortex that activate EPO production in the setting of local tissue hypoxia and these lead to decrease EPO production and cause anemia.

**Conclusion**

Human cytomegalovirus seemed to have relationship with chronic and acute renal failure and can affect patient’s immune status. Also, the decrease of erythropoietin hormone is the mean cause of anemia in renal failure patients.

**Ethical Clearance:** The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

**Funding:** Self-funding.

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Comparison between HSP70 Levels in Acute and Chronic Coronary Artery Diseases

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Abstract

Background: Heat shock proteins are a family of endogenous proteins that act as molecular chaperon and increase in different stress situations like heart disease and atherosclerosis. Therefore, the aim of this study was to investigate whether there is a difference between Hsp70 level in the blood of myocardial infarction patients and apparently normal population; and between recent myocardial infarction patients and previous myocardial patients using immunocytochemistry technique. Method: Peripheral blood sample was taken from 50 patients with history of myocardial infarction divided into two groups (11 with acute or recent myocardial infarction and 39 with old or chronic myocardial infarction. Another 50 apparently healthy individuals were taken as a control group. Heat shock protein 70 level was measured by immunocytochemistry technique. Results: There was significant rise of heat shock protein 70 in myocardial infarction patients as compared with control group. Also, a significant decrease in heat shock protein 70 level in the chronic myocardial infarction patients group as compared with the acute myocardial infarction patients group. Conclusion: this study showed that heat shock protein 70 increases in acute myocardial infarction patients' but its level decreases in chronic myocardial infarction. So heat shock protein 70 can be used as a biomarker to differentiate acute, from chronic, myocardial infarction and may be helpful as an indicator of acute myocardial infarction.

Keywords: acute myocardial infarction, heat shock protein 70, chronic myocardial infarction, immunocytochemistry.

Introduction

Cardiovascular disease is one of the global public health problems contributing to 10% of the global disease burden and 30% of global mortality (1). Myocardial infarction (MI) is one of the five main presentations of coronary heart disease, which include unstable angina pectoris, stable angina pectoris, MI, heart failure and sudden death, and is defined as myocardial cell necrosis due to remarkable and sustained ischemia. It is usually an acute manifestation of atherosclerosis-related coronary heart disease (2).

Atherosclerosis is a widely accepted risk factor for coronary heart disease and a well-known inflammatory disease (3). In addition to inflammation in the coronary plaque, there may be myocardial inflammatory response after acute myocardial infarction (AMI) as part of the healing and scar formation processes (4).

Heat shock proteins (HSPs) are a family of endogenous proteins responsible for different types of stresses. They are classified according to their molecular weight into families, e.g. HSP27, HSP70, etc (5). They have the capability to act as ‘molecular chaperones, since they guide protein folding, stabilize macromolecules, carry out refolding and get rid of irreversibly denatured proteins in the cell (6). In addition, HSPs can be overexpressed in various stressful conditions such as hyperthermia (7), hemodynamic stress caused by heart diseases (6), physical exercise (8) and atherosclerosis (9). After cell death or stress, HSP peptide complexes may be presented on the cell surface or released to the

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circulation leading to activation of the adaptive immune system cells in addition to activating innate immune system, thus; signaling cell stress or damage to the immune system (10). Among HSPs, HSP70 is a powerful endogenous activator of the innate immune system working as a putative Toll-like receptor (TLR) ligand and is capable of stimulating the production of cytokines by macrophages (Figure 1) (11).

Multiple previous studies indicated and confirmed the protective role of HSP especially in myocardial ischemia (9, 12). Hsp70 may suggest important mechanisms of ischemic injury including misfolding, unfolding or pathological changes of critical proteins (13). Therefore, the aim of this study was to investigate whether there is a difference between Hsp70 level in the blood of myocardial infarction patients and apparently normal population; and between recent myocardial infarction patients and previous myocardial patients using immunocytochemistry technique.

Figure 1 Schematic representation of potential role of HSP in atherogenesis. Various stressors induce HSP production in the arterial wall, soluble HSPs bind to TLR complex resulting in proinflammatory responses and autoimmune reactions which contribute to atherosclerosis. TLR: Toll-like receptor). MQ: macrophages (9).

**Materials and Method**

This cross-sectional study was approved by Al-Nahrain College of Medicine Ethical committee. All participants gave their informed consents prior to their inclusion in the study. The study was carried out from September 2018 till 31st, December 2018. There were three groups of participants, their age ranged from 42-80 years, first group (group A) included 11 patients (7 males and 4 females) with acute myocardial infarction who were admitted to the Cardiac Care Unit (CCU) at Al-Imamain Al-kadhymain Teaching Hospital in Baghdad. The second group (group B) included 39 patients (33 males and 6 females) who were attending the outpatient clinic of Ibn –Albitar Hospital in Baghdad with chronic MI (more than 4 weeks from diagnosis of MI) (14). Third group (group C) of 50 apparently healthy individuals, who were age-, and sex-, matched to patients participants, were included as control group.

The diagnosis of patients was done by a specialist in cardiology based on clinical presentation and history of ischemic heart disease, which was confirmed by ECG, cardiac enzymes and coronary artery catheterization.

**Blood samples collection:** A 3-ml venous blood sample was drawn from each participant, for group A, within less than 24 hours from the myocardial infarction, for group B, after 4 weeks from MI diagnosis. The blood sample was immediately transferred to sterile heparinized
vacutainer tubes for lymphocytes separation.

After rehydration of smears, peroxidase block and protein block were added respectively. Primary antibody (US biological company) was added on smears (20ml of diluted mouse monoclonal Ab specific human CD marker) and incubated for 1 hour at 37°C, washed then incubated with secondary antibody and peroxidase conjugate and substrate-DAB chromogen complex were added respectively with buffer washing between each addition. Finally, the slides were counter-stained with hematoxylin, and then slides were covered with cover slide using mounting media. The slides were examined under 400x magnification power of light microscope (Olympus). The dark brown cytoplasmic or membranous staining of cells were considered positive (Figure 2), the percentage of positive cells was calculated as follows:

Percentage of positive cells = (No. of positive cells / total No. of cells)*100%.

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS, version 15.01). Data analysis was done using independent sample $t$-test for tables with means and standard deviations. $P$ value of ≤0.001 was used as the level of highly significance. Descriptive statistics for the clinical and laboratory results were formulated as mean and standard deviation.

### Results

HSP70 expression on PBLs was detected by immunocytochemistry technique. A comparison between patients (groups A and B) and control groups and between group A and B were summarized in Table 1 and Table 2, respectively. Independent sample $t$-test revealed a high statistically significant difference between patients and control groups ($P=0.001$). In addition, there was a high statistically significant difference between acute and chronic MI patients group ($P=0.000$) in expression of HSP70 on PBLs.

#### Table 1 Expression of HSP70 in PBLs by MI patients and control group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP-70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (group C)</td>
<td>19.571</td>
<td>8.751</td>
<td>2.339</td>
<td>0.001</td>
</tr>
<tr>
<td>Patients (groups A+B)</td>
<td>31.327</td>
<td>17.376</td>
<td>2.482</td>
<td></td>
</tr>
</tbody>
</table>

Highly significant difference (P≤0.001)

#### Table 2 Expression of HSP70 in PBLs by recent and old MI patients groups

<table>
<thead>
<tr>
<th>Patients group</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP-70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>57.909</td>
<td>10.134</td>
<td>3.055</td>
<td>0.000</td>
</tr>
<tr>
<td>Group B</td>
<td>23.632</td>
<td>9.646</td>
<td>1.565</td>
<td></td>
</tr>
</tbody>
</table>

Highly significant difference (P≤0.001)

### Discussion

HSP70 proteins are central components of the cellular chaperone system associated with folding, assembly, disassembly and degradation of proteins (16). During the last decades, a number of studies had suggested that stress-induced overexpression of HSP70 may confer protection against ischemia and reperfusion injury of the heart (17).

Previous studies had evaluated the role of Hsp70 in heart failure (18,19), but few of them had investigated its...
role in myocardial infarction and none had compared between recent and old MI.

Our study revealed that Hsp70 was significantly increased in peripheral blood of myocardial infarction patients in comparison with healthy control group, these findings were similar to (20) who had measured serum level of Hsp70 in 24 patients with acute MI at the arrival, 6 hours later and in the next morning using ELISA method and found that serum HSP70 in patients with acute MI were significantly higher than in the control group at all-time points and concluded that there was a relation between HSP70 and the extent of myocardial necrosis measured by the increase in cTnT and CK-MB (known markers of myocardial necrosis), as well as by typical echocardiographic findings. Our study agreed with (21) who had investigated synthesis of HSP70 in the human heart (in vivo) after CABG and found that the increase in HSP70 occurred after at least 2 hours of stress induction by CABG. Similarly (22) had examined the immunohistochemical expression and distribution of HSP72 after various periods of ischemia (from 30 minutes to 7 days) and reperfusion in the non-stress-pretreated rat heart. Moreover, (23) also studied the protective role of HSP70 by direct HSP70 gene delivery in rabbits hearts (in vivo) and inducing ischemic condition. They concluded that HSP70 reduced the size of infarcts and thus reducing the severity of ischemic injury. HSP70 was studied by (24) in atherosclerotic plaques in aorta of humans and rabbits through immunohistochemistry and they found that HSP70 staining was most pronounced in the central portions of advanced atherosclerotic plaques.

Our method of HSP70 detection in blood by immunocytochemistry was never been reported in previous studies. Also, no previous similar studies had compared the difference between recent or acute MI from old previous MI in humans.

**Conclusion**

HSP70 may be used as a biomarker for myocardial infarction and as a confirmatory test to differentiate between acute and chronic MI.

**Ethical Clearance:** The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

**Funding:** Self-funding.

**References**


A Statistical Study to Determine the Factors of Vitamin D Deficiency in Men: the City of Baghdad as a Model

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Abstract

Background: Exposure to sun in the sunny days for 10 to 15 minutes a day and two to three times a week is enough to get vitamin D requirements in the majority, but people with darker skin need to be exposed to sun for longer periods to get their vitamin requirements. The purpose of this research was to achieve a statistical study to know and determine the factors behind deficiency of vitamin D among men in Baghdad city in addition to knowing the most important diseases associated with its deficiency in order to inform and educate the community as well as to raise awareness of the society about the role of this vital vitamin, prevention of its deficiency and to propose appropriate treatment for it. Methods: The research was conducted in several private clinics in Baghdad throughout 2017. The investigation was including study of 500 cases of men who were randomly selected. The cases were divided according to age groups into two groups: the first group was (20-40) years, while the other group was (more than 40 years). Also, the cases were classified according to vitamin D level to sufficient, insufficient and deficient. Results: The results obtained from the study showed that the percentage of vitamin D insufficient and deficient were 69.6% and 18.8%, respectively, while the sufficiency percentage was the lowest and scored 11.6%. Common chronic diseases, such as high blood pressure and diabetes as well as sleep disorders and muscle fatigue, were also followed in the cases. The results illustrated that 47.6% of men cases had diabetes and 39% had high blood pressure, while 28.2% and 17.2% of them had muscular fatigue and sleep disorders, respectively. These chronic diseases and symptoms were related in one way or another with Vitamin D deficiency. Conclusion: Vitamin D can be given for preventive and therapeutic purposes.

Keywords: vitamin D, diabetes mellitus, sun exposure, calcitriol.

Introduction

The mention of Vitamin D is directly related to sunlight, which is called the vitamin of sun rays, because this vitamin is made in sufficient quantities inside the body when exposed to the moderate sunlight with the association of cholesterol (¹). So, taking this vitamin is not considered a necessary food in the event of adequate exposure to sunlight. Exposure to sun in the sunny days for 10 to 15 minutes a day and two to three times a week is enough to get vitamin D requirements in the majority, but people with darker skin need to be exposed to sun for longer periods to get their vitamin requirements (²). Despite the easy access to the body’s needs of vitamin D through the sun, many people avoid sun exposure, or use sunscreen - which hinders the production of vitamin D in the skin - to protect the skin from damage caused by sun such as wrinkles and skin cancer (³). For the benefit of exposure to sunlight and avoid damage at the same time, the best thing to do is to use the sunscreen after exposure for a long time enough to get requirements of vitamin D. the latter, although called vitamin, is actually not a vitamin but a hormone produced in the body as a result of exposure to sunlight (⁴). The active form of this hormone

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is represented by 1, 25-dihydroxycholecalciferol, or 1alpha, 1, 25-dihydroxyvitamin D3 also called (Calcitriol). The manufacture of this hormone begins in the skin and then activated by two steps; the first in the liver and the second in the kidneys (5). People living in the Arctic cannot get their vitamin D needs through exposure to sun, especially in the winter, as well as people who do not leave the home or whose live confined to indoor areas and buildings and people living in crowded places where air pollution is high, which prevents the arrival of ultraviolet light adequately (6). Also dark-skinned people, as dense skin pigments can prevent up to 95% of ultraviolet rays from reaching deep layers of skin, the manufacture of vitamin D3. The use of sunscreen with a protective factor of 15 or more reduces skin’s ability to manufacture vitamin D for up to 99% (3). When talking about the importance of vitamin D and its functions in the body, the first thing that comes to mind is its importance in the absorption of calcium and phosphorus as well as bone health, but science is still discovering functions and other important roles of vitamin D (7). Vitamin D functions include maintaining balance of calcium and phosphorus in the body by stimulating the absorption of calcium and phosphorus as well as reabsorption in kidneys (1,2). It works with the hormone of parathyroid gland to promote release of calcium from bones. Also, it remove phosphorus through urine when the level of calcium in the blood is decreased, which thus maintains the concentration of calcium and phosphorus in the blood to allow the bones to precipitate them (1), guaranteed getting adequate amounts of vitamin D and calcium to preserve the level of calcium in the blood, thus preserving bone health (3). Moreover, it maintains normal growth, differentiation and proliferation rates of cells of many body tissues such as muscles, skin, immune system, parathyroid gland (1) as well as nervous system, brain, genital organs, cartilage pancreas, breast and colon (2). Furthermore, it contributes to prevention of abnormal reproduction of cells in the prevention of cancer (3). It contributes to the metabolism of muscles and affects their strength and constriction. In contrast, people who do not have sufficient vitamin D are at increased risk of muscle weakness, this includes weakness of the heart muscle (3). Some studies had found that the level of vitamin D (calcitriol) in the blood is inversely proportional to insulin resistance and reduces the risk of type 2 diabetes mellitus (4). Vitamin D contributes to the control of immune system responses that cause certain autoimmune disorders, such as type 1 diabetes mellitus, scleroderma, inflammatory bowel disease (3) and rheumatism caused by autoimmune defects (5). One of the major diseases caused by vitamin D deficiency is rickets in children, osteoporosis and osteomalacia in adults, because its paucity leads to a decrease in the intake of calcium from food, and as a result, calcium is released from bones in order to maintain a constant rate of calcium in the blood. Vitamin D deficiency affects not only bones of children and adults but also affects teenagers, preventing them from reaching the largest bone mass they can reach (6). In addition to the above, vitamin D deficiency has other effects such as increased chance of asthma as it was found to be associated with severe asthma in children (7,8). Vitamin D deficiency causes increasing risk of bacterial and viral respiratory infections (8), the chance of depression (9), the chance of weight gain and obesity (10) as well as the chance of hypertension (11). In addition, vitamin D deficiency increases the incidence of cognitive retardation in older persons (12) and increases the chance of death from any reason (11). Vitamin D deficiency is considered as a major cause for increasing the risk of death from heart and vascular diseases. One of the major reasons for increasing cholesterol level in the body is deficiency of vitamin D (11) and its deficiency is related with cancer (13). As well as all previously mentioned, deficiency of vitamin D leads to autoimmune diseases such as type 1 and 2 diabetes mellitus, multiple sclerosis and others.

The purpose of this research was to achieve a statistical study to know and determine the factors behind deficiency of vitamin D among men in Baghdad city in addition to knowing the most important diseases associated with its deficiency in order to inform and educate the community as well as to raise awareness of the society about the role of this vital vitamin, prevention of its deficiency and to propose appropriate treatment for it.

**Methodology**

Vitamin D, which is taken or synthesized in the skin, turns into a compound known as calcidiol, 25-hydroxycholecalciferol, or 25-hydroxyvitamin D (abbreviated 25(OH)D) (14). Thus, the best test of body content of vitamin D is analysis of total level of 25-hydroxyvitamin D. There is a variance in the definition of vitamin D deficiency or inadequacy. In general, a person is severely deficient vitamin D (Deficient) if the result of 25-hydroxyvitamin D test
is less than 10ng/ml, while it is deficient (Insufficient) in the case of result between 10-29ng/ml. If the result is between 30-100ng/ml, it is normal and good (Sufficient), however, levels higher than 100ng/ml may cause toxicity (Potential toxicity) \(^{(15)}\). The present study was carried out in five private clinics in different regions of Baghdad in 2017 (from 1/1/2017 to 31/21/2017), which covered 500 cases of men and was laboratory monitored by a special questionnaire including checking date, the clinical condition and laboratory tests. The investigations of study comprised two parts; the first part was laboratory analysis and testing of vitamin D (which is 25-OH Vitamin D Total) and tests for chronic diseases (diabetes and high blood pressure), while the second part included followed up of accompanying symptoms of sleep disorders and muscle fatigue periodically for all men cases.

**Statistical analysis**

Statistical analysis of study results of was carried out by dividing data into the two groups; the first group was analyzed according to laboratory tests and the second group was according to age groups of participated men.

**Results obtained according to laboratory tests**

The study cases were divided according to results of laboratory tests of vitamin D-25 total deficiency into the following three groups; Group 1: Normal level of vitamin D (Sufficient) ranged between 30-100ng/ml, Group 2: Low level of Vitamin D (Insufficient) ranged between 10-30ng/ml and Group 3: Very low level of vitamin D (Deficient) with a value >10ng/ml. The number of cases who studied in these five private clinics was 500 persons who were monitored periodically via a special questionnaire for one year.

**Table (1) Distribution of participants according to level of 25-OH Vitamin D Total**

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>Reference Range</th>
<th>Number of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufficient</td>
<td>30-100</td>
<td>94</td>
<td>18.8</td>
</tr>
<tr>
<td>Insufficient</td>
<td>10-29</td>
<td>348</td>
<td>69.6</td>
</tr>
<tr>
<td>Deficient</td>
<td>&gt; 10</td>
<td>58</td>
<td>11.6</td>
</tr>
</tbody>
</table>

From Table (1) was clear that the proportion of men with normal (Sufficient) level of vitamin D was the lowest, while the percentage of men with vitamin D deficiency was high and the highest proportion of participated men was with insufficient vitamin D.

**Table (2) Distribution of vitamin D levels according to age groups of participating men**

<table>
<thead>
<tr>
<th>Age group/yr</th>
<th>Number of cases</th>
<th>Percentage</th>
<th>Vitamin D Level</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sufficient</td>
<td>Insufficient</td>
</tr>
<tr>
<td>20-40</td>
<td>323</td>
<td>64.6</td>
<td>65</td>
<td>212</td>
</tr>
<tr>
<td>&gt;40</td>
<td>177</td>
<td>35.4</td>
<td>29</td>
<td>136</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>100</td>
<td>94</td>
<td>348</td>
</tr>
</tbody>
</table>

Table (2) showed that the percentage of men who were deficient of vitamin D was the lowest one in both age groups followed by the percentage of men with sufficient vitamin D level with a clear increase in number of men with low level of vitamin D (Insufficient).

Two types of chronic diseases (diabetes mellitus and hypertension) are associated with vitamin D deficiency and were studied in current study. Also, follow-up of some of the accompanying symptoms, such as sleep disturbance and muscle fatigue, were studied, noting that it was probable that patients were suffering from more than one disease at the same time (Table 3).
Table (3) Relation of Vitamin D Deficiency with Other pathological conditions

<table>
<thead>
<tr>
<th>Pathological condition</th>
<th>Number of Cases</th>
<th>Percentage</th>
<th>Vitamin D Level</th>
<th>Sufficient</th>
<th>Insufficient</th>
<th>Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>238</td>
<td>47.6</td>
<td></td>
<td>74</td>
<td>123</td>
<td>41</td>
</tr>
<tr>
<td>Hypertension</td>
<td>195</td>
<td>39.0</td>
<td></td>
<td>62</td>
<td>94</td>
<td>39</td>
</tr>
<tr>
<td>Sleep Disturbances</td>
<td>141</td>
<td>28.2</td>
<td></td>
<td>23</td>
<td>76</td>
<td>43</td>
</tr>
<tr>
<td>Muscle Fatigue</td>
<td>86</td>
<td>17.2</td>
<td></td>
<td>19</td>
<td>57</td>
<td>10</td>
</tr>
</tbody>
</table>

Table (3) showed that the number of men with diabetes mellitus and insufficient level of vitamin D was the highest among all associated diseases followed by those with hypertension, sleep disturbances and muscle fatigue, respectively. On the other hand, a high number of men with diabetes, high blood pressure and sleep disorders had vitamin D deficiency, both insufficient and deficient, indicating a close correlation between these diseases and vitamin D deficiency.

Vitamin D deficiency by months of the year

As previously illustrated, this research was conducted over a period of one year and follow-up of the cases during that period. All laboratory tests for vitamin D deficiency were performed for all the cases throughout the year (Table 4).

Table (4) Distribution of Vitamin D levels during months of the year

<table>
<thead>
<tr>
<th>Vitamin D Level</th>
<th>Months of the year</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Sufficient</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Insufficient</td>
<td>43</td>
<td>32</td>
</tr>
<tr>
<td>Deficient</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Total number of cases</td>
<td>60</td>
<td>48</td>
</tr>
<tr>
<td>Percentage of (Insufficient + Deficient) Vitamin D</td>
<td>80</td>
<td>81.25</td>
</tr>
</tbody>
</table>

Table (4) showed that the percentage deficiency (both Insufficient and Deficient levels) of vitamin D was very high in comparison with the normal cases along the year. In winter and spring months (i.e. from October to March) there was significant increase in vitamin D deficiency, both Insufficient and Deficient levels, while in the summer months, these level was lower. This is certainly due to the fact that the sun light in the summer months is stronger and available more than in the winter months, so the body can get its requirement from Vitamin D easily.

Conclusions

Deficiency of vitamin D is considered as an important factor in the development of a large number of diseases and possibly all diseases, which can be called “the disease of all diseases”. The following conclusions can be drawn from current study:

- A high percentage of patients are suffering from vitamin D deficiency making it a pandemic and is considered to be related to quality and quality of life.
• It is needed for wide and comprehensive surveys of vitamin D deficiency in children, women and men and all ages.

• It has become clear that vitamin D deficiency is indicated in front of any clinical exhibitor or a satisfactory condition.

• Vitamin D can be given for preventive and therapeutic purposes.

• Increase awareness of the benefits of exposure to sunlight, which represents a large energy away from all diseases.

• This study is considered as a first step in a series of steps to be taken in order to identify more comprehensively vitamin D deficiency among different population groups.

Recommendations

• Provision of free vitamin D in health centers for all who suffer from a shortage.

• Focusing on the scientific side by giving awareness lectures and seminars for medical staff and patients in health centers and hospitals similar to lectures and seminars on vaccines.

• All practitioners should consider vitamin D deficiency as one of the causes of chronic diseases and other clinical symptoms and perform a vitamin D deficiency analysis for patients in parallel to any other type of analysis.

• Exposure to sunlight directly for a period of 10-20 minutes 3 times a week and when sun is vertical (10-12 am), this is easy and available to sunny countries most of the year, like Iraq.

• Focus on eating foods rich in vitamin D such as fish (rosacea and tuna), egg yolks, liver, orange juice, legumes and mushrooms.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

Funding: Self-funding

References


Mutation Evaluation in $P_{53}$ exon 5 in Iraqi AML Patients with 4 Growth Levels

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Abstract

**Background:** The role of TP$_{53}$ is not limited to repairing damaged DNA and regulating its proliferation, it also activates other repairing genes and prevents mutated DNA from multiplying which prevents malignancy formation that’s why it’s called “DNA’s gatekeeper”. The aim of current study was to evaluate the role of Exon 5 of $P_{53}$ gene in the development of AML in Iraqi patients. **Method:** Sixty newly diagnosed AML patients at Baghdad (haematology national centre) were involved in current study. Peripheral blood samples were collected in EDTA tubes then they followed a month after receiving 3 and 7 AML treatment regimens to compare mutational status pre and post treatment. The patient divided into 4 age groups based on growth level (0-15 years, 16-40 years, 41-65 years and 66 years and above) in a 15 sample for each.

**Results:** We uncovered transcriptional downregulation of significant p53 acetyltransferases in both CN-AML and APL, joined by expanded Mdmx protein articulation and deficient Chk2 protein enactment. Mutation study on exon 5 of $P_{53}$ gene showed no differences in gene sequence from the standard sequence of NCBI geneBank sequence.

**Conclusion:** Exon 5 of $P_{53}$ gene not included in the AML causes since the patients of this study showed no alteration in sequence from the reference sequence.

**Keywords:** AML, $P_{53}$, exon 5, growth level, PCR.

Introduction

TP53 and correlations with AML

According to the last review paper published by (1) who explained the role of TP$_{53}$ as a producer for the protein called tumor protein suppressor that regulates cell division to keep it in order and do not allow fast or uncontrolled proliferation. This protein binds directly to DNA to evaluate if DNA is repairable after toxins, chemicals or UV exposure damage or the cell should apoptosised. The role of TP$_{53}$ is not limited to repairing damaged DNA and regulating its proliferation, it also activates other repairing genes and prevents mutated DNA from multiplying which prevents malignancy formation that’s why it’s called “DNA’s gatekeeper”(3).

Choosing the type of causative mutation of AML, helps decide treatment type. A previous study (3) investigated genes and DNA errors associated with TP$_{53}$ mutations and found that (hub genes) are responsible for it which are: $LEP, BMP_2, ITGA_2B, MNX_1, TRH, NMU, CDH_1, KDR, CASR$ and $APOE$. These genes may change the type of treatment received by patient and may increase his healing opportunity.

A pioneer researcher (4) found that MDM2 inhibitors are under investigation for therapy of acute AML patients in M3 clinical trials. To study resistance formation to MDM2 inhibitors in AML cells, we here established 45 sub-lines of the AML TP$_{53}$ wild-type cell lines MV4-11 (15 sub-lines), OCI-AML-2 (10 sub-lines), OCI-AML-3 (12 sub-lines) and SIG-M5 (8 sub-lines) with resistance to MDM2 inhibitor nutlin-3. The outcomes showed that all MV4-11 sub-lines harbored the same R248W mutation and all OCI-AML-2 sub-lines harbored the same Y220C mutation, indicating the selection of pre-existing TP$_{53}$-mutant subpopulations. In concordance, rare alleles harboring the respective

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mutations are detectable in the parental cell lines. The third and fourth sub-lines were characterized by varying TP$_{53}$ mutations or wild type TP$_{53}$, indicating the induction of de novo TP$_{53}$ mutations. For the most part, loss of p53 capacity was not associated with diminished affectability to cytotoxic medications. They suggested that loss of p53 capacity is associated with chemo obstruction in AML, nutlin-3-adapted sub-lines displayed, in the majority of experiments, similar or increased drug sensitivity compared to the respective parental cells. Hence, chemotherapy may remain an option for AML patients after MDM2 inhibitor therapy failure.

Nonsexual chromosomal mutation may give alarm to AML to be, even years before its diagnosis. Under this subject, (5) published their study to show the example of substantial changes seen at analysis of AML. Moreover, 2012 healthy women who eventually develop AML within average of 9.6 years, when compared their DNA sequence with healthy age-matched women gene mutations found in P$_{53}$, IDH$_1$ and 2, splecosome, TET$_2$ and DNMT$_3A$, all mutated subjects develop AML. That study proved that there is genetic predisposition for AML and it could be diagnosed early before AML becomes evident, which may be called latent AML.

In addition, (6) found that TP$_{53}$ changes were recognized in 18% of patients with AML that have mutated TP$_{53}$ with missense mutation (histidine to arginine) on different codons. These mutations correspond with unfavorable karyotyping (ch5, 7 and 17) which come with poor prognosis. Patients with mutated TP$_{53}$ showed mutations also in FLT$_{3}$, RAS and NPM$_{1}$ when compared to wild TP$_{53}$ and these mutations were the same for patients above and less than 60 years. On the other hand, remission differed between wild and mutated types of TP$_{53}$ with rates of 57% vs 41%, respectively, and overall survival rates of 24% vs 9%, respectively, for wild and mutated types.

Bioinformatics analysis by (7) uncovered that p53 is not practical in CN-AML and APL influences at initiating its most significant utilitarian results: cell cycle capture, apoptosis, DNA fix and oxidative pressure barrier. They uncovered transcriptional down guideline of significant p53 acetyltransferases in both CN-AML and APL, joined by expanded Mdmx protein articulation and deficient Chk2 protein enactment. It revealed that p53 pathway was differentially inactivated in various AML subtypes. Centered quality and protein examination of p53 pathway in CN-AML and APL patients suggested that useful inactivation of p53 protein can be credited to its disabled acetylation. Their investigations showed the need in further precise assessment of p53 pathway working and guideline in unmistakable subtypes of AML.

The aim of current study was to evaluate the role of Exon 5 of P$_{53}$ gene in the development of AML in Iraqi patients.

**Methodology**

**Samples collection**

One hundred and twenty peripheral blood samples were collected between 1$^{st}$, May to 20$^{th}$, September from AML patients attending Baghdad Special Nursing Center (which is the drainage of all Iraq to diagnose the tumor type) and AL-Yarmouk Hematology Center using EDTA tubes. Blood was centrifuged then plasma was gathered. In addition, 60 non-AML blood samples of healthy patients, within same age groups of the patients, were collected in EDTA to compare the results with the pre and post treatment groups.

**DNA extraction**

A volume of 200µl of whole blood placed into a sterile 1.5ml micro centrifugtube. Also, 400µl of whole blood lysis buffer was prepared (Qiagen® SV Lysis Buffer + 1% Triton® X-100 for every 200µl of whole blood. Then, Proteinase K solution prepared (20mg/ml) by re-suspending 100mg Proteinase K in 5ml nuclease-free water. Moreover, 40µl Proteinase K (20mg/ml) were added to the 200µl of whole blood in the micro-centrifuge tube, incubated at room temperature for 10 minutes. Invert the tube occasionally to mix. After that, 400µl of prepared whole blood lysis buffer was added to the Proteinase K-treated whole blood sample. Vortexed briefly to mix, incubated at room temperature for 10 minutes, vortexing occasionally to mix.

**DNA purification from Whole Blood Lysate**

The genomic DNA eluted 75–250µl nuclease-free water. The optimal elution volume depends on volume of original whole blood sample and the desired concentration of genomic DNA for downstream applications. Elution in smaller volumes will concentrate DNA but may lower total yield. Larger elution volumes will give optimal yields but a more dilute final DNA preparation. We recommend eluting in 100µl of nuclease-free water and
adjusting from there based on need.\(^{(8)}\)

**NanoDrop**

Logged into the computer next to the NanoDrop machine, on the desktop the ND1000 program was opened, in the pop-up window click “Nucleic Acid” for DNA samples, the NanoDrop pedestal cleaned (the little platform where the sample placed) with wipes and water. Then, 2μl of H\(_2\)O loaded and click “Okay”, clicked “Blank” to calibrate it where it says “Sample Type” click DNA-50 for DNA samples. After that, 2μl of sample loaded onto the pedestal, then click “Measure”. After the machine analyzes it, data saved. If it is a good reading, the graph will show a smooth curve. The absorbance readings at 230nm and 280nm should be about half the reading at 260nm, which means it is a pure DNA sample.

* Pedestal should cleaned between each reading\(^{(9)}\).

### P\(_{53}\) mutation-detection Primer Designing

Primers designed by Qiagen flank variants in exon 5 of P\(_{53}\).

**A-** Primers sequences

P53: Chromosome Position: 17p13 Exon5

US-5’ TACTCCCCTGCCCTCAACAA-3’

Ds-5’ CATCGCTATCTGAGCAGCAGCGC-3’

**B-** Polymerase Chain Reaction (PCR) programs

<table>
<thead>
<tr>
<th>Chemical Substances</th>
<th>Quantity (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dH(_2)O</td>
<td>14.875</td>
</tr>
<tr>
<td>10X PCR buffer Ammonium sulfate (NH(_4)) (2)SO(_4)</td>
<td>2.5</td>
</tr>
<tr>
<td>25mM MgCl(_2)</td>
<td>2</td>
</tr>
<tr>
<td>2 mM dNTP</td>
<td>1.5</td>
</tr>
<tr>
<td>20mM Forward primer</td>
<td>1</td>
</tr>
<tr>
<td>20mM Revers primer</td>
<td>1</td>
</tr>
<tr>
<td>5U/ML Taq DNA polymerase</td>
<td>0.125</td>
</tr>
<tr>
<td>DNA template</td>
<td>2</td>
</tr>
<tr>
<td>Mixture</td>
<td>25</td>
</tr>
</tbody>
</table>

**Table (2) Conditions of gradient PCR reaction.**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pre denaturation</td>
<td>95°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>2. Denaturation at</td>
<td>95°C</td>
<td>40 seconds</td>
</tr>
<tr>
<td>3. Primer annealing</td>
<td>55.2°C</td>
<td>30 seconds</td>
</tr>
<tr>
<td>4. Extension</td>
<td>72°C</td>
<td>30 seconds</td>
</tr>
<tr>
<td>5. Final extension</td>
<td>72°C</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td>0</td>
</tr>
</tbody>
</table>

The final products were analyzed by 2.0% agarose gel electrophoresis and stained with safe stain (Novel Juice). The gel was run at 100 volts for 45 minutes. The DNA fragments were illuminated by UV-light.

**Nucleotide Sequencing**

**PCR sequencing procedure**

Sixty DNA fragments of the P53 gene were excised.
from the agarose gel and used as source of DNA templates for PCR amplification. The ratios of other substrates were the same (Table 1). The total volume of PCR reaction mixture for each sample will be 25µL; and then run it on a thermo-cycler machine (Eppendorf, German) with the same conditions of PCR reaction (Table 2).

C- Pre-Sequencing Preparation procedure

Protocol of PCR production cleanup with ExoSAP

The ExoSAP mixture consists of sterile water, exonuclease I (10U/µl), shrimp alkaline phosphatase (1U/µl)). The Exonuclease I functions for degradation of primers and Shrimp Alkaline Phosphatase was for degradation of unincorporated nucleotides to prepare template for sequencing. The ExoSap mixture was prepared as follows:

5µl of PCR product+3µl of Exo/Sap.

The purification of DNA template was performed in the thermocycler (Eppendorf, German) according to the conditions indicated in Table (3).

Table (3) Conditions for PCR product cleaning up with ExoSAP

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Left over primers were degraded</td>
<td>37°C</td>
<td>30 minutes</td>
</tr>
<tr>
<td>2- Enzyme was degraded</td>
<td>85°C</td>
<td>15 minutes</td>
</tr>
<tr>
<td>3 - Hold</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

Cycle sequencing reaction

Since BigDye is highly sensitive to light, during preparation of cycles sequence reaction mix, all light sources must be turned off. The protocol of cycles sequencing was illustrated in Table (4)

Table (4) The protocol of cycles sequencing per reaction

<table>
<thead>
<tr>
<th>Chemical Substances</th>
<th>Quantity (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>1</td>
</tr>
<tr>
<td>Forward primer (0.8µM)</td>
<td>2</td>
</tr>
<tr>
<td>5X BigDye buffer</td>
<td>2</td>
</tr>
<tr>
<td>BigDye (v3. 0) Mix</td>
<td>1</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>4</td>
</tr>
</tbody>
</table>

Then DNA samples were placed in the thermocycler for amplification and the program was set as indicated in Table (5).

Table (5) Thermal cycling conditions for sequencing PCR reaction products

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pre denaturation</td>
<td>96°C</td>
<td>1 minutes</td>
</tr>
<tr>
<td>2. Denaturation at</td>
<td>96°C</td>
<td>10 seconds</td>
</tr>
<tr>
<td>3. Primer annealing</td>
<td>55.2°C</td>
<td>5 seconds</td>
</tr>
<tr>
<td>4. Extension</td>
<td>60°C</td>
<td>30 minutes</td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

Sephadex spin-column protocol for cleaning PCR products

Sephadex solution was indicated in Table (6)

Table (6) Components of sephadex spin–column for cleaning PCR products

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sephadex G-50 powder</td>
<td>4g</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>42ml</td>
</tr>
</tbody>
</table>

The solution was put on vortex for 45 minutes to mix it. The empty receiver column was placed in 1.5ml collecting tubes and 850µl of sephadex solution was added into each receiver column tube. The tubes were centrifuged at 3,800rpm for 2 minutes, then the receiver column was replaced in a new clean collecting tube. After that, 10µl of the PCR sample was added to a prepared column. Then the PCR samples were transferred and placed in the center of the receiver column matrix without touching it. Then, the tubes were placed in the centrifuge at 3,800rpm for 2 minutes.

Results and Discussion

This test was done at Turkey/Gaziantep University/Biology Department. We had ordered the primers for P53 gene from Qiagene.

DNA sequence of exon 5 of P53 gene was screened to find different genotypes. Both exons sequences of P53
gene were screened by genetics analyzer (nucleotide sequencing) (Figure 1).

The DNA sequences of \( P53 \) gene of \(^{(10)}\), to look at the subsequent DNA groupings of AML (Query Sequence) with the reference groupings.

![DNA sequence of \( P53 \) gene (exon 5 sequences were indicated in blue color).](image1)

No variety was found in the succession of PCR format for the objective areas in the wake of contrasting and the reference grouping (GenBank sequence). Figure (2) indicated and revealed the partial sequence results without any variation (Mutation).

![The partial sequence result of exon 5 of \( P53 \) gene.](image2)

According to the results of \(^{(11)}\) study, there was an overall reduction in the level of p53 expression in patients with AML, meanwhile p53 showed differential expression in AML subtypes and M3 subtype showed higher expression in comparison with other AML subtypes. It was suggested that p53 expression has a possible relation with granulocyte maturation and prognosis. Further investigations needed to clarify the exact role of p53 expression fluctuations in AML patients as basic molecular events in malignant cells.

In addition, \(^{(12)}\) uncovered noteworthy and differential changes of p53 pathway-related quality articulation in the greater part of AML subtypes. They found that p53 pathway-related quality articulation was not associated with acknowledged gathering of AML subtypes, for example, by cytogenetically-based guess, morphological stage or by sort of sub-atomic transformation. Their bioinformatics investigation uncovered that p53 was not practical in CN-AML and APL influences at prompting its most significant utilitarian results: cell cycle capture,
apoptosis, DNA fix and oxidative pressure guard. We uncovered transcriptional downregulation of significant p53 acetyltransferases in both CN-AML and APL, joined by expanded Mdmx protein articulation and deficient Chk2 protein enactment.

Also, (13) found that $TP_53$ transformations are free indicators of short survival and chemo-refractoriness, and that CLL giving $TP_53$ changes without del17p13 passage as inadequately as CLL conveying del17p13. Since CLL harboring $TP_53$ changes without del17p13 as of now not perceived by ordinary analytic methodologies, these outcomes might be pertinent for an exhaustive prognostic portrayal of CLL.

**Conclusion**

Exon 5 of $P_53$ gene not included in the AML causes since the patients of this study showed no alteration in sequence from the reference sequence

**Ethical Clearance:** The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

**Funding:** Self-funding.

**References**

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Assessment of Antagonistic Effect of Alcoholic Extract from Cyanophyta (Spirulina Platensis) Against Several Human and Plant Derived Pathogenic Fungi

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1Biology Department- Collage of Science/ Mustansiriyah University, Iraq

Abstract

Background: Blue-green algae have been proved to producing different bioactive compounds. For this reason Spirulina platensis isolates have been used in the present study as a biocontrol agent against several human and plant pathogenic fungi. Current study was aimed to investigate the use of crude extract of Spirulina platensis as antifungal agent by determining the antagonistic activity of methanolic extract of Spirulina platensis against several human and plant pathogenic fungi. Also, to determine the antifungal phytochemicals within algal alcoholic extract through GC-mass analysis.

Method: Four different concentrations of alcoholic extract were prepared (100, 75, 50 and 25mg/ml) from algal isolates and their antagonistic activity was investigated against molds isolated from spoiled fruits (two isolates of Aspergillus niger, Aspergillus flavus, Mucor sp. and Botrytis sp.) and against several Candiada spp. (Candida albicans, Candida glurbrate, Candida fameta, Candida lustrans) which were isolated from different clinical sources.

Results: The results showed that algal extract displayed wide range of antagonistic activity against tested fungi depending on applied algal extract concentration and tested fungal species. Also, GC-Mass data analysis had been performed for algal extract and 36 different bioactive chemicals have been identified including eight compounds authenticated as having antifungal activity.

Conclusion: Spirulina could be used as alternative drug to treat Candida sp. infections as well as added to food industries to enhance their nutritional value and simultaneously decreasing the possibility of food spoilage by molds.

Keywords: algal extract, bioactivity, onychomycosis, fungi, candida spp.

Introduction

Nature has been a source of therapeutic agents for thousands of years and a remarkable number of recent drugs have been isolated from environmental sources. In addition, biologically active constituents have recently received notable attention. Algae are rich sources of constituents that are novel in their structure and authenticated as biologically active metabolites, primary or secondary metabolic products produced by these organisms may exhibit potential bioactivity in pharmaceutical industry (1). Blue-green algae have been proved to produce different bioactive compounds. These comprise antibiotics which inhibited microorganisms responsible for human and plants diseases(2).

Spirulina is blue green algal genus belongs to Oscillatoriaeae which is characterized as free floating, spiral, multicellular and filamentous in appearance. The filaments are approximately 50-300μ long and 10μ in diameter and nitrogen-non fixing (3). Fungi cause a broad range of superficial mycoses diseases in both humans and animals, involving the outer layers of the skin and frequently leadings to chronic infections. The major etiological agents of mycoses infections are Candida spp. and dermatophytes (4). Also, fungal pathogens are important problem in agriculture, since most of the fungicides engaged exhibit lower effectiveness under field conditions and may cause chemical environmental pollution as well as poisoning of crops. In addition, some species, e.g. Aspergillus flavus can contaminate food and produce severe mycotoxins (5).

Spirulina as many other blue-green algae are producing varieties of antimicrobial agents, so they are
considered as suitable natural agents for manipulation as biocontrol mediators of plant pathogenic fungi and bacteria (1).

Therefore, current study was aimed to investigate the use of crude extract of *Spirulina platensis* as antifungal agent by determining the antagonistic activity of methanolic extract of *Spirulina platensis* against several human and plant pathogenic fungi. Also, to determine the antifungal phytochemicals within algal alcoholic extract through GC-mass analysis.

**Materials and Method**

**Source of algae**

Pre-isolated and characterized *Spirulina platensis* used in the present study was obtained from the laboratory of higher graduate at Biology Department in AL-Mustansiriyah University, Baghdad. The obtained isolate was cultured and maintained on BG-11 Medium and grown in an illuminated incubator.

![Figure 1](https://example.com/figure1.jpg)

**Figure (1) Microscopic view of Spirulina platensis (40X)**

**Preparation of algal Extract**

Algal extract was prepared according to (6). The *Spirulina platensis* materials were grounded to a fine powder and a weight of 40 gram from algal powder was extracted successively with 200ml of solvent (methanol) in Soxhlet extractor until the extract was clear. The extract was evaporated to dryness by reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4°C for future experiments.

**Collection of human pathogenic tested Yeast**

Four different species of *Candida* were obtained from the laboratory of higher graduate of Biology Department in AL-Mustansiriyah University, Baghdad. These species were (*Candida albicans, Candida glubrarete, Candida fameta* and *Candida lustrans*). These yeasts were isolated from different clinical sources and maintained using Sabouraud dextrose agar (SDA) medium according to (7).

Isolation and identification of tested plant pathogenic Molds

The examined plant-pathogenic molds used in this investigation were isolated from different local spoiled fruits according to (8) and were identified using cultural and morphological features such as conidial morphology, pigmentation and colony growth pattern, according to the technique of (9).

**Bioactivity test of hot alcoholic extract of Spirulina platensis against molds**

According to (10) technique that has been used for this test, different concentrations of hot alcoholic algal extract (100, 75, 50 and 25mg/ml) were incorporated into PDA medium (potato dextrose agar) before pouring in Petri dishes. Also, clotrimazole (10μg/ml) was tested against tested molds to compare between the antifungal activity of natural agent (*Spirulina extract*) and industrial antifungal (clotrimazole). PDA medium Petri dishes without additions have been used as negative controls. All experiments ran in three replicates. Moreover, 3mm discs of fungal plugs were inoculated in the center of Petri dishes and incubated at 28±2°C for (8 – 10) days. The radial growth of the colony was measured. Percentage of inhibition of mycelial growth was calculated as follows:

$$\% \text{Radial growth Inhibition} = \frac{(R1- R2)}{R1} \times 100$$

Where: R1 is the average of radial growth in control plates; R2 is the average of radial growth in plates treated with algal extract or clotrimazole.

The spore suspension was collected from above culture. Through suspensions of spores in sterile DW then centrifuged. A hemocytometer was used to calculate the percentage of sporulation inhibition using the formula given by (11):

$$\text{Sporulation inhibition ratio} = \frac{(X-x)}{X} \times 100$$

Where: X is the average number of spores in control plates; x is the average of spores’ number in plates treated with nanoparticles.
Bioactivity test of alcoholic extract of *Spirulina platensis* against *Candida* spp

Antagonistic activity of algal extract was estimated in vitro by using well diffusion method according to (12). Four concentrations of algal extracts had been tested (100, 75, 50 and 25 mg/ml) and 100 μL of each concentration was added to each well after spreading of each *Candida* sp. isolate by sterile loop and incubated at 37°C for 24 hrs. Also, 10 μg/ml clotrimazole was tested as positive control. All tests were performed in triplicate. Antifungal activity was determined by measuring the clear zone around the wells in millimeters.

Gas Chromatography-Mass Spectrometry

GC-Mass analysis was performed according to (13) using (SHIMADZU—Japan) and post run software. The phytochemical compounds have been identified by comparison of their mass with NIST library search and authentic standards.

Results and Discussion

Identification of isolated fungi

Five molds isolates were isolated from spoiled food and identified these molds were *Aspergillus niger* (two isolates), *Aspergillus flavus*, *Mucor* sp., *Botrytis* sp. These molds were used to estimate antifungal activity of *Spirulina platensis* crude extract. Some food-born fungi are implicated in human diseases. These fungal infections are categorized into systemic, subcutaneous, superficial and opportunistic (14). Spores propagules from *Aspergillus* and *Mucor* pose dangerous public health problems. For instance, *A. niger* causes aspergillosis which is common amongst employees who inhale soil dust particles (14,15). Inhalation of spores of *Mucor* spp. and *Aspergillus* spp. produces allergic reactions in human. These diseases are difficult to treat. Furthermore, *A. flavus* have been reported as dangerous to humans through causing aspergillosis that’s associated high human mortality rates and producing serious toxins (16).

So that, there is a serious needs to improve naturally antagonistic products to eliminate food spoilage by these molds.

Bioactivity test of hot alcoholic extract of *Spirulina platensis* against molds

In current study, results of antagonistic activity of *Spirulina* methanolic extract revealed that algal extract displayed potential inhibition percentage against tested molds as compared to controls. Data from current study revealed that algal extract gave 89%, 78% and 95% inhibition in 100mg/ml against *Botrytis*, *Aspergillus flavus* and *Aspergillus niger* 2, respectively. In case of *Aspergillus niger* 1, there was 94% inhibition caused by algal extract in (75mg/ml), while 85% inhibition caused by fungicide (Figure 2). However, no growth inhibition was noticed against *Mucor* for both algal extract and clotrimazole.

![Figure 2](image_url) Growth inhibition percentages of different concentrations of *Spirulina platensis* methanolic extracts and clotrimazole against isolated plant-pathogenic molds.

As shown in Figure (3), spore germination was inhibited by *Spirulina* extracts. Spore germination of *Aspergillus flavus* was the most inhibited by algal extract (55%) at (100mg/ml), while *Botrytis* spore germination was less inhibited by algal extract (17.5%) at concentration (25mg/ml). Also, for *Botrytis* data, Figure (3) revealed that *Spirulina* extract (100mg/ml) inhibited spore germination for this mold by (44%). This percentage was higher than that caused by clotrimazole (28%).

![Figure 3](image_url) Spore germination inhibition percentages BY different concentrations of *Spirulina platensis* methanolic extracts and clotrimazole against isolated plant-pathogenic molds.
These findings agreed with other authors. The previous authors tested *Spirulina* methanolic extracts against several fungi as *Candida* spp., *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* spp. Their results showed that extract exhibited strong antifungal activity against these fungi. In addition, reported that since the algal extracts are considered natural products which may have efficacy for fungal diseases management, a directed search is needed to understand culture conditions enhancing production of biologically active chemicals. During the present study, *Spirulina* have cultivated using BG-11 medium. The results of indicated that *Spirulina* exhibited the highest antimicrobial effects when being cultured in BG-11 medium against tested microbes. For *Mucor* spp., algal extracts did not exhibit antagonistic activity against it. On the other hand, reported that *Spirulina* extract exhibited stimulation of *Geotrichum* and *Cladosporium* growth. The author suggested that this growth stimulation probably due to *Spirulina* nutritional value properties.

**Bioactivity test of alcoholic extract of *Spirulina platensis* against *Candida* spp**

Algal extract displayed variable zones of inhibition against tested candida species compared to clotrimazole. In terms to *Candida fameta Spirulina*, extracts exhibited higher inhibition zone (18mm) against it than clotrimazole (15mm). Also, *Spirulina* methanolic extract gave (19 and 20mm) inhibition zones against *Candida lustrans* as compared with (18mm) caused by clotrimazole. No inhibition zone was detected by DMSO-containing well against all tested *Candida* spp. (Figures 4 and 5).

These tested species of *Candida* may cause serious infections such as progressively common nail infection (Onychomycosis) which is mainly caused by dermatophytes fungi. The disease is extremely difficult to treat. Since that Onychomycosis is not threatening human life, this type of nail infection is an essential public health concern due to its high incidence, low response to therapy as well as suggestive clinical, social and economic impacts.

**Gas Chromatography-Mass Spectrometry**

As a result of GC-Mass analysis, a total of 36 phytochemical constituents were detected for *Spirulina* alcoholic extract. These compounds possess various biological activities. Eight chemical compounds are authenticated to exhibits antifungal effect. These constituents were listed in Table (1).
Several screening studies have been achieved over the past years to record new antibiotic metabolites from microalgae particularly green and blue-green algae \(^{(28)}\). *Spirulina platensis* is known to produce a wide range of bioactive molecules, making them a rich natural source of various types of medications \(^{(1)}\). This alga showed many therapeutic properties, i.e. the ability to prevent cancer, decreasing blood cholesterol levels, decrease toxicity of kidneys and protection against the harmful effects of radiation \(^{(29)}\).

### Conclusions

The data obtained from this study indicated that *Spirulina platensis* alcoholic extracts act as a potential source of antifungal constituents against plant and human diseases caused by fungi. This activity depends on fungal species. GC-Mass data analysis clearly revealed the presence of many documented antifungal chemical constituents. So that, authors suggested that *Spirulina* could be used as an alternative drug to treat *Candida* spp. infections as well as added to food industries to enhance their nutritional value and simultaneously decrease the possibility of food spoilage by molds.

#### Ethical Clearance

The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

#### Conflict of interest

The authors declare that they have no conflict of interest.

#### Funding

Self-funding.

#### Acknowledgment

The authors would like to thank AL-Mustansiriyah University (www. uomustansiriyah.edu.iq), Baghdad, Iraq for providing support in the current work.

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The Differentiation between Premenstrual Tension Syndrome and Dysmenorrhea in Young Age Group in Kerballa City: Cross-Sectional Study

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¹Obstetric and Gynecology Department, College of Medicine, Kerbela University, Iraq

Abstract

Background: The repeated psychological and physical symptoms that occur during the luteal phase of menstrual cycle and relief with menstruation called Premenstrual syndrome.

The aim of study is to identify the symptoms of dysmenorrhea and premenstrual tension syndrome in young age group female and how can differentiate between these two symptoms.

Material & Method: The study was across sectional which was conducted with 259 students randomly selected from Karbala secondary nursery school of girls, Their ages ranged from (range 15-29 years) with mean age 17.85±1.920 years. A questionnaire was used to collect the data, and was distributed randomly to all students in 3 classes in period from October 2017 to May 2018.

Results: 259 girl shared in this study with mean age 17.85±1.920 years (range between 15-29 years). Out of 259, 205 had PMS (79.15%) with different symptoms. Most patients with PMS, developed dysmenorrhea (77.5%) There was significant difference between them p value (<0.001).

Conclusion: high rate of young girl had premenstrual syndrome. The diagnosis of PMS is generally undervalued due to different features for PMS are used in different research. Though dysmenorrhea would distort the physicians about the actual giving symptoms from these women.

Keywords: rate of young; diagnosis; dysmenorrhea.

Introduction

The repeated psychological and physical symptoms that occur during the luteal phase of menstrual cycle and relief with menstruation called Premenstrual syndrome. (1) PMS is a public health problem among young girls. (2)

The main symptoms of PMS includes the symptoms connected to mood and physical conditions. (3) The symptoms are severe and very disabling in some women and affect with their career, social function and family relations. (4)

Dysmenorrhoea a painful cramps that start before the onset of menstruation and may continue for hours to days. Dysmenorrhea may be primary, with no recognizable cause, or secondary due to pelvic diseases. (5)

The PMS begins before the MC and stops shortly after menstruation. (6)

The prevalence rates of PMS between 16% to 93% In the Middle East. (7) PMS was the collaboration between hormonal, neural, environmental and psychosocial factors. (8,9) the underlying pathophysiology of PMS remains uncertain. (10)

The diagnosis of PMS mostly depends on the patients complains and symptoms, which over Two MC using a symptom record.

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To achieve that diagnosis 3 key element must be present: (1) symptoms should happen during the luteal phase and resolution occur within 1 to 2 days after the start of menstruation; (2) symptoms should be recognized for several MC; (3) no medical or psychological disorders behind these symptoms. According to ACOG diagnostic criteria the diagnosis of PMS was done when at least one somatic symptom and one emotional symptom had been occurred.

Estrogen and progesterone effect on Serotonin receptors, and selective serotonin reuptake inhibitors (SSRIs) are proven to reduce PMS symptoms. The relationship between Prolactin levels, hypoglycemia or vitamin deficiencies and PMS had not been established.

Materials and Method

The study was across sectional which was conducted with 259 students randomly selected from Karbala secondary nursery school of girls. Their ages ranged from (range 15-29 years) with mean age 17.85±1.920 years. A questionnaire was used to collect the data, and was distributed randomly to all students in 3 classes in period from October 2017 to May 2018.

The study was approved by the Ethics Committee of the University of Karbala and informed consent was obtained from all participants.

The students were instructed to fill out the questionnaire over 2 menstrual cycle.

All of the questionnaire related to symptoms asking to them before include week before, week after and others. Also include the severity of symptoms which include mild, moderate, severe.

For severity of dysmenorrhea we select MMS criteria (Multi dimensional Scoring system criteria).

The MSS grading of pain is as follows:

Grade 0: not painful Menstruation and un affected daily activities.

Grade 1 (mild): not painful Menstruation and rarely affected daily activities.

Grade 2 (moderate): moderate painful Menstruation and affected daily activities. Grade 3 (severe): extreme painful Menstruation and inhibited daily activities.

Result

259 girls participate in this study with mean age 17.85±1.920 years (range between 15-29 years).

The mean BMI 22.40±5.285 kg/m² (range 17.1-29.3 kg/m²), Most of them 65.3% had normal BMI as shown in table (1).

Table (1): BMI in studied group

<table>
<thead>
<tr>
<th>Classification</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under weight</td>
<td>54</td>
<td>20.9</td>
</tr>
<tr>
<td>Normal</td>
<td>165</td>
<td>63.7</td>
</tr>
<tr>
<td>Overweight</td>
<td>40</td>
<td>15.4</td>
</tr>
<tr>
<td>Total</td>
<td>259</td>
<td>100.0</td>
</tr>
</tbody>
</table>

In table (2), most of these females hadn’t use COC (combined oral contraceptive pills) 62.5%, and had regular period 64.5% with no history of chronic disease 96.2%.

Table (2): The history of studied group

<table>
<thead>
<tr>
<th>History points</th>
<th>Classification</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of children</td>
<td>Yes</td>
<td>18</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>241</td>
<td>93.1</td>
</tr>
<tr>
<td>Marital status</td>
<td>Divorced</td>
<td>9</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Married</td>
<td>31</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Single</td>
<td>219</td>
<td>84.6</td>
</tr>
<tr>
<td>C.O.C pills user</td>
<td>NO</td>
<td>191</td>
<td>73.7</td>
</tr>
<tr>
<td></td>
<td>Other types</td>
<td>28</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>YES</td>
<td>40</td>
<td>15.5</td>
</tr>
<tr>
<td>History of COC use</td>
<td>NO</td>
<td>180</td>
<td>69.5</td>
</tr>
<tr>
<td></td>
<td>YES</td>
<td>69</td>
<td>30.5</td>
</tr>
</tbody>
</table>
Out of 259, 205 had PMS (79.15%) with different symptoms. Only 11.6% (30 patients) needed doctor advice for the symptoms, and 17% (44 patients) took drugs for relief symptoms.

During the period, Out of 259, 191 (73.7%) had different symptoms in the first 2 days of the period as shown in Table (3).

### Table (3): Symptom during 2 days of period of studied group

<table>
<thead>
<tr>
<th>Symptom during 2 days of period</th>
<th>Classification</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Menstrual Cramps) 2 days before</td>
<td>Mild</td>
<td>39</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>58</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>Sever</td>
<td>96</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>66</td>
<td>25.5</td>
</tr>
<tr>
<td>(Menstrual Cramps) 2 days after</td>
<td>Mild</td>
<td>60</td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>72</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>Sever</td>
<td>89</td>
<td>34.4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>38</td>
<td>14.7</td>
</tr>
<tr>
<td>(Menstrual Backache) 2 days before</td>
<td>Mild</td>
<td>38</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>59</td>
<td>22.8</td>
</tr>
<tr>
<td></td>
<td>Sever</td>
<td>93</td>
<td>35.9</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>69</td>
<td>26.6</td>
</tr>
<tr>
<td>(Menstrual Backache) 2 days after</td>
<td>Mild</td>
<td>56</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>77</td>
<td>29.7</td>
</tr>
<tr>
<td></td>
<td>Sever</td>
<td>83</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>43</td>
<td>16.6</td>
</tr>
</tbody>
</table>

According to BMI, Most patients with dysmenorrhea had normal BMI (137) as shown in Table (4). There was insignificant difference between them (p value = 0.2).
Table (4): Dysmenorrhea in different BMI groups

<table>
<thead>
<tr>
<th>BMI</th>
<th>NO</th>
<th>YES</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>33</td>
<td>21</td>
<td>54</td>
</tr>
<tr>
<td>Normal</td>
<td>28</td>
<td>137</td>
<td>165</td>
</tr>
<tr>
<td>overweight</td>
<td>7</td>
<td>33</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>191</td>
<td>259</td>
</tr>
</tbody>
</table>

P value: 0.20

According to age, Most patients with dysmenorrhea with age group 16-20 years as shown in table (5). There was significant difference between them (p value <0.001).

Table (5): Dysmenorrhea in different age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>NO</th>
<th>YES</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤15</td>
<td>5</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>16-20</td>
<td>39</td>
<td>145</td>
<td>184</td>
</tr>
<tr>
<td>21-25</td>
<td>13</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>26-30</td>
<td>11</td>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>

P value: <0.001

Most patients with PMS, developed dysmenorrhea (77.5%), There was significant difference between them p value (<0.001). as shown in table (6).

Table (6): PMS and dysmenorrhea in studied group

<table>
<thead>
<tr>
<th>NO</th>
<th>Dysemorrhea</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>PMS</td>
<td>No</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>68</td>
</tr>
</tbody>
</table>

P value: <0.001**

Discussion

Out of 259, 205 had PMS (79.15%) with different symptoms, while the Royal College of Obstetrics and Gynecology published in her Green-top Guideline No. 48 (15) that the prevalence of PMS is 40% depending on Pearlstein et al study (16) this percentage is not the same in all the world and according to Sanctis, et al (17) study which is held in 2016 he found that the prevalence of dysmenorrhea varied greatly from different countries it was 94% (Oman), 59.8% (Bangladesh), 34% (Egypt) to 0.9% (Korea). This prevalence varied according to many factors which influence the disease occurrence and severity of the disease like the type of food, degree of knowledge.

Only 11.6% (30 patients) of these girls ask for doctor advice to control their symptoms and only 17% (44 patients) took drugs to relieve symptoms. This finding is compatible to that reported by Chan et al. (18).
Pain is the most frequent symptom experienced by those girls and from the total 259 participants, 191 (73.7%) describe pain and dysmenorrhea in the first 2 days of period.

During the period, Out of 259, 191 (73.7%) developed symptoms in first 2 days of period as shown in table (3), cramps and backache are the main symptoms developed during the 1st two days of the cycle.

We found in our study that females weight and BMI did not affect the symptoms of pain and dysmenorrhea. There was insignificant difference between groups (p value =0.2), this result differ from that obtained from Madhubala and Jyoti Kala in 2012 in Rajasthan – India which reveal a significant relationship between dysmenorrhea and BMI (19) . he advice to reduce weight because decrease adipose aromatase activity and increase estrogen metabolism and excretion which may modulate the pain during the cycle(20).

According to age, Most patients with dysmenorrhea with age group 16-20 years as shown in table(6). There was significant difference between them (p value <0.001). In comparison with Dawood (21) recognized dysmenorrhea in half of young women.

58% of studied group between the age group of 11 years to 22 years had dysmenorrhea and 22% and 17% had severe and moderate constituted respectively. This indicates that dysmenorrhea had negative effect on social environment, work and psychological status (22).

Most patients with PMS, developed dysmenorrhea (77.5%). There was significant difference between them p value (<0.001) as shown in (table 7). Orhan Derman et al., which propose that the diagnosis of PMS is usually underestimated and he advice primary care physicians to be alert of this health insult in order not to underestimate the disease and its consequences on patients life(23). Backache which usually perceived by the patient as pain correlated to menstrual cycle found to be mild in 17.4%, moderate in 24.3% and sever in 32.4% in the week before the cycle this figure changed to mild 30.1, moderate 26.3, sever 26.6, the severity of backache slightly decrease after menstruation which may be due to the relief of menstrual tension while mild pain increased which may be correlated to dysmenorrhea.

Conclusion

high rate of young girl had premenstrual syndrome. The diagnosis of PMS is generally undervalued due to different features for PMS are used in different research. Though dysmenorrhea would distort the physicians about the actual giving symptoms from these women.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References


Bacteriological and Pathological Study on Kidneys of Slaughtered Sheep in Fallujah City

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¹University of Fallujah, College of Veterinary Medicine, Department of Pathology and Poultry diseases/Iraq, ²University of Fallujah, College of Veterinary Medicine, Department of Parasitology/Iraq.

Abstract

This study was carried out to determine the prevalence and type of renal lesions in sheep that were slaughtered at Fallujah abattoir. A total number of 100 clinically normal sheep of different sex and age randomly selected, renal samples were taken for , bacteriological, macro and micro study. One hundred swabs were taken from cortex and medulla were cultured on blood, nutrient, and MacConkey agars, then biochemical tests were done for identification of these bacteria. Bacteriological examination of kidney revealed that (65%) of samples show positive results in bacterial isolation including (15%) Bacillus, (10%) Escherichia coli, (11%) Klebsiella, (18%) Staphylococcus, (8%) streptococcus, (3%) Corynebacterium spp., (3%) pseudomonas aeruginosa, (1%) Salmonella and (1%) Proteus. Pathological study showed that 24 (24%) and 38 (38%) gave positive results in macroscopic and microscopic lesions respectively, the results of gross pathology were renal hemorrhage 8%, pale kidneys 4%, enlarged kidneys 4% white spotted kidneys 3%, renal fibrosis 2%, pigmented kidney 2% and renal cyst 1%. Meanwhile the microscopic lesion were nephritis 24%, nephrosis 5%, kidney fibrosis 3%, infarcted kidneys (2%), amyloidosis 2% hemosiderosis 2%. From the presented study, it was concluded that most kidneys are infected with many gram negative and gram positive bacteria and many kidney problems such as renal hemorrhage and interstitial nephritis are the most common renal lesions in sheep.

Keywords: Kidney, sheep, bacteria, pathological.

Introduction

Slaughter houses and butchery shops afford an excellent opportunity to detect animals health problems so it is a potential source of information for both economic and public health (1). Sheep as food animals act as worthy strength to the economy of the nation (2), besides economic losses, diseases of sheep might constitute an epidemiologic and zoonotic threat (3).

Kidneys are paired organs structures and with their physiological functions such as excretion of waste materials, regulation of acid-base balance, body fluid balance and hormonal effects, it plays a vital role in the life of animals for close inspection, the information resulting from abattoir data are a good source for evaluation and monitoring of renal disease in livestock (4, 5, 6).

Renal diseases are common and they are significant important problems and causes illness and death in many species of animals (3).

Renal diseases are not detected until they become generalized, leading to renal failure and death so collected evidence from abattoir studies remain a good source for evaluation and control of renal diseases in animals (7).

Many researches were done for bacterial isolation from the offal especially the kidneys in abattoir, from these a study of Abdul-Hadi (2010), who showed that the prevalence of microorganisms identified in kidneys...
and other offal were Staphylococci, Corynebacterium, Streptococci, Micrococcus, Salmonella, *Escherichia coli* and Yeast (8).

The goal of this study was a bacterial isolation and pathological examination of kidneys of sheep slaughtered in Fallujah City/ Iraq.

**Materials and Method**

The study was conducted on 100 kidney samples of sheep collected from local abattoir during 5 months (November 2018 to March 2019). Swabs were taken from every sample (cortex and medulla) and inoculated in a nutrient broth, then incubated aerobically at 37°C for 24-48h. The primary growth were sub-cultured on nutrient, blood agar, EMB and MacConkey agars. The pure cultures obtained were subjected to gram stain and biochemical tests including catalase, oxidase, TSI, indole, Citrate and carbohydrate fermentation for characterization and identification, these were done according to 9. Markey et al. (2013) (9).

For pathological study, gross pathological lesion of each sample was established and recorded as described by Gracey (1985) (10). The Sectioning and staining were done according to Luna (1968) (11).

**Results and Discussion**

Out of 100 samples swab taken from cortex and medulla of sheep kidneys, total bacterial isolated (65%), while negative samples were (35%). Table (1) shows the distribution of percentage of finding bacteria according to isolates culturing media.

The bacteriological examination of kidney were the following microorganisms isolated from kidney sheep include *Bacillus spp.* was (15%), *Klebsiella spp.* was (11%), *Escherichia coli* was (10%), *Staphylococcus aureus* was (9%), *Staphylococcus spp.* was (9%), *Streptococcus spp.* was (8%), *Corynebacterium spp.* was (3%), *pseudomonas aeruginosa* was (3%), *Salmonella spp.* was (2%), *Proteus spp.* was (1%) (Table 1).

<table>
<thead>
<tr>
<th>Isolated germs</th>
<th>Number/total (n ) %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus spp.</em></td>
<td>10/100 (10%)</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>11/100 (11%)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>10/100 (10%)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>9/100 (9%)</td>
</tr>
<tr>
<td><em>Staphylococcus spp.</em></td>
<td>9/100 (9%)</td>
</tr>
<tr>
<td><em>Streptococcus spp.</em></td>
<td>8/100 (8%)</td>
</tr>
<tr>
<td><em>Corynebacterium spp.</em></td>
<td>2/100 (3%)</td>
</tr>
<tr>
<td><em>pseudomonas aeruginosa</em></td>
<td>3/100 (3%)</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>2/100 (2%)</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>1/100 (1%)</td>
</tr>
<tr>
<td>Negative</td>
<td>35/100 (35%)</td>
</tr>
<tr>
<td>Total</td>
<td>100/100 (100%)</td>
</tr>
</tbody>
</table>

It has been reported that the most common causative organisms in kidney disorders were *Klebsiella* and *Escherichia coli*, bacteria that is responsible of ascending infection causing kidney disease (12). Also other bacteria are involved such as *Proteus*, *Staphylococcus* and *Streptococcus*. The kidney infection can also occur through blood in the case of *Salmonella* and *Pseudomonas*. Kidney infection via the hematogenous route (suppurative embolic nephritis) is much less common but may result from bacteremia with such agents as *Salmonella* species (13, 14). These results were in compatible with our results.
The current study were also in agreement with results of Mohammed (2009) who found that *Escherichia coli, Staphylococcus aureas, streptococcus spp., Proteus spp. and Klebsilla spp.* were the most bacteria isolated (15). Also, Hirsbrunner *et al.* (1996) reported that Corynebacterium species is an obligate pathogen of the urinary mucosa and isolated this bacteria from cow suffered from unilateral pyelonephritis (16).

Out of one hundred animal’s kidneys randomly collected from clinically healthy sheep, thirty four kidneys showed affection with different pathological conditions include 24 macroscopic and microscopic lesions table (2) and also 12 kidneys with microscopic lesions, table (3).

<table>
<thead>
<tr>
<th>Table (2) Percentages of macroscopic changes in sheep kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
<tr>
<td>4.</td>
</tr>
<tr>
<td>5.</td>
</tr>
<tr>
<td>6.</td>
</tr>
<tr>
<td>7.</td>
</tr>
<tr>
<td>8.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table (3) Histopathological changes results of kidneys in sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
<tr>
<td>4.</td>
</tr>
<tr>
<td>5.</td>
</tr>
<tr>
<td>6.</td>
</tr>
<tr>
<td>7.</td>
</tr>
<tr>
<td>8.</td>
</tr>
<tr>
<td>9.</td>
</tr>
<tr>
<td>10.</td>
</tr>
</tbody>
</table>

The results of this study indicated that the highest incidence of occurrence of gross pathology were renal hemorrhage 8% , pale kidneys 4%, enlarged kidneys 4% white spotted kidneys 3%, renal fibrosis 2% , pigmented kidney 2% and renal cyst 1%.

The most common gross lesions were the hemorrhages in sheep kidneys with incidence of (8%) and it varied from pin point petechia to ecchymosis, renal hemorrhages may occur due to acute nephritis or with septicemia and bacterial intoxication (17,18).
The paleness of kidney also recorded, with prevalence (4%) paleness may be due to, amyloidosis, infarction and obstruction of blood vessel at the base towards the periphery, nephrosis (17, 19). While renal enlargement showed prevalence of (4%), it was occur as a result to accumulation of blood, edema, fluid, fat and urine in pelvis or tubules, moreover enlarged kidneys with pale grey color may be due to Interstitial nephritis, glomerulonephritis and tubular nephritis (20). This results agreed with the result stated by Ali and Aljeboori, (2017) (21).

White spotted kidney prevalence were (3%), It is possible that these results are due to several pathogens especially bacteria e.g. Leptospirosis spp., Escherichia coli septicemia, also other pathogens cause this lesion (20, 22, 23).

kidneys with severe fibrous tissue formation (renal fibrosis) prevalence were (2%), fibrous tissue formation appear grossly as enlarged, fibrosed, hard and cut with difficulty, similar changes were observed in sheep kidney by several investigators (2).

Otherwise the presence of cyst in the kidney was (1%), this might be due to congenital malformation or obstructive lesion or as fundamental change of unknown origin may occur in the tubular basement membrane and result in formation of sacculation or fusiform dilatation of tubules (hydronephrosis), (17, 19, 24). These results are in agreement with those obtained by Ali & Aljeboori, (2017)(21).

All these histopathological changes were also recorded by other researchers, Jibat et al. (2008) and Woube (2008) reported nephritis as the major pathological observation and it is the principal cause for kidney condemnation in sheep(25, 26). Also Ali and Aljeboori, (2017) who reported renal infarction was 28.57% followed by nephritis 26.53%, renal haemorrhages 20.40%, renal amyloidosis 14.28% and hydronephrosis 10.20%(21).

Our histopathological examination revealed that nephritis were the highest incidence.

The most common observed histological nephritis was Interstitial nephritis 9% which were characterized by lymphocyte and plasma cell infiltration in the interstitium with degenerative changes in the Proximal convoluted tubules (Fig.1). Interstitial nephritis occurred from bacterial and viral septicemias or following ingestion of certain irritants, poisons or toxins (4, 20, 27), also it were occurred due to side effects reactions to drugs, there were more than 100 different medications may trigger Interstitial nephritis like Antibiotics and NSAIDs (28).

Our Histopathological study showed prevalence of glomerulonephritis (6%) This finding corroborated with the findings of Sastry and Rao (2006) and Jones et al. (2006) (29, 30). Also Mahouz et al. (2015) recorded the incidence of glomerulonephritis were (6.2%)(3).

Histologically, glomerulonephritis characterized by increase cellularity of glomerular tuft in size and number of endothelial and mesangial cells. Glomerulonephritis mainly occur due to antigen- antibody reactions to the foreign proteins and as a sequel to bacterial and viral diseases (29, 31, 32). Caseous lymphadenitis may cause glomerulonephritis (33).

Tubular nephritis in microscopic examination coms with prevalence of (5%), kidney showed degeneration and necrosis of renal tubular epithelium with pyknotic nuclei (Fig.2). Sastry and Rao (2006) and Jones et al. (2006) also described degenerative and necrotic changes renal tubular epithelium(29, 30). All these changes accrued due to various irritant toxic substances which act directly to produce degenerative changes and necrosis of delicate epithelial cells lining the tubules (19).

Acute Tubular Necrosis (hydronephrosis) also recorded with prevalence of 5%, This finding is in agreement with the findings of Sastry and Rao (2006) and Jones et al. (2006) (29, 30), hydronephrosis in microscopic view showed scattered atrophic glomeruli remain in the thin and fibrous mass with presence of polymorphs and monomorphic cell infiltration (Fig. 3). The obstruction produced by the stasis of urine causing its back pressure leading to atrophy of renal parenchyma was the primary cause (20, 24).

On histopathological examination of infarcted kidneys with prevalence (2%), showed glomeruli and the tubules were discerned through the whole area taking a homogenous pink stain showing coagulative necrosis. Such lesions in kidney were also described by Sastry and Rao (2006) and Jones et al. (2006) (29, 30). All these histological alteration occurred due to the occlusion of main or branches of renal artery (20, 24, 27).

Our results showed prevalence of amyloidosis was (2%). Histologically, kidney showed presence of pinkish amyloid material in Bowann’s space of hypercellular
glomeruli. Similarly, Mensua et al. (2003) also described the renal amyloidosis in sheep and goats (34).

The histomorphologic results showed the presence of hemosiderin pigment with prevalence of (2%). The pigment deposited in the epithelial cells of renal tubules and glomeruli, resulted from degradation of resorbed hemoglobin (17, 24).

**Conclusion**

It was concluded that most kidneys are infected with many gram negative and gram positive bacteria and the pathological examination revealed that the most common observed lesions were renal hemorrhage at macroscopic exam and interstitial nephritis at microscopic exam.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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Prevalence of Dental Anomalies (Mesiodens and Enamel Hypoplasia) Among Primary School Children in Badra/Iraq

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Abstract

Background: Dental anomalies considered as one of the developmental defects during teeth formation caused by both genetic and environmental factors which responsible for notable deviation from normal size, color, number, contour and developmental degree. The aim of this study was to determine prevalence of defect in number (hyperdontia or mesiodens teeth) and structural defect (enamel hypoplasia, localized and generalized,) among primary school children.

Material and methods: A cross sectional study that comprises data from 403 boys and 411 girls; a primary school students aged from 5-12 years with a total of 814 student; was conducted in Badra/ Iraq.

Results: The study shows a prevalence rate of a mesiodens from 814 students was 0.49% and it represented more frequently in males more than females with a ratio of 3:1. While, the prevalence of enamel hypoplasia was estimated to be 0.86 % among the observed population, it observed more in girls than in boys, with the proportion being around 1: 1.333 male: female ratio.

Conclusion: the prevalence of dental anomalies was found to be more in males than in females and most of the cases found in age group between 8-10 years old. Routine checkup for these anomalies during primary and mixed dentation which can help for early diagnosis and detection of these disorders that will help minimizing future complications and give well prognosis.

Key words: prevalence, Dental anomalies, mesiodens, enamel hypoplasia.

Introduction

Dental anomalies are brought about by both hereditary and environmental factors; the deformities in specific genes are the most compelling etiological factors in the prenatal and postnatal periods which responsible for abnormality in tooth measurement, morphology, position, number and structure ¹, ², ³.

Supernumerary teeth (Fig.1) are extra teeth in comparison to normal condition, is one of the developmental problems in children ⁴. Mesiodens are one the most common type of these defects, it mostly present in the midline between the two central incisors. ⁵. It may occur as single, multiple, unilateral or bilateral. It happens in 82% of cases in the maxilla, specifically in the premaxillary district only a few numbers of studies have revealed the occurrence of mesiodens in the mandible ⁵, ⁶, ⁷. The incidence of mesiodens in primary dentition is uncommon in contrast to permanent dentition ⁸, with a two-time risk of appearance in the male when compared to the female ⁹. Morphologically; mesiodens may have various structures cone-like or peg structure, tuberculate and supplemental (tooth like) have been recorded, of which the cone form is the most popular ⁶, ¹⁰. Mesiodens may erupt into oral cavity spontaneously or they stay impacted and not erupted, which intervene with permanent teeth eruption and may be give rise to malocclusion. In general, mesiodens may cause different types of oral problems such as malocclusion, impaction of food, aesthetics problems and formation of cyst ¹¹, ¹².

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Enamel defects are divided into four forms which include: pit, plane, linear, and enamel hypoplasia (Fig. 2). Enamel hypoplasia is one of structural abnormalities, caused by defect in formation of enamel matrix. Both primary and permanent teeth susceptible for this deformity. It is two types, either hypo-mineralization, which is a diminution in mineral substance of tooth enamel or hypo-calcification, which is a distortion of tooth enamel in which normal amount of enamel are formed but hypo-calcified, in both types, the enamel is weak than normal.

Studies have recommended that enamel hypoplasia, especially in anterior teeth, is related with poor esthetics; tooth sensitivity; malocclusion and susceptibility to dental caries.

Early identification and treatment of this anomalies is important to prevent malocclusion, delay eruption, function, esthetic and psychological problems.

The aim of this study was to determine the prevalence of defect in number, hyperdontia (mesiodens teeth) and structure defect enamel hypoplasia among primary school children in Badra- Wasit/Iraq. Data will be useful to evaluate the effectiveness of dental services and preventive programs among population in this small city.

Materials and Method

A cross sectional study that comprises data from 403 boys and 411 girls; a primary school students aged from (5-12) years with a total of 814 student; was conducted from (8) primary schools found in Badra city /Wasit governorate/Iraq. The total number of people population of that city was about 10000 people during time of examination. The examination was done by a well-trained dentist inspector and as a part of the governmental preventive program named “oral and dental care program” to control dental caries and enhance oral health among primary school children in whole of Iraqi cities after obtaining approval from the school administrations and the students’ families for the examination.

The presence of mesiodens and enamel defect were recognized by direct visual assessment using a torch to enhance visualization and disposable dental mirror and probe for each child, radiograph not taken at this study because the field of survey was a classroom of school. All obtained data were statistically analyzed using Microsoft excel sheet to determine the mean and percentage of occurrence.

Results

According to this study, only four patients were recorded with a mesiodens from 814 students as shown in table 1 and 2 with a prevalence to be 0.49% of Badra’s primary school children population (Fig. 3). It occurs more frequently in males more than females with a ratio of 3:1. All mesiodens seen was conical shape, single and concerning to position all mesiodens was palatal placed causing space between the central incisors.

The same tables show that the prevalence of enamel hypoplasia was estimated to be 0.86 % among the observed population (Fig. 3). It happened more much of the time in young female than in young male with approximately proportion being around 1: 1.333 male: female. Most of the anomalies found in age group (8-10) years as shown in table 1.
Table 1 distribution of study sample according to age

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesiodens</td>
<td>Enamel Hypoplasia</td>
<td>Total</td>
<td>Mesiodens</td>
<td>Enamel Hypoplasia</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>5-7 years</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>78</td>
<td>158</td>
</tr>
<tr>
<td>6-8 years</td>
<td>0</td>
<td>0</td>
<td>77</td>
<td>0</td>
<td>0</td>
<td>87</td>
<td>164</td>
</tr>
<tr>
<td>7-9 years</td>
<td>0</td>
<td>0</td>
<td>71</td>
<td>0</td>
<td>2</td>
<td>73</td>
<td>146</td>
</tr>
<tr>
<td>8-10 years</td>
<td>3</td>
<td>2</td>
<td>59</td>
<td>0</td>
<td>2</td>
<td>77</td>
<td>136</td>
</tr>
<tr>
<td>9-11 years</td>
<td>0</td>
<td>1</td>
<td>73</td>
<td>1</td>
<td>0</td>
<td>74</td>
<td>143</td>
</tr>
<tr>
<td>10-12 years</td>
<td>0</td>
<td>0</td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>43</td>
<td>86</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>3</td>
<td>403</td>
<td>1</td>
<td>4</td>
<td>411</td>
<td>814</td>
</tr>
</tbody>
</table>

Table 2 prevalence of mesiodens and enamel hypoplasia observed

| Types of Anomalies | Total cases examined | Males | | | | | | Females | | | | Total cases observed |
|-------------------|----------------------|-------|-------|--------|-------|--------|--------|
|                   | N | % | | | | | | N | % | | | |
| Mesiodens | 814 | 3 | 0.36855 | 1 | 0.1228 | 4 | 0.4914 |
| Enamel hypoplasia | 814 | 3 | 0.36855 | 4 | 0.4914 | 7 | 0.85995 |

Fig. 3 Pie chart showing prevalence of mesiodens and Enamel Hypoplasia in Badra’s primary school children population

Discussion

The present study was conducted for searching dental anomalies “mesiodens and enamel hypoplasia” from 814 primary school students in Badra which is small oil city in the middle and far East of Iraq.

The type of mesiodens found in this study was a single conical tooth found in the midline of the maxilla, with prevalence 0.49% of total sample, this finding is agreed with other studies like: Najm and Younis 19 in Missan governorate\Iraq 0.36%, Al-Nori & Talabani 20 in Baghdad city\Iraq which was 0.4% and Abdullah 21 in Anbar governorate (Fallujah city)\Iraq 0.3%, and less than the prevalence of other studies that reported by Sirkis 22 in AlRadwaniya village in Baghdad which was 0.93% , Bashir 23 in 2006 in Khartoum 7.4% and Peedikayil et al 6 in India 0.71%.

According to this study the male to female ratio was 3:1 which is agreed with Peedikayil et al 6, Rajab and Hamdan 10 and Najim and Younis 19. The findings of the present study are disagreed with Abdullah 21 which found male to female ratio of about 1:1.67 and Najim et al 24 where they found a ratio of 1:3 male to female.

The differences in prevalence of mesiodens teeth in this population as compared to other studies may be due to variation in age of study group, sample size, location of study and nutritional status in addition to that in this study no radiograph was taken so impacted mesiodens were excluded.
The main causes of mesiodens still unclear; but it was suggested to have a genetic origin, higher rate of hyperdontia have been noticed between related families 25.  

Treatment choice includes normal extraction of extra tooth at early of mixed dentition so as to encourage spontaneous eruption and alignment of the incisors, which may be reduce the need for orthodontic treatment 5. Authors suggested that “postponed remove of mesiodens tooth about the ten of age when the central incisor apex almost forms may complicate the treatment plan in which intricate surgical and orthodontic treating may be needful “. Therefore early detection permit the most suitable treatment. 26.

For enamel hypoplasia, the result of the present study found a prevalence of (0.86 %) among the surveyed population which considered less than prevalence of enamel hypoplasia in studies done in different areas in Iraq as they found a percentage of (9.04%) in Fallujah 21; (6.61%) in Mosul 27; (5.8%) in Al-Radwaniya 22; (2.07%) in Sulimania 28 and 2.04% in Missan 19. The findings of this study were also less than other several studies done in different countries which was found (21%) according Enwonwu 29, (6%) of children examined had enamel hypoplasia as stated by Slayton et al 30 in Iowa, USA.

In this study enamel hypoplasia found more recurrent in girls than in boys, with male: female ratio 1: 1.333. There is a controversy in the enamel defect prevalence in relation to the gender, many studies show that there is no significant difference in the prevalence of enamel defect between males and females (Slayton et al 30, and Soviero Soviero et al 31). Other studies recorded greater prevalence among males than females (Malliville 32, Steinberg & Luckas 38), while another studies show the opposite as a higher prevalence of enamel defect found among females as compared to males (Al-Nori &Al-Talabani 20).

The presence of enamel defects may be attributed to local factor such as trauma or infection to the primary tooth which leads to development of defect in permanent successor 34, in addition to general factors such as systemic diseases, like “chickenpox, measles, hypocalcemia, congenital syphilis, and may be due to birth injury or premature birth” 35. On other hand the prevalence of enamel defects may be increases with increasing levels of fluoride ingested 36. Rugg-Gunn recorded that “boys of 14 years classed as malnourished, by height for age percentage had a higher prevalence of enamel defects than those classed as well-nourished” 37. Hover, the deficiency of vitamin D has been reported as a cause of enamel hypoplasia 38. Psychological stress during tooth development may be associated with defect of enamel and enamel hypoplasia 39.

Acknowledgement: We are thankful to all children; their parents, school administration and local government for participating and providing support during this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References


Evaluation of the PCR Method for the Diagnosis of Cutaneous Leishmaniasis in AL Hawija District, Kirkuk, Iraq

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Abstract

Males (191) and females (229) with skin lesions were enrolled in this investigation with the highest incidence of the disease in both sexes being in the age group (0-5) years. The clinical features of the lesions were covered by firmly adherent crust and some were edematous. By using classical microscopic examinations, the amastigotes were detectable in 143 cases out of 200 lesion samples. On the other hand, the use of specific PCR system and DNA sequences provided evidence that Leishmania tropica is the only etiologic agent of cutaneous leishmaniasis in AL Hawija district, Kirkuk province, Iraq.

Key words: Cutaneous leishmaniasis; Polymerase chain reaction (PCR); Diagnosis, AL Hawija

Introduction

Cutaneous leishmaniasis (CL) is the most widespread form of anthropozoonotic disease caused by hemoflagellate of the genus Leishmania, which infect the vertebrate host after a bite by infected sandfly of the genus Lutzomyia in the New World and phlebotomus in the Old World (¹). Human cutaneous infections display a clinical spectrum ranging from primary lesions (oriental sore) that often selfheal to severe mutilating mucocutaneous lesions (Chilcero ulcer, espundia) to diffuse cutaneous leishmaniasis (²). In Iraq CL (Baghdad boil) is widely distributed in the central parts of the country with most cases being caused by L. tropica (urban, dry CL) or L. major (rural, wet CL) (³). During the past decade polymerase chain reaction (PCR) approaches have been developed as sensitive alternatives (⁴) to augment existing an early diagnostic methods based largely upon microscopic examination of Giemsa stained smears, culture of lesion biopsies, histopathology and immunological tests (⁵). The kinetoplast DNA (kDNA) in members of the genus Leishmania represent an ideal PCR target since contains 10000 small minicircular DNAs and have conserved and variable regions that allow differentiation between Leishmania species (⁶). In the present study our validation of kDNA amplification for the diagnosis of CL as part of an epidemiological surveys confirmed the high current rate of infection in an area of endemicity in AL Hawija district, Kirkuk province, Iraq.

Method

Dermal scraping specimens were collected by slitting the border of a skin lesion from 420 patients with clinically suspected CL, who had admitted to AL Hawija hospital from August 2018 to July 2019. The specimens were divided into three samples. The first sample was used for microscopic smear examination. The tissue aspirate was smeared on to glass slide, fixed with methanol and stained with Giemsa. The second sample was used for in vitro culture which was performed by inoculation of tissue fragment in Nove-MacNeal – Nicole (NNN medium) and incubated at 26 C. After 5 days, the samples were transferred to fresh medium and observed for at least 6 weeks before they were diagnosed as negative culture. The third sample was processed for PCR.

Extraction of DNA. DNA as purified using the Genomic Prep Cells and Tissue DNA isolation kit (Bioneer, Korea) according to the protocol of the manufacturer. Frozen tissue sample (20 mg) were lysed by incubate 30 μl of lysis buffer (10 mM Tris –HCl, pH 8.0, 10 mM EDTA, 1 mg/ml of protease K) at 65 C for 2 hours with subsequent heating at 100 C for 30 minutes to denature the enzyme. Cell debris and proteins were removed by centrifugation (10000 Xg at 4 C for 10 minutes) and the supernatant fraction containing the DNA used for PCR. Five microliters of the DNA solution was added to the PCR mixture.
Polymerase chain reaction amplification. The PCR amplification was performed using the oligo primers forward T2b(5'-CGGCTTCGCACCATGCGGTG-3') and reverse B4 (5'-ACATCCCTGCCCACATACGC-3') which were previously reported to specifically amplify the entire 750-bp minicircle kDNA of Leishmania species. The PCR reaction mixture (25µl) containing 10 mM Tris – HCl, 50 mM KCl, 0.1 mg of gelatin per ml, 1.5 mM Mg CL2, 0.2 mM each deoxynucleoside triphosphate, 25 pmol of each appropriate primer and 2.5 U of Taq DNA Polymearse (Promega, USA) was amplified using Perkin-Elmer thermocycler following thermal profile which composed 32 cycles (preincubation at 94 C for 5 minutes, denaturation at 94 C for 1 minute, annealing at 60 C for 1 minute, extension at 72 C for 1 minute and extra incubation at 72 C for 10 minutes). Aliquots (10µl) of amplified PCR mixture were subjected to electrophoresis on 1% agarose gel using X TBE running buffer (0.045 M Tris borate, 1 mM EDTA) and PCR product bands visualized by staining with ethidium bromide (0.4 µg/ml). Positive control DNA was purified from promastigote of L.tropica and from human monocyte and amplified by PCR using the same protocol as described above.

Sequence analysis. PCR was purified using GFX PCR DNA product and Gel band purification Kit (Amersham, UK) following the manufactures instruction. The purified PCR product was sequenced by Macrogen Corporation Korea. Multiple sequence alignment was done using Clustal W method (Bioedit DNA analysis software).

Result

AL-Hawija district, approximately 70 km from the center of Kirkuk province, was chosen as a site for field studies because it is an area of alluvial plains with scattered irrigation channels moderately cultivated. People of this area mostly poor farmers living in mud houses in small scattered villages and are in close association with domestic animals. Four hundred twenty patients with skin lesions were enrolled in the study; 191 were males and 229 females. The age distribution of skin lesions (Table 1) showed the highest peak incidence of the disease in both sexes was in the age group (1-5) years. The median duration of the lesions was 6 months with the highest frequency being presented in September (19%) followed by October (15.4%), November (13%) and December (11.9%). Seventy nine patients (18.79%) presented with multiple lesions (4-7 lesions). It is interesting to note that one patient was with 19 lesions. The lesions were located primarily on the lower extremity (8.8%), face (10.7%) or upper extremity (11.9%). Of the 200 lesion samples 143 lesions were positive by Giemsa – stained smear (figure 1), Yielding a sensitivity of (7% 5%) and only 12 were culture positive with NNN medium. Total DNA was extracted from 24 lesion samples and assessed by electrophoresis on the agarose gel. The extracted DNA was used as templates in PCR reaction. Molecular characterisation of the isolates revealed amplification of the characteristic 600 bp minicircle band (figure 2). The PCR of human monocyte DNA samples from healthy control with no previous exposure to CL-endemic area showed amplification of a band of a size (340-bp) which confirm the fact that the amplicon of 600-bp of lesion isolate being not derived from host DNA (figure 3). The sequencing of the PCR product of 600-bp for each sample after correction and filling the missed nucleotide depending on reverse sequence using chromat Pro, the multiple sequence alignment was done by Clustal W method using Bioedit (DNA analysis program) and compared with previously reported references of Leishmania gentypes was due to L. major starins.

Figure (1) Amastigotes within macrophages. 100X

Figure (2). Representing agarose gel of PCR products obtained from biopsy samples with primers specific for L.tropica. Lane M, represent 123 bp DNA Lader; Lane 1-6 represent positive control.
The phylogenetic relationship using the neighboring – joining method by (NCBI) the nucleotide sequence data reported have been submitted to the gen bank data base with accession number of Leishmania to show the phylogeny tree

Table 1: Age and sex distribution of 420 cases of cutaneous leishmaniasis recorded in AL-hawija district, Kirkuk province, Iraq during 2018 – 2019.

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Male</th>
<th>% infection</th>
<th>Female</th>
<th>Infection%</th>
<th>Total</th>
<th>Infection%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-0</td>
<td>57</td>
<td>13.49</td>
<td>67</td>
<td>15.94</td>
<td>124</td>
<td>29.43</td>
</tr>
<tr>
<td>10-6</td>
<td>45</td>
<td>10.7</td>
<td>48</td>
<td>11.42</td>
<td>93</td>
<td>22.1</td>
</tr>
<tr>
<td>15-11</td>
<td>30</td>
<td>7.1</td>
<td>41</td>
<td>9.7</td>
<td>71</td>
<td>16.9</td>
</tr>
<tr>
<td>20-16</td>
<td>22</td>
<td>5.23</td>
<td>29</td>
<td>6.9</td>
<td>51</td>
<td>12.1</td>
</tr>
<tr>
<td>25-21</td>
<td>17</td>
<td>4.04</td>
<td>24</td>
<td>5.7</td>
<td>41</td>
<td>9.7</td>
</tr>
<tr>
<td>30-26</td>
<td>20</td>
<td>4.76</td>
<td>20</td>
<td>4.76</td>
<td>40</td>
<td>9.5</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td>45.4</td>
<td>229</td>
<td>54.5</td>
<td>420</td>
<td>99.75</td>
</tr>
</tbody>
</table>
Discussions

Rural and urban forms of cutaneous leishmaniasis are known to be prevalent in Iraq probably since 3000 - 2000 BC (6). During the last ten years there have been an outbreak of CL in Alhawija district, Kirkuk province of Iraq. As a result of the second gulf war, the increased human mobility and long distance travel may contribute to the spread of CL in areas where the disease was not endemic. This could possibly make diagnosis and species critical for the importance of establishing control strategies. Prevalence of infected female phlebotomine sand flies in urban and suburban areas of Iraq where USA military personal have been stationed could possibly confirm emergence of new Leishmania foci and the coexistence of multiple Leishmania species in the same geographical local (7). In addition, accurate diagnosis of CL is often difficult because some of the dermal disease have some resemblance to those found in patients with CL. Direct microscopic smear examination of tissue sample for the presence of Leishmania is cheap and simple but rarely successful and require great expertise. Axenic culture of parasites from biopsy specimens or lesion aspirates is sensitive but it is time consuming and is subject to contamination. Therefore, there has been a great need for a more reliable laboratory test for diagnosis of CL in this area. The present study reports the application of PCR as a tool for the identification of Leishmania in lesion aspirates attained from patients suspected to have CL. In the present study we use PCR primers based on sequences present in World Health Organisation reference strain (8). Although, they also anneal to DNA from strains from ALQadessia district, Iraq (9), this had not formally demonstrated in isolate from regions of endemicity in Kirkuk. Thus typing of Leishmania isolated from patients with CL by PCR yielded results in agreement with thus obtained by typing cultured Leishmania tropica (10). The evidence available at present incriminates L. tropica as the only causative agent of CL in Alhawija, Kirkuk district of Iraq and this is in agreement with results reported from neighbouring Iran and Turkey. (11). Thus PCR is more sensitive than conventional methods of diagnosis and is likely to be valuable tools not only for species identification but also for investigation of relationships between causative agents and the clinical manifestation and epidemiology of the disease.

Conclusions

This study showed that PCR technique is the sensitive methods in the diagnosis of leishmaniasis. And from DNA sequencing have found that the main causes of cutaneous leishmaniasis in AL Hawija district is Leishmania tropica.

Acknowledgment: Thanks due to university of Kirkuk for financial support.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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

Funding: Self-funding

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Evaluation of Some Blood Parameters in Anemic Patients in Relation to Periodontal Condition

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Abstract

Background: Periodontal diseases are the inflammatory process result from microbial-host interaction in the tissues surrounding the teeth. Gingivitis and periodontitis are the most common type of periodontal diseases. Anemia of the chronic disease (ACD) is common health problem occur in patients with acute or chronic activation of immune system and production of inflammatory cytokines, so it is resemble to periodontal diseases from this aspect.

Aims of the study: To compare the clinical periodontal parameters (plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL)) and the levels of hemoglobin (Hb), mean cell volume (MCV), and Mean corpuscular hemoglobin (MCH) in blood, and correlate them in patients with gingivitis, chronic periodontitis (CP) and clinically healthy periodontium.

Materials and method: 90 subjects included in the study with age range from 30-50 years old. There were divided into three groups: 30 patients with chronic periodontitis, 30 patients with gingivitis, and 30 subjects with clinically healthy periodontium. Blood samples were collected from the subjects for automated blood analyzer to determine the levels of Hb, MCV, and MCH, after clinical periodontal examination was done for (PLI, GI, BOP, PPD, CAL).

Results: statistically highly significant differences among the groups (Gingivitis and chronic periodontitis) in PLI, GI and BOP score. The highest mean value of Hb shown in control group (15.33) while the highest mean value of MCV demonstrated in gingivitis group (87.44) and the highest mean value of MCH demonstrated in control group (32.09). The correlation of Hb, MCV, and MCH with clinical periodontal parameter shown almost weak negative correlation.

Conclusion: the periodontal diseases like other inflammatory diseases can lead to development of anemia type (anemia of chronic disease) and the severity of disease increase with increase the severity of periodontal diseases.

Key Words: periodontal diseases, Anemia of the chronic disease, Hb, MCV, MCH.

Introduction

Periodontal diseases (PD) are group of inflammatory diseases result from the interaction of periodontal pathogenic bacteria with host, which cause damaging in the tooth supporting tissues[1]. Dental plaque is the main etiological factor of the periodontal diseases, which is a diverse community of microorganisms found on the tooth surfaces as a biofilm[2]. Gingivitis is the reversible inflammatory and more stable type of periodontal disease, characterized by the inflammation of soft tissues surrounding the teeth without formation of pocket and loss of attachment[3]. Periodontitis is a chronic irreversible inflammation caused by specific periodontic pathogenic bacteria mainly anaerobic that accumulate subgingivally in the periodontal pocket. These bacteria and their product (protease, hyaluronidase collagenase, and other endotoxin) cause destruction of alveolar bone and periodontal ligament (PDL)[4,5], that result periodontal pocket or gingival recession or both and may be lead to loss of tooth[6]. Anemia of the chronic disease (ACD) is the second most prevalent type of anemia after iron deficiency anemia, as a result of neoplastic...
conditions, or inflammatory or chronic infection where the bone marrow do not affected and there is a sufficient amount of vitamins and iron that stored[7]. This type of anemia characterized by increase production of pro-inflammatory cytokines like: interferon, tumor necrosis factor(TNF-α) and interleukin 1(IL-1)[8]. The microorganisms or their toxin of the periodontal diseases penetrate the protective barrier of sulcular and junctional epithelium into blood stream cause direct systemic effect (dysfunction or injury of endothelial) or indirectly by their lipopolysaccharides(LPS) which provoke the immune response and produce inflammatory cytokines, so the periodontal diseases resemble other chronic infection can be lead to systemic disease like anemia. This elevation in the production of pro-inflammatory cytokines, lead to change in the iron homeostasis, erythroid progenitor proliferation, production of erythropoietin and the life span of erythrocytes, all of these can be lead to development of ACD[9].

The aim of the present study, evaluate and compare the relationship between the different types of periodontal diseases and anemia by estimation the levels of some blood parameters look like hemoglobin(HGB), mean cell volume(MCV),and mean corpuscular hemoglobin(MCH), to find if the periodontal diseases with different kind of severities can lead to ACD.

Materials and Method

This study is a case-control study, included 90 samples(males and females), age range from(30-50) years old. They were selected from the department of periodontology at Teaching Hospital of College of Dentistry, University of Tikrit and from laboratories of Salah Al-Din Hospital in Tikrit for 3 months from January to March. The inclusion criteria included in the study were: all teeth included in the measurement except third molar teeth, there were at least twenty teeth exist for each patients, should be systemically healthy subjects, the patients with chronic periodontitis must had a probing pocket depth at least ≥4mm for four sits and clinical attachment loss at least 1-2mm, while the patients with gingivitis should had signs and symptoms of gingival inflammation(redness, swelling, bleeding on the probing, other) without loss of the alveolar bone, all of these designed according to international classification of periodontal diseases[10,11]. The subjects that classified, as a healthy periodontium should had no signs and symptoms of gingival inflammation and no pocket formation or clinical attachment loss. The exclusion criteria of the study were: smokers, alcoholism, patients subjected to periodontal treatment and/or received course of antimicrobial, anti-inflammatory, or other medication for treatment of anemia in the three month prior to the study and had other systemic diseases. Subject’s agreement documented by signing on specially designed informed consent. Patients shared in the study had been divided into three groups: group of 30 patients had chronic periodontitis, group of 30 patients had gingivitis and control group consist of 30 subjects with clinically healthy periodontium. Full information for all participants were taken through specially designed case sheet. 5ml venous blood was collected by using 20-gauge needle with 5 ml syringe under aseptic field from ante - cubital fossa for each subject after the measurement of clinical periodontal parameters by using graduated Michigan O periodontal probe that included (plaque index (PLI)[12], Gingival index (GI)[12], bleeding on the probing (BOP)[3], probing pocket depth(PPD)[3], clinical attachment loss[13]), for four sits (mesial, distal, lingual/palatal, buccal/labial ). The collected blood put in the Ethylene diamine tetra acet acid tube then blood analysis were done for Hb, MCV, MCH. The statistical analysis used in the study were mean, mean percentage, standard deviation(SD), Kruskal-Walis H test, and Simple person’s correlation coefficients (r). In the statistical tests, the levels of significant(S) when 0.05≥P≥0.01, non-significant (NS) when P>0.05, while highly significant (HS) when P≤ 0.01. We certify that this study involving human subjects is in accordance with the Helsinki declaration of 1975 as revised in 2013 and that it has been approved by the relevant institutional Ethical Committee[14].

Results

The results in table(1) shows that the high mean value of age parameter appear in CP group(42) and the lowest mean value in control group(31).Table (2) revealed that there are statistically highly significant differences among the groups(Gingivitis and chronic periodontitis) in PLI, GI and BOP score1, also table(3) shown statistically highly significant differences among the groups in Hb, MCV, and MCH. The highest mean value of Hb shown in control group (15.33) and the lowest mean value shown in CP group(10.45). The highest mean value of MCV demonstrated in gingivitis group(87.44) and the lowest mean value demonstrated in CP group(81.43).The highest mean value of MCH shown in control group(32.09) and the lowest mean value demonstrated in control group(22.54) in table(3).
Table (4) revealed, almost non-significant weak correlation of Hb with PLI, GI, BOP, PPD as well as CAL, however the control group in GI show weak positive correlation with Hb. Almost non-significant weak correlation of MCV in all groups with PLI, GI, BOP, PPD and CAL, demonstrated in table (5). Weak positive correlation of MCH with PLI shown in chronic periodontitis group while the other index revealed weak negative correlation with MCH in table (6).

Table (1): Descriptive statistics of age parameter for the study group and control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>Mean</th>
<th>SD ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic periodontitis</td>
<td>30</td>
<td>42</td>
<td>0.24</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>30</td>
<td>35</td>
<td>0.36</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>31</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Table (2): Statistical analysis of clinical periodontal parameters for gingivitis and chronic periodontitis group.

<table>
<thead>
<tr>
<th></th>
<th>PLI</th>
<th>GI</th>
<th>BOP score1</th>
<th>PPD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>±SD</td>
<td>mean</td>
<td>Mean %</td>
<td>±SD</td>
</tr>
<tr>
<td>Chronic periodontitis</td>
<td>2.03</td>
<td>0.32</td>
<td>1.73</td>
<td>0.37</td>
<td>35.1</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>0.81</td>
<td>0.63</td>
<td>1.23</td>
<td>0.45</td>
<td>12.43</td>
</tr>
<tr>
<td>Control group</td>
<td>0.28</td>
<td>0.12</td>
<td>0.42</td>
<td>0.13</td>
<td>-</td>
</tr>
<tr>
<td>Kruskal-Wallis H test</td>
<td>89.34</td>
<td>67.21</td>
<td>55.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>without control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sig.</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (3): Statistical analysis of hemoglobin(g/dl), MCV(FL.), and MCH(Pg.) in blood for gingivitis, chronic periodontitis and control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>HB</th>
<th>MCV</th>
<th>MCH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>±SD</td>
<td>mean</td>
</tr>
<tr>
<td>Chronic periodontitis</td>
<td>10.45</td>
<td>1.09</td>
<td>81.43</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>12.61</td>
<td>1.32</td>
<td>87.44</td>
</tr>
<tr>
<td>Control group</td>
<td>15.33</td>
<td>1.29</td>
<td>85.21</td>
</tr>
<tr>
<td>Kruskal_Wallis H test</td>
<td>0.453</td>
<td>0.765</td>
<td>0.829</td>
</tr>
<tr>
<td>without control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Sig.</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
</tr>
</tbody>
</table>
Table (4): Correlation between hemoglobin (g/dl) level with clinical periodontal parameter of gingivitis, chronic periodontitis and control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>PLI r</th>
<th>PLI P</th>
<th>PLI Sig.</th>
<th>GI r</th>
<th>GI P</th>
<th>GI Sig.</th>
<th>BOP score1 r</th>
<th>BOP score1 P</th>
<th>BOP score1 Sig.</th>
<th>PPD r</th>
<th>PPD P</th>
<th>PPD Sig.</th>
<th>CAL r</th>
<th>CAL P</th>
<th>CAL Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic periodontitis</td>
<td>-0.321</td>
<td>0.604</td>
<td>NS</td>
<td>-0.631</td>
<td>0.419</td>
<td>NS</td>
<td>-0.381</td>
<td>0.421</td>
<td>NS</td>
<td>-0.406</td>
<td>0.316</td>
<td>NS</td>
<td>-0.241</td>
<td>0.094</td>
<td>NS</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>-0.036</td>
<td>0.123</td>
<td>NS</td>
<td>-0.143</td>
<td>0.106</td>
<td>NS</td>
<td>-0.345</td>
<td>0.230</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>-0.007</td>
<td>0.074</td>
<td>NS</td>
<td>-0.019</td>
<td>0.053</td>
<td>S</td>
<td>-0.213</td>
<td>0.187</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (5): Correlation between MCV (FL) level with clinical periodontal parameter of gingivitis, chronic periodontitis and control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PLI r</th>
<th>PLI P</th>
<th>PLI Sig.</th>
<th>GI r</th>
<th>GI P</th>
<th>GI Sig.</th>
<th>BOP score1 r</th>
<th>BOP score1 P</th>
<th>BOP score1 Sig.</th>
<th>PPD r</th>
<th>PPD P</th>
<th>PPD Sig.</th>
<th>CAL r</th>
<th>CAL P</th>
<th>CAL Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic periodontitis</td>
<td>-0.213</td>
<td>0.091</td>
<td>NS</td>
<td>-0.670</td>
<td>0.061</td>
<td>NS</td>
<td>-0.421</td>
<td>0.083</td>
<td>NS</td>
<td>-0.502</td>
<td>0.134</td>
<td>NS</td>
<td>-0.321</td>
<td>0.170</td>
<td>NS</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>-0.401</td>
<td>0.321</td>
<td>NS</td>
<td>-0.431</td>
<td>0.291</td>
<td>NS</td>
<td>-0.351</td>
<td>0.122</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>-0.451</td>
<td>0.241</td>
<td>NS</td>
<td>-0.356</td>
<td>0.153</td>
<td>NS</td>
<td>-0.241</td>
<td>0.042</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (6): Correlation between MCH (Pg.) level with clinical periodontal parameter of gingivitis, chronic periodontitis and control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PLI r</th>
<th>PLI P</th>
<th>PLI Sig.</th>
<th>GI r</th>
<th>GI P</th>
<th>GI Sig.</th>
<th>BOP score1 r</th>
<th>BOP score1 P</th>
<th>BOP score1 Sig.</th>
<th>PPD r</th>
<th>PPD P</th>
<th>PPD Sig.</th>
<th>CAL r</th>
<th>CAL P</th>
<th>CAL Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic periodontitis</td>
<td>-0.390</td>
<td>0.056</td>
<td>S</td>
<td>-0.179</td>
<td>0.533</td>
<td>NS</td>
<td>-0.152</td>
<td>0.654</td>
<td>NS</td>
<td>0.530</td>
<td>0.171</td>
<td>NS</td>
<td>0.232</td>
<td>0.543</td>
<td>NS</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>-0.033</td>
<td>0.752</td>
<td>NS</td>
<td>-0.102</td>
<td>0.732</td>
<td>NS</td>
<td>-0.219</td>
<td>0.123</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>-0.019</td>
<td>0.024</td>
<td>NS</td>
<td>-0.185</td>
<td>0.321</td>
<td>NS</td>
<td>-0.233</td>
<td>0.932</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The results showed that the lowest mean value of age parameter appear in the control group while the highest mean value demonstrate in chronic periodontitis group and this may be due to that the incidence of CP is usually associated with old age [15]. The highest mean value of PLI showed in CP group compared to that in other study group with highly significant differences among the study and control group. Dental plaque has important role in the pathogenesis of periodontal diseases, because it is considered the primary cause of gingivitis and with subsequent accumulation of biofilm in inadequate oral hygiene and absence of mechanical plaque control lead to progression of gingivitis to periodontitis [16].

The highest mean value of GI and BOP score1 revealed in CP group with highly significant difference among the study and control group, and this due to the accumulation of plaque result in alteration of gingival index and beginning of disease process with all signs and symptoms of gingivitis and subsequent development of CP [17]. The lowest mean value of HB, MCV and MCH showed in CP group with highly significant difference among the study group and control group, this due to that the periodontitis caused by specific types of microorganisms mainly gram negative, anaerobic bacteria with high percentage of spirochetes accumulate in gingival sulcus in the periodontal pocket [18], the actual active sits of connective tissue loss showing have a high percentage of P.gingivalis(P.gingivalis), Aggrigatibacter actinomctecomitance(A.a), Campylobacter rectus(C. rectus), and etc., when it is compared with non-active sits that do not have loss of attachment[19].

These Mo. liberate toxic substances such as lipopolysaccharides, protease, collagenase and other that motivate the innate and adaptive immune system of the host[20]. Antigen presenting cells such as dendritic cells, B cells, and macrophage begin to interact with T cells that lead to differentiation of plasma cells and produce of chronic lesion with beginning the signs and symptoms of inflammation [21]. All these process lead to release of inflammatory cytokines in blood such as TNF-α, IL-6, IL-8 and other from various cells like monocytes, macrophage, fibroblast. The liberation of these cytokines (interleukins’ and TNF-α) lead to bone and attachment loss and construes the pathogenesis of periodontal diseases [22,2].

Previously chronic inflammation or infection may be interpret the underlying cause of ACD, and increase some of inflammatory cytokines such as TNF-α, IL-1 are observed in ACD. These cytokines lead to decrease the life span of RBC and impair erythroids development and reduce erythropoietin response to anemia and abnormality of iron store, in addition the increase production of inflammatory cytokines inhibit the maturation and differentiation of erythrocytes[23,24].

Because of the same cytokines observed in ACD and periodontitis and the treatment of periodontal diseases show the improvement of anemia, can proposed the relation between these two diseases, and this agree with Yamamoto et al. [25], and Naik et al. [26], this determine the association between blood parameters and periodontitis which the aim of the study. While the almost weak non-significant correlation showing in the study may be due to the small human sample size of data.

So, we can concluded that the periodontal diseases like other inflammatory diseases can lead to development of anemia type(anemia of chronic disease) and the severity of disease increase with increase the severity of periodontal diseases because decrease erythroid proliferation in bone marrow and life cycle of red blood cells decrease because the cytokines prevent release the erythropoietin from kidney[27].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References


Effect of Algal Extracts on the Growth of Tow Bacterial Types Isolated from Pollutants Discharge

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¹College of Education for Pure Sciences- Ibn- Al-haitham, University of Baghdad, Iraq,
²Ministry of Education, Open Educational College, Iraq

Abstract

Effect of Chlorococcum humicola alcoholic algae extract was studied on the growth of, Pseudomonas aeruginosa, and Klebsiella pneumonia, which were isolated from contaminated water. The extract of Ch. humicola showed a high efficiency in reducing the numbers of the two types of bacteria. The removal rate of K. pneumonia were 0.0, 48.4 and 57.0, The removal rate of P. aeruginosa were 63.1, 79.8 and 82.9% after 24, 48, 72 h respectively. The results improved that the K. pneumonia is more sensitive than P. aeruginosa for algae extract concentrations used in study, and the best effective time is 24h for the two bacterial species. The aim of the study was to eliminate microorganisms using the Alcoholic algae extract. Especially P. aeruginosa and K. pneumonia of bacterial isolates that cause many diseases for human and animals.

Keywords: Alcoholic algae extract, toxicity, polluted water

Introduction

Klebsilla pneumonia infections may occur at almost all body sites, but the highest incidence was found in the urinary and respiratory tracts. The main population at risk are neonates and patients predisposed by prior surgery, diabetes, malignancy, etc. [1]. Also it is found in mammalian mucosal surfaces and opportunistic pathogens and the principal pathogenic reservoirs of infection are the gastrointestinal tract of patients and the hands of hospital personnel organisms can spread rapidly. Often leading to nosocomial [2]. As well as K. pneumonia occurs in the lungs. Where they cause destructive changes inflammation, hemorrhage and sometimes producing a thick, bloody, mucoid sputum described as currant jelly sputum [3].

Pseudomonas aeurginosa causes disease in healthy persons. Most infection occurs in compromised hosts or only when they reach tissue outside the intestinal tract, particularly the urinary tract, biliary tract, lung, eye, kidney, ear intestine and damaged or burned skin and causing inflammations at these sites [4].

P.aeurginosa infection occurs in three stages the bacterial attachment and colonization followed by local invasion and dissemination and systemic disease, And two mechanisms contribute to the complex pathogenesis of these respiratory infections with P.aeurginosa: first the bacterial adhesion to the respiratory epithelial cells initiates infections. And secondly inhibition of phagocytosis by macrophages and poly morph leucocytes far ours progression. And during both mechanisms. Bacterial cells become mucoid by secreting an exopoly-saccharide containing alginate [5].

From another view the water environment has a great importance in the world through covering an area of about 75% of the world. Therefore, attention to water resources is necessary for the purpose of ensuring the requirements of human use in all fields.

Water Sources, especially surface water, is exposed to the dangers of pollution, which leads, directly or indirectly, to the ecosystem. Water pollution means any change in the physical, chemical and biological characteristics of water, as well as the pathogens resulting from the effects of microorganisms such as bacteria, fungi and viruses [6].

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The rivers are the most water bodies susceptible to pollution because of the discharges resulting from the various human activities that make them unusable only after the adoption of additional treatment and liquidation units of high and cost technology.

In addition to the use of chemical sterilizers, the algae are microorganisms that are highly efficient in inhibiting the growth and effectiveness of various microorganisms [7].

It also contributes to the process of self-purification in the water bodies through the photosynthesis process, where they release the dissolved oxygen gas, which causes the gas balance between oxygen and carbon dioxide between the atmosphere and water, that have a biological, medical and economic importance and are necessary to sustain life [8].

As well as algae have recently received a lot of attention as a new biomass source for the production of a new energy [9]. Some of the main characteristics which set algae apart from other biomass sources are that algae can have a high oil or starch content do not require agricultural land. Fresh water is not essential and nutrients can be supplied by waste water and CO₂ by combustion gas [10]. The first distinction that needs to be made is between macroalgae or microalgae [11].

Therefore, the aim of the study was to eliminate some microorganisms, Pseudomonas aeruginosa, and Klebsiella pneumoniae using the Alcoholic algae extract.

**Material and Method**

1. Isolation and diagnosis of bacterial

The bacteria, P. aeruginosa, and K. pneumoniae bacteria were isolated from the drainage water of the Rustmiya Sewage Treatment Plant in Baghdad using Prescott method [12]. They were grown on the Nutrient agar medium, then 1ml were taken from pure isolation into glass vials containing broth nutrient medium and incubated at 37°C.

2. Preparation of dried algae

Chlorococcum humicola was isolated from small stream near the University of Baghdad - Jadriya, from different places where several samples were collected, according to [13].

Then the algae was laboratory diagnosed using a microscope based on the diagnosis method [14].

The algae was cultured and purified in 10ml test tube with Chu13 medium by using dilution method and streaking plating under constant laboratory conditions (temperature of 25±2 °C, 16:8h light:dark, 3000 LUX) [15]. The axenic algal culture was incubated in the incubation room for 18 days, the harvesting where by Centrifuge with of 3000 rpm/minute speed for 15 minute. Then the sediment dried in 45°C for 48h and saved in dark until used.

3. Extraction of active substances from algae

1.5 g of the dried algae was dissolved in 250 ml of Chloroform and then placed in shaking incubator at 25 °C with 70 rpm/minute for 15 min and then dried at 40 °C [16].

4. Preparation of concentrations of algae extract

(1) 1ml of bacterial suspension was taken for each isolation of bacterial isolates and placed in a sterile glass flask containing different concentrations of the extract: (0.07, 0.15, 0.31, 0.62, and 1.25) (mg / l) prepared from the primary concentration by the dilutions and then the volume was completed to 100 ml of sterile sewage water with (2) repeated of each bacterial isolation, In addition to the control treatment which was free of the algae extract and incubated at 37°C for 72 hours [17].

The number of bacterial cells was calculated in (1) ml of bacterial suspension using the method of Hemocytometer (Chamber counting) where the calculation was daily for 72 hours [18].

**Results and Discussion**

The results showed that the algae extract showed a clear effect on the microorganisms used, where the numbers of were decreased that treated with different concentrations of C. humicola extract (1.25, 0.62, 0.31, 0.15, and 0.07) mg / l within 72 hours.

The algae extract showed no effect on the number K. pneumoniae in 24 hours. On the 48 hours, the number of bacteria decreased from 470 cells/ml to 422, 400, 386, 330, and 290, cells/ml. On the 72 hours, the number of bacteria was 350, 300, 290, 276, and 200 cells/ml. As shown in Fig.(1).
The effect of algae extract on *P. aeruginosa* bacteria, using different concentrations (0.07, 0.15, 0.31, 0.62, 1.25) mg / l, was the decrease in the number of cells on the first day of the total 410 to (390, 280, 220, 176, 155) cell / ml. The decrease in the number of bacteria on the 48 hours was (350, 264, 230, 100, 85) Cell / ml. On the 72 hours, the numbers were as follows: (300, 210, 120, 83, and 72) cell / ml. As shown in, Fig.(2)

The study results showed the effect of algae extract towards bacteria *P. aeruginosa*, while the *K. pneumoniae* showed resistance to the effect of the extract was low.

As compared to other types of bacteria. This is because they contain complex layers in their walls, making them more difficult to penetrate into the cell walls [19].

The studies also indicated that the algae extract had a clear inhibitory effect on *P. aeruginosa* and, and the reason is due to their contain of the peptides rings and alkaloids, in addition to polysaccharides [20].

The study agrees with [2], who pointed to the ability of the algae extract to reduce the bacterial numbers, where these fatty acids interact with membrane proteins causing deformation in the structure and effectiveness of the membrane.

Also the study agrees with [21], which indicated the ability of *Cladophora phare* extract to reduce the number of *E. coli* bacteria, the reason is due to the secretion of many substances such as Ferpenoids, fatty acids. amino acids, which have inhibitory effect on bacteria [22]. And they produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other selling organisms [24]. These active metabolites also known as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, terpenoids, are produced by several species of marine macro and macroalgae and have antibacterial, anti-algal, and anti-fungal properties which are effective in the prevention of bio fouling and have other uses in therapeutics [24].

The algae extract has an inhibitory effect against the positive and negative bacteria (Gram stain) by inhibiting the Enzyme of phosphatase protein, which plays an important role in the process of inserting substances into the body of the organism [25].

The active substances found in the algae extract have an effect on the positive bacteria of (Gram stain) rather than on the negative bacteria [26].

As indicated by [27]. The reason is that the negative bacteria are less sensitive than the active compounds found in positive bacteria of (Gram stain) due to their cell wall structure, which consists of many layers.

The results of the study showed that the concentration of 1.25 mg / l was the most concentrated effect on bacterial cell count, where *Klebsiella pneumoniae* was the most resistant to the effect of the extract, and the effect of the algae extract was more effective on *E. coli*. [28]

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding
References


Prevalence of Aerobic Bacterial Vaginosis and Trichomonas Vaginalis Associated with Socioeconomic Factors among Women in Misan Governorate

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Abstract

Vagina is an ecosystem balance, human vagina is dominated by lactobacillus spp which creates a vaginal acidity environment(3.8-4.5) to protect vagina from Vaginitis pathogen. Vaginitis is an inflammation of vagina caused by bacterial vaginosis and Trichomonas vaginalis. The aims of this study were investigated aerobic bacterial vaginosis, lactobacillus spp, and T.V in women with vaginitis. Study the clinical feature and demographic factors with vaginitis. 345 samples were collected from women with vaginitis. Study a demographic variables such as age, education level, socioeconomic state, residence, parity, in pregnant and non-pregnant women.

Keywords: Aerobic bacterial vaginosis, Vaginosis, Trichomonas vaginalis, Socio demographic, Lactobacillus spp

Introduction

Aerobic bacterial vaginosis is abnormal vaginal bacteria derived from bacterial vaginosis types. It is caused by replacement lactobacillus spp with Escherichia coli, Staphylococcus aureus, Streptococcus agalactiae. Aerobic bacterial vaginosis caused to increase in Vaginal pH>4.5 and inflammation with WBCs infiltration. Bacterial vaginosis causes abnormal vaginal secretions. Trichomonas vaginalis is an extracellular single cell flagellated parasite of different in shape, with active motility.

Materials and Method

Patients: Three hundred and forty five sample were obtain from females with different age (15-45) year attending to gynecology Out-patients department in Al-Sadder hospital( Missan city).

Measurement of ph: For ph measurement, vaginal secretion be occurred by placed on ph paper. The ph paper color after that compared to the pH value on a standard chart.

Whiff test: Examination a whiff test is positive when a (10% )KOH is added to discharge and release a bad odor as a positive indicator.

Microscopic examination : Which include a wet amount technique for vaginal discharge to detected Trichomonas vaginalis, bacteria, clue cells, and WBCs. Clue cells is a large epithelial vaginal cells adhesion coated with bacilli bacteria. Gram stain used for examining aerobic bacterial vaginosis.

Cultivation of vaginal specimens: All vaginal swabs were cultured immediately on Blood, MacConKey Chocolate agar for bacterial isolates and MRS agar as a selective media for lactobacillus spp. about three vaginal swabs from each infected woman, first swabs for cultivation laboratory media and second swabs for cultivation on MRS agar with or without (3-5)% CO₂ for lactobacillus spp grow. Third swabs for wet amount technique and gram stain.

Biochemical tests: Some biochemical test was used for diagnosing bacterial vaginosis such as Vitex-2, Catalase test and Oxidase test.

Identification of Trichomonas vaginalis: To identify of Trichomonas vaginalis in swabs by taking vaginal discharge from women with vaginosis. Then take one drop from PBS and put on glass slide and put a cover slide on it this technique called wet a mounting technique and giemsa stain to determinate a flagellated
parasite positive results was a motility of parasite \(^{(10)}\).

**Results**

**Socio Demographic characteristics of population:**

In table (1) high percentage was age groups F range from (40-45) year were 68(80%). The second variable was education level and related with vaginal infection is an important part due to education may help to decreased a diseases. The socio-economic level was taken the high percentage was in women with a middle social level of 114(76%), in this study rural was recorded a high percentage 157(79.3%). As for the parity of the study, the high ratio with multiparity 158 (77.1%). The pregnancy state effect on women with vaginosis but highest ratio was documented in non-pregnant women 277 (75.9%).

<table>
<thead>
<tr>
<th>Variable factors</th>
<th>No. of cases</th>
<th>Frequency</th>
<th>Positive cases%</th>
<th>Negative cases %</th>
<th>( \chi^2 )</th>
<th>( P )-value R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups)(years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.135 R=0.071</td>
</tr>
<tr>
<td>GA: 15-19</td>
<td>49/345</td>
<td>14.2%</td>
<td>33(67.3%)</td>
<td>16(32.7%)</td>
<td></td>
<td>5.16</td>
</tr>
<tr>
<td>GB: 20-24</td>
<td>56/345</td>
<td>16.2%</td>
<td>46(82.1%)</td>
<td>10(17.9%)</td>
<td>6.044*</td>
<td>P=0.05* R=0.017</td>
</tr>
<tr>
<td>GC: 25-29</td>
<td>59/345</td>
<td>17.1%</td>
<td>41(69.5%)</td>
<td>18(30.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD: 30-34</td>
<td>60/345</td>
<td>17.4%</td>
<td>42(70%)</td>
<td>18(30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE:35-39</td>
<td>36/345</td>
<td>10.4%</td>
<td>30(83.3%)</td>
<td>6(16.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GF: 40-45</td>
<td>85/345</td>
<td>24.6%</td>
<td>68(80%)</td>
<td>17(20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education levels</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>97/345</td>
<td>28.1%</td>
<td>73(75.3%)</td>
<td>24(24.7%)</td>
<td>6.044*</td>
<td>P=0.05* R=0.017</td>
</tr>
<tr>
<td>Primary</td>
<td>130/345</td>
<td>37.7%</td>
<td>100(76.9%)</td>
<td>30(23.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>93/345</td>
<td>27%</td>
<td>64(68.8%)</td>
<td>29(31.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Higher</td>
<td>25/345</td>
<td>7.2%</td>
<td>23(92%)</td>
<td>2(8.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socioeconomic classes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>145/345</td>
<td>42%</td>
<td>104(71.7%)</td>
<td>41(28.3%)</td>
<td>3.07</td>
<td>P=0.10 R=0.092</td>
</tr>
<tr>
<td>Middle</td>
<td>150/345</td>
<td>43.5%</td>
<td>114(76%)</td>
<td>36(24%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>50/345</td>
<td>14.5%</td>
<td>42(84%)</td>
<td>8(16%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>198/345</td>
<td>57.4%</td>
<td>157(79.3%)</td>
<td>41(20.7%)</td>
<td>3.86*</td>
<td>P=0.025* R=0.106</td>
</tr>
<tr>
<td>Urban</td>
<td>147/345</td>
<td>42.6%</td>
<td>103(70.1%)</td>
<td>44(29.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Cont... Table (1): Socio-demographic characteristics of respondents in vaginosis women

<table>
<thead>
<tr>
<th></th>
<th>Nullipara</th>
<th>46/345</th>
<th>13.3%</th>
<th>30 (65.2%)</th>
<th>16 (34.8%)</th>
<th>P=0.11</th>
<th>R=0.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unipara</td>
<td>94/345</td>
<td>27.2%</td>
<td>72 (76.6%)</td>
<td>22 (23.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multipara</td>
<td>205/345</td>
<td>59.4%</td>
<td>158 (77.1%)</td>
<td>47 (22.9%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Woman state**

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>46/345</th>
<th>13.3%</th>
<th>33 (71.7%)</th>
<th>13 (28.3%)</th>
<th>P=0.25</th>
<th>R=0.03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Pregnant</td>
<td>299/345</td>
<td>86.7%</td>
<td>277 (75.9%)</td>
<td>72 (24.1%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P≤0.05* (p≤0.01)**

**Clinical manifestations of vaginal infection** : Vaginal discharge was the most common sign in Vaginitis women. The highest percentage of discharge was recorded 247(76%), than the lower percentage of pelvic inflammatory was documented 38(77.6%) as in table (2).

Table (2): A Clinical manifestations of vaginitis women

<table>
<thead>
<tr>
<th>Symptoms and signs</th>
<th>No. of cases N=345 %</th>
<th>No. of positive cases N=260</th>
<th>No. of negative cases</th>
<th>χ²</th>
<th>P-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal discharge</td>
<td>325(94.2%)</td>
<td>247(76%)</td>
<td>78(24%)</td>
<td>1.228</td>
<td>0.134</td>
<td>0.060</td>
</tr>
<tr>
<td>Profuse itching</td>
<td>317(91.9%)</td>
<td>239(75.4%)</td>
<td>78(24.6%)</td>
<td>0.002</td>
<td>0.48</td>
<td>0.002</td>
</tr>
<tr>
<td>Malodor</td>
<td>298(86.4%)</td>
<td>226(75.8%)</td>
<td>72(24.2%)</td>
<td>0.268</td>
<td>0.30</td>
<td>0.028</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>173(50.1%)</td>
<td>131(75.7%)</td>
<td>42 (24.3%)</td>
<td>0.024</td>
<td>0.43</td>
<td>0.008</td>
</tr>
<tr>
<td>Pelvic inflammatory</td>
<td>49(14.2%)</td>
<td>38(77.6%)</td>
<td>11(22.4%)</td>
<td>0.147</td>
<td>0.35</td>
<td>0.021</td>
</tr>
</tbody>
</table>

* (In present trichomonas vaginalis, aerobic bacterial and lactobacillus spp)

Effect of ph measurement with vaginosis pathogen:

Change in ph help to encourages the growth of pathogenic microorganisms which prefer alkaline ph. This study showed the effect of ph with lactobacillus spp when ph was acidity. In Trichomonas vaginalis, the highest percentage was observed 62(78.5%). The high percentage of aerobic bacterial when ph was 156(60%) as in table (3) and figure (1) show amoeboid shape of Trichomonas vaginalis and lactobacillus spp.
Table(3): A correlation ph level with vaginosis pathogen

<table>
<thead>
<tr>
<th>Microorganism ≤ 4.5</th>
<th>Vaginal pH &gt; 4.5</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus spp</td>
<td>No. 187</td>
<td>113</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>% 62.3%</td>
<td>37.7%</td>
<td>100%</td>
</tr>
<tr>
<td>T. vaginalis</td>
<td>No. 17</td>
<td>62</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>% 21.5%</td>
<td>78.5%</td>
<td>100%</td>
</tr>
<tr>
<td>Aerobic bacterial vaginosis</td>
<td>No. 104</td>
<td>156</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>% 40.0%</td>
<td>60.0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*(P ≤ 0.01), **(P 0.001), NS = no significance

Figure (1): A- Amoeboid shape for *Trichomonas vaginalis* staining giemsa stain

B- Microscopic examination of *Lactobacillus spp* by gram stain under 1000X magnification

Types of isolates of aerobic bacteria which causes vaginosis:

Different types of aerobic bacterial had been identified. The bacteria were diagnosed by vitex-2 and some biochemical test. The following results were shown in table (4) high incidence of bacteria vaginosis was recorded *Escherichia coli* 36(13.8%).

Table(4): Types of isolates aerobic bacterial vaginosis

<table>
<thead>
<tr>
<th>No.</th>
<th>Aerobic bacterial vaginosis</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td><em>Escherichia coli</em></td>
<td>36</td>
<td>13.8%</td>
</tr>
<tr>
<td>2-</td>
<td><em>Staphylococcus aureus</em></td>
<td>27</td>
<td>10.4%</td>
</tr>
<tr>
<td>3-</td>
<td><em>Enterobacter spp</em></td>
<td>25</td>
<td>9.6%</td>
</tr>
<tr>
<td>4-</td>
<td><em>Streptococcus spp</em></td>
<td>23</td>
<td>8.8%</td>
</tr>
<tr>
<td>5-</td>
<td><em>Klebsiella spp</em></td>
<td>20</td>
<td>7.7%</td>
</tr>
<tr>
<td>6-</td>
<td><em>Proteus spp</em></td>
<td>17</td>
<td>6.5%</td>
</tr>
</tbody>
</table>
Discussion

Socio-Demographic characteristics of population

Women in age groups F (40-45)years as a higher rate 68(80%). These results was disagree with Bhalla and Chawla.,2007 were recorded a highest percentage among age groups (25-49) year was 57(32.5%) which had a high sexual activity and high hormones activity with bacterial vaginosis (11). In Sulaimania ( Kadir and Fattah .,2010) were documented a high percentage between age group (26-35)year was 5(2.48%) these disagree with this results (12). However, the age groups F (40-45)years more effect than young age . This age groups may be relation with low activity of hormones as estrogen hormone and low secretion of glycogen ,elevated ph to alkaline when advance in age which courage pathogens . Primary education was recorded a highest rate 100(76.9%)and this results in line with report in Nigeria (13). This results agree with Singh and Kanti .,2016 (14).In Iraq were documented that illiterates women were more effected (15).The most common causes related to neglect their health ,lack of education, bad body hygiene and lack of health are women’s awareness programs (16). In socio-economic level a highest percentage in middle state 114(76%). This study also agrees with Yasmeen and Mohamoud.,2011 (17).The main reasons for the role of socioeconomic stranded to infected by many women below the average in level of nutrition and health neglect (18).A current study reported a high percentage in rural residence157(79.3%).This results disagree with Mahmoud .,2017 due to lack of awareness of health neglect and low education and poor life style (19). In parity  observed that multipara had a higher rate of 158(77.1%).(Singh.,2015) study a parity as parameter with Trichomonas vaginalis was recorded a high percentage in multipara 31(26.7%) (20). These results related to abortion ,vaginal delivery , douching uses and having oral contraceptive ,intrauterine device use and sexual activity those similar to Victor.,2016) (21). This related to low education, poor feeding, bad .Current study showed vaginitis based on women  states with non-pregnant women state this agree with Yasmeen.,2011 (17).

Clinical Manifestations of vaginal infection :

Vaginal discharge recorded a high percentage 247(76%) . The percentage of vaginal discharge was 247(76%) with aerobic bacterial and Trichomonas vaginalis. Others studies had shown a percentage of

<table>
<thead>
<tr>
<th>Types of isolates aerobic bacterial vaginosis</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus epidermidis</td>
<td>14</td>
<td>5.4%</td>
</tr>
<tr>
<td>Acrobacter spp</td>
<td>14</td>
<td>5.4%</td>
</tr>
<tr>
<td>Enterococcus faeciimi</td>
<td>12</td>
<td>4.6%</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>12</td>
<td>4.6%</td>
</tr>
<tr>
<td>Leuconostoc mesenteroidis</td>
<td>12</td>
<td>4.6%</td>
</tr>
<tr>
<td>Staphylococcus albus</td>
<td>9</td>
<td>3.5%</td>
</tr>
<tr>
<td>Serratia spp</td>
<td>8</td>
<td>3.1%</td>
</tr>
<tr>
<td>Acinetobacter spp</td>
<td>6</td>
<td>2.3%</td>
</tr>
<tr>
<td>Kocuria spp</td>
<td>4</td>
<td>1.5%</td>
</tr>
<tr>
<td>Pantoea spp</td>
<td>4</td>
<td>1.5%</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>4</td>
<td>1.5%</td>
</tr>
<tr>
<td>Citrobacter spp</td>
<td>4</td>
<td>1.5%</td>
</tr>
<tr>
<td>Peptostreptococcus spp.</td>
<td>4</td>
<td>1.5%</td>
</tr>
<tr>
<td>Providencia spp</td>
<td>2</td>
<td>0.8%</td>
</tr>
<tr>
<td>Lactococcus gravies</td>
<td>2</td>
<td>0.8%</td>
</tr>
<tr>
<td>Staphylococcus haemolyticus</td>
<td>1</td>
<td>0.4%</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>100%</td>
</tr>
</tbody>
</table>

Cont...
discharge with bacterial vaginosis 11(34.4%) and Trichomonas vaginalis 13(13.1%) (22). An abundant itching was documented 239(75.4%) and this results different with Mateus.,2016 were documented a vaginal discharge 56(0.18%) (23). Also Ranjit.,2018 in her studies was reported ,itching as a vaginal signs 13(23.2%) and no vaginal itching 78(75%) (24). (Nzyomo.,2013) was reported a high rate abdominal pain (25), his study agree with this results . Bacterial vaginosis had a special bad odor. This study had another clinical manifestations as a malodor that was reported 226(75.8%) and agreement with Ranjit.,2018 was study a foul odor as one of a clinical manifestations was 31(29.9%) in non- present (24). A bad odor with Trichomonas vaginalis in this study similar to Al-Samarraie.,2002 was documented a bad odor (88.09%) and discharge (11.09%) this causes may be related to metabolic by products of aerobic bacterial (26). Fishy odor may related to volatilization of amine such as putrescine and cadaverine which produce by bacterial (27). Abdominal pain in this study was reported 131(75.7%) this agreement with Bhallo.,2012 (28). Ranjit .,2018 was study abdominal pain as symptoms of vaginosis was a 22(26.6%) agree with this results(24). Pelvic inflammatory, was recorded 38(77.6%), this study agrees with (Yasmeen.,2011) observed a pelvic inflammatory and reported 53(74.6%) and its similar with this data (17) this result may be related to douching use, that enhance to growth bacteria(29).

**Correlation of pH levels of Vagina with vaginosis pathogen:**

PH is complementary in diagnosis vaginitis as an evidence of inflammation. Lactobacillus spp was recorded a higher pH level at less than 4.5 was 187(62.3%). The study was observed and agreement with Tachedjian and others.,2017. Estrogen hormone also rise during puberty leading to increase glycogen deposition in vaginal epithelial cells as well as increased colonization of lactobacillus spp and pathogenic microorganisms. Lactobacillus spp breakdown glycogen into glucose and maltose , then convert to lactic acid and led to decrease pH (30). Trichomonas vaginalis and bacterial vaginosis, which were preferable to alkaline pH at expense of lactobacillus spp. Valadkhani ,2004 showed the role of pH in adhesion and colonization of trichomonas with lactobacillus spp at 47% and 35%. Trichomonas vaginalis, was recorded a highest proportion with alkaline pH 62(78.5%) (31). Also this study documented a high pH in alkaline pH 156(60%) with aerobic bacterial vaginitis and this identical with Liston had shown a suitable pH is an important with development of Trichomonas vaginalis. Some factors that help to elevate pH level like antibiotics treatment, seminal fluid and intercourse. The vaginal acidity related to increase estrogen hormone and lead to increase a thick of stratified epithelium during sexually maturity which help lactobacillus spp to colonize epithelium and increase of glycogen.

**Distribution of Aerobic bacterial vaginosis in vaginitis women:**

This study documented a high rate of aerobic bacterial vaginosis was E.coli 36(13.8%). Adane was studied some bacterial Vaginitis like Escherichia coli was a high rate 43(28.5%), therefore his data agree with our data which reported Escherichia coli a high rate (32). In McDonald work, on a vaginal discharge and associated with aerobic bacterial and recorded it ,he founded Streptococcus spp group, Staphylococcus aureus and Escherichia coli. were important aerobic bacterial vaginosis related with pregnancy. Escherichia coli is a causative agent of vaginosis because Escherichia coli is a normal flora in GI and may be transferred to genital tract and causes bacterial vaginosis. Also washing under wear with strong detergent poor body hygiene.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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Synthesis, Characterization of New Tris Hydrazones based on Cyanuric Acid and Studies the Biological Activity as Antibacterial and Antifungal

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Directorate of Education Dhi Qar, Ministry of Education, Iraq

Abstract

In this study synthesis of new Tris Hydrazones derived from Cyanuric acid, these compounds synthesized by reaction of Cyanuric acid with Chloro ethyl acetate to product [I], then converted it to acid Hydrazide [II] by reacted with Hydrazine hydrate(80%) in absolute ethanol as a solvent. Compound [II] refluxed with different aromatic aldehyde in present of Glacial acetic acid (GAA) as a catalyst to formation new Tris Hydrazones [III]a-f. All new compounds were synthesized characterization by FT-IR and $^1$H,$^{13}$CNMR spectroscopy. The biological activity of new Tris Hydrazones studies against to positive and negative Gram bacteria also test it against to Candida albicans as a sample of Fungi.

**Keywords:** Cyanuric acid, Hydrazones, antibacterial, 1,3,5-Triazinane-2,4,6-trione, antifungal.

Introduction

The 1,3,5-triazines or s-triazines, as represented by Cyanuric acid are among the oldest recognized organic compounds. Cyanuric acid was known to Scheele as early as 1776, as “pyro-uric” acid (1). This compound is found in two tautomers are known the keto and hydroxy forms

Although the keto structure is generally more stable than the hydroxy structure. Only a few reaction of cyanuric acid occur by replacement of hydrogen of oxygen in alkaline solution the hydroxyl structure is formed over the keto structure (2).

While many derivatives of cyanuric acid synthesis by replacement of hydrogen of nitrogen atom such as N- chlorinated isocyanurates, which are employed most importantly in swimming pool disinfectants, household bleaches, industrial and institutional cleaners, dishwasher detergents (3) and the ether derivatives are uses as antioxidant (4), rust inhibitor (5), light stabilizer, cross-linking agent for vinyl polymers (6), insecticidal activity (7). The hydrazones are obtained from the condensation of hydrazides with aldehyde or ketones (8,9). These compounds have been used in the various chemical synthesis (10) and wide application in biology and medicine as antiviral (11), antimalarial (12), antibacterial (13), antifungal (14,15), antitumor (16), anticancer activity (17). In this work synthesis and characterization new tris hydrazones by condensation reaction of tris acid hydrazide derived from cyanuric acid with different aromatic aldehyde and study the biological activity as antibacterial and antifungal.

**Materials:** The chemical compounds were supplied from Merck, Himedia and CDH chemicals Co. and used as received.

**Instruments:** FT-IR spectra were recorded using KBr disc on a Shimadzu (Ir prestige-21) FTIR spectrophotometer. $^1$H-NMR spectra were obtained with Bruker spectrophotometer model ultra shield at 400 MHz using tetramethylsilane (TMS) as internal standard and DMSO-d$_6$ as solvent at 298 k. Uncorrected melting points were determined by using Hot-Stage Gallen Kamp melting point apparatus. Thin layer chromatography (TLC) was carried out on aluminum sheets. Experimental Synthesis of Tris (ethoxycarbonyl methyl) isocyanurate [I].
In the round bottom flask (500mL) put 250 mL of anhydrous dimethyl formamide (DMF) and dissolved a mixture of (10.002 g, 0.0775 mol) of cyanuric acid and anhydrous potassium carbonate (32.132 g, 0.2325 mol) then added ethyl-2-chloro acetate (28.492, 0.2325 mol) the reaction mixture was stirred and heated under reflux for 12hrs then allowed to cool at room temperature and poured onto cold water and cooled at 5 ºC overnight. The precipitated solid was filtered, washed and recrystallized from ethanol to product white needle, mp.: (74-75)ºC ; yield (59%) ; FT-IR(KBr, ν(cm⁻¹) : 2985-2877(C-H aliph.), 1697(C=O endocyclic), 1759(C=O ester), 1211(C-O), 1100(C-O) ; ¹H NMR (DMSO-d₆) δ(ppm): 4.59(s,6H,3N-CH₂), 4.14-4.18(quart,
6H,3O-CH₂), 1.20 -1.23(t,9H,3CH₃ ); ¹³C NMR (DMSO-d₆) δ(ppm):166.4(C=O ester),147.51(C=O amide), 60.87(OCH₃), 42.93(N-CH₃), 13.35(CH₃).

**Synthesis of Tris (Hydrazinecarbonyl methyl) isocyanurate[III]**

A solution of tris ester(3.8735 g, 0.01 mol) [I] dissolved in 12.5 mL of absolute ethanol and hydrazine hydrate (80%) 7.5 mL was heated under reflux condition after half hour the white precipitate was formed for 24hrs and the reaction mixture followed by thin layer chromatography (TLC)(4:6) ethylacetate:n-hexane. The mixture was then cooled at room temperature and the solid obtained was filtered and recrystallized from DMF/ethanol(¹⁹) to product white powder, mp. : >300ºC ; yield (81%); FT-IR(KBr , ν(C=O endocyclic)) : 3194(NH), 3062(C-H endocyclic), 2990-2885(C-H aliph.), 141.67(C=N), 123.39,127.53 , 129.01, 139.58 (C=C aliph.), 43.06 (CH₃) , 166.4(C=O amide endo),43.10(CH₂).

The general procedure from synthesis Tris hydrazones [III]a-f The compounds [III]a-f were synthesized by dissolving (0.03 mol) of an aromatic aldehyde in 10 mL of absolute ethanol and some drops of glacial acetic acid , then(3.4528 g , 0.01 mol) of tris hydrazide [II] was added and the reaction mixture was refluxed for 12 hrs. (²⁰). Afterword the mixture was cooled in ice bath. The resulting precipitate was filtered, dried and recrystallization from DMF/ethanol.

**Synthesis of 2,2',2''-(2,4,6-trioxo-1,3,5-triazinane-1,3,5-triyl)tris(N'-(E)-4-hydroxy-3-methoxybenzylidene)acetohydrazide) [III]a** White powder, mp. : >300ºC ; yield (88%); FT-IR(KBr , ν(cm⁻¹) : 3194(NH), 3062(C=H aromatic), 2962-2854(C=H aliph.), 1712(C=O endocyclic), 1681(C=O hydrazide), 1597(C=N), 825(C=Cl) . ¹H NMR (DMSO-d₆) δ(ppm):11.66(s, 3H,3NH), 8.63(s,3H,3CH=N), 6.45-7.98(m, 12H, aromatic), 4. 97 (s,6H,3N-CH₂), 3.39(s,9H,3OCH₃). ¹³C NMR (DMSO-d₆) δ(ppm): 166.25 (C=O amide exo), 148.38 (C=OH aromatic), 148.18(C=O amide endo), 144.27(C=N), 108.68 , 114.90 ,121.12 , 127.76(C=C aromatic), 55.02(OCH₃), 43.06(CH₂).

**Synthesis of 2,2',2''-(2,4,6-trioxo-1,3,5-triazinane-1,3,5-triyl)tris(N'-(E)-4-methoxybenzylidene)acetohydrazide) [III]b** Beige Light powder, mp. : >300ºC ; yield (95%); FT-IR(KBr , ν(cm⁻¹) : 3209(NH), 3070(C=H aromatic), 2954-2831(C=H aliph.), 1712(C=O endocyclic), 1681(C=O hydrazide), 1604(C=N), 1249(C-O). ¹H NMR (DMSO-d₆) δ(ppm):11.62(s, 3H,3NH), 9.54(s,3H,3OH) , 8.09(s,3H,3CH=N), 6.43-7.92(m, 9H,aromatic), 4. 97 (s,6H,3N-CH₂), 3.39(s,9H,3OCH₃). ¹³C NMR (DMSO-d₆) δ(ppm): 166.18 (C=O amide exo),148.38 (C=OH aromatic), 148.18(C=O amide endo), 144.27(C=N), 108.68 , 114.90 ,121.12 , 127.76(C=C aromatic), 55.02(OCH₃), 43.06(CH₂).

**Synthesis of 2,2',2''-(2,4,6-trioxo-1,3,5-triazinane-1,3,5-triyl)tris(N'-(E)-4-nitrobenzylidene)acetohydrazide) [III]c** Yellow powder, mp. : >300ºC ; yield (97%); FT-IR(KBr , ν(cm⁻¹) : 3186(NH), 3086(C=H aromatic), 2962-2800(C=H aliph.), 1705(C=O endocyclic), 1681(C=O hydrazide), 1589(C=N), 1458 and 1342(NO₂) . ¹H NMR (DMSO-d₆) δ(ppm):11.89(s, 3H,3NH), 8.63(s,3H,3CH=N), 6.45-7.98(m, 12H, aromatic), 4. 94 (s,6H,3N-CH₂), 3.82(s,9H,3OCH₃). ¹³C NMR (DMSO-d₆) δ(ppm): 166.25 (C=O amide exo), 160.39(C=OCH₃), 148.15 (C=O amide endo), 143.81(C=N), 113.84,127.77 , 129.44(C=C aromatic), 54.02(OCH₃), 43.07(CH₂).

**Synthesis of 2,2',2''-(2,4,6-trioxo-1,3,5-triazinane-1,3,5-triyl)tris(N'-(E)-4-nitrobenzylidene)acetohydrazide) [III]d** Beige Yellow powder, mp. : >300ºC ; yield (95%); FT-IR(KBr , ν(cm⁻¹) : 3186(NH), 3086(C=H aromatic), 2962-2800(C=H aliph.), 1705(C=O endocyclic), 1681(C=O hydrazide), 1589(C=N), 1458 and 1342(NO₂) . ¹H NMR (DMSO-d₆) δ(ppm):11.89(s, 3H,3NH), 8.63(s,3H,3CH=N), 6.45-7.98(m, 12H, aromatic), 4. 94 (s,6H,3N-CH₂), 3.82(s,9H,3OCH₃). ¹³C NMR (DMSO-d₆) δ(ppm): 166.25 (C=O amide exo), 160.39(C=OCH₃), 148.15 (C=O amide endo), 143.81(C=N), 113.84,127.77 , 129.44(C=C aromatic), 54.02(OCH₃), 43.07(CH₂).

**Synthesis of 2,2',2''-(2,4,6-trioxo-1,3,5-triazinane-1,3,5-triyl)tris(N'-(E)-4-nitrobenzylidene)acetohydrazide) [III]e** Yellow powder, mp. : >300ºC ; yield (97%); FT-IR(KBr , ν(cm⁻¹) : 3186(NH), 3086(C=H aromatic), 2962-2800(C=H aliph.), 1705(C=O endocyclic), 1681(C=O hydrazide), 1589(C=N), 1458 and 1342(NO₂) . ¹H NMR (DMSO-d₆) δ(ppm):11.89(s, 3H,3NH), 8.63(s,3H,3CH=N), 6.45-7.98(m, 12H, aromatic), 4. 94 (s,6H,3N-CH₂), 3.82(s,9H,3OCH₃). ¹³C NMR (DMSO-d₆) δ(ppm): 166.25 (C=O amide exo), 160.39(C=OCH₃), 148.15 (C=O amide endo), 143.81(C=N), 113.84,127.77 , 129.44(C=C aromatic), 54.02(OCH₃), 43.07(CH₂).
Synthesis of 2,2’,2”-(2,4,6-trioxo-1,3,5-triazinane-1,3,5-triyl)tris(N’-(E)-3,4-dimethoxyacetohydrazide) [III]<sub>a-f</sub>Yellow powder, mp.: >300°C; yield (94%); FT-IR(KBr, ν (cm<sup>-1</sup>): 3201(NH), 3062(C-H aromatic), 2962-2839(C-H aliph.), 1712(C=O endocyclic), 1681(C=O hydrazide), 1597 (C=N), 1265(C=O). <sup>1</sup>H NMR (DMSO-d<sub>2</sub>) δ(ppm): 11.68(s, 3H,3NH), 8.13(s,3H,3CH=N), 6.48-7.95(m, 9H, aromatic), 4.99(s,6H,3N-CH<sub>2</sub>), 3.80(s,18H,6OCH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ(ppm): 166.30(C=O amide), 159.77(C=N), 126.50(C-4), 148.18(C=O amide endo), 143.97(C=N), 107.84,110.93, 55.01(OCH<sub>3</sub>), 54.17(CH<sub>2</sub>). Biological activity

The antibacterial activity of new hydrazones [III]<sub>a-f</sub> were synthesized studies by agar disc diffusion method against four types of pathological bacteria, two (G<sup>+</sup>)(Staphylococcus aureus) and (Bacillus subtilis), So that two(G<sup>-</sup>)(Escherichia Coli) and (Klebsiella pneumonia) whereas the antifungal activity from new hydrazones [III]<sub>a-f</sub> screening against to strains fungal Candida albicans. DMSO was used as a solvent The test was preform at (100 mg/ ml) conc. and The bacteria and fungi were carried out in agar and potato dextrose agar medium and these plate were incubated for 24hrs. at 37°C.

Results and Discussions

The synthetic routes for synthesized hydrazone compounds [III]<sub>a-f</sub> are outlined in Scheme 1. The initial step including replacement of hydrogen on nitrogen from cyanuric acid by potassium carbonate anhydrous then Nucleophilic attack to chloro ethyl acetate to formation cyanuric acid by potassium carbonate anhydrous then endocyclic as well as elucidate the absence of signals carbon atoms from ethyl groups. The end step including reaction of hydrazide [II] with virus substituted aromatic aldehyde under Schiff base conditions to product the hydrazones [III]<sub>a-f</sub> correspond. These compounds characterization by FT-IR spectroscopy which show disappearance of stretching vibration bands of amino group so that exhibited the stretching vibration bands of C-H aromatic ring in the region at 3109-3062 cm<sup>-1</sup> also appearance the ν(C=N) in the region at 1604-1589 cm<sup>-1</sup>. On the ether hand the <sup>1</sup>HNMR spectra of these compounds show the singlet signal in the region at δ (11.59-11.89) ppm attributed to three protons of (3NH) in addition appear the singlet signal in the region at δ (8.09-8.80) ppm refer to proton azomethine (3HC=N) groups in addition appearance the multiplet signals in the region at δ (6.43-8.35) ppm attributed to aromatic protons. Moreover the <sup>13</sup>C NMR spectra from these compounds showed the signal of carbon azomethine group in the region at δ (141-67-144.27) ppm (22).

Antimicrobial activity:

In this study uses the Amoxicillin drug as a standard antibacterial and antifungal for compared with the new hydrazones [III]<sub>a-f</sub> activity. The results tests of these compounds are summarized in table (1). All the compounds show moderate activity against to Staphylococcus aureus except compounds III<sub>a</sub>, III<sub>b</sub> while exhibited high activity toward Escherichia Coli and Candida albicans except compound III<sub>f</sub> do not show any activity. On the ether hand compound [III]<sub>b-f</sub> showed any activity.
showed high inhibition zone against Klebsiella pneumoniae while only compounds III_d, III_f have been high activity to Bacillus subtilis.

### Table 1: Antimicrobial evaluation of compounds.

<table>
<thead>
<tr>
<th>Synthesized compound</th>
<th>Inhibition zone (mm) at 100 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grampositive</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>[III]_a</td>
<td>-</td>
</tr>
<tr>
<td>[III]_b</td>
<td>-</td>
</tr>
<tr>
<td>[III]_c</td>
<td>12</td>
</tr>
<tr>
<td>[III]_d</td>
<td>11</td>
</tr>
<tr>
<td>[III]_e</td>
<td>14</td>
</tr>
<tr>
<td>[III]_f</td>
<td>13</td>
</tr>
<tr>
<td>Amoxillin</td>
<td>34</td>
</tr>
</tbody>
</table>

### Conclusion

The tris hydrazones were synthesized in very good to excellent yields about 81-97% in our work characterized by FT-IR, ¹HNMR, ¹³CNMR Spectrometry and showed that their compositions matched their proposed structures. The study of biological activity of these compounds shown good activity toward Gram-negative bacteria comparative with Gram-positive bacteria of most compounds also good inhibition zone against to Fungi (Candida albicans) comparative with Amoxillin.

### Ethical Clearance:

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

### Conflict of Interest:

The authors declare that they have no conflict of interest.

### Funding:

Self-funding

### References

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bis(4-methoxy phenyl)-1,3,5-triazin-2,4-diamine carbamic acid ester derivatives. Ind J Chem. 2012; 51B :1020-1026.


Assessment of Hyperuricemia in Patient with End Stage Chronic Kidney Disease

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Abstract

Chronic kidney disease (CKD) has become a global public health problem because of its high prevalence and the accompanying increase in the risk of end-stage renal disease, cardiovascular disease, and premature death. Progression of CKD is associated with a number of serious complications including increased incidence of cardiovascular disease, hyperlipidemia, anemia and metabolic bone disease. Hyperuricemia may be a major contributor to the development of progression of chronic kidney disease (CKD). Although there is no clear cutoff uric acid (UA) value associated to the risk for kidney damage, it appears to be an increased risk as UA rises.

Key words: Chronic kidney disease (CKD), Uric acid (UA), Hyperuricemia.

Introduction

CKD is characterized by progressive deterioration of kidney function, which develops eventually into a terminal stage of chronic kidney failure (CKF). CKF has traditionally been categorized as mild, moderate, or severe [1].

During the last few years, an international consensus has emerged categorizing CKF into five stages according to the glomerular filtration rate (GFR) and presence of signs of kidney damage: stage 1: GFR > 90 ml/min and signs of kidney damage, stage 2: GFR = 60-89 ml/min and signs of kidney damage, stage 3: GFR = 30-59 ml/min, stage 4: GFR = 15-29 ml/min, and stage 5: GFR < 15 ml/min [2]. Stage 5 represents the total inability of kidneys to maintain homeostasis, and this metabolic state is incompatible with life. Thus, at this stage, it is necessary to use methods that substitute for kidney function to ensure patient survival; these methods include peritoneal dialysis & hemodialysis [3].

CKF is associated with many kinds of metabolic changes caused by the kidney disease and also attributable to dialysis treatment. Phenomena such as accumulation or deficit of various substances and dysregulation of metabolic pathways combine in the pathogenesis of these changes [4]. In the process of accumulation, decreased urinary excretion plays a crucial role and leads to retention of metabolites in the organism (e.g. creatinine, urea, uric acid, electrolytes, water). The increased formation of metabolites through catabolic processes and alternative metabolic pathways also exerts an influence. Regular dialysis treatment partly decreases this accumulation, but cannot avert the overall deficit [5].

Chronic kidney disease leading to chronic kidney failure is an urgent medical problem in the context of demographic trends. In addition to the basic kidney disease, many metabolic disorders develop in the course of CKF. Particularly, patients in the terminal stage of CKF are endangered [6]. Regular dialysis treatment decreases the accumulation of metabolites; however, it contributes to a deficit of some important metabolic regulators and to the development of a chronic inflammation state. These factors can lead to serious secondary complications in CKF, including atherosclerosis and related cardiovascular disease, malnutrition, anemia, renal bone disease, and other problems [7]. These complications markedly and negatively affect the prognosis and quality of life of patients with CKF and increase costs for their treatment. The prognosis of CKD patients can be improved if kidney disease is diagnosed early and...
properly cured, including secondary complications. Appropriate treatment encompasses consistent control of blood pressure, prevention of malnutrition, anemia, and hyperparathyroidism, and treatment of metabolic disorders [8].

Because humans lack uricase, they cannot convert the uric acid generated during purine metabolism into a soluble form. This can lead to an increased risk for hyperuricemia and monosodium uric acid crystallization in joints and tissues [9]. Because approximately 70% of uric acid is excreted from the kidney, hyperuricemia occurs when renal function deteriorates. Hyperuricemia is defined as a level of serum uric acid greater than or equal to 7.0 mg/dl (420 μmol/l) in man and 6.0 mg/dl (360 μmol/l) in women. CKD is associated with decreased excretion of uric acid and resultant hyperuricemia. Other mechanisms may be implicated in CKD since variations in serum uric acid do not account for most of the risk for developing gout [10]. Hyperuricemia can be caused by the overproduction of uric acid, but is more often the result of insufficient kidney uric acid excretion. Observational data also suggest that hyperuricemia, even in the absence of gout, may independently worsen CKD, possibly via a pathogenic role in hypertension (Figure 1), and diabetic nephropathy, the two leading causes of CKD [11,12].

Hyperuricemia may be a consequence of decreasing of glomerular filtration rate (GFR), reflecting tissue hypoxia or cell lysis associated with renal disease [13].

Materials and Method

Subjects: Between 1st October 2019 and 1st December 2019, 100 subjects were recruited to this study, 50 consecutive patients aged between 25-70 years old, diagnosed by expert physicians as having CKF from kidney dialysis unit (KDU) in Al-kafeel hospital in Karbala City, in addition to age and sex matched (50 subjects) without CKF with similar risk factors considered as control group. This study was matched for gender, the ratio of male: female was 2:1 for both sexes. This study was performed in the Biochemistry laboratory in Pharmacy Department in Alsafwa collage.

Ethical Issues: The objectives and methodology were explained to all participants in the current study and their verbal consent was gained.

Sample collection: Five ml of blood were obtained from each subject by vein puncture in sitting or lying position, and then pushed slowly into disposable tubes containing separating gel. Blood in the gel containing tubes was allowed to clot at room temperature for 2 hours and then centrifuged at 1000 ×g for approximately 15 minutes then the supernatant were obtained and stored at -20°C until analysis.

Materials: Uric acid biochemical kit (Biolab).

Methods: uric acid serum level assayed by Biolab biochemical kit.

Statistical analysis: The collected data were tabulated and analyzed by using the Statistical Package for Social Sciences (SPSS) for Windows version 20th version. Data were expressed as (mean ± SD). Independent sample t-test was used to compare means between two groups. P values less than (0.05) were considered significant.

Results

a. Differences of Patients with Chronic Kidney Failure and control by Socio-Demographic Characteristics:

The overall mean age of patients with ACS and control were (40.28±12.02) and (38.21±11.61) years old, respectively and the extremes were 25 to 70 years old. There was no significant mean difference between the mean age of patients and control. This age matching helps to eliminate differences in parameters’ results. This study was matched for gender, the ratio of male: female was 2:1 for both sexes.

b. Differences of patients and control by uric acid serum level

There were significant differences of UA serum level by patients and control subjects as shown in table (1).
Table (1): Mean differences of patients and control by UA serum level

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>S.D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid</td>
<td>case</td>
<td>50</td>
<td>17.78</td>
<td>7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>50</td>
<td>7.3</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

*p value ≤ 0.05 is significant

Discussion

This study revealed that hyperuricemia is clinically significant in the setting of CKD, especially when drugs used for their management can further impair kidney function. The established role of CKD as an independent risk factor for hyperuricemia may therefore warrant screening for CKD when gout is first diagnosed, as presented by Juraschek SP et al. The role of hyperuricemia as an independent risk factor for CKD, however, is still being debated. Large randomized controlled trials can provide definitive answers about its relationship to CKD, and how its treatment might forestall CKD progression in populations such as those with hypertension and diabetic nephropathy. The finding of this study agreed with Tae Ryom Oh et al. who reported that in the case of CKD, renal excretion of uric acid is decreased, resulting in hyperuricemia. It is thought that the interstitial accumulation of sodium urate induces the deterioration of the disease. This suggests that patients with renal impairment are more likely to be exposed to higher uric acid concentrations, which means that the multisystem effect of uric acid can be further strengthened. A recent study by Verzola D et al. utilizing immortalized proximal tubular epithelial cells from normal adult human male kidney has demonstrated that increasing levels of uric acid cause NAPDH-dependent oxidative changes which promote apoptosis. This finding sheds light on the connection between hyperuricemia and tubule-interstitial renal damage. The association of hyperurecemia with CKD also was reported by Jalal DI et al. & Momeni A. Momeni (2012) who concluded that hyperuricemia is frequently found in patients who are found having chronic renal failure. Epidemiological studies have detected an association of hyperuricemia and heart and vascular disease in the general population and in chronic renal insufficiency patients.

Conclusion

- There is significant elevation of serum uric acid in CKD patients.
- The challenge remains that the significance of elevations in uric acid is difficult to assess in those with chronic kidney disease because, as clearance decreases, the levels of serum uric acid naturally increase.
- Hyperuricemia may be used as a disease marker for the potential to develop renal disease in the future as well as predict risk for a patient with renal disease to develop worsening renal function.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

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Reduced Glutathione, Lipid Peroxidation and Malondialdehyde Status in Women with Mild and Severe Preeclampsia for Babylon Governorate

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¹University of Babylon, College of Medicine, Hilla, Iraq

Abstract

The presented study aims to evaluate the levels of serum reduced glutathione, lipid peroxidation and malondialdehyde status with Mild, severe preeclampsia and healthy pregnant control in the case – control study. Twenty five patients with severe preeclampsia, twenty five patients with mild preeclampsia and fifty healthy pregnant as control in Babylon province / Iraq were enrolled in this study. Results of this study were shown The median serum reduced GSH concentration in the preeclampsia group was significantly lower than that in the group with normal pregnancy, indicating that there is more extensive oxidative stress in preeclamptic pregnancy. Also In this result, there was increased in malondialdehyde level in preeclampsia pregnant women groups when compared with non-preeclampsia pregnant women group.

Keywords: Babylon Governorate ; Severe Preeclampsia ; Glutathione, Lipid

Introduction

Pregnancy complicated by gestational hypertension is managed based on its severity, presence of preeclampsia, and gestational age. Increases in systolic and diastolic blood pressure can be either normal physiological changes or signs of developing pathology. Heightened surveillance permits more prompt recognition of ominous changes in blood pressure, Critical laboratory findings, and clinical signs and symptoms (1).

Pre-eclampsia (PE) is defined when BP ≥140/90 mm Hg and proteinuria (≥300 mg per 24 hours) after 20 weeks of gestation with an incidence of 2-8%. Its complications lead to placental abruption, eclamptic fits. Moreover, PE has been strongly associated with increased risk of later-life death due to liver insults and cardiovascular disease (2). It is a major cause of preterm birth, accounting for 15% of all preterm Deliveries (3).

Oedema is no longer included as a diagnostic criterion for preeclampsia because its occurrence is expected in pregnancy and has not been shown to be discriminatory. If untreated, the symptoms get progressively worse (4). Although PE is associated with abnormal trophoblast invasion in the first half of pregnancy, it is not until later in the pregnancy that the clinical syndrome of PE is seen. The timing of onset and the clinical course are unpredictable. In some, hypertension and proteinuria are the only manifestation, while others may present with severe renal or liver impairment, and in yet others the most prominent feature might be intrauterine fetal growth restriction secondary to placental disease.

The term pregnancy-induced hypertension (PIH) suggests a disorder of blood pressure that arises because of the presence of pregnancy. Pregnancy induced hypertension and PE rarely develop before 20 weeks gestation unless associated with trophoblastic disease or fetal triploid (5).

Mild and Severe Pre-eclampsia:

Pre-eclampsia is divided into mild and severe forms, depending on the severity of the hypertension, the
amount of proteinuria, and the degree to which other organ system are affected (6).

**Mild Pre-eclampsia:**

Is defined as the presence of hypertension (BP ≥140/90 mm Hg) on 2 occasions, at least 6 hours apart, but without evidence of end-organ damage in the patient (7).

**Severe Pre-eclampsia:**

Is defined as the presence of the following:(2)

1. Severe hypertension (systolic blood pressure ≥160 mm Hg, or diastolic blood pressure ≥ 110 mm Hg) at rest, on two occasions at least 6 hr apart.

2. Heavy proteinuria (at least 5 g in a 24-hr collection or a qualitative value of 3+ in urine samples collected 4 hr apart.

3. Oliguria (<500 mL in 24 hr).

4. Cerebral or visual disturbances.

5. Pulmonary edema or cyanosis.


7. Impaired liver function (elevated liver enzymes).

8. Thrombocytopenia.


**Materials and Method**

**Ethical Issues**

a- Approval by scientific committee of the Clinical Biochemistry Department, College of Medicine/University of Babylon, Iraq.

b- Approval by Babylon Health Directorate, Ministry of Health and Information centre for Research and Development of Babylon Province.

c- The objectives and methodology were explained to all subjects and verbal consent had been taken.

**Date and Durations**

The period extended from October 2018 to April 2019. This work was done in the Department of Biochemistry, College of Medicine University of Babylon and in Hilla city. Iraq.

**Study Design**

This study design was a case – control study.

**Patients and control**

A total of 50 patients divided to 25 sever and 25 mild with preeclampsia were enrolled in this study. 50 subjects who were apparently healthy control group of pregnant.

**Exclusion Criteria:**

Age over 35 years.

Previous history of PE.

Conditions in which the placenta is enlarged like multiple pregnancy

Pre-existing hypertension or renal disease.

Pre-existing vascular disease (as diabetes or autoimmune vasculitis.

**Determination of Serum Reduced Glutathione**

Determination of serum reduced glutathione levels in patient and control group were depends on the action of sulfhydryl group (8).

**Determination of Lipid peroxidation**

Determination of lipid peroxidation was done by Elabscience (USA) kits and according to Colorimetric method.

**Determination of MDA**

Determination of MDA was done by Elabscience ELISA kits and according to Competitive-ELISA principle.

**Results**

According to the clinical and laboratory examination the patients participating in this study divided in to two main groups illustrated below in Table 1
Table 1: Number of the different stage among preeclampsia patients.

<table>
<thead>
<tr>
<th>Stage of cancer</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 . mild preeclampsia</td>
<td>25</td>
</tr>
<tr>
<td>G2 . sever preeclampsia</td>
<td>25</td>
</tr>
</tbody>
</table>

In this study serum reduced glutathione was significantly lower in preeclampsia patients groups compared with normal patients CG (Control group) P-value < 0.001. In this study, the level of (GSH) decreases more with increase the severity of preeclampsia. Serum reduced glutathione was not significantly lower in G2(sever preeclampsia) compared with G1 (mild preeclampsia) P-value > 0.05 Table 3.

Table 3: The mean ±SD of (GSH) in preeclampsia compared to control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects</th>
<th>No.</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced Glutathione (ng/ml)</td>
<td>G1 sever preeclampsia</td>
<td>25</td>
<td>15.6±5.5</td>
</tr>
<tr>
<td></td>
<td>G2 mild preeclampsia</td>
<td>25</td>
<td>18.4±4.9</td>
</tr>
<tr>
<td></td>
<td>CG Control</td>
<td>50</td>
<td>26.3±6.3</td>
</tr>
</tbody>
</table>

P-value
- mild preeclampsia versus Control group (P < 0.001)
- sever preeclampsia versus Control group (P < 0.001)
- mild preeclampsia versus sever preeclampsia group (P > 0.05)

In this study MDA was significantly higher in preeclampsia patients groups compared with normal patients CG (Control group) P-value < 0.05. In this study, the level of MDA increase more with increase the severity of preeclampsia. Serum MDA was significantly higher in G2(sever preeclampsia) compared with G1 (mild preeclampsia) P-value < 0.05 Table 3.

Table 4: The mean ±SD of MDA in preeclampsia patients compared to control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects</th>
<th>No.</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (ng/ml)</td>
<td>G1 sever preeclampsia</td>
<td>25</td>
<td>477.5±33.1</td>
</tr>
<tr>
<td></td>
<td>G2 mild preeclampsia</td>
<td>25</td>
<td>418.2±23.6</td>
</tr>
<tr>
<td></td>
<td>CG Control</td>
<td>50</td>
<td>203±27.2</td>
</tr>
</tbody>
</table>

P-value
- mild preeclampsia versus Control group (P <0.05)
- sever preeclampsia versus Control group (P < 0.01)
- mild preeclampsia versus sever preeclampsia group (P < 0.05)

In this study Lipid peroxidation was significantly higher in preeclampsia patients groups compared with normal patients CG (Control group) P-value < 0.05. In this study, the level of Lipid peroxidation increase more with increase the severity of preeclampsia. Serum Lipid peroxidation was not significantly higher in G2(sever preeclampsia) compared with G1 (mild preeclampsia) P-value >0.05 Table 3.
Table 4: The mean ±SD of Lipid peroxidation in preeclampsia patients compared to control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects</th>
<th>No.</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (ng/ml)</td>
<td>G1 sever preeclampsia</td>
<td>25</td>
<td>4.2±0.69</td>
</tr>
<tr>
<td></td>
<td>G2 mild preeclampsia</td>
<td>25</td>
<td>4.0±0.82</td>
</tr>
<tr>
<td></td>
<td>CG Control</td>
<td>50</td>
<td>3.7±0.76</td>
</tr>
</tbody>
</table>

P-value: mild preeclampsia versus Control group (P > 0.05)
          sever preeclampsia versus Control group (P < 0.05)
          mild preeclampsia versus sever preeclampsia group (P > 0.05)

Discussions

The median serum reduced GSH concentration in the preeclampsia group was significantly lower than that in the group with normal pregnancy, indicating that there is more extensive oxidative stress in preeclamptic pregnancy. Reactive oxygen species cause decreases in antioxidants. In patients with preeclampsia placental angiogenic activity is significantly lower than in the placenta of normal pregnancy(9). Other studies are similar, as shown by the significant decreases in the mean value of endogenous antioxidants such as GSH, superoxide dismutase, and glutathione peroxidase in preeclampsia cases as compared with normotensive pregnant women.

In this study extremely low level of reduced glutathione in sever preeclampsia go with the idea that says increase production of ROS In conjunction to increase the severity of preeclampsia the matter that will extremely increase the consumption of glutathione to neutralize the ROS and other free radicals. Glutathione plays essential roles in T cell function and proliferation (10). Reported that Activation of T helper cell results in decreased levels of reduced glutathione (GSH) and production of ROS. In sever preeclampsia there is a demand to increase activation of T helper cell, that may contribute the consumption of reduced glutathione pool in the serum of preeclampsia.

Lipid peroxidation is oxidative degradation of lipid by which, free radicals steal electron from lipid in cell membrane and resulting in cell damage (11). Lipid peroxidation represent a crucial and causative role in the pathogenesis of atherosclerosis and it involve also, in the oxidative modification of LDL which lead to initiation of hypertensive and preeclampsia (12). Excessive lipid peroxidation occurring in preeclampsia can be attributed to hypercholesterolemia. Hypercholesterolemia promotes the formation of free radicals.

Increased oxygen demand to meet the bodily functions in pregnancy is also a contributory factor for the oxidative stress that results in the formation of free radicals. Thus, lipid alterations observed may promote oxidative stress, leading to endothelial dysfunction in preeclampsia(13).

In this result, there was increased in malondialdehyde level in preeclampsia pregnant women groups when compared with non-preeclampsia pregnant women group. Products of lipid peroxidation are the candidate factor that mediate disturbance of the maternal vascular endothelium and may inhibit prostacyclin synthesis and also stimulate smooth muscle contraction that lead to widespread vasospasm, a prominent feature of preeclampsia(14). The decrease of red cell deformability due to the increase of plasma malondialdehyde level may be the underlying mechanism of pregnancy induced hypertension(15).

Conclusion

Preeclampsia patients in Babylon province have low reduced glutathione level comparing to normal subjects and this level decrease more as the preeclampsia progress to advance severity. As regard to glutathione antioxidant and immune supporting properties, glutathione consumed during elimination of ROS that produced by lipid peroxidation processes. Also, preeclampsia patients in Babylon province have high Malondialdehyde and Lipid peroxidation level comparing to normal subjects and this level increase more as the the preeclampsia progress to advance.
severity. The oxidative stress underlying the occurrence of systemic inflammation in patients with preeclampsia indicates that there is an imbalance between free radicals and the antioxidant defense mechanism in the course of pregnancy.

**Acknowledgment:** Authors would like to thank the staff of the Biochemistry Department at College of Medicine, University of Babylon, Iraq, for their efforts and facilities to complete the task of this study. Also, authors would like to thank the staff of Maternity and children Hospital, Hilla city, Iraq, for their assistance in the collection of samples.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


Association of Autoimmune Thyroiditis and Type 1 Diabetes Mellitus With Severity of Children with Celiac Disease

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Pediatric Dept., Hammurabi faculty of Medicine, Babylon University, F.I.B.M.S, C.A.B.P General pediatrician.

Abstract

Purposes of study: analysis and measurement of incidence and association between autoimmune thyroiditis (AIT) and type 1 Diabetes mellitus (T1DM) with clinical and serological severity of patients with Celiac disease.

Design and Methods: This is prospective study, All children (107 children with 41 male and 66 female) with celiac disease are admitted and follow up in Hospital. Age groups (1-12 years) are arranged into two groups (1-6 and 7-12 years old). The celiac disease are divided into: Group1= 25-50 IU/ml, Group2 = 50-100 IU/ml, Group3 >100 IU/ml. All patients with celiac disease are follow up for AIT and type 1 T1DM.

Results: (107) patients with celiac disease, mean age is 6.78±2.85, median age is 8, and female :male ratio are 1.6:1. All celiac patients are divided into: group 1 (43 patients), group 2 (50 patients), and group 3 (14 patients). Incidence of T1DM in all patients is 8.4% while AIT 2.8%. T1DM incidence in group 2 is 3.7% and in group 3 is 12.3%. But AIT incidence in group 3 is 4.6%. All cases with AIT have hypothyroidism with TSH > 100 µU/ml. All diabetic patients have random blood sugar > 450 mg/dl. Age group are divided into two groups, 1-6 and 7-12 years old. Incidence of T1DM are 6.25% and 11.6% in these two age groups respectively, while AIT is 7% in age group 7-12 years. The association between celiac patients groups with T1DM and AIT is insignificant association (P value is 0.265 and 0.717 respectively). Also age groups have no significant association with T1DM and AIT (P value is 0.48 and 0.062 respectively).

Conclusion: follow up of celiac disease is important step for diagnosis AIT and T1DM. These two autoimmune diseases are increasing especially when there high concentration of IgA anti-tissue transglutaminase level (>100 IU/ml) and advanced children age group. AIT may presented initially with hypothyroidism.

Key words: Celiac disease, autoimmune thyroiditis, type 1 diabetes mellitus.

Introduction:

Celiac disease is an initially described as bowel disease in 1st century A.D. It is also called gluten sensitive enteropathy or celiac sprue (1). Sex distribution have also different rates of disease involvement, female is increased 1.5-2 folds in compare to male(3).

Celiac disease is intestinal autoimmune disease which is triggered by gluten ingestion in genetically liable persons. The main distinctive features of celiac disease are systemic symptoms, serologically autoimmune antibodies, and lastly, there is genetic association (2). Since, there is commonly unclear and wide variation clinical symptoms of celiac disease and so the real prevalence of this disease is not accurate(4-6). Consequently, patients with celiac disease is underdiagnosed in about 90%(7-9).

Gastrointestinal symptoms of celiac disease are malabsorption with diarrhea and subsequently, it is leading to weight loss. The intestinal biopsy shows mucosal inflammation of proximal small bowel with variable level of inflammatory extension to jejunum and...
ileum\(^{(10)}\).

The prevalence distribution of this disease is 1%-2% in North and South America, North Africa and Middle East with first suggestion in the same degree in Asian population\(^{(11)}\). Family history of 1st degree people is also rising the incidence of celiac disease to about 10%-15%\(^{(12)}\).

European society of pediatric gastroenterology, hepatology and nutrition (ESPGHAN) have diagnostic principles and recommendation for diagnosis of celiac disease.

Celiac diagnostic criteria is yet depending on intestinal biopsy that have typical findings with positive response to gluten free diet but further biopsies (2\(^{nd}\) and 3\(^{rd}\)) are done only when first biopsy findings is unclear and/or gluten free diet ingestion is not changing clinical outcome of patients. Most recent diagnostic criteria (ESPGHAN-2012) is depending on characteristic symptoms, titer level of transglutaminase antibodies (ten times greater than upper normal value), and presence of associated HLA-genotype\(^{(13)}\).

Regarding serology, patients is testing by serum IgA anti-tissue transglutaminase antibodies\(^{(14)}\). If they have IgA antibodies deficiency, both serum IgG anti-tissue transglutaminase and IgG anti-deaminated gliadin antibodies can use\(^{(15)}\).

Incidence of celiac disease is increasing in previous fifty years and the mechanism of these changes is not completely identified but it might be related mostly to environmental factors in addition to effect of genetic abnormalities\(^{(16)}\).

Type 1 diabetes mellitus (T1DM) is common endocrine disease that is appearing in children adolescence age group. T1DM is a result as outcome of defect in patient genes, immunity, and environmental stimulation factors, and these factors result in stimulation of T-cell autoimmunity against beta cells of pancreas\(^{(17)}\).

Thus, T1DM is resulting from decrease insulin secretion from pancreas, and it is associated frequent urination and thirst. Diagnostic standardization of world health organization standardization of T1DM are fasting blood glucose \(\geq 7\) mmol/L or blood glucose \(\geq 11.1\) mmol/L in addition to diabetes mellitus symptoms or when oral glucose tolerance test is resulting two hour blood glucose \(\geq 11.1\) mmol/L\(^{(18)}\).

The T1DM is rising in incidence and it is risk could be increase to double level in children by 2020\(^{(19)}\).

T1DM are associated other autoimmune diseases and so with firstly autoimmune thyroiditis and then celiac disease\(^{(20)}\).

Autoimmune thyroiditis (AIT) is a widespread autoimmune disorder, it is affecting 0.2% and 2% of men and women respectively\(^{(21)}\).

Autoimmune hypothyroidism is a common etiology for acquired hypothyroidism that presenting from children to adult age group, AIT prevalence is 1%-2% and female is four folds than male\(^{(22)}\). Autoimmune hypothyroidism is commonly associated with systemic lupus erythematous, celiac disease, and other diseases\(^{(23)}\).

Antithyroglobulin and antithyroid peroxidase autoantibodies is found in about 60% and 95% respectively in patients with both diffuse goiter and hypothyroidism or one of them\(^{(24)}\).

Aim of study is to evaluate incidence and association between autoimmune thyroiditis and type 1 Diabetes mellitus with clinical and serological severity of patients with Celiac disease.

**Materials and Method**

This is prospective study, All children (107 children with 41 male and 66 female) with celiac disease are admitted and follow up in Babylon teaching hospital for gynecology and pediatrics, in period between April/2017 to February/2019. Age group involved is 1-12 years old that arranged into two groups (1-6 and 7-12 years old). The diagnosis of celiac disease is depending on symptoms, serum anti-tissue transglutaminase IgA (TTGA) and intestinal biopsy. The celiac disease are divided into three groups according to concentration of IgA anti-tissue transglutaminase: (1) Group1 = 25-50 IU/ml; (2) Group2 = 50-100 IU/ml; (3) Group3 >100 IU/ml.

All patients with celiac disease are follow up for AIT and T1DM.

T1DM is diagnosis by random blood sugar with clinical symptoms and fasting blood sugar. Also, autoimmune thyroiditis is followed with antithyroglobulin and antithyroid peroxidase autoantibodies in addition to
presence of goiter with other thyroid disorder symptoms. Then, if the children has AIT, we do thyroid serology for thyroid stimulating hormone (TSH) and free thyroxine (FT4) hormones.

In Hospital laboratory, the serology of TSH and FT4 is measured with cobas e 411 analyzer. anti-tissue transglutaminase IgA, antithyroid peroxidase antibodies, and antithyroglubin antibodies measured by Chorus trio machine.

Laboratory normal reference for following data are: FT4: 10-35 pmol/L, TSH: 0.25-5 µU/ml, anti-tissue transglutaminase IgA titer < 12 IU/ml (negative) and 12-18 IU/ml (equivocal), antithyroid peroxidase antibodies titer < 35 IU/ml, antithyroglubin antibodies titer: 2.6-25 ng/ml.

SPSS version 22 program was using for Statistical evaluation. Mean ± SD and median are represented the continuous variable. Pearson’s chi square (X2) and Fisher’s Exact Test are assessed the association between categorical variables, and Significant P value is ≤ 0.05.

**Results**

107 patients are diagnosis with celiac disease, mean age is 6.78±2.85, median age is 8, and female :male ratio are 1.6 : 1.

All patients with celiac disease are divided according to level of TTGA into group 1 (43 patients), group 2 (50 patients), and group 3 (14 patients).

During period of follow up of those patients, the incidence of T1DM in all patients is 8.4% while AIT 2.8%. T1DM incidence in group 2 is 3.7% and in group 3 is 12.3%. But AIT incidence in group 3 is 4.6% as in table1 and Fig.1.

**Table 1 : Compare incidence and assiciation between autoimmune thyroiditis and type 1 diabetes mellitus according to severity of celiac disease.**

<table>
<thead>
<tr>
<th>Celiac disease</th>
<th>Group 1 (mild cases)</th>
<th>Group 2 (moderate cases)</th>
<th>Group 3 (sever cases)</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA anti-tissue transglutaminase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>concentration</td>
<td>25-50 IU/ml</td>
<td>50-100 IU/ml</td>
<td>&gt; 100 IU/ml</td>
<td></td>
</tr>
<tr>
<td>cases N(% from total patients)</td>
<td>15 (14%)</td>
<td>27(25%)</td>
<td>65(61%)</td>
<td></td>
</tr>
<tr>
<td>T1DM N(% from each group)</td>
<td>0</td>
<td>1(3.7%)</td>
<td>8(12.3%)</td>
<td>0.265</td>
</tr>
<tr>
<td>AIT N(% from each group)</td>
<td>0</td>
<td>0</td>
<td>3(4.6%)</td>
<td>0.717</td>
</tr>
</tbody>
</table>

*Fisher exact test
all cases with AIT have hypothyroidism with TSH > 100 µU/ml. All diabetic patients have random blood sugar > 450 mg/dl.

Also, age group are divided into two groups, 1-6 and 7-12 years old. Incidence of T1DM are 6.25% and 11.6% in these two age groups respectively, while AIT is 7% in age group 7-12 years as shown in table 2.

Table 2: Compare incidence and association between autoimmune thyroiditis and type 1 diabetes mellitus according to age group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>1-6 years</th>
<th>7-12 years</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases N(% from total patients)</td>
<td>64 (60%)</td>
<td>43 (40%)</td>
<td></td>
</tr>
<tr>
<td>T1DM N(% from each group)</td>
<td>4 (6.25%)</td>
<td>5 (11.6%)</td>
<td>0.48</td>
</tr>
<tr>
<td>AIT N(% from each group)</td>
<td>0</td>
<td>3 (7%)</td>
<td>0.062</td>
</tr>
</tbody>
</table>

*Fisher exact test

The association between celiac groups with T1DM and AIT is insignificant association (P value is 0.265 and 0.717 respectively). Also age groups have no significant association with T1DM and AIT (P value is 0.48 and 0.062 respectively).

Discussion

In this study, we study association of AIT and T1DM in patients with originally diagnosis as celiac disease, all these diseases have an autoimmune origin, and many studies support their relationship.

Celiac disease is commonly associated with T1DM, AIT, and Sjogren’s syndrome.

Kahaly et al indicated in their research that type 1 diabetic patients are fifty percentage that may progress to autoimmune thyroiditis and forty percentage are presented with celiac disease.

This present study is accomplished in hospitalized children, mean age is 6.78 predominant female (female : male ratio is 1.6 : 1). Antonella et al. study was done in 324 children that had always celiac disease, mean age is 6.6 years old, and female to male ratio is 2:1.
Our study shows percentage of T1DM (8.4%) is more than autoimmune thyroiditis (2.8%). Antonella et al. \( (27) \) and Collin et al. \( (28) \) exhibited that autoimmune thyroiditis were found 10.5% and 3.5% respectively in patients with celiac disease.

Also, T1DM was diagnosis about 3.5%-10% in patients having celiac disease\(^{(29-31)}\).

Study of PEKKA et al. \( (32) \) was diagnosis of both autoimmune thyroid disease and T1DM with equal evidence (2%-5%) in celiac disease.

High IgA anti-tissue transglutaminase level (\( >100 \) IU/ml) in group 3 has more (in compared to other groups) association to develop T1DM and AIT, but their association is not reach to significant level (\( P \) value is 0.265 and 0.717 respectively).

Age factor in celiac disease responsible for prediction of developing autoimmune diseases, rather than the period of gluten ingestion as a registered by authors\(^{(33)}\)

Two age groups in present study are insignificant associated with AIT and T1DM in this present study, but age group 7-12 years are higher frequency with AIT(7%)in compare to age group 1-6 years (0%), similar study of Giuseppina et al.\(^{(34)}\) is 4.3% for age group < 6 years in compare to 27.7% age group > 6 years old.

Also, T1DM is occurring in both age groups (6.25% and 11.6% respectively) that have celiac disease, and as known that T1DM is common disease in both children and teenager\(^{(35)}\).

In conclusion, follow up of celiac disease is important step for diagnosis AIT and T1DM. These two autoimmune diseases are increasing especially when there high concentration of IgA anti-tissue transglutaminase level (\( >100 \) IU/ml) and advanced children age group. AIT may presented initially with hypothyroidism.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


Role of PSA in Diagnosis of Chronic Prostatitis

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Abstract

Background: Prostatitis is the poor cousin of prostatic cancer and Benign hyperplasia. The prevalence of prostatitis in general population was estimated to be 0.5 to 14.2% in developed countries. Publishing in Iraq was concern mainly with treatment of prostatitis.

Objective: This work was carried out to comment on the concept of prostatitis in clinical practice.

Method: A total of 275 males was included in this study. They were complaining of urinary symptoms (frequency, urgency, hesitancy, straining, difficulty in initiation urinary stream). All cases were confirmed to have chronic prostatic after excluding prostatic carcinoma by abdominal ultrasound, free to total PSA and urinary Prostatic carcino-antigen-3. Their age was 45.6 ± 9.6 year. Urine samples were proceeded immediately after collection. Centrifuged and non-centrifuged specimen were examined.

Results: PSA was affected significantly by age (p = 0.002). A significant high level was observed among those complaining of pain during ejaculation (p = 0.0001). Those showed epithelial cells in urine had significant high level of PSA (p = 0.0001). Uric acid in urine was associated with significant high level of PSA (p = 0.03).

Conclusion: The diagnosis of CP / CPPS might be outside the traditional urologic practice and might consider PSA level too.

Key words: Prostatitis, PSA, urinary symptoms, chronic pelvic pain syndrome, Iraq.

Introduction

Prostatitis is a microscopic inflammation of the prostate gland that covers a wide range of clinical conditions in relation. Prostatitis is the “poor cousin” of prostate cancer and benign prostate hyperplasia (BPH) which is the most common urologic diagnosis in younger than 50 years, and the 3rd most common diagnosis in men older than 50 years. Factors identifiable with prostatitis are microorganism, urine reflux and high voiding pressure, immunological status and mental stress.

Prostatitis was classified by National Institute of Health (NIH) into four categories, I, II, III, and IV, representing acute prostatitis (CP), chronic prostatitis/chronic pelvic pain syndrome (inflammatory and non-inflammatory) (CPPS) and asymptomatic inflammatory prostatitis, respectively.

Acute prostatitis is mostly caused by several different types of bacterial infections and represented as a medical emergency. 5% of patients might end up with chronic bacterial prostatitis, which is mostly caused by E. coli and other gram negative Enterobacteriaceae.

Chronic nonbacterial prostatitis, also known as chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), is long term pelvic pain and symptoms with urination without evidence of a bacterial infection.

Asymptomatic inflammatory prostatitis is a painless inflammation of the prostate gland where there is no evidence of infection.

PSA was first identified in human prostatic tissue extracts in 1970, purified and characterized by Wang and associates in 1979, and detected in human serum by Papsidero and associates in 1980.

PSA is a single chain, 240–amino acid glycoprotein
with a molecular weight of 33 kDa. The human PSA gene is located on chromosome 19 (544). The mRNA of PSA, like other cytoplasmic serine proteases, is translated as an inactive pro-PSA/pro-PSA precursor. Following passage through the intracellular secretory pathway, the signal peptide is cleaved, yielding the proform of the protein. Evidence suggests the conversion of pro-PSA to the mature enzymatically active PSA requires the action of human kallikrein 2 (hK2) 9.

PSA shares sequence homology with the human kallikreins and, in fact, a 78% homology with hK2. PSA possesses chymotrypsin-like activity and has a weak interaction with the plasma inhibitor, aprotinin. PSA also has modest overlapping homology with urokinase-like plasminogen activator and is capable of facilitating the degradation of the extracellular matrix. PSA is primarily produced by intraluminal or secretory cells of the prostate and the epithelial lining of the periurethral glands 10.

The basal cells of the prostate do not express this protein. It is androgen regulated and one of the most abundant serine proteases in the seminal plasma (1,000,000 ng/mL). Its major physiologic role is to promote the liquefaction of seminal clot. Seminal clotting is due to the presence of semenogelin 1 and semenogelin 2 and fibronectin. PSA targets the semenogelin component, thus liquefying the seminal clot, which in turn facilitates sperm motility. The half-life of PSA is 2.2 to 3.2 days 11.

Low levels of PSA and/or PSA-gene expression have been detected in various tissues, particularly those that have constitutive expression of the steroid receptor superfamily, including the uterine endometrium and amniotic fluid, normal lactating breast tissue and the milk of lactating women, breast cancer, perianal/periurethral glands, salivary glands, adrenal/renal neoplasms, and various other malignant tumors 10.

The prevalence of prostatitis in general population was estimated to be 5.0 to 14.2%. The figure was for developed countries (America and Europe). No publish figure on prevalence of prostatitis in Iraq which might be endemic like other endemic diseases in Iraq like hydatid cyst and brucellosis, and publishing were mainly concerned with treatment with antibiotics.

The aim of this study is to comment on the role of PSA in chronic prostatitis in clinical practice.

---

**Materials and Method**

During the study period July 2016 to April 2018, a total of 275 males referred to Lagash laboratory for general urinalysis because pain and urinary symptoms (frequency, urgency, hesitancy, straining, difficulty in initiation of urinary stream and incomplete bladder emptying). After excluding prostatic carcinoma by abdominal ultrasound, free to total PSA values, and estimating the level of Prostatic carcinoma-3 in urine by ELSA method (PCA-3), the diagnosis of CP / CPPS was according to NIH criteria. Their age was 45.6 ± 9.6 year.

Urine samples were processed immediately after collection. The centrifuged and the non-centrifuged specimen were examined under 10x and 40x of bright field microscope to find out the presence of pus cells, RBCs, casts, crystals and bacterial cells. Blood samples to obtain serum PSA levels and was processed by Snibe Maglumi 800 Immunoassay Analyzer chemiluminescence immunoassay system.

Data was dichotomized for age (≤ 50 and > 50 year), pain on ejaculation (yes and no), epithelial cell in urine (present and not present), pus cells in urine (present or not present), bacteria in the urine (high density or low density), uric acid (present or not), seminal fluid pus cells (present or not present), seminal fluid uric acid (present or not present) and history of smoking (positive or negative).

Student’s t test was carried out to examine the differences in means of PSA between the dichotomized variables. P value < 0.05 was considered significant.

**Results**

PSA among those aged ≤ 50 and > 50 year, were 1.6 ± 0.1 and 1.4 ± 0.1, respectively. A significant difference in PSA between the two groups (t = 3.1, d.f. = 273, p = 0.002). (Table 1). PSA was increasing with age (Fig.1).

Men complaining of pain on ejaculation had significantly higher PSA (6.6 ± 1.5) than those with no pain on ejaculation (5.1 ± 1.2) (t = 6.6, d.f. = 273, p = 0.0001).

Those men showed epithelial cells on urinalysis had a significant higher PSA (9.2 ± 1.7) than those showed no epithelial cells on urinalysis (6.2 ± 1.5) (t = 5.1, d.f. = 273, p = 0.0001).
Pus cells in urine was significantly associated with higher PSA (6.4 ± 1.5) than those without pus cells in urine (4.7 ± 1.1) \((t = 3.8, \text{ d.f.}= 273, p = 0.0001)\).

Men showed bacteria in urine with a significant higher PSA (6.7 ± 1.7) than those without bacteria in urine (6.2 ± 1.4). There was a significant difference in PSA between the two groups \((t = 2.6, \text{ d.f.} = 273, p = 0.006)\).

Men with uric acid in urine showed higher PSA (6.6 ± 1.8) than those without uric acid in urine (6.2 ± 1.4). A significant difference was observed between the two groups \((t = 2.1, \text{ d.f.} = 273, p = 0.03)\).

Smokers showed a level of PSA (6.4 ± 1.6) and non-smokers showed (6.3 ± 1.6). No significant difference in PSA due smoking was noticed \((t = 0.7, \text{ d.f.} = 273, p = 0.4)\).

### Table 1. PSA distribution among patients according to studied factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>PSA mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\leq 50)</td>
<td>194</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>(&gt; 50)</td>
<td>81</td>
<td>1.4</td>
<td>0.1</td>
</tr>
<tr>
<td>(t = 3.1, \text{ d.f.} = 273, p = 0.002)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain on ejaculation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>225</td>
<td>6.6</td>
<td>1.5</td>
</tr>
<tr>
<td>No</td>
<td>50</td>
<td>5.1</td>
<td>1.2</td>
</tr>
<tr>
<td>(t = 6.6, \text{ d.f.} = 273, p = 0.0001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>9.2</td>
<td>1.7</td>
</tr>
<tr>
<td>No</td>
<td>268</td>
<td>6.2</td>
<td>1.5</td>
</tr>
<tr>
<td>(t = 5.1, \text{ d.f.} = 273, p = 0.0001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pus cells (urine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>262</td>
<td>6.4</td>
<td>1.5</td>
</tr>
<tr>
<td>+</td>
<td>13</td>
<td>4.7</td>
<td>1.1</td>
</tr>
<tr>
<td>(t = 3.8, \text{ d.f.} = 273, p = 0.0001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria (urine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>98</td>
<td>6.7</td>
<td>1.7</td>
</tr>
<tr>
<td>+</td>
<td>166</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
<td>(t = 2.6, \text{ d.f.} = 273, p = 0.006)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>101</td>
<td>6.6</td>
<td>1.8</td>
</tr>
<tr>
<td>+</td>
<td>174</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
<td>(t = 2.1, \text{ d.f.} = 273, p = 0.031)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminal fluid pus cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>262</td>
<td>6.4</td>
<td>1.5</td>
</tr>
<tr>
<td>+</td>
<td>13</td>
<td>4.7</td>
<td>1.1</td>
</tr>
<tr>
<td>(t = 3.8, \text{ d.f.} = 273, p = 0.0001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminal fluid uric acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>275</td>
<td>6.3</td>
<td>1.6</td>
</tr>
<tr>
<td>History of smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>72</td>
<td>6.4</td>
<td>1.6</td>
</tr>
<tr>
<td>No</td>
<td>203</td>
<td>6.3</td>
<td>1.6</td>
</tr>
<tr>
<td>(t = 0.7, \text{ d.f.} = 273, p = 0.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Prostatitis refers to a fascinating urologic diagnosis with spectrum of etiologies and varying natural histories. The term Chronic prostatitis (CP) / chronic pelvic pain syndrome (CPPS) were usually used as (CP/CPPS).

PSA was not mentioned in diagnosis of CP/CPPS in the recent published data. This report was done to comment on the role of PSA in diagnosis of the CP/CPPS.

This study revealed that PSA was significantly elevated in men with CD/CPPS. This finding is in agreement with that reported recently. This finding is intriguing in the light of general practice in Iraq. However, recently a postulation that genetic variations in the promoter of gene of PSA might contribute to the individual variations in the serum PSA levels in men without prostatic diseases.

The study revealed that PSA was significantly higher among those ≤ 50 years than among those > 50 years old (p=0.002). This finding is similar to that reported in Jordan.

In the line with that in literature, PSA was showing a positive significant association with age in the above normal levels. Recently, it was reported that ageing is usually characterized by a mild chronic pro-inflammatory state. This phenomenon is called inflammageing. Therefore, the finding that PSA level was associated with age might be attributed to inflammatory process.

The study showed that markers of inflammation (epithelial cells, pus cells and bacteria cells) in urinalysis were associated significantly with elevated PSA level than that showing no inflammatory markers (p = 0.0001 for each marker) which could indicate that PSA is part of the pathophysiological inflammatory reaction.

Presence of pus cells in seminal fluid was significantly associated with higher PSA level than that in patients with no pus cells in their seminal fluid (p =
0.0001). Literature documented that chronic bacterial infection of prostate has been identifiable with virulent microorganism in prostatic secretion. The finding shows the PSA as a reflection to the inflammatory state of prostate.

It was stated that painful ejaculation in men address prostatitis and. This study showed that painful ejaculation was associated with elevated level of PSA which is it non malignant than that not complaining of painful ejaculation (p = 0.0001). The finding might be suggesting that PSA reflects prostatitis again.

**Conclusion**

The diagnosis of CP / CPPS might be outside the traditional urologic practice and might consider PSA level too as an acute reactant protein. PSA increase in Chronic prostatitis indicates it’s an inflammatory marker not only a tumor marker.

**Limitation of detection:**

Free PSA was not included.

**Acknowledgement:** I am grateful to the authorities of College of Pharmacy\ Anbar University, Lagash laboratories personel and all urologist and patents whom allowed for their data to be collected and analyzed.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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Treatment and Experimental Infection with *Klebsiella pneumoniae* in Rats

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Abstract

So many diseases are caused by *Klebsiella* species including urinary tract infections (UTIs), pneumonia, sepsis, diarrhea and bacteremia. Also *Klebsiella* is responsible for a significant number of community-acquired infectionssuch as pneumonia that results in severe injury in the lung and responsible for a high death ratein children. The intranasal and left lung route of *K. pneumoniae* infection causes pathological change in the lungs tissues due to acute and chronic injury. Conducted to detect the histological and immunological changes in experimental rat’s lung infected by different routes with *K. pneumoniae*.

Twenty-seven Albino Swiss male rats (Rattus rattus) were infected with 0.2ml of *K. pneumonia* suspension in different routes. After seven days from the last injection *K. pneumoniae*, the Lung and spleen are examined for histopathology changes. The blood vessels congestion with dispersed lymphocytic cells, infiltration throughout pulmonary parenchyma parts, edema formation along with hemorrhages Early neutrophils distribute into the Broncho-alveolar space, were detected. An acute splenitis was present in rat’s infection by left lung injection route after 10 days, were observed in the Spleen tissue with mild white pulp within pulp hyperplasia.

What makes *K. pneumoniae* infections more difficult to treat is that they gradually became more virulent and antibiotic resistant through time. The early *K. pneumoniae* infection -induced secretion of tumor necrosis factor alpha as pro-inflammatory cytokines. The level of cytokines has been related to severity of pulmonary inflammatory process. TNF-α is important for the acute phase response as proinflammatory responses.

**Keywords**: Acute lung injury, acute splenitis and TNF-α.

Introduction

*K. pneumoniae* is a gram-negative, non-motile, rod-shaped, lactose-fermenting, facultative anaerobic bacteria with a prominent polysaccharide capsule. Some strains of *K. pneumonia* have been implicated in bloody diarrhea, such cases became not easily treated as a result of the developing capability of *K. pneumonia* to resist the effects of various antibiotics (1).

*Klebsiella* known of their ability to produce enterotoxin that is heat stable and can be considered as a cause of their pathogenicity, the major virulence factors of *K. pneumonia* which play essential roles in pathogenesis include capsular polysaccharide, lipopolysaccharide, type 1 and type 3 fimbriae, and siderophores (2).

Recently, *K. pneumoniae* are emerging worldwide as a Leading cause of nosocomial infections, including pneumonia, bacteremia, urinary tract infections and wound infections (3). *K. pneumoniae* causes the pneumonia disease which is recognized by an excessive macrophage and neutrophil infiltration associated with exacerbated inflammatory response, severe lung injury and high production of pro-inflammatory cytokines (4).

Local inflammation gives an advantage in such infections by avoiding pathogen spreading, long lasting hyper-inflammation usually comes together with death and chronic inflammatory disorders (5).

Other researchers determined that *Klebsiella pneumoniae* taken from bloody diarrhea has the ability to bind to cytoskeletal proteins and Henrietta lacks
cells, such as the actin that gathers at the bacterium-host contact point (6).

Antibiotic resistance of G- bacteria is related to raising death, long periods of hospitalization and hospital expenses (7). *Klebsiella pneumoniae* and other species may inhabit the pharynx, intestinal tract and the skin of humanand it also colonizes in urine, sterile wounds and might presents as normal flora in many organs such as the intestine, biliary tract and colon (8).

TNF-α(cachectin) has significant functionality as an antitumor, inflammation, anorexia, immune modulation, viral multiplication, cachexia, septic shock and platelets agglutination. Many factors stimulating the secretion of cachetin by macrophages such as bacterial infection and it can be produced by many other types of cell such as NK cells, CD4<sup>+</sup> lymphocytes, mast cells, neutrophils, eosinophils, and neurons (9).

Biofilm of *K. pneumoniae* that is found on Contaminated medical devices such as catheters is the major cause of infections in catheterized patients (10). *K. pneumonia* is a major cause of bacteraemia, and abscesses. These infections may be nosocomial, healthcare-related or community-acquired (11).

**Materials and Method**

The specimens were collected carefully ways to avoid contamination. The specimens were transported by sterile transport swabs to the bacteriological laboratory, and each specimen was inoculated on blood agar, MacConkey agar, then inoculated at 37°C under aerobic condition for 18-24 hrs.

**Culture of Clinical specimens**

The bacterial sample was obtained by taking a colony from MacConkey and blood agar and repeating it’s growth to gain a pure culture and was diagnosed according to it’s cultural and morphological characteristics, microscopic characteristics after Gram’s stain, then further identified by biochemical tests (12). The final identification was performed with automated VITEK-2 compact system using G-ve ID cards.

**Animals Study:**

Twenty-seven Albino Swiss male rats (*Rattus rattus*) were used as the experimental animals. their ages ranged between eight to ten weeks and 230-270 g in weight. Rats were divided into three groups as following:

**Group A:** 3 rats treated with normal saline as control suspension.

**Group B:** 12 rats were infected with 0.2ml of *K. pneumonia* suspension in different routes. 3 rats infected orally, 3 rats infected intranasal, 3 rats intra-dermally and 3 rats infected inklung.

**Group C:** 12 rats infected with *K. pneumonia* and divided in to four sub-groups to treated with antibiotics (Tetracycline, Ciprofloxacin, Amoxicillin, amoxicillin and ciprofloxacin).

**Histopathological examination**

Lung and spleen were removed after seven days from the last injection and fixed in 10% formalin in PBS for 24 h, dehydrated in ethanol, cleared with xylene, infiltrated and embedded in paraffin. The histological section is cut with rotary microtome. Without knowing the origin of the tissue slides, hematoxylin and eosin stained slides were scored by a pathologist for inflammatory parameters. The sections are conveyed into water bath (52°C) to plain the tissues then fixed on slides containing Glycerin-Albumin mixture, as a thin film and placed for drying in the oven at 40°C for 24 hrs. Harris hematoxylin and Eosin are used in the tissue section staining while Southgate’s Mucicarmine and Periodic Acid Schiff-(PAS) stains are used to stain the polysaccharide capsule according to (13). Then Paraffin-embedded sections from each specimen are cut into 3-5 μm thick, sections are placed on positively charged slides, and left over night to dry at room temperature, or put slides in oven 60°C for 60min for Dewaxing. according to (14). The Slides are deparaffinized by heating in an incubator at 37°C Cover night.

**Immunological Assay:**

TNF-α was measured in serum, plasma and other biological fluids of rats by Sandwich enzyme-linked immunosorbent assay. the ELISA kit are prepared according to the manufacturer’s instructions.

**Statistical Analysis**

All statistical analysis was performed by using SPSS v.24 software program. Also, it was drawing histograms by Microsoft Excel 2013.

**Results**

**Samples collection and examination:**
140 clinical specimens were collected from stool, urine, burns, wounds and bone inflammation. 115 specimens are positively bacterial growth (18 specimens are *k. pneumonia*, 97 specimens are other types of bacteria) and 25 specimens were not growth. Only 10 *k. pneumonia* isolates were obtained from urine and 8 *k. pneumonia* isolates were obtained from stool. The initial identification of bacteria depended on some characteristics such as morphological colonies, Grams stain, and biochemical diagnosis.

**Histopathological examination after induce infection by *k. pneumonia* from different infection routes:**

In present study, there was significantly (p < 0.05) between different infection routes (Intranasal, Left lung, Orally, and Intra-dermal), *K. pneumonia* causes acute inflammation in the rats lungs through the intranasal and left lung routes. See figures (1, and 2).

The microscopic examination of lungs showed normal histological features (normal alveoli and interstitial parenchyma portion’s) in control group, whereas infected group by *K. pneumonia*, the lung sections showed pathological changes in the rats lung, which is characterized by blood vessels congestion with dispersed lymphocytic cells, infiltration throughout pulmonary parenchyma part’s, lymphocytes are distribute in the pulmonary tissue, and lymphoid aggregates, edema formation along with hemorrhages, congestion.
and severe lymphocyte and macrophage infiltration were detected. Histological examination exposed that at 10 days after injection of *K. pneumonia*, the rats had moderate to severe multifocal bronchopneumonia characterized by a cellular infiltrate composed almost exclusively of neutrophils and a few macrophages. These cells filled alveolar and bronchiolar spaces of affected areas. Alveolar spaces at the margin of a lesion were filled with eosinophilic proteinaceous edema fluid.

**The comparison between types of cytokines with different infection routes**

The comparison between different cytokines types with route infections showing TNF-α, IL6 and IL10 levels was different increases in *K. pneumonia* dose $10^5$ CFU/ml in comparison to infection routes and slightly significant increased other level compared to control group, selection left lung injection and intranasal routs continuously research and experiments working as showed in Table (1).

**Table (1): Levels of pro-inflammatory TNF-α**

<table>
<thead>
<tr>
<th>Mean±SD TNF-α</th>
<th>Control(n=3)</th>
<th>Intrasinal(n=3)</th>
<th>Left lung(n=3)</th>
<th>Orally(n=3)</th>
<th>Intra-dermal(n=3)</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>189.4±1.21 *</td>
<td>1184.7±0.52 *</td>
<td>55.1±0.87</td>
<td>120.3±1.21</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Significant differences = p<0.05

**Histopathological observations after infection by *K. pneumonia* from different infection routes and treatment by different antibiotics:**

A histological study showed that the intranasal and intraperitoneal routes of infection with *K. pneumonia* have caused pathological changes in the lung tissues, which is characterized by congestion of blood vessels with dispersed lymphocytic cells, infiltration throughout pulmonary parenchymal components, lymphocytes are distribute in the pulmonary tissue with blood vessels blocking, and lymphoid masses(Figures 3 – 4).

**Fig. (3)** Rat lungs after intra nasal introduction of predatory bacteria *K.penumoniae* & Lung treated with Amoxicillin. (Lung tissue with normal alveolar wall thickness and architecture and moderate congestion of blood vessels. H&E. 40X).

**Fig. (4)** Rat lungs after left lung introduction of predatory bacteria *K.penumoniae* & Lung treated with Ciprobloxacin. (Lung tissue with normal alveolar wall thickness and architecture, with a focal area of acute inflammatory cell infiltration around pulmonary venules (arrow). H&E. 40X.)
Effect of antibiotics on Pro-inflammatory cytokines production.

-TNF-α (pg/ml):

The results in the Table (2) showed decreased considerable variations p<0.05 in TNF-α concentration of treated rats with different antibiotics when compared with Infected rats, therefore the results indicated to decrease significant in Treated rats groups with different antibiotics CIP, AX, TET, AX+CIP when compared between them.

Table (2): levels of TNF-α cytokine before and after treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Infected rats N=12</th>
<th>Treated rats N=27</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AX</td>
<td>CIP</td>
</tr>
<tr>
<td>Left lung injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1185*</td>
<td>757*</td>
<td>80*</td>
</tr>
<tr>
<td>0.52</td>
<td>1.73</td>
<td>0.70</td>
</tr>
<tr>
<td>0.31</td>
<td>1.00</td>
<td>0.41</td>
</tr>
<tr>
<td>Intranasal cavity injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>156*</td>
<td>54*</td>
<td>94.2*</td>
</tr>
<tr>
<td>0.90</td>
<td>0.17</td>
<td>0.61</td>
</tr>
<tr>
<td>Collected from – blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.52</td>
<td>0.10</td>
<td>0.35</td>
</tr>
<tr>
<td>Intranasal cavity injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>184*</td>
<td>825*</td>
<td>714.1*</td>
</tr>
<tr>
<td>- Collected from secretion</td>
<td>1.73</td>
<td>18.95</td>
</tr>
<tr>
<td>1.00</td>
<td>10.95</td>
<td>4.00</td>
</tr>
<tr>
<td>Control</td>
<td>120 ± 0.00</td>
<td>Mean ± Std. Deviation</td>
</tr>
</tbody>
</table>

*LSD 10.031

*considerable variations = p<0.05 between control and study groups.

** Considerable variations = p<0.05 between them groups.

Discussion

One of four scores was the lung pathology which was assigned according to their severity of inflammation as follows: 1- mild focal inflammation, 2-normal histology, 3- moderate to severe focal inflammation with areas of normal tissue, 4- severe inflammation to necrosis (15).

K. pneumoniae is a vital cause of community-acquired and nosocomial infections, so gaining attention due to the high morbidity and mortality rates and the rising number of resistant strains to the antibiotic effects (16)(23).

Lungs considered as important organ of respiratory system, is susceptible to many agents smoking, genetics, and microbial infection that are responsible for numerous pathological conditions (17).

Pulmonary infection can cause a number of histological changes in the architecture of lung, as well as in the extrapulmonary organs as in the case of bacterial dissemination via blood stream, and these changes can impact the result of disease (18).

Pulmonary infection can cause a number of pathological changes in the lung, as well as in the extra-pulmonary organs as in the case of bacterial dissemination, and these changes can impact the outcome of disease. One of the greatest limitations to the study of microbial virulence factors is the availability of relevant animal models (19).

The present study, states that TNF-α has considerable differences in levels between control and study groups and significant differences between these groups that are shown in table (1) In comparison with (20) who found significantly increased levels of TNF-α in groups of animals under acute stress and chronic mild stress due to bacterial LPS compared to non-stressed control groups.

TNF-α is a major pro-inflammatory cytokine mediate the development of many inflammatory lung diseases and its concentrations significantly higher 4 to 6 fold after 6 and 24 hours respectively from infection (16). Moreover, TNF-α mediate lung inflammatory diseases by enhances macrophage inflammatory protein, KC secretion, play an important role in the up-regulation of cellular adhesion molecules, vasodilatation and increasing the vascular permeability (21).

TNF-α, IL-1β and IL-6 main functions are to attract neutrophils to the site of inflammation, induce the acute phase response, i.e. starting inflammatory processes such as production of APPs in the liver. Stimulated macrophages by (lipopolysaccharide LPS) secret wide array of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8 (22).
Conclusions

*Klebsiella pneumoniae* infection and lung diseases are important topics regarding public health interest. Neutrophils are one type of the first innate immune cells recruited to the site of infection to prevent bacterial colonization and clear bacteria.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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Abstract

Objectives: The purpose of this in vitro study was to evaluate post-cementation vertical marginal seating of full contour zirconia crown restorations using different finish line designs (deep chamfer and shoulder) with different occlusal reduction schemes (planar and flat).

Materials and Method: Thirty-two sound maxillary first premolar teeth freshly extracted for orthodontic purposes were collected to be used in this study. Teeth were divided into two main groups according to the design of finishing line used (n=16): Group A: Deep chamfer; Group B: Shoulder. Each group was then subdivided into two subgroups according to the scheme of occlusal reduction used (n=8): (A₁, B₁) Planar; (A₂, B₂) Flat. Standardized preparation for full contour zirconia crown restorations was carried out with finishing lines depth 1.0 mm, total convergence angle of 6 degrees and axial height 4 mm (buccally and palatally).

Results: The results of this study showed that there were statistically highly significant differences among different groups (p < 0.01) using one-way ANOVA analysis and Student’s t-test. Additionally, comparison of significance between each pair of subgroups at pre and post-cementation intervals using Paired-Samples t-test showed a statistically highly significant differences.

Conclusions: Deep chamfer with planar occlusal reduction scheme provided better marginal fit compared to that obtained with shoulder. On the other hand, shoulder with flat occlusal reduction scheme provided better marginal fit compared to that obtained with deep chamfer. Concerning the effect of the cementation procedure, the marginal gap was increased post-cementation as compared with the pre-cementation gap for all subgroups, but still within the clinically acceptable limit.

Key words: Chamfer and shoulder finishing line, Digital impression, Full contour zirconia, Marginal fit, Planar and flat occlusal reduction scheme.

Introduction

The success of all ceramic restorations strongly depends upon marginal adaptation. A well fitted margin is expected to reduce plaque accumulation, recurrent caries which result in damage to the tooth with its supporting periodontium, and potentially decreases the longevity of the restoration (¹). The clinically acceptable limit of the marginal discrepancies is reported to be less than 120 μm (²).

One of the CAD/CAM technology is a digital impression which provides speed, accuracy, high quality of restoration as its designing is based on materials characteristics, ability of storing captured information indefinitely and transferring digital images between the dental office and the laboratory (³).

The designs of tooth preparation can affect the success of the crown restoration (⁴). Cementation of an indirect dental restoration is the final step after finishing all the clinical and laboratory stages and it is considered
to be an equally important stage that can affect the longevity of the restoration (5).

**Materials and Method**

Thirty-two sound human maxillary first premolar teeth of comparable size and shape extracted for orthodontic purposes were selected and collected to be used in this study.

The teeth samples were divided into two main groups according to the design of finishing line used (n=16):

- **Group A**: Deep chamfer design.
- **Group B**: Shoulder design.

Each main group was, then, subdivided into two subgroups according to the scheme of the occlusal surface reduction (n=8):

- **Subgroup A₁ & B₁**: Anatomical occlusal reduction.
- **Subgroup A₂ & B₂**: Non anatomical occlusal reduction.

All the teeth were prepared for full ceramic crown restorations with the following preparation features: A total convergence angle of 6 degrees, the depth for both deep chamfer and shoulder finishing line of 1.0 mm and a standardized axial height of 4 mm (buccally and palatally), these dimensions were checked using a modified digital caliper (Fig. 1).

After an axial reduction of each tooth according to its respective group, occlusal surface of all teeth was prepared with diamond wheel to produce flat occlusal reduction scheme. According to the scheme of occlusal surface reduction, all teeth samples in subgroups A₁ and B₁ received further occlusal reduction to change the design into planar using rugby ball bur. All sharp angles or internal line angles were rounded to prevent stress concentration on the crown restoration.

A three dimensional digital image for each tooth sample was taken by AC Omnicam intra-oral scanner (Sirona Dental Systems, Bensheim, Germany) (powder free). Full contour zirconia crown restorations were then fabricated using In-Lab MC X5 milling device used (Sirona InCoris TZI C blank).

A custom made holding device was especially fabricated in this study to be used during seating of the zirconia crown, it serve as a screw that secured the zirconia crowns to the natural tooth sample. Furthermore, it hold the specimens on the horizontal table of the microscope to allow for viewing the references points during measurement of vertical marginal gaps (Fig. 2).

The measurements were made at two intervals (before and after cementation) at four points determined...
on each surface of the tooth (two at the edges of the line, two points drawn in the mid of the surface by permanent marker, while the other two points were at a distance of (1mm) from the previous one, on both (left and right sides). Sixteen measurements were obtained from each tooth sample; highest one was selected to represent the maximum marginal gap of that sample \(^6\) (Fig. 4).

Figure 4: Four points of measurement at a magnification of 50X.

The measurement of the marginal gap of four different points at four areas (mid-buccal, mid-mesial, mid-distal and mid-palatal) was used by Khdaier and Ibraheem (2015) \(^7\) and Abdulkareem and Ibraheem (2016) \(^8\).

Statistical methods were used in order to analyze and assess the results included:

- Descriptive statistic.
- Inferential statistics.

1. One-way ANOVA (analysis of variance).
2. Paired-Samples t-test.

### Results

Table (1) showed the descriptive statistics of vertical marginal gap for the four subgroups measured in \(\mu\)m at pre and post cementation intervals. At Pre-cementation interval, the table showed that the lowest mean of vertical marginal gap values was scored by subgroup \(A_1\) (38.837±9.30), while the highest mean of vertical marginal gap values was belonged to subgroup \(B_1\) (66.636±8.57), however on the other hand, at Post-cementation interval, the table showed that the lowest mean of vertical marginal gap values was scored by subgroup \(A_1\) (79.281±10.25), while the highest mean of vertical marginal gap values was belonged to subgroup \(B_1\) (110.082 ±9.63).

Paired-Samples t-test was applied in (Fig. 5), showed that there was highly significant differences between the subgroups, this mean that cement have significant negative effect on the amount of vertical marginal gap of the crown restorations.

**Table (1): Descriptive statistics of vertical marginal gaps for the four different subgroups in micrometer (pre and post-cementation)**

<table>
<thead>
<tr>
<th>Types of finishing line</th>
<th>Subgroups</th>
<th>Descriptive Statistics</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-cementation</td>
<td>Post-cementation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Mean (±)SD</td>
<td>Mean (±)SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep chamfer finishing Line</td>
<td>A</td>
<td>(A_1)</td>
<td>8</td>
<td>38.837 (±9.30)</td>
<td>79.281 (±10.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(A_2)</td>
<td>8</td>
<td>63.199 (±9.22)</td>
<td>105.618 (±10.11)</td>
<td></td>
</tr>
<tr>
<td>Shoulder finishing Line</td>
<td>B</td>
<td>(B_1)</td>
<td>8</td>
<td>66.636 (±8.57)</td>
<td>110.082 (±9.63)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(B_2)</td>
<td>8</td>
<td>50.763 (±12.88)</td>
<td>91.990 (±14.02)</td>
<td></td>
</tr>
</tbody>
</table>

HS: \((p < 0.01)\) (Highly significant).
Discussion

The results of this in vitro study showed statistically highly significance difference but still within the clinically acceptable limit (<120 µm) for all tested subgroups. It is worth to mention that when reviewing the available literature, no previous studies concerning the effect of both finishing line designs with different occlusal reduction schemes on the marginal fit of full contour CAD/CAM zirconia crown restoration was found.

Statistical analysis of the results of this study showed that the teeth prepared with deep chamfer finishing line and planar occlusal reduction showed less mean marginal gap values than teeth prepared with flat occlusal reduction. This might be due to that the occluso-axial line angle is a right angle in the case of flat occlusal reduction scheme that may impede proper seating of the crown restoration.

However, the phenomenon was the opposite when the finishing line changed to the shoulder design where the mean marginal gap values of the teeth prepared with flat occlusal reduction was less than of that teeth prepared with planar occlusal reduction. This might be due to that the flat occlusal surface might produce smaller surface area than that produced with planar occlusal surface which might lead to even distribution of the load that going to be applied on the crown restoration during seating.

Furthermore, teeth prepared with shoulder finishing line and flat occlusal reduction showed less mean marginal gap values than the teeth prepared with deep chamfer finishing line. This might be due to that the force applied on the axio-gingival angle of the shoulder design was perpendicular on the surface of the margin, while in the chamfer design the force applied at the line angle of that surface was smaller and the applied pressure was thus smaller, so the seating of the crown restoration was not as good as in the shoulder design.

In addition to that, the teeth prepared with deep chamfer finishing line and planar occlusal reduction showed less mean marginal gap values than the teeth prepared with shoulder finishing line. This might be due to that deep chamfer finishing line design has a more round angle between the axial and gingival seat which enables more accurate seat for the crown restoration. Furthermore, the stress concentrated at the area of the finishing line during the crown seating is more evenly distributed. This is in total agreement with what has been stated by Rosenstiel et al. (2016) (9) stated that “The occluso-axial line angle of the tooth preparation should be a replica of the gingival margin geometry”. In addition this is total agreement with: Wostmann et
al. (2005) (10) concluded that the lowest mean value of marginal gap was obtained for the chamfer preparation, while the 90° shoulder finishing line always produced the highest mean value. Comlekoglu et al. (2009) (11) reported that the cervical finish line type had an influence on the marginal adaptation of the tested zirconia crown restorations. This is also in agreement with Alzubaidy and Alshamaa (2015) (12) stated that the deep chamfer finishing line is more preferable than shoulder finishing line for full contour CAD/CAM zirconia crown restorations.

However, this disagrees Subasi et al. (2012) (13) revealed that the finish line design had no influence on the marginal adaptation of zirconia crown restorations.

On the other hand, the quality of the three dimensional image of a tooth preparation might be a factor that affect the marginal adaptation of the final crown restoration (14). The scanning accuracy have the limitation of finite resolution; which can result in edges that are slightly rounded and leads to interfering contacts at the incisal/occlusal edges (15). Furthermore, Reich et al. (2005) (16) reported the scanning system that depend on optical impression, experience problems with rounded edges and positive error (which simulates virtual peaks near the edges, so-called ‘over-shooters’). The ‘rounded edges’, “point clouds” and ‘over-shooters’ phenomena have been described for the CEREC intraoral camera. In addition, the scanning process based on the principle of “not at the same plane surface” that is obtained in the scanning area were the scanning process transformed into smooth continuous surface (17).

Kunii et al. (2007) (18) reported the anisotropic shrinkage of zirconia blanks during construction procedure might play a role in the vertical marginal gap of different subgroups in this study, as a result, sintering shrinkage in the vertical axis was smaller than that in the horizontal axis due to this shrinkage property.

Effect of cement

The result of this in vitro study showed that the luting cement and cementation procedure play an important role in the final accuracy of the marginal fit for all ceramic crown restorations and this clearly shown when compare the result of pre-cementation with that recorded of post-cementation for all tested subgroups. Furthermore the statistical analysis of the results has proven the presence of those significant differences between each two subgroups at these two intervals.

The closing angle between tooth preparation and restoration becomes smaller, the flow of the cement is inhibited and its escapement becomes more difficult. Resin cement increased viscosity too rapidly to flow toward the cervical area and extruded from the margins of the crown, this will create the problematic discharge of excess cement and hydraulic pressure that is going to push the cement upward, this will result in great amount of luting cement to be accumulated on the occlusal surface of the prepared tooth that might interfere with proper seating of crown restoration after cementation procedure (19).

The luting cement and cementation procedure play an important role in the final accuracy of the marginal fit for all ceramic crown restorations, so, the marginal gap values were increased significantly post-cementation but within the limits of clinical acceptability (<120 μm), this is in total agreement with other previous studies: Okutan et al. (2006) (20).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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References


Effect of the Different Disinfectants on the Microbial Contamination of Alginate Impression Materials

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Abstract

Objectives: This study aimed to evaluate the efficacy of different solutions used to disinfect Alginate impression material.

Materials and Method: Twenty Alginate impressions for the maxillary arch of five patients recently completed their treatment (four impressions for each patient) were taken swabbed before and after disinfecting them with Chlorhexidine, Desident CaviCide and Alcohol and one just washed with tap water as a control. Swabs were cultured to detect bacteria and fungi and count them.

Results: Only Streptococcus bacteria were detected with no fungal contamination. Tap water reduced the bacterial count while other disinfectants killed the bacteria completely.

Conclusions: Chlorhexidine, Desident CaviCide and Alcohol can be used to disinfect the dental impression effectively.

Key words: Disinfection, infection control, dental impression.

Introduction

There is a great danger nowadays about transferring of infectious organisms to the workers in dental field’s treatment as orthodontists, prosthodontists, oral surgeons and their assistants whom dealing with dental impression for patients required prosthesis ¹, ².

Disinfection can be defined as the procedure that kills vegetative organisms, in the same context; sterilization is the procedure of terminating spores too³.

There are many researches that study the effect of different disinfectant on irreversible hydrocolloid dental impressions and they monitor that effect on the measurements of the dental cast produced from a disinfected impression ⁴, ⁵.

Alcohol is not highly recommended for sterilization since it lacks the sporicidal activity while it works well in the category of antimicrobial activity against bacteria, fungi and viruses (cell lysis and proteins denaturation) in the range of 60-90% but it demonstrates low activity below 50% ⁶.

Chlorhexidine had a wide range anti-microbial activity against many micro-organisms. Jani et al. ⁷ in 2010 used chlorhexidine from different manufacturers to disinfect alginate dental impressions and found that Corsodyl was the most potent one in killing Streptococcus Mutans and Lactobacilli.

Desident CaviCide is a disinfected solution with broad anti-microbial activity used in recent Iraqi study to disinfect the contaminated clamping tweezers. It showed potent antibacterial activity but poor antifungal effect ⁸.

This study aimed to test the effect of different solutions in disinfecting irreversible hydrocolloid alginate dental impressions.
Materials and Method

Sample

Five orthodontic patients (3 males and 2 females), whose had just finished their orthodontic treatment that lasting more than 12months, will be carefully selected with fair oral hygiene and full complement permanent teeth.

Method

The patients or patients’ parents were asked for permission to participate in this research and a written consent form was signed by the patient or the parent. A total of 20 alginate impressions were taken (four maxillary dental impressions taken successively for each patient) using Hydrogum soft mint scent alginate, Zhermack, Germany). Alginate was mixed according to manufacturer instructions (2 spoons 18 gm mixed with 2 levels 36 ml of water loaded into a disposable dental tray and left to set for two and half minutes). The impressions were grouped according to the disinfectant used as followed:

Group A: represented the first impression that will be taken, swabbed (A1) then washed with running tap water and swabbed again (A2).

Group B: represents the second impression that will be taken, swabbed (B1) then washed and immersed in Desident CaviCide (Spofa Dental Co., Czech Republic) for five minutes and swabbed again (B2).

Group C: represents the third impression that will be taken, swabbed (C1) then washed and immersed in Alcohol 96% (Teeba Co., Iraq) for five minutes and swabbed again (C2).

Group D: represents the fourth impression that will be taken, swabbed (D1) then washed and immersed ten minutes in Corsodyl (Chlorhexidine 0.2%, Omega Pharma Manufacturing GmbH & Co. KG, Germany) mouth wash and swabbed again (D2).

The surface of each impression was swabbed with sterile cotton swab and dipped in normal saline then sent to the laboratory for incubation and culturing on three agar media Blood agar, McConkey agar, and Sabouraud dextrose agar (Hi-Media Co., India) to detect the aerobic and facultative anaerobic bacteria of both types Gram positive and negative, in addition to candida species before and after disinfections using manual colony forming counting.

Statistical Analyses

Data were managed statistically using SPSS version 25 software. Means, standard deviations were obtained for each disinfection solutions in addition to the tap water.

Findings

Table 1 showed the means and standard deviations of the colony forming units of Streptococcus bacteria isolated from five successive dental impressions of the five patients.

Generally, the highest CFU was decreased after washing in tap water but still there is growth in contrary to other disinfectants that kill all bacteria. No fungal contamination was detected.

Table 1: Descriptive statistics of colony forming units (CFU) before and after disinfection with different disinfectants

<table>
<thead>
<tr>
<th>Tap water</th>
<th>Desident CaviCide</th>
<th>Alcohol 96%</th>
<th>Corsodyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
<td>B1</td>
</tr>
<tr>
<td>A1</td>
<td>1140</td>
<td>950</td>
<td>1000</td>
</tr>
<tr>
<td>A2</td>
<td>1150</td>
<td>900</td>
<td>900</td>
</tr>
<tr>
<td>B1</td>
<td>1200</td>
<td>850</td>
<td>950</td>
</tr>
<tr>
<td>B2</td>
<td>1000</td>
<td>950</td>
<td>900</td>
</tr>
<tr>
<td>C1</td>
<td>1100</td>
<td>1000</td>
<td>850</td>
</tr>
<tr>
<td>C2</td>
<td>1118</td>
<td>930</td>
<td>920</td>
</tr>
<tr>
<td>Mean</td>
<td>1118</td>
<td>930</td>
<td>920</td>
</tr>
<tr>
<td>S.D.</td>
<td>74.967</td>
<td>57.009</td>
<td>57.009</td>
</tr>
</tbody>
</table>
Discussion

One of the major infection control procedure in the dental practice is disinfecting the dental impressions as the microorganisms can be transmitted effortlessly by saliva and blood to the dental staff and technicians, so a high standard of hygiene and disinfection of dental equipment, including dental impressions is recommended.

Generally, chemicals are broadly used in dental practice because of their easy application although their actions is influenced by many factors like the numbers and types of the organisms, disinfectant concentration, presence of blood and mucus which act as a insulating layer preventing the disinfection from contact with surface, time of contact with the disinfectant and the nature of the surface weather it is porous or not.\(^9\)

In this study swabs from Alginate dental impression materials were obtained from five successive impressions for five patients completed their fixed orthodontic therapy that lasts more than one year. The main aim of this study was to evaluate the efficacy of different agents in disinfecting the dental impressions. Tap water was used as a negative control and had been tested previously in two studies\(^7,11\), while Desident CaviCide is tested for the first time in this study. It possessed wide range anti-microbial activity in short period of exposure reaching to 30 seconds.

Immersion not spraying technique of disinfection is used to ensure uniform contact between the disinfectant and dental impression. Disinfection with spraying method decreases the possibility of distortion, particularly in hydrocolloids impression materials, but will not reach the areas of undercuts and may releases air that leads to occupational exposure\(^10\).

Correia-Sousa et al.\(^12\) found a 48.5% reduction in the microbial load after washing alginate impression with tap water. The present study reported lesser percentage of reduction (Table 1). Jani et al.\(^7\) reported that tap water fail to kill the Streptococcus Mutans and Lactobacilli while Chlorhexidine kill Streptococcus Mutans completely. Chlorhexidine is a positively charged molecule that binds with the negatively charged sites of the bacterial cell wall so interferes with the osmosis. Moreover, it assaults the cytoplasmic membrane and leaks the components that lead to cell death. It has been shown that Chlorhexidine at high concentration of 2% demonstrated antimicrobial activity against S. aureus, E. coli, and B. subtilis, but not C. albicans\(^13\).

Desident CaviCide contains low concentration of Alcohol and enable to inactivate the bacterial growth in the impression by alkylating the amino and sulfhydral groups of bacterial proteins, the present findings come in agreement with that of other studies\(^8,14,15\).

Isopropyl alcohol has high bactericidal activity in concentration as high as 99% but is relatively inefficient in the presence of blood and saliva. It lacks sporcidal activity and also causes corrosion of metals. Ethyl alcohol has more bactericidal than bacteriostatic activity in addition to tuberculocidal, fungicidal, and virucidal activity against enveloped viruses but has no effect against bacterial spores and non-enveloped viruses. They work by denaturation the bacterial proteins and lipids and leads to cell membrane disintegration so, inactivating the microorganisms. The optimum bactericidal concentration in water is 60% to 90%, and the lethal activity falls when diluted below 50% concentration. Ethanol has shown clear bacterial growth inhibition, especially when used in high concentrations against S. mutans and S. aureus\(^16,17\). In this study, Alcohol was used in a concentration of 96% and it as able to kill all detected bacteria (Table 1).

In a previous study, Mohammed et al.\(^11\) tested the effect of sodium hypochlorite, Biosanitizer M and Zeta plus 7, used as disinfectant for Alginate impression materials, on the teeth and dental arch measurements and the results proved non-significant effect. Further studies are needed to check the efficacy of the tested disinfectants in this study on the viral and fungal contaminations and their effect on the dimensional stability of the resultant model.

Conclusions

Disinfecting dental impressions is far important procedure to control the transmission of microorganisms among the dental staff. Tap water was able to reduce the microorganism while Desident CaviCide, Alcohol and Chlorhexidine eradicated the bacteria completely.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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**References**


Role of Respiratory Syncytial Virus and Some Bacteria Causes Tonsillitis among Children Under 5 Years Old in Duhok City

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Abstract

Background: Acute infection of the respiratory tract is a prevalent cause of death in children under the age of 5 years, particularly in developing nations. Infection with respiratory syncytial virus is a causative factor for bacterial co-infection with an increase in the incidence of respiratory disease.

Objective: This research was intended to clarify the connection between the Respiratory Syncytial Virus RSV and the tonsillitis-causing bacteria.

Method: In 120 patients with acute and chronic tonsillitis, swabs were obtained from the tonsils to detect pathogenic and commensal bacteria in the throat, and blood samples collected to identify RSV infection by identifying the antibody value in the serum.

The results: Culture results showed that 78.53% of the bacteria isolated belonged to the Gram-positive group and that only 21.47% belonged to the Gram-negative group, (51.98%) were positive for pathogenic bacteria while (48.02%) were positive for commensal bacteria and showed the highest rate of pathogenic bacteria isolated from tonsillitis patients was to Staph. aureus and Strep. pyogenes. Serological testing using ELISA showed that respiratory syncytial virus infection increases tonsillitis co-infection. Type of antibodies showed a change depending on the sex group and the stages of immune response maturity.

Key word: Respiratory syncytial virus RSV, Tonsillitis, Bacteria, ELISA, Co-infection, IgG, IgM.

Introduction

Recently, acute respiratory tract infections (ART) have been found to be among children’s most common disease. Recent research by the World Health Organization (WHO) has shown that acute respiratory infections are responsible for 22% of child deaths. Under the age of five, which represents more than 2 million children, 99% of these deaths happen in developing nations, the primary cause of which is RSV (1).

ARI can be categorized into two kinds depending on its anatomical place: upper respiratory infections and lower respiratory infections. The most prevalent infectious diseases such as rhinitis (common cold), sinusitis, ear infections, acute pharyngitis, and tonsillitis are acute upper respiratory infections. Infections of the ear and pharyngitis lead to more serious problems such as acute rheumatic fever and deafness (2).

A community of mucosal dwelling microorganisms (the microbiota) colonizes the healthy URT, which includes both commensals and prospective pathogens kept under host immune system control. There is growing evidence that viral respiratory infections can significantly increase bacterial load (3).

In children as well as adults, many viruses and bacteria can cause pharyngitis and acute tonsillitis. About 75% of pharyngitis is caused by viruses (4). Group Aβ hemolytic Streptococci and Staphylococcus aureus are one of the primary causes of tonsillitis, particularly in the event of acute and chronic tonsillitis (5,6).

Physiologically, the lower respiratory tract is usually sterile. However, there is a prevalent connection between bacterial infection and respiratory infection,
and bacterial co-infection development enhances the severity of infection \(^\text{7}\).

The RSV virus is one of the most frequently recognized variables in young children with ARI and is the main reasons for hospital admission. Where the elevated incidence of RSV infection is a severe result of increasing the risk of RSV-associated hepatitis infection among children \(^\text{8}\).

Studies have shown that some respiratory viruses alter the colonization of bacteria and increase the likelihood of secondary bacterial diseases. The immune response to respiratory viruses may rely on certain bacteria spreading \(^\text{9}\).

Antibodies have a major part of protective immunity and that cellular immunity is important for clearing the infection. The protective contribution of antibodies in human disease is probably best demonstrated by the interconnection between Superior levels of maternally acquired antibodies and less serious disease in infants and the ability of high tittered RSV intravenous immunoglobulin (Ig) to decrease the risk of serious disease when administered prophylactically \(^\text{10}\). Therefore, the objective of this study was to detect the role of Respiratory Syncytial Virus and some bacteria in children causing tonsillitis and to identify the relationship between RSV infection and secondary bacterial colonization.

### Material and method

The groups in this study consisted of 120 throat swab and serum samples from acute and chronic tonsillitis patients and 30 people were collected as a control group at the Hivi Pediatric Teaching Hospital in Duhok city. For the period from the middle of December (15/12/2018) to the beginning of April (8/4/2019) and for children less than five years age for both sexes. Ethical approval for this study was obtained from Duhok Directorate General of Health, Department of planning, Scientific Research Division. (Approval reference number /27112018-9). The samples were taken with a swab from the tonsils. Were cultured on blood agar, MacConkey agar and chocolate agar and incubated at 37°C for 24-48 hours \(^\text{11}\). The growth diagnosed using biochemical tests and the Vitek 2 compact system 5ml venous blood was collected from all patients. Blood was placed in a test tube and Centrifuge and immediately stored in an Eppendorf tube and frozen at -20°C until assayed for serological diagnosis of RSV (Respiratory syncytial virus) was assayed by EIISA kits which performed according to the instruction of the manufacturing company (Vircell company Spin).

### Results

**Table (1) Seroprevalence of RSV antibodies, bacterial pathogens and (bacterial & viral) co-infection among patient with Tonsillitis.**

<table>
<thead>
<tr>
<th>Study group</th>
<th>Results</th>
<th>RSV antibodies</th>
<th>Bacterial &amp; viral infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteria isolated from Tonsillitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G+ Isolates No.</td>
<td>%</td>
<td>G-I isolates No.</td>
</tr>
<tr>
<td>Tonsillitis patient</td>
<td>139</td>
<td>78.53%</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>177</td>
<td>100</td>
<td>177</td>
</tr>
<tr>
<td>control</td>
<td>44</td>
<td>86.28%</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100</td>
<td>51</td>
</tr>
</tbody>
</table>
Among the bacteria isolated, 78.53% belonged to Gram positive group and only 21.47% belonged to Gram negative group. The rate of RSV-IgG and RSV-IgM with RSV- IgG highest than RSV-IgM among tonsillitis patients compared with control group which showed RSV-IgM highest than RSV-IgM with RSV- IgG. Bacteria with RSV co-infection was 41.67%

Table-(2) Distribution of seropositive RSV antibodies in relationship to gender tonsillitis patient by using ELISA technique.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Seropositive of RSV antibodies</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSV-IgM</td>
<td>RSV-IgG</td>
<td>RSV-IgM &amp; IgG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>57.14%</td>
<td>21</td>
<td>61.77%</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>42.86%</td>
<td>13</td>
<td>38.33%</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>100</td>
<td>34</td>
<td>100</td>
</tr>
</tbody>
</table>

The results of serological diagnosis of RSV infection indicate that the highest rate of RSV-IgG 61.77% was found in male tonsillitis patients, while 38.23% was fond in female. The highest rate57.14 % of RSV-IgM was found in male. Also, the highest rate 61.77% of both RSV-IgG and RSV-IgM together was found in male tonsillitis patients

Table (3) the percentage of pathogenic and commensal bacteria isolated from patient with tonsillitis.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Throat swab</th>
<th>Case yielding that organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>I. Pathogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>+</td>
<td>46</td>
</tr>
<tr>
<td>Strept. pyogenes</td>
<td>+</td>
<td>36</td>
</tr>
<tr>
<td>E. coli</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>II. Commensal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strept. Viridians</td>
<td>+</td>
<td>28</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>+</td>
<td>28</td>
</tr>
<tr>
<td>Strept. Pneumonia</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Micrococcus ssp</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>Non-Coagulase – Staph.</td>
<td>+</td>
<td>13</td>
</tr>
<tr>
<td>Corynebacterium diphtheria</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>177</td>
</tr>
</tbody>
</table>
In this study, the rate of pathogenic bacteria was (51.98%) which represent 92 isolates, while commensal bacteria represent 85 isolates with rate 48.02%. The most common types of pathogenic bacteria which causes tonsillitis in children are *Staph. aureus* 25.99% and *GAβ hemolytic Streptococcus pyogenes* 20.33% which have been found to be Co-infection in cases of respiratory Syncytial Virus.

**Discussion**

The development of the immune response in children depends on the early exposure to respiratory viruses and bacteria, where acute viral respiratory infections lead to increase the readiness for bacterial infection (12).

This study included the collection of 120 throat swabs and serum samples from children with respiratory diseases and recorded bacterial co-infections with the RSV which was diagnosed by measuring IgG and IgM and there are different types of gram positive and gram negative microorganisms were detected by throat swab culture of which gram positive bacteria constituted (78.53%) and gram negative one were (21.47%); these findings of bacterial percentage goes with that of (4,5). These results agreement with (13) Which indicated how severe diseases like infant virus acute respiratory infections can help us better comprehend secondary bacterial infections and long-term respiratory results of predisposition. In a study conducted by (9) they postulate that local bacterial ecosystems modulated the immune response of the host to RSV infection severity.

The results of serological diagnosis of RSV infection indicate that the highest rate of RSV-IgG was found in male tonsillitis patients, while 38.23% was found in females. The highest rate of RSV-IgM was found in male. Also, the highest rate of both RSV-IgG and RSV-IgM together was found in male tonsillitis patients, our results agreed with the results of (14) in terms of numbers of infected males, That was greater than females. This is in line with some research on gender relationships which suggest that the reasonable to compare immune reactions among males and females because many early-stage infections predominate among males (15). In our study, the limitation includes the small cohort size. Because of this study’s restricted cohort size, substantially induced immunological factors following RSV infection Could have been undervalued. Following the outcomes of this research, in-depth research would elucidate real protein correlates against RSV diseases by following a bigger cohort, particularly examining the correlation of clinical seriousness and immunological variables. Thus, in this study gender-specific antibody response profiles against RSV infection are considered as a hypothesis, and should be confirmed by further clinical data in future study.

In the present study, 177 bacterial isolates were obtained (51.98%) of which were pathogenic bacteria and (48.02%) were commensal bacteria. These results were agreed with (16) who obtained (55%) pathogenic bacteria, while (34.2%) were found with similar study in KSA (17).

The microbial content of the infant’s pharynx is usually low (18). The isolate of *Staphylococcus aureus* was proved to be the major causative microorganism of tonsillitis which constituted (25.4%) of total isolates. While the rate of *strep. pyogenes* are 20.33%, which represents 36 isolates. These results agreed with (4,5) and other studies (6,19) have described *Staphylococcus* is one of the most predominant genera, while (20,21) reported that main tonsillitis factors were *Strep. pyogenes*, followed by *Staph. aureus*.

The structure of airway microbiota is extremely vibrant and various environmental factors have been shown to affect colonization patterns. Vaccinations and antimicrobial use, as well as exposure to tobacco smoke, may result in changes in the airway microbiota (22).

**Conclusion**

Our results suggest that 41% of respiratory tract viral infections, which caused by RSV are associated with different microbial profiles that causes tonsillitis.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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21- Alnori, H. A., Mahmood, K. A., & Mohammed, A. A. M. Bacteriological , Serological and

Broad-Spectrum Cytotoxic Effect of *Calendula officinalis L* Against Breast Cancer Cells

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**Abstract**

**Background:** *Calendula officinalis L* used in Iraqi folklore medicine for several medical applications. This research evaluated the leaves extract as an anti-breast cancer agent in in-vitro cancer cell line systems and studies its active compounds. Crystal violet viability assay was used to determine the cytotoxicity of the leave methanolic extract of *Calendula officinalis L* against diverse breast cancer cell lines. Human breast cancer MCF7, AMJ13, MDAMB, and CAL51 cells were treated with different concentrations of extract for 72 hours. Morphological study for the exposed cell was done by examination under a phase-contrast inverted microscope. High-performance liquid chromatography (HPLC) analysis was performed to measure the concentrations of each component of phenols and flavonoids in the *Calendula officinalis L* extract.

**Results:** It was found that methanolic extract of *Calendula officinalis L* inhibits the proliferation of all breast cancer cells significantly at the meantime; it does not affect normal embryonic cells. Additionally, it induced the cytopathic morphological changes in cancer cells. Furthermore, HPLC study revealed that *Calendula officinalis L* extract contained an important component of flavonoids. **Conclusions:** *Calendula officinalis L* leaves extract inhibited the proliferation of breast cancer cells especially MDAMB cells with no effect on normal cells. This work showed that *Calendula officinalis L* is a possible natural source as broad-spectrum anti-breast cancer drug.

**Keywords:** Cytotoxicity; HPLC analysis; Flavonoids; Iraq; Clonogenic assay

**Introduction**

Cancer disease is a highly complex condition that hard to treat (1). Its incidence is increased globally and especially in Iraq due to several factors mainly related to environmental pollution for several years of war conflicts (2). Breast malignant tumours are ranking second causing of mortality in Iraqi females. Conventional cancer treatments such as chemotherapy, radiation, targeted therapy; immunotherapy, etc, have their unwanted side effects. Therefore, herbal medicine has shown as a useful alternative for the present therapies (3, 4). Herbal medicine widely used for the treatment for several types of diseases such as viral infections and cancer as it has fewer side effects that may be caused by conventional cancer therapeutics (5-7). Marigold (*Calendula officinalis L.*) belong to Asteraceae family and was considered among the most important medicinal and garden plants (8). Several species of this plant are widely distributed in different Mediterranean countries. Marigold is an aromatic annual, seldom biennial. It grows between 30 and 50 cm height and has about 20 cm long tap root and numerous thin secondary roots (9). The stem is erect, angular, down, and branched from the base up or higher. The alternate leaves are almost spatulate at the base, oblong to lanceolate above and are all tomentosae. Several phytochemical studies have been established to investigate the presence of numerous classes of chemical compounds. The main
compounds are terpenoids, flavonoids, coumarines, quinones, volatile oil, carotenoids, and amino acids (10, 11). Pharmacological studies have confirmed that C. officinalis shows a wide range of biological effects such as anti-inflammatory, antioxidant, hepatoprotective, and immunostimulant activities (12, 13). Cytotoxic effect of C. officinalis on tumour cell lines in vitro and its anticancer efficacy in an in vivo briefly outlined 20 years ago (14). According to the most active compounds that have high bioactivity this research was conducted to evaluate the leaves extract as an anti-tumor agent in in vitro cancer cell line systems.

Materials and Method

Collection of plant samples:

Flowers of the marigold plant were collected from medical plants garden at the college of pharmacy. The collected flowers were authenticated and did the formal identification of the plant material by the National Herbarium Centre in Abu – Graib countryside/Iraq and a specimen of this material has been deposited in its data base. Furthermore, the flowers were washed under running tap water to remove the surface pollutants, dried for two weeks at room temperature in the shade, then after, grinded to fine powder, weighed, and stored for future studies at room temperature.

Plant extraction:

100 grams of flower powder was extracted by using soxhlet apparatus in the presence of ethanol 90 (500 ml) till exhaustion. The extract was concentrated by using rotary evaporated, then mixed with 50 mL of distilled water and extracted with 30 mLX 3 of ethyl acetate. The upper layer which was ethyl acetate layer was separated by a reparatory funnel, then dried by using anhydrous sodium sulphate, and labelled as Ethyl acetate extract (15).

Method of Analysis

Phenols & Flavonoids in Calendula Extract:

Analysis of phenols & flavonoids in Calendula officinalis was performed by HPLC for the detection of flavonoid. A 3 micrometre particle size Column (50*4.6 mm 1.D ) Shimpack C-18 with a mobile phase of 0.1% phosphoric acid : acetonitrile (52:24, V/V), and a detection UV set at 285nm. The flow rate is 1.5 ml / mainland at a temperature of 25°C. The concentration for each compound were quantitatively determined by comparison the peak area of standard with that of the sample (16).

Maintenance of cell cultures

The human breast cancer cell lines AMJ13 (17), MCF7, MDAMB, CAL51 and the mouse embryo fibroblast (MEF). The AMJ13 cell line was cultured in an RPMI-1640 medium (USbiological, USA) with 10% fetal bovine serum (FBS) (Capricorn- Scientific, Germany), 100 units/mL penicillin, and 100 μg/mL streptomycin. A human breast cancer cell lines MCF7, MDAB, CAL51, were cultured in MEM medium (USbiological, USA) supplemented by 10% fetal bovine serum (FBS) (Capricorn- Scientific, Germany), and 100 μg/mL streptomycin, 100 units/mL penicillin. The cells were incubated at 37 °C in a humidified environment and 5% CO2 (17) for 72 hours.

Cytotoxicity Assays

Crystal violet cell viability assay was employed to measure the cytotoxic effect of plant extract. Human Breast cancer cell lines (MDAMB, AMJ13, MCF7, and CAL51), as well as normal mouse embryonic cells (MEF), were seeded at 7000 cells/well in 96-well plates (Santa Cruz Biotechnology, USA), after 24hr or until confluent monolayer is achieve. Cells were treated with (extract) at 2 fold dilutions from 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15 μg of culture media. The assay was done in triplicate and the cell viability was determined after 72h of exposure by staining with 50 μl of Crystal violet (Sigma Aldrich, USA) and incubated at 37°C for 2h. The stain was aspirated, and PBS used to wash the wells. The microplate reader (Biochrom, UK) was used to measure the absorbency at 492 nm; Results were shown percentage proliferation with respect to control cells (18, 19).

Morphology analysis

The treated and untreated cells were photographed at a magnification of 200x at four haphazardly selected cultured fields using an inverted light microscope (Leica-microsystems, Germany) and a digital colour camera (Leica-microsystems, Germany) (20).

Statistical Analysis

The data of the current study are presented as means ± standard error of the mean. One-way analysis of variance was used for data comparison between treatment groups. Data were considered statistically
significant at P < 0.05. A GraphPad Prism 6 software was used for the analysis (GraphPad Software, Inc. San Diego, California).

Results

Chemical structure analysis *Calendula officinalis* L:

Table 1: HPLC results for *Calendula officinalis* L flower extract

<table>
<thead>
<tr>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>Retention time (min)</td>
</tr>
<tr>
<td>vitexin</td>
<td>2.005</td>
</tr>
<tr>
<td>Rutin</td>
<td>2.910</td>
</tr>
<tr>
<td>Qercetin-3-galactoside</td>
<td>4.510</td>
</tr>
<tr>
<td>Luleolin-7-glucoside</td>
<td>5.515</td>
</tr>
<tr>
<td>Quercetin-3-glucoside</td>
<td>6.683</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>8.018</td>
</tr>
<tr>
<td>Myricetin</td>
<td>9.587</td>
</tr>
<tr>
<td>Luteolin</td>
<td>10.793</td>
</tr>
<tr>
<td>Apigenin</td>
<td>11.745</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>12.695</td>
</tr>
</tbody>
</table>

Cytotoxicity assay

The current study investigated the selective cytotoxic effect of the Calendula officinalis L. extract in breast cancer cells. In this study, four human breast cancer cell lines were used, CAL51, MCF7, AMJ13, and MDAMB, and the normal mice embryonic cells, MEF. All cell lines were exposed to Calendula officinalis L. extract at 2-fold concentrations started from 0.0 µg/mL to 4mg for 72 h, and cytotoxicity was determined using crystal violet assays. As shown in Figure-2a, the Calendula officinalis L. extract had no cytotoxic effect on the normal cells as the IC50 was very high dose (4440mg/ml) compared to the IC50 on cancer cells which were 2088µg, 1737µg, 3081µg and 4.732µg for the AMJ13, MCF7, CAL51, and MDAMB, respectively. These results indicate that Calendula officinalis L. extract is very effective against MDAMB cells as revealed by Figure-3.
Cytopathological observation showed that Calendula officinalis L extract-treated cells had lower cell count due to detachment in compare to the control (not treated cells). Furthermore, there were condensed nuclei which refer to early apoptosis in the treated cell compared to untreated cells and this photo is shown in the highest concentration used of exposure. Untreated cancer cells continue to proliferate to form monolayers. Early apoptotic cells that have condensed nuclei and stained darker along with normal lightly stained cells (Figure-4).

Figure-2, The Calendula officinalis L extract cytotoxicity assay. A) showed no cytotoxicity against normal mouse embryonic cells as the IC50 was very high dose 4440mg, while against breast cancer cell lines were very low IC50 values. B) AMJ13 the IC50 value is 2088 µg/ml. C) MCF7 IC50 is 1737 µg/ml. D) IC50 value in CAL51 cells was 3081 µg. E) IC50 in MDAMB cells was 4.732 mg/ml. F) the comparative study for IC50 values showed that cancer cells are very sensitive to the Calendula officinalis L extract in comparison to the normal embryonic cells.
Figure-3, Comparison between cancer cell lines according to their sensitivity to the Calendula officinalis L. extract. The figure indicates that Calendula officinalis L. extract is very effective against MDAMB cells, and this cell line is very sensitive to the extract more than other cells types.

Figure-4, Cytomorphology of treated and control cells. A) MCF-7, A1 control, A2 treated cells showing the extract induces cell death. B) CAL51, B1 control, B2 treated, showing the extract induce cell shrinkage. C) MDA MB-468, D1 control, D2 treated, the extract reduced cancer cells number due to detachment. E) MEF, E1 control, E2 treated, E3 image analysis showing a high dose of extract was cytotoxic on normal cells. 400xg (Crystal violet stain)

Discussion

HPLC analysis for Calendula officinalis L. extract revealed the presence of active compounds mainly flavonoids. Active compounds have been recognized and isolated to be used in cancer therapy (21). Flavonoid compounds were founds to be the major constituent of the extract as revealed by HPLC analysis. There is over 4000 type of flavonoids; several of them are accountable for the beautiful colors of fruits, flowers, and leaves (22). The scavenging of oxygen-derived free radicals is a significant effect of flavonoids that also showed anti-carcinogenic properties (23). The antioxidative effect is the top-defined feature of nearly every group of flavonoids. The flavones and chaechins are flavonoids that protect the body from reactive oxygen species (ROS). Cells organelles and components can be damaged by ROS, and free radicals, that are induced by exogenous damage or produced during the metabolism of oxygen (24, 25). Throughout injury, production elevation of reactive oxygen species results in consumption and exhaustion of the endogenous scavenging compounds. Flavonoids may have an additive effect on the endogenous scavenging compounds (26). Our experiments outcomes of cytotoxicity assay revealed that Calendula officinalis L. extract have antiproliferative and cytotoxicity against breast carcinoma cell lines, especially the MDAMB breast cancer cells. Other researchers found that Calendula officinalis extracts also had cytotoxicity on human melanoma and epidermoid carcinoma cells (27). The cytotoxic effect of Calendula officinalis L. extract was explained by the presence of the major flavonoids, which are flavone and luteolin-7-O-β-glucoside (28). Other research found that that luteolin-7-O-β-glucoside is promising anti-cancer molecule, that possesses anti-breast adenocarcinoma (29). C officinalis described as important agent for developing novel cancer therapeutics, moreover, it used to reduce the side effects of radiotherapy (8).

Conclusions

In Conclusion, we reported for the first time that Iraqi Calendula officinalis L. extract is selective broad spectrum anti-breast cancer agent different type breast
cancer cells such as estrogen progesterone positive or triple negative breast cancer and has no toxic effect on normal cells which make it very promising candidate as cancer therapy for clinical application.

**Abbreviations**

(HPLC) High-performance liquid chromatography,  
(IC50) 50% Inhibition of cell lines growth,

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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The Role of c-ERBB2(Her-2) and P53 in Differentiating Low Grade from High Grade Urothelial Carcinoma of Urinary Bladder

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College of Medicine, University of Babylon /Iraq

Abstract

Background: Urothelial carcinoma of the urinary bladder, represents 90% of all primary tumors of this organ, and one of the most common ten malignancy in Iraq and world wide. These tumors can range from low grade papillary neoplasms to less frequent more aggressive and invasive solid tumors. More than 70% of superficial tumors recur, and about one-third of the patients have tumor progression, which can affect the quality of their life. HER-2/neu receptor as an important growth factor. This receptor possesses intrinsic tyrosine kinase activity and all members of this family are frequently overexpressed or their respective genes are amplified in human neoplastic tissue. Its overexpression has been associated with high grade and advanced stage of bladder carcinoma specially TCC. Altered expression of the p53 gene has also been associated with transitional cell carcinoma. Materials and Methods: 62 patients with TCC were included in this study and conducted in the Department of Pathology, Faculty of Medicine, Kufa University during the period from January 2012 through April 2013. EnVision method (polymer based immunohistochemistry) was used for the immunohistochemical detection of p53 and Her_2/neu. Results: HER-2/neu overexpression was found in 62.9% of bladder carcinoma, and no expression in benign bladder lesions (cystitis) was detected, (P value < 0.05). HER-2/neu overexpression and its intensity were well correlated to grade of tumor indicating that HER-2/neu positive bladder carcinoma are biologically aggressive and detected more frequent in grade III than grade I or grade II (P value < 0.05, R = 0.0181). P53 was expressed in (66.12%) of bladder carcinoma, and no expression in benign bladder lesions (cystitis) was detected, (P value < 0.05). P53 overexpression and its intensity were well correlated to grade of tumor indicating that P53 positive bladder carcinoma are biologically aggressive and detected more frequent in grade III than grade I or grade II (P value < 0.05, R = 0.0821). Conclusions: From the above results we concluded that p53 and Her_2/neu over-expression play an important role in pathogenesis of urothelial carcinoma evolution, as their positivity associated with higher tumor grade.

Introduction

Urothelial carcinoma, formerly known as transitional cell carcinoma is a common malignancy, representing approximately 90% of all primary tumors of this organ. In Iraq it is one of the ten most common cancers according to Iraqi cancer registry of 2005. Urothelial carcinomas range from papillary to flat, noninvasive to invasive and low grade to high grade. Low-grade carcinomas are always papillary and are rarely invasive, but they may recur after removal. Tumor progression with increasing degrees of cellular atypia and anaplasia are associated with an increase in the size of the lesion with increasing tendency of invasion into deeper structures of the bladder wall. Occasionally, these cancers show foci of squamous cell differentiation; only 5% of bladder cancers are true squamous cell carcinomas (SCC). Adenocarcinomas account for less than 2% of primary bladder tumors. The urinary bladder may also be affected rarely by neuroendocrine tumors (including small cell carcinoma), carcinosarcoma, malignant lymphoma, rhabdomyosarcomas (mainly in children), and leiomyosarcoma. The latter is the most common sarcoma of the bladder. Aims of the study: to assessment of P53 and Her/2neu expression in different grades of urothelial carcinoma, correlation between the expression of both markers and the use of P53 and Her/2 as prognostic markers.
Materials and Method

This study was carried out from January 2012 through April 2013. Sixty two patients with TCC of the urinary bladder were included in this study, fifteen specimens of benign bladder lesions taken from patients with cystitis were considered as control group. The specimens were formalin-fixed, paraffin embedded tissue blocks, from these blocks, 5 micrometer-thick tissue sections were obtained and stained with hematoxylin eosin staining method and immunohistochemical Envision staining method. Scoring according to Sophia KA et al at objective 40 as in P53 Scoring system and HER2/neu scoring system. Statistical analysis was done with SPSS software version 18 (SPSS Inc, Chicago IL). Significant differences between non-parametric variables were done by Chi squared test and between parametric variables by independent t-test. Pearson Correlation was calculated to evaluate the relations between parameters. P values ≤ 0.05 were considered statistically significant and P values ≤ 0.01 were considered statistically higher significant.

Results

Sixty two cases of bladder carcinoma were included in this study 50(80.65%) patients were males and 12 (19.35%) were females (Table 1), showing highly significant difference between male and female.

Table 1 : Immunohistochemically expression of Her_2/neu and P53 in relation to patient gender:

<table>
<thead>
<tr>
<th>Positive</th>
<th>Her2_neu expression</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4(33.34%)</td>
<td>8(66.66%)</td>
</tr>
<tr>
<td>Male</td>
<td>35(70%)</td>
<td>15(30%)</td>
</tr>
<tr>
<td>Total</td>
<td>39(62.90%)</td>
<td>23(37.10%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>P53 expression</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>negative</td>
</tr>
<tr>
<td>Female</td>
<td>10(83.33%)</td>
<td>2(16.67%)</td>
</tr>
<tr>
<td>male</td>
<td>31(62%)</td>
<td>19(38%)</td>
</tr>
<tr>
<td>total</td>
<td>41(66.12%)</td>
<td>21(33.88%)</td>
</tr>
</tbody>
</table>

P>0.05 not significant

Assessment of age presentation of patients, there is a significant difference between age group (Table 2). The intensity of immunostaining of HER-2/neu protein was assessed in relation to the grade of tumor, and there is a significant difference between the intensity of HER-2/neu and the grade of tumor (R= 0.0181) (Table 2). The intensity of p53 was assessed in relation to the grade of tumor, and there is a significant difference between the intensity of HER_2/neu and the grade of the tumor (p <0.05) (R=0.691).
Table 2: Correlation between intensity of HER-2\textsuperscript{neu} and P53 with grade of bladder carcinoma.

<table>
<thead>
<tr>
<th>Grade</th>
<th>HER-2\textsuperscript{neu} intensity (Score)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>Grade I</td>
<td>6</td>
<td>33.33%</td>
</tr>
<tr>
<td>Grade II</td>
<td>5</td>
<td>25%</td>
</tr>
<tr>
<td>Grade III</td>
<td>1</td>
<td>4.16%</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>19.35%</td>
</tr>
</tbody>
</table>

P<0.05 , R=0.691

Immunohistochemical analysis of HER-2\textsuperscript{neu} protein overexpression in relation to the age group of bladder carcinoma patients, there was no relationship between the age group and HER-2\textsuperscript{neu} overexpression (P > 0.05) (Table 3). Immunohistochemically analysis of p53 protein overexpression in relation to the age group of bladder carcinoma patients, there was no relationship between the age group and p53 overexpression (P > 0.05) (R= 0.025).

Table 3: Immunohistochemical expression of HER-2\textsuperscript{neu} and P53 in relation to the age group in bladder carcinoma.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Immunostaining of HER-2\textsuperscript{neu}</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>40-49</td>
<td>4</td>
<td>44.45%</td>
</tr>
<tr>
<td>50-59</td>
<td>12</td>
<td>85.72%</td>
</tr>
<tr>
<td>60-69</td>
<td>11</td>
<td>61.12%</td>
</tr>
<tr>
<td>70-79</td>
<td>12</td>
<td>63.16%</td>
</tr>
<tr>
<td>80-89</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>60.90%</td>
</tr>
</tbody>
</table>

P > 0.05 not significant

There was well correlation in expression of both markers in relation to grade of tumor (R = 0.8956) but no significant difference among these grades (P = 0.464) (Table 4).

Table 4: Correlation between Her2\textsuperscript{neu} and P53 immunostaining regarding grades of the presented bladder tumor.

<table>
<thead>
<tr>
<th>Grade of tumor</th>
<th>Immunostaining of HER2</th>
<th>Immunostaining of P53</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Grade I</td>
<td>7 (38.88%)</td>
<td>11 (61.22%)</td>
</tr>
<tr>
<td>Grade II</td>
<td>10 (50%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Grade III</td>
<td>22 (91.66%)</td>
<td>2 (8.33%)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (62.91%)</td>
<td>23 (37.09%)</td>
</tr>
</tbody>
</table>
HER-2/neu in the presented benign and malignant bladder tissue. Regarding the assessment of the control group (cystitis), all cases were negative for HER-2/neu immunohistochemical staining this finding agrees with that reported by Sanna E., et al., (2004)\(^8\). Truls G., et al., (2005)\(^7\), showed high percentage of (80%) HER-2/neu overexpression in bladder carcinoma. Wester K., et al., (2002)\(^9\), found that HER-2/neu was overexpressed in 81% of the bladder carcinoma. While Rafael E., et al., (2001)\(^10\), found that the HER-2/neu overexpression were 28% of bladder carcinoma. Mohammed Reza N., et al., (2004)\(^11\). Regarding the intensity of HER-2/neu in correlation with TCC type of urinary bladder carcinoma the finding agreed with Vildan C., et al., (2008)\(^12\), the majority of HER-2/neu–positive patients (93%) were detected by IHC, and a minority (26%) were positive by FISH or serology (23%). This is very important in the new research, in studying the role of trastuzumab. This often requires eligible patients to have either +3 overexpression by IHC or +2 expression by IHC with positive FISH results\(^13\). The immunohistochemical analysis of the results revealed that HER-2/neu overexpression agreed with Regina E., et al., (2002)\(^15\) showed that the HER-2/neu expression was significantly higher in high grade muscle invasive bladder carcinoma. Coogan CL., et al., (2004)\(^16\), had found that overexpression of HER-2/neu was significantly increased in grade III and invasive tumors than grade I, II or superficial tumors, Gokhan Atis, et al., (2007)\(^17\) had found that overexpression of HER-2/neu increased as the grade increased, Mohammed Reza N., et al., (2004)\(^11\), found that the overexpression of HER-2/neu has a direct significant relationship with grade of TCC of urinary bladder. Mohammad R. Jalali, et al., (2007)\(^18\), found that HER2/neu was positive in 7.1% of grade I, 28.6% of grade II and 65.4% of grade III, \(P=0.002\). Our findings state that there was no relationship between age group and HER-2/neu overexpression in bladder carcinoma patients (\(P > 0.05\)). This is in agreement with Gokhan Ates, et al., (2007)\(^17\) and Mohammad R. Jalali, et al., (2007)\(^19\). Our results state that in TCC, there is male increasing significantly than female in relation to HER-2/neu protein overexpression (\(P < 0.05\)).

The immunohistochemical analysis of the results revealed that P53 overexpression was increasing as the grade increased, with significant difference among these grades (\(P = 0.014\)). However, it is significantly correlated to the grade of tumor (\(R = 0.821\)). Venyo A., et al., (2012)\(^24\) found that 28.88% of grade I, 66.66% of grade II and 80.7% of grade III were p53 positive, Salah A. Ali et al., (2012)\(^20\) show that the p53 expression was increasing with increasing grade of tumor. There was statistically a highly significant correlation between p53 expression and grading of cases of TCC (\(P < 0.001\)), Hong-Lin Cheng, et al., (2002)\(^25\) showing that p53 immunostaining was positive in 26% of grade I, 57% of grade II, and 42% of grade III. Findings of the current work also agreed by Nurcan Kilicli-Camur, et al., (2002)\(^25\), Ming-Lan Lu et al., (2002)\(^22\), and Sunanda J. et al., (2004)\(^23\). While Underwood MA et al., (1996)\(^26\) found no predictive prognostic value for p53 immunohistochemistry over grade value and this may be due to the usage of polyclonal antibody to p53 in their study. In the presented study there was no relation between p53 expression and age \(p>0.05\) in agreement with Salah A. Ali et al., (2012)\(^20\). In our study, positive immunostaining of Her2 was reported in 38.88% in grade I transitional cell carcinoma, while P53 was positive in 44.44% of the same grade. In grade II Her2 was positive in 50% of cases compared with 60% positive P53 in the same grade. Finally Grade III showed 91.66% positive Her2 while P53 showed 87.5%. Most of positive Herr bladder cancers were positive for P53. There was strong positive correlation between Her2/neu and P53 regarding all the three grades. Yuh-Shyan T. et al., (2005)\(^18\) show similar correlations between HER2/neu with P53 overexpression in transitional cell carcinoma although Gunia S., et al., (2011)\(^27\) found a positive correlation between the two markers while evaluating their role in separating CIS from benign flat lesions of urinary bladder.

### Discussion

From the above results we concluded that p53 and Her_2/neu over-expression play an important role in pathogenesis of urothelial carcinoma evolution, as their positivity associated with higher tumor grade.

### Ethical Clearance:
The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

### Conflict of Interest:
The authors declare that they have no conflict of interest.
Funding: Self-funding

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Serum Secretagogin and Focal Adhesion Kinase as Markers for Type 2 Diabetes Mellitus and Beta Cells Function

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Abstract

Diabetes mellitus (DM) is one of the world’s major public health problems. The increasing incidence of DM worldwide makes it a leading cause of morbidity and mortality for the anticipated future. Secretagogin (SCGN) is a protein enriched and secreted from pancreatic islets, it demonstrates protective effects on β-cell function. Focal adhesion kinase (FAK) plays a critical role in β-cell survival and is a vital regulator of insulin secretion. In this research, serum SCGN, FAK, fasting blood glucose (FBG), HbA1c, C-peptide, lipid profile, blood urea nitrogen (BUN) and creatinine were measured in patients diagnosed with type 2 diabetes mellitus (T2DM) and in healthy volunteers. The results showed that there was a significant increase in the levels of serum SCGN in patients with T2DM compared to the control group. There was a significant decrease in levels of FAK and C-peptide in T2DM patients compared to the control group. In addition, a significant increase was seen in FBG and HbA1c levels in T2DM patients compared to the control group. The lipid profile of T2DM patients was altered compared to the control group. There was no significant difference in the levels of BUN and creatinine among the study subjects. The results of this study suggest that both SCGN and FAK may serve as potential biomarkers reflecting the state of islet cells dysfunction in T2DM patients which may present additional markers for the diagnosis of T2DM.

Keywords: Serum secretagogin, focal adhesion kinase, C-peptide, glucose, HbA1c, type 2 diabetes mellitus.

Introduction

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders commonly associated with hyperglycemia resulting from insulin secretion disorders, insulin action, or both[1]. DM is a common public health issue worldwide and continues to increase its prevalence[2, 3]. DM is of the top five major death causes in developed countries. The International Diabetes Federation (IDF) estimated that in 2015, the global population of people with diabetes was 415 million and is expected to increase to 642 million by 2040[4].

Secretagogin (SCGN) is a Ca2+ sensor protein that has six EF-hand helix-loop-helix motifs for binding calcium. Originally, SCGN was cloned with pancreatic β cells[5]. It has been revealed recently that SCGN also plays key roles in insulin secretion in pancreatic β-cells by interacting with vesicle fusion, protein trafficking[6] and actin cytoskeleton[7]. The human protein atlas shows by systematic comparison of expression in various human tissues that SCGN has its largest amount of protein expression in Langerhans islets relative to other tissues[8].

Focal adhesion kinase (FAK) is a largely conserved 125 kDa non-receptor tyrosine kinase that plays a critical role in motility, survival and proliferation of adhesion-dependent cells in responding to signals of receptors of integrin and growth factor[9]. FAK protein is overexpressed in cancers such as ovarian, cervical, kidney, pulmonary, pancreatic, brain, colon, breast, and skin cancer[10]. In addition, FAK plays a critical role in regulating F-actin remodeling and insulin secretion[11]. In pancreatic β-cells, integrin β1-mediated intracellular signaling activates and phosphorylates FAK and paxillin upon glucose stimulation. The present study aims to investigate the role of SCGN and FAK in the pathogenesis of T2DM and the possibility of using these proteins as markers that may reflect β-cells dysfunction and thus aid as additional and more accurate markers in the diagnosis of T2DM.
Materials & Method

Study subjects:

The study subjects were divided into two groups. The first group included 40 patients diagnosed with T2DM (21 males and 19 females) with an age range of (70 – 36 and 65 – 44 respectively) and the second group included 40 healthy individuals (23 males and 17 females) with an age range of (70 – 25 and 70 – 26 respectively) serving as the control group. The patients enrolled in the present study were attending the Al-Imameen Al-Kademen Medical City and Al-Kindi Hospital. This study was approved by the Department of Chemistry, College of medicine, Al-Nahrain University, Baghdad, Iraq.

Exclusion criteria:

Patients were excluded from the study if they had one or more of the followings; treatment with statins or any hyperlipidemia drugs, type 1 diabetes, liver or pancreatic inflammation and any type of cancers or tumors.

Samples collection:

Five milliliters of venous blood were taken from each patient and healthy control. (3 mL) of the blood was transferred into a gel tube to separate the serum. The remaining (2 mL) of the blood was transferred into an EDTA tube to prevent the blood from clotting. The obtained samples were stored at (-20 ºC) until assayed.

Biochemical Analyses:

Human serum secretagogin and FAK were measured by enzyme linked immunosorbent assay (ELISA) using Human secretagogin and Human FAK ELISA kits purchased from (Mybiosource/ USA) following the manufacturer’s instructions. C-peptide was measured by cobas e411 using C-peptide kit purchased from (Roche Diagnostics/ Switzerland) following the manufacturer’s directions. HbA1c, FBG, lipid profile, creatinine and blood urea nitrogen were measured by cobas c111 using HbA1c, FBG, lipid profile, creatinine and blood urea nitrogen kits purchased from (Roche Diagnostics/ Switzerland) following the manufacturer’s directions.

Statistical Analyses:

Biochemical data were analyzed using statistical package for social sciences (SPSS) version 25. T-Test was used to calculate mean ± standard deviation (SD) and the p value.

Results & Discussion

Tables (1) show that there was a significant increase in the levels of SCGN in the sera of patients diagnosed with T2DM )104.52 ± 1.8 pg/mL) compared to the control group (59.1 ± 2.73 pg/mL) (p > 0.000). A significant decrease of FAK serum levels was also observed T2DM patients )402.82 ± 76.1 pg/mL) compared to the control group (1312.35 ± 163.35 pg/mL) (p > 0.000), there was a significant decrease in the levels of C-peptide in the sera of patients diagnosed with T2DM )0.7 ± 0.12 ng/ml) compared to the control group (3.54 ± 0.95 ng/ml) (p > 0.000). There was a significant increase in the levels of FBG in the sera of patients diagnosed with T2DM )203.15 ± 82.88 mg/dl) compared to the control group (97.70 ± 4.82 mg/dl) (p > 0.000). There was also a significant increase in the levels of HbA1c in the sera of patients diagnosed with T2DM )8.82 ± 2.11 %) compared to the control group (5.37 ± 0.30%) (p > 0.000). There was a significant increase in the level of Triglyceride in the sera of patients diagnosed with T2DM )189.26 ± 80.39 mg/dl) compared to the control group (101.07 ± 31.58 mg/dl) (p > 0.000). The result showed a significant increase in the level of cholesterol in the sera of patients diagnosed with T2DM (185.74 ± 53.56 mg/dl) compared to the control group (156.42 ± 24.44 mg/dl) (p > 0.003). There was a significant decrease in the levels of HDL in the sera of patients diagnosed with T2DM (40.64 ± 14.26 mg/dl) compared to the control group (49.21 ± 13.53 mg/dl) (p > 0.007). There was a significant increase in the levels of LDL in the sera of patients diagnosed with T2DM (106.21 ± 45.56mg/dl) compared to the control group (86.84 ± 22.37 mg/dl) (p > 0.018). There was also a significant increase in the levels of VLDL in the sera of patients diagnosed with T2DM (37.79 ± 16.09 mg/dl) compared to the control group (20.20 ± 6.32 mg/dl) (p > 0.000). In this study, there was no significant difference in the levels of creatinine and BUN among the study subjects.
SCGN appears to have an extra role in cytoskeleton reorganization during insulin release, it affects the dynamics of F-actin to promote the transportation of the vesicle to the periphery and the remodeling of the focal adhesions\(^7\). In the present study, there was a significant increase in the levels of the SCGN in the sera of patients diagnosed with T2DM compared to the control group, and the results were in accordance with the results of a recent study that found increased levels of SCGN in T2DM patients\(^12\). The study showed that SCGN release was not fluctuating in relation to insulin release and that it could enable SCGN to reflect a certain disease condition that cannot be captured by insulin, C-peptide or proinsulin levels. These results support that SCGN release from the islets was sufficient to create quantifiable levels in the plasma as part of islet failure (i.e. increased islet stress)\(^12\). Another study supported that intracellular SCGN protected β-cells from apoptosis, in which apoptosis was inhibited by overexpression of SCGN\(^13\). In addition, a recent study suggested that intracellular SCGN promoted the survival of pancreatic β-cells and reduced Endoplasmic reticulum (ER) stress by stabilizing deubiquitinating proteins\(^14\).

FAK is an exercise-sensitive protein that plays a part in skeletal muscle morphology, metabolism and insulin sensitivity\(^15, 16\). FAK regulates insulin-mediated cytoskeletal rearrangement in skeletal muscle cells, which is essential to normal glucose transportation and glycogen synthesis\(^17\). FAK’s role in regulating glycogen synthesis was also revealed in HepG2 cells and hepatic insulin signaling in vitro\(^17, 18\). In the current study, there was a significant decrease in the levels of the FAK in the sera of patients diagnosed with T2DM compared to the control group. This study represents the first attempt to measure the levels of FAK in the blood of T2DM patients however, there are a number of previous studies describing the role of FAK in tissues. A previous study showed that activated FAK-paxillin complexes were integrated into nascent focal adhesions when main rat β-cells were stimulated by glucose. Focal adhesion remodeling in response to glucose is Ca\(^{2+}\) -dependent, fast, reversible and connected to short-term glucose-induced signaling pathway activation of ERK1/2. Finally, these glucose-mediated occurrences are crucial for controlled β-cell insulin secretion\(^19\). Another study showed that FAK is needed to maintain both pancreatic β-cell mass and in vivo function so that glucose homeostasis is disturbed in its absence. The study also showed that deletion of FAK in β-cells has been shown to result in impaired cell proliferation, survival and function. In the absence of FAK, deficiencies in actin dynamics related to impaired focal protein dynamics and insufficient trafficking of insulin granules resulted

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>T2DM patients Mean ± SD</th>
<th>Controls Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCGN (pg/mL)</td>
<td>104.52 ± 1.8</td>
<td>59.1 ± 2.73</td>
<td>0.000*</td>
</tr>
<tr>
<td>FAK (pg/mL)</td>
<td>402.82 ± 76.1</td>
<td>1312.35 ± 163.35</td>
<td>0.000*</td>
</tr>
<tr>
<td>C-peptide (ng/ml)</td>
<td>0.7 ± 0.12</td>
<td>3.54 ± 0.95</td>
<td>0.000*</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>203.15 ± 82.88</td>
<td>97.70 ± 4.82</td>
<td>0.000*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.82 ± 2.11</td>
<td>5.37 ± 0.30</td>
<td>0.000*</td>
</tr>
<tr>
<td>S. Cholesterol (mg/dl)</td>
<td>185.74 ± 53.56</td>
<td>156.42 ± 24.44</td>
<td>0.003*</td>
</tr>
<tr>
<td>S. Triglyceride (mg/dl)</td>
<td>189.26 ± 80.39</td>
<td>101.07 ± 31.58</td>
<td>0.000*</td>
</tr>
<tr>
<td>S. HDL (mg/dl)</td>
<td>40.64 ± 14.26</td>
<td>49.21 ± 13.53</td>
<td>0.007*</td>
</tr>
<tr>
<td>S. LDL (mg/dl)</td>
<td>106.21 ± 45.56</td>
<td>86.84 ± 22.37</td>
<td>0.018*</td>
</tr>
<tr>
<td>S. VLDL (mg/dl)</td>
<td>37.79 ± 16.09</td>
<td>20.20 ± 6.32</td>
<td>0.000*</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td>28.04 ± 8.17</td>
<td>29.93 ± 8.03</td>
<td>0.301</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.78 ± 0.18</td>
<td>0.77 ± 0.17</td>
<td>0.889</td>
</tr>
</tbody>
</table>

*Significant at the levels of (p ≤ 0.05).
in decreased insulin exocytosis. The study concluded that FAK has a critical dual role in controlling both the viability of β-cells and cell functions and can be a prospective therapeutic target for T2DM(20).

C-peptide is a helpful and commonly used technique for evaluating the function of pancreatic β-cells(21). The concentration of C-peptides has been shown to decrease over decades with diabetes length(22). Lower concentrations of C-peptide and lower function of β-cells were associated with higher rates of glucose variability(23). In the current study, FBG and HbA1c levels were higher in the T2DM patients compared to the controls. Our findings were supported by a number of previous studies that showed increased levels of FBG and HbA1c in T2DM patients compared to controls(24).

In the present study, levels of serum triglycerides were higher in the patients diagnosed with T2DM compared to the control group. Previously reported studies had suggested that the levels of TG in serum were positively associated with DM. Moreover, it has also been shown that increased serum TG levels over time enhanced the risk of developing DM in various populations(25). In the current study, serum TC, VLDL and LDL in the patients diagnosed with T2DM were higher than the control.

**Conclusion**

The results of the present study suggest that both SCGN and FAK may serve as potential biomarkers reflecting islet cell dysfunction in T2DM patients and that they may aid as additional markers for the diagnosis of T2DM. SCGN and FAK may differentiate β-cells dysfunction T2DM from insulin resistant T2DM. The patients of the present study suffered from β-cells dysfunction. Alterations in the lipid profile of the patients was associated with T2DM. The kidney function reflected by BUN and creatinine levels was not affected by T2DM in patients of the present study.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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**References**


Thyroid Nodules of Iraqi Patients with Acromegaly

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Abstract

Acromegaly is a chronic endocrine disorder caused by excessive secretion of growth hormone. The incidence of malignancy like colorectal carcinoma is well documented to increase in patients with acromegaly. While the real incidence of thyroid neoplasm in these patients are not well known.

**Aims:** Find out the rate of structural thyroid disorders in a sample of Iraqi patients with acromegaly; Identify the rate of thyroid malignant transformation in patients with acromegaly.

**Settings and Design:** A case control study

**Methods and Material:** seventy patients with acromegaly were enrolled compared with 70 controls from 2 endocrinology centers from Jan. - Dec. 2017. Their mean age 46 +/- 11 years; 41 (58.5%) males. Thyroid ultrasound was conducted for all participants while fine needle aspirate conducted for those with nodule diameter ≥1 cm.

**Results:** Goiter was founded more in patients with acromegaly versus control group (81.4% vs 18.5% respectively; P<0.0001). The overall nodular thyroid disease were found in (61.4%) acromegalic patients of those: (11.4%) had single thyroid nodule and (50%) had multiple thyroid nodules. The ultrasonic feature of thyroid nodule were suspicious in (13.9%) of acromegaly group and none in control; P<0.0001. The fine needle aspirate results were suspicious in (9.3%) acromegaly group versus none in control group, P<0.05). One patient (2.3%) with acromegaly had papillary thyroid carcinoma and none in control; P=0.31).

**Conclusions:** Nodular thyroid disease were more prevalent in Iraqi patients with acromegaly but the risk of malignancy is not significantly different from normal population.

**Keywords:** thyroid nodule, acromegaly, goiter, multinodular goiter, ultrasonography

Introduction

Acromegaly is a chronic endocrine disorder caused by excessive secretion of growth hormone (GH) [¹] and subsequently Insulin –like Growth Factor 1 (IGF-1) [²]. IGF1 is consider as a potent mitogen for different cells [³]. Some report appear that these patients are at a high risk of developing neoplastic disease [⁴]. The incidence of some types of malignancy like colorectal, breast & prostatic carcinoma are well documented in patients with acromegaly [⁵]. While The real incidence & prevalence of thyroid neoplasm in patients with acromegaly are not well known[⁶]. However this study designed to identify the prevalence of thyroid tumors in Iraqi patients with acromegaly.

Subjects and Method

This is a case control study in which 70 patients with acromegaly were enrolled from January - December 2017 from 2 endocrinology specialized centers compared with 70 (age and sex matched) healthy control.

This study have been accepted by research ethics committee of Iraqi Board of Medical specialization before starting a study. An informed consent have been obtained from all patients, signed and dated before participation in...
this study. The enrolled subjects were already diagnosed as acromegaly clinically, biochemically, and radiologically before their enrollment in the study. The diagnosis of acromegaly was based on unsuppressed GH level after OGTT (>1.2 mUI/L) and increased IGF-1 value for age and sex; fortified by detection of pituitary adenoma by MRI either macroadenoma (>10mm) or microadenoma (<10mm).

The collection of data in regard of age per years, sex, duration of acromegaly, size of tumor (micro- or macroadenoma), history of hypophysectomy, radiotherapy, medical therapy (total dose for each patient) GH level in 1st and last visits (by chemiluminescent immunoassay), IGF1 in 1st and last visits (according to sex and age matched limits measured by immunochemiluminometric assay), disease activity (assessed as described in the 2009 international consensus statement [7]), history of smoking, family history of thyroid disease, family history of malignant disease.

The thyroid ultrasonography conducted to all patients and control subjects by to assess the morphology of thyroid gland (size of thyroid gland and thyroid nodule(s) -if any- its/their characteristics. According to American thyroid association guidelines 2015 [8] suspicious thyroid nodule(s) sent for Fine Needle Aspirate (FNA). Furthermore, according to the cytology report patients with suspicious nodules sent to thyroid surgeon for thyroidectomy; followed by histopathological examination of the removed specimen.

Continuous variables were expressed by mean +/- SD while categorical variables as percentage. All these collected data & variables analyzed by using Chi square test or Student’s unpaired t-test. The value of <0.05 considered as statistically significant.

Results

Seventy cases of acromegaly were enrolled in this study with a mean age 46 +/-11 years. Forty one (58.5%) males and 29 (41.5%) females. Duration of disease ranged from 1 year -30 years (average 8 +/- 6). A growth hormone producing macroadenoma was the cause of acromegaly in 62 patients (88.5%) and in the remaining 8 (11.5%) had microadenoma. Thirty six (51.4%) underwent hypophysectomy. Six patients (8.5%) were exposed to gamma knife & another one to conventional radiotherapy. All of our patients received monthly Octreotide (Sandostatin (LAR)). Twelve (17.2%) had inactive disease while the remainder 58 (82.8%) had active disease.

Hypophysectomy, radiotherapy and/or medical therapy for acromegaly induced a significant reduction of mean GH and IGF-1 levels (27.83 +/- 25.69 vs. 4.73 +/- 6.72 ng/ml, p< 0.001 and 772.15 +/- 521.9 vs. 404.2 +/- 278.6 ng/ml, p < 0.001 respectively).

Thirteen patients (18.5%) were smoker.

Family history revealed thyroid disease or goiter in 17 (24.2%). While 12 (17.1%) had family history of malignancy (of those: 2 of them had family history of thyroid malignancy).

The control group was composed of 70 healthy subjects 47 (67%) female and 23 (33%) with the mean age 42.9 +/-14.3.

Ultrasound characteristics

Goiter discovered in 57 (81.4%); of these 14 (20%) had diffuse and 43 (61.4%) had nodular goiter (both single 8 (11.4%) and multinodular goiter (MNG) 35 (50%)).

Cytological & histopathological diagnosis

Out of 43 patients with nodular goiter; FNA performed in 23 (53.4%) patients while none of nodules in control group required FNA because all of their ultrasound characteristics were either benign looking or purely cystic.

The FNA results were benign follicular cells without any metaplasia or dysplasia in 19 patients with acromegaly (44.2% out of all nodular disease and 82.6% out of all cytological examined sample); P value < 0.05. While 4 of patients with acromegaly (9.3% out of all nodular disease and 17.4% out of all cytological examined sample) had suspicious for dysplasia, metaplasia or malignancy, (P value < 0.05).

Seven patients sent to thyroid surgeon for thyroidectomy (all 4 patients of suspicious FNA cytology & 3 had large multinodular goiter with obstructive symptoms). One patient had malignant histopathology in form of papillary thyroid carcinoma (2.3% out of patients with thyroid nodules and 1.4% out of the entire group of patients with acromegaly) that was statistically non-significant compared with control group (P value = 0.3). While the others had benign histopathological results.
**Correlation study**

In table (1) the patients subdivided according to thyroid ultrasound findings.

There is a significant differences in gender with male predominance in diffuse goiter group and female predominance in MNG group (13(97%) male vs 1(3%)female, (19(54%) female versus 16(46%) male ; respectively \( P <0.05 \) in both). There is significant predominance of MNG among patients with macroadenoma versus microadenoma (34(97%) vs 1(3%); \( P <0.05 \) ). There is also a significant development of MNG in patient who had active disease versus inactive disease (32(91.4%) vs 3(8.6%); \( P <0.05 \) ). While there is a significant difference in development of both diffuse goiter and MNG in non-smoker patient versus smokers(8(57%) vs 6 (43%), and 10 (91.5%) versus 3(8.5%)respectively; \( P <0.05. \))

We could not found statistically significant differences disease duration, mean last GH , mean last IGF1, history of hypophysectomy or family history of thyroid disease between patient with diffuse goiter ,single thyroid nodule or MNG( table 2).

In table (3) the patients who had thyroid nodule (whether single or multiple) are subdivided according to ultrasonic feature and cytological examination .There is a significant female predominance in patients with benign ultrasonic feature group (20(54%) female versus 17(46%) male, \(<0.05\)).

The mean last IGF1 has significant relationship with development of both suspicious ultrasonic feature group and suspicious FNA cytology group(\( P \) value <0.05 respectively).

In patients with suspicious FNA group there is a significant occurrences in patients with past history of hypophysectomy versus those without surgery (\( P \) value=0.05).

While there is no significant relationship between any nodular thyroid characteristics from one side and duration of disease of acromegaly ,size of pituitary adenoma, activity of the disease and mean last GH level on the other side.

**Discussion**

In our study of 70 patients with acromegaly we searched for structural thyroid abnormalities, we found an increase prevalence of goiter(81.4%) both diffuse and nodular types ,thyroid nodules were(61.4%), MNG were(50%). These result of nodular disease are similar to newer studies that shows prevalence 62% in Gullu et al \[9\], 63% in Baldys-Waligórska et al \[10\], 57.6% in Tita et al \[11\] and 74.1% in Anagnostis et al. \[12\].

In our study we found that diffuse goiter was in 20% which was slightly higher than what is founded in Turkish study; Dugan S. et al \[13\] which shows approximate results of 14.1%& in Brasil study \[14\] were 10.4% however the differences can be accepted because of individual and environmental factors. While in comparison with these studies we found that MNG were 50 % in our patients ;which is similar to Turkish study \[13\] that shows 48.6% ,while on the other side our results was lower than other study in Brasil \[14\] that shows (32.5%) this could be explained by similarity of geographical ,environmental factors that had an effect on our patients &Turkish patients

The suspicious FNA cytology was detected in 9% of the FNAs specimens in our study which were lower than the finding in other studies that was 13% \[14\]. However the differences between our study and the other study can be accepted.

We found a significant relationship between MNG and activity of acromegaly which is similar to what is found in other study like Uchoa et al. \[14\] We also found a significant relationship between MNG and the size of pituitary macroadenoma.

We fail to found a relationship between goiter development ,thyroid nodule and the last GH,IGF1 ,and disease duration that are similar to other studies\[13,15\]. The prevalence of thyroid cancer was (2.3%)in our study which is like other studies 0.8% \[16\] and 2.9% \[10\], but these results are much less than other studies which found thyroid cancer in 11.8 % \[17\] and 7.2% \[11\].
Table (1) shows the positive relationship between structural thyroid disease and different variables:

<table>
<thead>
<tr>
<th>variable</th>
<th>all</th>
<th>normal</th>
<th>diffuse goiter</th>
<th>single thyroid nodule</th>
<th>MNG</th>
</tr>
</thead>
<tbody>
<tr>
<td>patients No.</td>
<td>70 (100%)</td>
<td>13 (18.6%)</td>
<td>14 (20%)</td>
<td>8 (11.4%)</td>
<td>35 (50%)</td>
</tr>
<tr>
<td>Age</td>
<td>46.2±10.9</td>
<td>44.6+/−10</td>
<td>46.5+/−8.4</td>
<td>45.8+/−9.6</td>
<td>46.7+/−12.5</td>
</tr>
<tr>
<td>Female</td>
<td>29 (41.5%)</td>
<td>4 (31%)</td>
<td>1 (7%)</td>
<td>5 (62%)</td>
<td>19 (54%)</td>
</tr>
<tr>
<td>Male</td>
<td>41 (58.5%)</td>
<td>9 (69%)</td>
<td>13 (93%)</td>
<td>3 (38%)</td>
<td>16 (46%)</td>
</tr>
<tr>
<td>P value ⃰</td>
<td>0.38</td>
<td>0.003</td>
<td>0.19</td>
<td>0.028</td>
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</tr>
<tr>
<td>Macroadenoma</td>
<td>62 (88.5%)</td>
<td>10 (77%)</td>
<td>12 (85.7%)</td>
<td>6 (75%)</td>
<td>34 (97%)</td>
</tr>
<tr>
<td>Microadenoma</td>
<td>8 (11.5%)</td>
<td>3 (23%)</td>
<td>2 (24.3%)</td>
<td>2 (25%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>P value ⃰</td>
<td>0.14</td>
<td>0.7</td>
<td>0.19</td>
<td>0.02</td>
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<tr>
<td>Active disease</td>
<td>58 (82.8%)</td>
<td>9 (70%)</td>
<td>11 (78.5%)</td>
<td>7 (87.5%)</td>
<td>32 (91.4%)</td>
</tr>
<tr>
<td>Inactive disease</td>
<td>12 (17.2%)</td>
<td>4 (30%)</td>
<td>3 (21.5%)</td>
<td>1 (12.5%)</td>
<td>3 (8.6%)</td>
</tr>
<tr>
<td>P value ⃰</td>
<td>0.14</td>
<td>0.63</td>
<td>0.7</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>smokers</td>
<td>13</td>
<td>2 (15%)</td>
<td>6 (43%)</td>
<td>2 (25%)</td>
<td>3 (8.5%)</td>
</tr>
<tr>
<td>Non smokers</td>
<td>57</td>
<td>11 (85%)</td>
<td>8 (57%)</td>
<td>6 (75%)</td>
<td>32 (91.5%)</td>
</tr>
<tr>
<td>P value ⃰</td>
<td>0.74</td>
<td>0.008</td>
<td>0.61</td>
<td>0.03</td>
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</table>

*Done by Chi square test
Table (2): The negative relationship between thyroid disease and different variables:

<table>
<thead>
<tr>
<th>Variable</th>
<th>all</th>
<th>normal</th>
<th>diffuse goiter</th>
<th>single thyroid nodule</th>
<th>MNG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients No.</td>
<td>70 (100%)</td>
<td>13(18.6%)</td>
<td>14(20%)</td>
<td>8(11.4%)</td>
<td>35(50%)</td>
</tr>
<tr>
<td>Last GH</td>
<td>4.7 +/- 7.7</td>
<td>5.1 +/- 9</td>
<td>4.7 +/- 5.4</td>
<td>4.5 +/- 5.7</td>
<td></td>
</tr>
<tr>
<td>( P \text{ value}^† )</td>
<td>0.489</td>
<td>0.42</td>
<td>0.49</td>
<td>0.818</td>
<td></td>
</tr>
<tr>
<td>Last IGF1</td>
<td>343 +/- 235</td>
<td>412.4 +/- 308.4</td>
<td>403.9 +/- 329</td>
<td>423 +/- 278</td>
<td></td>
</tr>
<tr>
<td>( P \text{ value}^† )</td>
<td>0.166</td>
<td>0.45</td>
<td>0.49</td>
<td>0.563</td>
<td></td>
</tr>
</tbody>
</table>

**Duration of disease**

<table>
<thead>
<tr>
<th></th>
<th>≤10 years</th>
<th>&gt;10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients No.</td>
<td>52(74%)</td>
<td>18(36%)</td>
</tr>
<tr>
<td>Last GH</td>
<td>11(85%)</td>
<td>3(15%)</td>
</tr>
<tr>
<td>( P \text{ value}^ceries )</td>
<td>0.34</td>
<td>0.1</td>
</tr>
<tr>
<td>history of hypophysectomy</td>
<td>4(30%)</td>
<td>6(43%)</td>
</tr>
<tr>
<td>( P \text{ value}^ceries )</td>
<td>0.09</td>
<td>0.47</td>
</tr>
<tr>
<td>Family history of thyroid disease</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>Patients No.</td>
<td>17(24%)</td>
<td>53 (76%)</td>
</tr>
<tr>
<td>Last GH</td>
<td>0(0%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>( P \text{ value}^acies )</td>
<td>0.14</td>
<td>0.7</td>
</tr>
</tbody>
</table>

\\(^*\text{Done by Chi square test}\)
\\(^†\text{Done by Student’s unpaired t-test}\)
Table (3): The relationship between nodular thyroid disease characteristics and different variables:

<table>
<thead>
<tr>
<th>variable</th>
<th>benign nodule by US*</th>
<th>suspicious nodule by US</th>
<th>benign nodule by FNA †</th>
<th>Suspicious nodule by FNA</th>
<th>Malignant after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (% out of all nodular thyroid disease)</td>
<td>37(86%)</td>
<td>6(14%)</td>
<td>19(44.2%)</td>
<td>4(9.3%)</td>
<td>1(2.3%)</td>
</tr>
<tr>
<td>Age</td>
<td>46.5 +/- 11.5</td>
<td>47.1 +/- 15.5</td>
<td>49.4 +/- 10.7</td>
<td>46.7</td>
<td>46</td>
</tr>
<tr>
<td>Female</td>
<td>20(54%)</td>
<td>4(66.7%)</td>
<td>10(52%)</td>
<td>2(50%)</td>
<td>1</td>
</tr>
<tr>
<td>Male</td>
<td>17(46%)</td>
<td>2(33.3%)</td>
<td>9(48%)</td>
<td>2(50%)</td>
<td>0</td>
</tr>
<tr>
<td>P value ‡</td>
<td>0.02</td>
<td>0.18</td>
<td>0.24</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>family history of thyroid disease</td>
<td>present</td>
<td>13(35%)</td>
<td>1(17%)</td>
<td>6(31%)</td>
<td>1(25%)</td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td>24(65%)</td>
<td>5(83%)</td>
<td>13(69%)</td>
<td>3(75%)</td>
</tr>
<tr>
<td>P value ‡</td>
<td>0.02</td>
<td>0.64</td>
<td>0.38</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>family history of malignancy</td>
<td>present</td>
<td>6(16%)</td>
<td>1(17%)</td>
<td>4(21%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td>31(84%)</td>
<td>5(83%)</td>
<td>15(79%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>P value ‡</td>
<td>0.82</td>
<td>0.01</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last IGF1</td>
<td>454.1 +/- 289</td>
<td>209 +/- 113</td>
<td>483.9 +/- 341</td>
<td>208 +/- 143</td>
<td>137</td>
</tr>
<tr>
<td>P value §</td>
<td>0.11</td>
<td>0.003</td>
<td>0.28</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>history of hypophysectomy</td>
<td>21(56.7%)</td>
<td>5(83%)</td>
<td>10(52%)</td>
<td>4(100%)</td>
<td>1(100%)</td>
</tr>
<tr>
<td>P value ‡</td>
<td>0.34</td>
<td>0.1</td>
<td>0.9</td>
<td>0.04</td>
<td>0.32</td>
</tr>
<tr>
<td>Microadenoma</td>
<td>3(8%)</td>
<td>1(17%)</td>
<td>1(5%)</td>
<td>1(25%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>macroadenoma</td>
<td>34(92%)</td>
<td>5(83%)</td>
<td>18(95%)</td>
<td>3(75%)</td>
<td>1(100%)</td>
</tr>
<tr>
<td>P value ‡</td>
<td>0.35</td>
<td>0.67</td>
<td>0.32</td>
<td>0.37</td>
<td>0.71</td>
</tr>
<tr>
<td>Active disease</td>
<td>33(89%)</td>
<td>5(83%)</td>
<td>16(84%)</td>
<td>3(75%)</td>
<td>0</td>
</tr>
<tr>
<td>Inactive disease</td>
<td>4(11%)</td>
<td>1(17%)</td>
<td>3(16%)</td>
<td>1(25%)</td>
<td>1</td>
</tr>
<tr>
<td>P value ‡</td>
<td>0.13</td>
<td>0.97</td>
<td>0.85</td>
<td>0.66</td>
<td>0.07</td>
</tr>
<tr>
<td>Last GH</td>
<td>4.5 +/- 5.6</td>
<td>4.8 +/- 5.9</td>
<td>3.89 +/- 5.1</td>
<td>6 +/- 7</td>
<td>0.2</td>
</tr>
<tr>
<td>P value §</td>
<td>0.796</td>
<td>0.94</td>
<td>0.59</td>
<td>0.82</td>
<td></td>
</tr>
</tbody>
</table>

* US: ultrasound † FNA: Fine Needle Aspirate ‡ done by Chi square test § Done by Student’s unpaired t-test

Conclusion

The prevalence of structural thyroid disease are increased in Iraqi patients with acromegaly compared to the general population in form of goiter [both diffuse & nodular types] mainly MNG, increased thyroid nodules of suspicious ultrasound & cytological characteristics. While the rate of thyroid cancer is not significantly different from that in general population.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of
both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**

Study on the Role of Interleukin-2 and Human Cytomegalovirus in Cases of Recurrent Spontaneous Abortion of Women in Wasit Province

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¹Wasit University / College of Science / Department of Biology/Iraq

Abstract

Recurrent spontaneous abortions (RSA) is the loss of pregnancy for two consecutive times at least before the arrival of the fetus for 24 weeks gestation. This study was conducted to investigate the role of IL-2 and human cytomegalovirus in recurrent spontaneous abortions women in Wasit province. to conduct immunological studies and viral by 90 women who suffer from abortion frequently (At least two abortions) and two sets of control (pregnant and healthy women), The average age of the groups ranged between (17-45) years, the results showed a high significant increase (≤0.001) at the level of IL-- 2 patients compared with pregnant women and healthy women, according to different age groups. And also it showed the results of a study of opposites immune IgM, IgG-virus human cytomegalovirus rise significantly (≤0.05) for patients when compared with pregnant women and healthy women in the cases of chronic and acute infection.

Key words : Recurrent spontaneous abortions (RSA) , Interlukin-2, CMV, IgM, IgG , Enzyme Linked Immunosorbenent Assay (ELISA).

Introduction

Recurrent spontaneous abortion (RSA) which known as repeated pregnancy loss ,it has no clear define at now Opinions differed about it, The American Reproductive Medicine Organization depend the tow pregnancy loss as (RSA) , While the European Organization of Embryology and Reproduction is considered three successive abortions and above as repeated spontaneous abortions¹. It is one of the most important and complications of pregnancy, especially in Iraq they have increased significantly in recent years, The causes are multiple, but most of the previous studies concentrate on parasitic and Viral causes². Studies have shown a number of factors causing spontaneous abortion But the most common causes are immune disorders, Hormonal and metabolic disorders, anatomical uterine anomalies and infectious causes The most common are toxoplasmosis and cytomegalovirus However, more than 50% of the cases are still unclear³.

The role of interleukin 2

In general, about 5% of couples are believed to have recurrent spontaneous abortions which are immunological reasons and increase this percentage may reach up to 40% after four losses of pregnancy⁴. The success of any pregnancy must be recognized and accepted by the mother’s immune system Otherwise, it is considered as foreign and be expelled, Here comes the role of cytokines, which are low molecular weight proteins they are used to allow connections between cells⁵ They are particularly important in both innate and adaptive immune responses. For its central role in the immune system, Cytokines are involved in a variety of immune, inflammatory and infectious diseases. Its functions are not limited to the immune system, but it has a major role in many developmental processes during the stage of embryonic development and during pregnancy⁶. Interleukin 2 is a monomer has 15.5 kD consisting of 133 amino acids, discovered more than 30 years ago in active T cells. It is mainly produced by cells (CD4-CD8-T) and a little produce it DCs and NK cell, IL-2 acts as a growth factor and promotes the proliferation and differentiation of natural killer cells to increase the normal functions of cells, It is also an effective stimulant for cell proliferation (T)If absent, T cells tend to go into hibernation or inactivity, It is therefore important to induce T cell proliferation and in maintaining both the regulatory and inflammatory role of T cells⁷.
The role of cytomegalovirus

The cytomegalovirus (CMV) came from the Greek word (cyto), meaning cell, and (megalo) large or overgrown. This is a viral genus from the group of human herpes viruses known as human herpes virus (HCMV)\(^8\). It’s one of the main causes of abortions and birth defects. Their clinical manifestations and symptoms in 90% of cases vary from severe fetal damage to death due to abortion\(^9\). The prevalence of herpes virus (CMV) infection among women of childbearing age ranges from 35% to 95% in different countries, and increases with age\(^{10}\). Cultural awareness as well as socio-economic status have a role in the spread of CMV infection and transmission\(^{11}\). A recent study in Baghdad\(^{12}\) indicates a high seroprevalence of cytomegalovirus in different age groups for men, women and children. The percentage was (68.78%) among the Iraqi patients and the rate of increase and rise compared to another study in Iraq as well was 57.2%\(^{13}\).

Materials and Method

This study was conducted in the College of Science / Department of biology / Wasit University, In cooperation with Laboratories of Al Karama Teaching Hospital, Al Zahra Teaching Hospital, Al-Kut Hospital for Pedantries and Gynae obstetrics and some private clinics during the period from of 2019. The study included 90 women with recurrent spontaneous abortion and two control groups (pregnant and healthy). There were (60) women, (30) pregnant women and (30) healthy women who are not pregnant. The average age of the two groups ranged between (17-45) years. All information included: age, weight, height, housing, number of previous births, number of abortions and body mass index (BMI) value was calculated for all samples.

Interleukin-2

The work was done according to a company Cusabio Biotech is of Chinese origin, since this test uses Sandwich Enzyme Immunoassay.

Measuring the level of cytomegalovirus infection

We have been working in accordance with\(^{14}\), detecting the presence of immunoglobulins (IgG / IgM) in the serum of both infected and two control groups, by using the ELISA method based on the principle of color change resulting from the binding of specific antibodies. Where (100 ml) from each diluted samples (Control Samples) and control solutions (Controls groups sample) of each (NC –CC –PC), The additives are double for control solutions to ensure accurate results and are single for samples. Then read in a short time not exceeding half an hour the results are recorded by the absorbance reading on along a wavelength (450 nm).

Statistical Analysis

Data were analyzed statistically using the program (Discovery Gen Stat) for statistical analysis of variance (ANOVA) was used. To find out the least significant difference (LSD). Comparisons were made between patients and control groups for the same age groups to find the significant differences between them was the least significant difference for the examinations studied below the probability level (P <0.05 )\(^{15}\).

Results and Discussion

rate of changes in the level (interleukin 2) by Age Groups

The significant differences (P <0.001) shown in Table (1) between the groups of aborted women and the control groups of pregnant and healthy. Interleukin 2 has been shown to play a major role in recurrent miscarriages and for all ages, it has also been shown to decrease its production during pregnancy as cytokines are originally regulatory immune proteins. It may affect some endocrine and placental activities and control the levels of secreted hormones. It also plays a major role in maintaining optimal conditions for fetal development and continuity of pregnancy or termination of pregnancy by abortion\(^{16}\). Because it is categorized based on its inflammatory reactions to inflammatory cytokines and anti-inflammatory cytokines since inflammatory cytokines include interleukin 2, interferon IFN-γ and tumor necrosis factor (TNF-α), And its action in the cell are reactions delays hypersensitivity reactions it may also attack the fetus and trophoblast\(^{17}\). So I found very high levels in our current study and for all age groups. The main act of increasing abortions is to increase the contractility of the uterine muscles by interleukin-2, as confirmed by a previous study\(^{18}\). Therefore, the value of interleukin-2 for pregnant and normal women is much lower than abortifacients. This is proven by our study as the normal state is the balance between TH1 / TH2 cytokines, Turns towards the production of TH2 cytokines for successful and continuing pregnancy or tends to increase the production of inflammatory cytokines TH1, including interleukin 2, therefore,
pregnancy fails and ends with miscarriage or death of the fetus and this is confirmed by\(^{(19,20)}\), the results of our current study are identical to a study in Iraq by\(^{(21)}\).

**Table (1) Rate of Changes in Level (IL-2) of Women with Repeated Abortions Compared to Control Groups by Age Groups**

<table>
<thead>
<tr>
<th>IL-2 Cases</th>
<th>Age Parameters</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>L.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients  n=90</td>
<td>1st. category (15-24 year)</td>
<td>A</td>
<td>101.249 ± 118.3</td>
<td>A</td>
<td>109.511 ± 118.3</td>
</tr>
<tr>
<td>pennants n=30</td>
<td>2nd. category (25-34 year)</td>
<td>B</td>
<td>53.0 ± 11.06</td>
<td>B</td>
<td>47.4 ± 5.491</td>
</tr>
<tr>
<td>Controls n=30</td>
<td>3rd. category (35-44 year)</td>
<td>B</td>
<td>44.7 ± 5.960</td>
<td>B</td>
<td>12.18 ± 57.5</td>
</tr>
</tbody>
</table>

*** Averages with different uppercase letters indicate a significant difference P < 0.05 (vertical) for each criterion.

**Table (2): IgG and IgM infection rates for women with recurrent miscarriages by age groups**

<table>
<thead>
<tr>
<th>Age parameters</th>
<th>Cases</th>
<th>CMV IgG mIU/L</th>
<th>1st. category (15-24 year)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>L.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients n=80+ve 2-ve</td>
<td>A</td>
<td>1.652 ± 0.365</td>
<td>A</td>
<td>1.682 ± 0.418</td>
<td>A</td>
<td>1.869 ± 0.493</td>
<td>0.2683</td>
</tr>
<tr>
<td>pregnant n=30+ve 0-ve</td>
<td>B</td>
<td>1.30 ± 0.678</td>
<td>B</td>
<td>1.18 ± 0.545</td>
<td>B</td>
<td>1.352 ± 0.533</td>
<td></td>
</tr>
<tr>
<td>controls n=28+ve 2-ve</td>
<td>B</td>
<td>0.74 ± 0.330</td>
<td>B</td>
<td>1.063 ± 0.637</td>
<td>B</td>
<td>1.067 ± 0.501</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age parameters</th>
<th>Cases</th>
<th>CMV IgM mIU/L</th>
<th>1st. category (15-24 year)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>L.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients n=42+ve 48-ve</td>
<td>A</td>
<td>0.951 ± 0.561</td>
<td>A</td>
<td>1.250 ± 0.629</td>
<td>A</td>
<td>1.660 ± 0.991</td>
<td>0.4428</td>
</tr>
<tr>
<td>pregnant n=6+ve 24-ve</td>
<td>A</td>
<td>0.93 ± 0.476</td>
<td>A</td>
<td>0.930 ± 0.496</td>
<td>B</td>
<td>1.09 ± 0.399</td>
<td></td>
</tr>
<tr>
<td>Controls n=3+ve 27-ve</td>
<td>A</td>
<td>0.764 ± 0.419</td>
<td>B</td>
<td>0.551 ± 0.209</td>
<td>B</td>
<td>0.70 ± 0.481</td>
<td></td>
</tr>
</tbody>
</table>

*** Averages with different uppercase letters indicate a significant difference P < 0.05 (vertical) for each criterion.

*** CMV IgG Chronic infections of cytomegalovirus

*** CMV IgM Acute infections of cytomegalovirus
Influence of Cytomegalovirus Infection by Age Groups

The significant increase found in our study Table (2) in the case of chronic CMV IgG infection among women with miscarriage and control groups of pregnant and healthy and for all age groups. As well as acute infection CMV IgM where the result was a significant increase for the third category with pregnant women, and a significant increase for the second and third categories with healthy. Clear evidence of the prevalence of CMV infection for women in the province of Wasit It is known according to recent statistics prevalence of CMV infections widely, especially among Iraqi women. The cytomegalovirus has a major role in repeated spontaneous abortions. Our study showed that the rate of chronic infection is higher than that of acute infection, these results corresponded to a study (22) in the province of Dhi Qar and also a study (23) in the province of Baghdad. In another study on abortifacients in Baghdad and the role of cytomegalovirus, seroprevalence of IgM from cytomegalovirus was the highest and most infections were in the first trimester of pregnancy, Given that pregnant women are the most affected category of viral infection, especially if the infection occurred during pregnancy, it either end the life of the fetus or be born deformed. So most studies have focused on the time of infection and infection in the first months of pregnancy is the most harmful. As the cytomegalovirus has the ability to alter the host’s immune response, it changes the cellular nature of the placenta and weakens the immune mechanism of the fetus and its mother. This leads to the life of the fetus (24). The results of the present study are consistent with (25,26).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References


Single Nucleotide Polymorphism of Follicle Stimulating Hormone Receptor Gene in Iraqi Infertile Men

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1Biotechnology Dept. College of Science- University of Baghdad/Iraq, 2Kamal Al-Samarie IVF Hospital- Ministry of Health, Baghdad, Iraq

Abstract

The study was directed to determine Single Nucleotide Polymorphism (rs6166 A>G) of follicle stimulating hormone receptor gene and their association as a risk factor with male infertility in Iraqi sterile patient. In the present study after seminal and serum analysis 50 patient (25 Azoospermia and 25 oligozoospermia) and 50 healthy fertile control were joined. The samples were collected from the Biotechnology Department/college of science/Baghdad university and Kamal Al-Samarie IVF Hospital, Baghdad, Iraq, through the period of two months from November 2018 to January 2019. The SNP (rs 6166) determination was carried out by using real-time polymerase chain reaction (qPCR) of blood samples. The difference in the mean of genotype showed a significantly different (p<0.05) in infertile patients group likened to corresponding means infertile control group. Survey of follicle stimulating hormone receptor gene SNP genotypes and allele frequencies in Azoospermia and oligozoospermia patient groups with the control group, showed that there was a significant variation in the heterozygous (AG) and homozygous mutant (GG) genotype frequencies in (rs 6166). It concluded that this SNP may have a role in an Azoospermia and oligozoospermia Iraqi patients complaining from idiopathic infertility.

Keywords: Follicle stimulating hormone receptor gene, Single nucleotide polymorphisms (SNPs), Azoospermia, Oligozoospermia, Iraqi sterile patients.

Introduction

Infertility is a disabling issue that affects public health and typically defined as the inability to conceive after a year of regular, unprotected sex 1,2. It is experienced by 10–15% of couples and is on the rise. It has previously estimated that half of these cases are due to male infertility 3. In the other hand there only a significant improvement in the diagnostic workup of infertile men 4.

The causes behind infertility are unexplained in 50% of all cases, this is described as idiopathic male infertility and frequently characterized by sperm dysfunction and spermatogenic failure 5. It is widely accepted that many genetic and environmental factors interact and are involved in the deficiency of spermatogenesis and consequent infertility 6. Genetic factors including chromosomal aberrations and single gene mutations account for 10-15% of severe male infertility. However recent research has submitted that idiopathic infertility may be produced in part by mutations or alterations in genes involved in spermatogenesis 7.

Spermatogenesis is a finely controlled process, exquisitely regulated by two gonadotropins secreted in the pituitary, namely FSH and LH 8,9.

Understanding the mechanisms of how FSH and LH regulate spermatogenesis is a very important goal in the area of male reproductive endocrinology, infertility, and contraception. FSH stimulates testicular development and spermatogenesis in the testis and folliculogenesis and steroidogenesis in the ovary 10,11. The role of FSH and its receptor (FSHR) is extensively considered in the setting of infertility and it may be relevant for impacting on ethnicity-related human reproductive achievement 12.

The FSHR is a G protein-coupled receptor with 76 kDa, part of the rhodopsin-like receptor subfamily and made of 695 amino acids 13. Located on the chromosome 2.p21, lengths more than 190 Kbases with 10 exons and 9 introns. Exons 1 to 9 encodes for the extracellular domain
allotted to ligand binding, while the largest one exon 10 that encodes part which called “hinge region”, for the seven transmembrane-spanning domains and for the intracellular C-terminal tail. FSHR is expressed mainly in granulosa and Sertoli cells, in which it mediates steroid synthesis and gametogenesis. Upon ligand binding, the receptor undergoes a conformational change, leading to the simultaneous activation of multiple signaling pathways at the intracellular level. More than two thousand SNPs located within coding and non-coding regions of the FSHR gene were described. Two of them displaying strong relationship uncertainty were largely studied in the role of infertility: rs6165 replaces threonine by alanine, it results in a change from a polar (T) to a nonpolar, hydrophobic (A) amino acid and removes a potential O-linked glycosylation site; rs6166 exchanges asparagine for serine in the intracellular domain of the receptor, introducing a potential phosphorylation site both located in the exon 10 an amino acid alteration in the hinge region and in the intracellular domain of the protein receptor are due to these SNPs.

A large body of evidence available previously suggests that FSH signaling is essential for the induction and maintenance of normal spermatogenesis. The modulatory activity of the FSHR p.As680Ser SNP on fertility was recently proposed in men, in which a slight correlation between the Ser/Ser homozygous state and a lower testicular volume was found.

For its critical role in spermatogenesis and sperm function, polymorphisms in the FSHR gene might disturb normal spermatogenesis and affect male reproductive ability. However, little is known about the FSHR gene polymorphisms in Iraq.

In this study, we explored the possible effects SNP of the FSHR gene on male infertility and serum FSH levels in Iraq by the case-control study, which included well-defined idiopathic infertile males and fertile controls.

2. Patients, materials, and methods

2.1. Patients

This study was approved by the Ethics Committee of Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq. Study subject was fifty Iraqi infertile male included in this study and 50 healthy fertile men from the same ethnicity without any systemic diseases were served as control. The patients were recruited from Kamal Al-Samaraie Hospital, Baghdad, Iraq. The healthy fertile men were volunteers with at least one child. With average age between (17 – 47) years old. The patient’s group was divided according to the semen analysis into 25 infertile men with azoospermia (Z) and 25 infertile men with oligozoospermia (O). In addition to fifty fertile male as a control (C) to this study.

Excluded criteria included: patients with secondary infertility, abnormal karyotype, obstructive azoospermia, varicocele.

2.2. FSHR gene SNPs

Primers and probes for FSH receptor gene (rs6166 A>G) were designed by Li, (2011) they provided in a lyophilized state by Macrogen Company (Korea) stored at (-23°C). The sequences of each of the probes and primers used in the allelic discrimination experiments are shown in table 1.

<table>
<thead>
<tr>
<th>FSHR (Primer for SNP Genotyping)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>5'-GGAATGGCCACTGCTTCA-3'</td>
<td></td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-GGGCTAAATGACTTAGGGACAA-3'</td>
<td></td>
</tr>
<tr>
<td>probe</td>
<td>FAM 5'-AGTCACCaaTGGTTC-3'</td>
<td></td>
</tr>
<tr>
<td>probe</td>
<td>VIC 5'-AGTCACCAGTGGTTC-3'</td>
<td></td>
</tr>
</tbody>
</table>

ReliaPrep™ Blood gDNA Miniprep System is used in this study to extract whole genomic DNA from leukocytes pellets of the blood samples according to the manufacturer instructions. Genotypes were detected by TaqMan allelic discrimination Assay on (MIC-4 Real-time PCR System, Australia).

The amplification reaction components and their final concentrations are 5µl Go Taq qPCR Master Mix (Promega/USA), 0.5µl of each primer, 0.5µl of each probe, 1.5 µl DNA, and 1.5µL nuclease-free water. The mix was transferred to a real-time thermocycler (MIC-4 Real-time PCR System, Australia).

The Real-time PCR System was programmed for optimized cycles shown in Table 2.
Table (2): The program for FSH receptor gene SNPs detection.

<table>
<thead>
<tr>
<th>Steps</th>
<th>°C</th>
<th>m:s</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95</td>
<td>05:00</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>00:30</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>60</td>
<td>00:30</td>
<td>100</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>00:30</td>
<td></td>
</tr>
</tbody>
</table>

**Statistical analysis**

Data analysis was done by utilizing SPSS for Windows, version 17 (SPSS Inc. Chicago, IL, United States). Data appeared as mean ± standard deviation. Shapiro–Wilk normality test was used to determine whether the studied parameters followed a Gaussian distribution.

Variables in which the distribution of data did not conform to normality were first log transformed for analysis and then converted back to standard units for presentation. Categorical variables were analyzed by the Chi-square test. Tukey’s, Dunnett, and Bonferroni Post Hoc test for multiple comparisons were applied after ANOVA tests.

Hardy-Weinberg equilibrium calculated using a web tool 18. The difference in frequencies of genotypes and alleles between the patient groups and the control group were analyzed using the Chi-square test. Odds ratios (ORs) with a 95% confidence interval (CI) were calculated for measuring the strength of the association between the studied gene SNP and male infertility. The association degrees between variables were analyzed by Pearson correlation analysis. A two-tailed p-value less than 0.05 (p<0.05) was considered significant 19.

**Results**

3.1. FSHR gene SNPs

The SNP of the FSHR gene (A > G; rs6166) was presented with three genotypes (AA, AG, GG) and two alleles (A and G).

Analysis of Hardy-Weinberg equilibrium (HWE) in (Z) group and (C) revealed that the genotypes were consistent with equilibrium. And significant differences (p <0.01) were detected between the observed and expected genotype frequencies in both control and patients groups (table 3A).

Inspecting FSHR gene genotypes and Allele Frequencies in (Z) group and (C) group revealed that there was significant variation between these frequencies. Although a decreased frequencies of G allele (84 vs. 65%) and an increased frequencies of A allele (16 vs. 35%) were observed in patients compared to control (Table 3 B).

In both GA and GG Polymorphisms, the odds ratio for the GA genotype was 0.09(0.01 -1.45) with p=0.025 and the odds ratio for the GG genotype was 4.12(1.26 -13.49) with p=0.021 indicating that heterozygous genotype GA and homozygous GG was a higher risk of (Z) group than the wild type AA.

**Table 3A. Number and percentage frequencies of FSHR gene genotypes and their Hardy-Weinberg equilibrium (HWE) in (Z) group and (C) group.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Z (no=25)</th>
<th>C (no=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>AA</td>
<td>4</td>
<td>16.0</td>
</tr>
<tr>
<td>AG</td>
<td>0</td>
<td>___</td>
</tr>
<tr>
<td>GG</td>
<td>21</td>
<td>84.0</td>
</tr>
</tbody>
</table>

HWE Analysis $X^2=25$ $p=0.000001$ Significant

<table>
<thead>
<tr>
<th></th>
<th>$X^2=18.264$</th>
<th>$p=0.000019$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Significant</td>
<td>Significant</td>
</tr>
</tbody>
</table>
Table 3B. Genotype and allele frequencies of the FSHR gene in (Z) group and (C) group.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Z (No=25)</th>
<th>C (No=50)</th>
<th>OR (95.0% CI)</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>4 16.0</td>
<td>13 26.0</td>
<td>0.54(0.16 1.84)</td>
<td>0.393</td>
</tr>
<tr>
<td>AG</td>
<td>0 __</td>
<td>9 18.0</td>
<td>0.09(0.01-1.45)</td>
<td>0.025</td>
</tr>
<tr>
<td>GG</td>
<td>21 84.0</td>
<td>28 56.0</td>
<td>4.12(1.26-13.49)</td>
<td>0.021</td>
</tr>
<tr>
<td>Total</td>
<td>25 100%</td>
<td>50 100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Allele frequency

<table>
<thead>
<tr>
<th>Allele</th>
<th>Z (No=25)</th>
<th>C (No=50)</th>
<th>OR (95.0% CI)</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>42 0.84</td>
<td>65 0.65</td>
<td>2.83(1.20-6.63)</td>
<td>0.021</td>
</tr>
<tr>
<td>A</td>
<td>8 0.16</td>
<td>35 0.35</td>
<td>0.35(0.15-0.83)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval;

Analysis of Hardy-Weinberg equilibrium (HWE) in (O) group and (C) revealed that the genotypes were consistent with equilibrium. And significant differences (p < 0.01) were detected between the observed and expected genotype frequencies in both control and patients groups (table 4A).

Inspecting FSHR gene genotypes and Allele Frequencies in (O) group and (C) group revealed that there was significant variation between these frequencies, Although a decreased frequencies of G allele (84 vs. 65%) and an increased frequencies of A allele (16 vs. 35 %) were observed in patients compared to control (Table 4 B).

In both GA and GG Polymorphisms, the odds ratio for the GA genotype was 0.09(0.01 -1.45) with p=0.025 and the odds ratio for the GG genotype was 4.12(1.26 -13.49) with p=0.021 indicating that heterozygous genotype GA and homozygous GG was a higher risk of (O) group than the wild type AA.

Table 4A: Number and percentage frequencies of FSHR gene genotypes and their Hardy-Weinberg equilibrium (HWE) in (O) group and (C) group.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Z (no=25)</th>
<th>C (no=50)</th>
<th>HWE Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
<td>Observed</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>AA</td>
<td>4 16.0</td>
<td>0.6</td>
<td>2.4</td>
</tr>
<tr>
<td>AG</td>
<td>0</td>
<td>__</td>
<td>6.7</td>
</tr>
<tr>
<td>GG</td>
<td>21</td>
<td>84.0</td>
<td>17.6</td>
</tr>
<tr>
<td>HWE Analysis</td>
<td>$X^2$=25 $p=0.000001$ Significant</td>
<td>$X^2$=18.264 $p=0.000019$ Significant</td>
<td></td>
</tr>
</tbody>
</table>
Table 4B: Genotype and allele frequencies of the FSHR gene in (O) group and (C) group.

<table>
<thead>
<tr>
<th>Genotype No</th>
<th>Z</th>
<th>C</th>
<th>OR (95.0% CI)</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>4</td>
<td>16.0</td>
<td>13</td>
<td>26.0</td>
</tr>
<tr>
<td>AG</td>
<td>0</td>
<td>18.0</td>
<td>9</td>
<td>18.0</td>
</tr>
<tr>
<td>GG</td>
<td>21</td>
<td>84.0</td>
<td>28</td>
<td>56.0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100%</td>
<td>50</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>G</th>
<th>A</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>42</td>
<td>0.84</td>
<td>65</td>
</tr>
<tr>
<td>A</td>
<td>8</td>
<td>0.16</td>
<td>35</td>
</tr>
</tbody>
</table>

 Discusses

4-1 - FSHR gene SNPs

Male infertility is common, especially in developing countries as a result of the complicated interaction between genetic and environmental factors. Due to the importance of FSH signaling for the pubertal initiation of spermatogenesis and maintenance of quantitatively normal sperm production in adults, genetic abnormality of the FSHR, as well as FSH, would be involved in some form of male infertility, such as azoospermia or oligozoospermia. However, to date little is known about mutations or polymorphisms of the FSHR gene in Iraqi infertile men. In the present study, we established the hypothesis that the polymorphism in the FSHR gene might be the reason behind idiopathic male infertility and related to the serum FSH levels of them. The result was observed significant differences in the distribution of FSHR genotypes between infertile men and fertile males in the control group. The proportions of Asn/Asn and Ser/Ser were 16% and 84% respectively in patients groups and 26% and 56% correspondingly infertile men, while Asn/Ser genotype was not observed in the patient group through its proportion, was 18% in the control group, and there was a significant difference between the two. This result disagrees with some studies that failed to prove the difference in the prevalence of FSHR genotype infertile or infertile men. Recent study among Iranian population found that FSHR gene polymorphism might increase the risk factor of azoospermia, which similar to our findings. Recent study among Iranian population found that FSHR gene polymorphism might increase the risk factor of azoospermia, which similar to our findings. The association between FSH levels within the sera and A919G and A2039G alleles and genotypes were also investigated. MATERIALS AND METHODS This case control study was performed on 212 men with azoospermia (126 non-obstructive and 86 obstructive.

Despite the fact that multi unidentified factors might increase susceptibility to male infertility, the outcomes of this study showed that single nucleotide polymorphisms in FSHR gene might account as one of the susceptible factors for the etiology of idiopathic male infertility. In conclusion, the present study findings demonstrated that the genetic polymorphisms in the FSHR gene might have a role in an Azoospermia and oligozoospermia Iraqi patients complaining from idiopathic infertility. However, additional investigations are recommended to be directed on other ethnic populations to approve the results of this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and
scientific research ministries in Iraq

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**Funding**: Self-funding

**References**


Follicle –Stimulating Hormone Receptor Polymorphisms in Iraqi Women with Primary Amenorrhea

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1Biotechnology Department College of Science- Baghdad University/Iraq,
2Educational laboratories, City of Medicine, Baghdad/Iraq

Abstract

The study was carried to determine Single Nucleotide Polymorphism (rs6165) of Follicle Stimulating Hormone Receptor (FSHR) gene in blood samples of 62 Iraqi women with primary amenorrhea and 40 healthy control females. The research included chromosomal study and serum analysis of 62 patient samples. The samples were collected from Educational laboratories, City of Medicine, Baghdad and Biotechnology Dept. College of Science- Baghdad University through the period from October 2018 to March 2019. The determinations of SNP (rs6165) were carried out by real-time PCR. Results of rs6165 genotyping showed significant variations between PA patients and controls, inspecting FSHR gene genotypes and allele frequencies in PA patients groups with the control group, revealed that there was significant variation in the heterozygous (AG) and homozygous mutant type (AA) genotype frequencies in (rs6165). the SNP of target gene may have a role in PA patients complaining from idiopathic puberty problems.

Keywords: primary amenorrhea, Follicle Stimulating Hormone Receptor (FSHR) gene, SNP, Iraqi women patient.

Introduction

Primary amenorrhea (PA) could be defined as the absence of menses by 14 years of age in the absence of growth or development of secondary sexual characteristics or absence of menses by 16 years of age regardless of the presence of normal growth and development including secondary sexual characteristics and1. According to WHO, amenorrhea stands as a 6th largest major cause of female infertility and affects 25% of women in the reproductive age. During normal female menstruation cycle, Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus, and it works on the pituitary to release FSH and LH and these 2 hormones from the pituitary act on ovaries that finally make estrogen and progesterone to work on the uterus to carry out the follicular and secretory phase of menstrual cycle. Any failure at any level of this normal physiology can cause amenorrhea2. The absence of menses in a female of reproductive age is related to the disturbance of normal hormonal, physiological mechanism or female anatomic abnormalities. The normal physiological mechanism works by balancing hormones and providing feedback between the hypothalamus, pituitary, ovaries, and uterus 3. According to the ministry of health of Iraq statistics for 2017 and 2018, 9.68% and 17.78% of women respectively suffering from primary amenorrhea and problems with menarche.

The FSHR is a 76 kDa G protein-coupled receptor, consisting of 695 amino acids and belonging to the rhodopsin-like receptor subfamily. The FSHR gene is located at chromosome 2.p21, spans more than 190K bases and embeds 10 exons and 9 introns. Exons 1-9 encode for the extracellular domain deputed to ligand binding, while the large exon 10 for part of the so-called “hinge region”, for the seven transmembrane-spanning domains and for the intracellular C-terminal tail. FSHR is expressed mainly in granulosa and Sertoli cells, in which it mediates steroid synthesis and gametogenesis. Upon ligand binding, the receptor undergoes a conformational change, leading to the simultaneous activation of multiple signaling pathways at the intracellular level and4. More than two thousand
SNPs falling within coding and non-coding regions of the FSHR gene were described so far. One of them displaying strong linkage disequilibrium were largely studied in the setting of infertility: p.Thr307Ala (c.919 G > A; rs6165). This SNP is located in the exon 10 of the FSHR gene and leads to an amino acid change in the hinge region protein receptor5. The biggest difference between SNPs and mutations is that SNPs are inherited. Some of these genetic differences have proven to be very important to human health. If those inherited SNPs are high-risk candidates, they deserve our attention, and their investigation will lead to further mechanistic research to develop new treatment programs6.

Patients, materials, and methods

This experimental work was carried out in the Educational laboratories, City of Medicine, Baghdad, from October 2018 till February 2019 and at the University of Baghdad, College of Sciences, Department of Biotechnology to investigate molecular parameters. The healthy control group included 40 apparently healthy females of different ages ranged from 12-35 years. Abnormalities were not found in selected blood samples taken from blood donors and therefore being used as controls for comparison with blood samples taken from primary amenorrhea patients. A total number of 62 patients, attended to the Educational laboratories, City of Medicine, Baghdad, were diagnosed with primary amenorrhea. Patient’s ages were ranged from 12-35 years. All patients were suffering from primary amenorrhea symptoms. Meanwhile, all blood samples were subjected to molecular studies. Venous blood samples (5ml each) were collected from affected individuals. Six patients were excluded from this study due to chromosomal abnormalities.

FSHR gene SNP

TaqMan fluorescent oligonucleotide probes and primers sequences for FSH receptor gene SNP (rs6165 G>A )designed by7 which mediates the effects of FSH, is essential for normal spermatogenesis and male reproduction. This study aimed to investigate the effects of the FSHR polymorphisms on idiopathic male infertility and serum FSH levels in Han-Chinese population. Methods: A case-control study was conducted with 364 idiopathic infertile patients (97 nonobstructive azoospermic, 79 oligozoospermic and 188 normozoospermic. And they provided in a lyophilized state by Macrogen Company (Korea), stored at (-23°C). The sequence of probes, forward and reverse primers are listed in the table (1):

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>codon 307-F</td>
<td>5’-TGTCTTCTGCCAGAGAGGATCTC-3</td>
<td>60°C</td>
</tr>
<tr>
<td>codon 307-R</td>
<td>5’-TCTGAGCTTCATCCAATTTGCA-3</td>
<td></td>
</tr>
<tr>
<td>codon 307-P/T</td>
<td>FAM 5’-CCCTAGtCTGAGTCATA-3</td>
<td></td>
</tr>
<tr>
<td>codon 307-P/C</td>
<td>VIC 5’-CCCCTAGcCTGAGT-3</td>
<td></td>
</tr>
</tbody>
</table>

The Genomic DNA was extracted from blood using the Wizard genomic DNA purification kit (Promega, USA) according to the manufacturer instructions, then the samples were subjected to a real-time polymerase chain reaction(qPCR). The reaction mix was adjusted to a final volume of 10 µl as suggested by the manufacturer and included: 5µl GoTaq Probe qPCR Master Mix (Promega/USA), 0.5µl of each primer (each lyophilized primer was dissolved in free nuclease water to prepare the stock solution in a concentration of 100 pmole/µl. Then the working solution is prepared by adding 10µl of the stock solution to 90µl of free nuclease water), 0.5µl of each prob, 1.5 µl DNA, and 1.5µL nuclease-free water. The mix was transferred to a real-time thermocycler (MIC-4 Real-time PCR System, Australia ), which was programmed for the following optimized cycles: initial denaturation for 5 min at 95°C (one cycle), 40 cycles of denaturation (20 sec at 95°C), annealing (30 sec at 60°C) and extension (30 sec at 72°C), and finally one cycle of melt curve at 65–90°C.
Table(2): The program for FSH receptor gene SNPs detection

<table>
<thead>
<tr>
<th>Steps</th>
<th>°C</th>
<th>m:s</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95</td>
<td>05:00</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>00:30</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>60</td>
<td>00:30</td>
<td>95</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>00:30</td>
<td></td>
</tr>
</tbody>
</table>

Statistical Analysis

Data analysis was done by utilizing SPSS for Windows, version 17 (SPSS Inc. Chicago, IL, United States). Data appeared as mean ± standard deviation. Shapiro–Wilk normality test was used to determine whether the studied parameters followed a Gaussian distribution. Variables in which the distribution of data did not conform to normality were first log transformed for analysis and then converted back to standard units for presentation. Categorical variables were analyzed by the Chi-square test. Tukey’s, Dunnett, and Bonferroni Post Hoc test for multiple comparisons were applied after T-tests.

Table(3): Number and percentage frequencies of FSHR gene genotypes and their Hardy-Weinberg equilibrium (HWE) in (C) group and (PA) group.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient (no=55)</th>
<th>Control (no=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>GG</td>
<td>25</td>
<td>45.4</td>
</tr>
<tr>
<td>AG</td>
<td>16</td>
<td>29</td>
</tr>
<tr>
<td>AA</td>
<td>14</td>
<td>25.4</td>
</tr>
<tr>
<td>HWE Analysis</td>
<td>p=0.003</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Inspecting FSHR gene genotypes and Allele Frequencies in (PA) group and (C) group revealed that there was significant variation between these frequencies, Although an increased frequencies of G allele (60 vs. 61%) and a decreased frequencies of A allele (40vs. 38.7 %) were observed in patients compared to controls (Table 4).

Results

FSHR gene SNP

The SNP of FSHR gene (G>A rs6165; located on Chromosome2p21-16:242193529 bp) was presented with three genotypes (GG, AG, AA) and two alleles (G and A).

Analysis of Hardy-Weinberg equilibrium (HWE) in (control) group and (patients) group revealed that the genotypes was consistent with the equilibrium, and significant differences (p <0.01) were observed between the observed and expected genotype frequencies in control and significant (p>0.05) in patients group (Table 3).

In AG Polymorphism, the odds ratio for the AG genotype was 0.25 (0.10 - 0.58)with P value =0.002 indicating that heterozygous genotype AG was a protective factor to (PA) group and mutant type AA which considered as a risk factor for primary amenorrhea according to the odds ratio 4.21 (1.14 - 15.58) with p-value 0.03.
Table (4): Genotype and allele frequencies of the FSHR gene in (C) group and (PA) group.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. patients</th>
<th>Control</th>
<th>OR (95.0% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG (Wild type)</td>
<td>25</td>
<td>30.0</td>
<td>1.94 (0.83 - 4.55)</td>
<td>0.142</td>
</tr>
<tr>
<td>GA (heterozygous mutant type)</td>
<td>16</td>
<td>62.5</td>
<td>0.25 (0.10 - 0.58)</td>
<td>0.002*</td>
</tr>
<tr>
<td>AA (homozygous mutant type)</td>
<td>14</td>
<td>7.5</td>
<td>4.21 (1.14 - 15.58)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Allele frequency

<table>
<thead>
<tr>
<th>Allele</th>
<th>No.</th>
<th>%</th>
<th>OR (95.0% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>66</td>
<td>60.0</td>
<td>0.95(0.53-1.71)</td>
<td>0.882</td>
</tr>
<tr>
<td>A</td>
<td>44</td>
<td>40.0</td>
<td>1.05(0.59 - 1.89)</td>
<td>0.882</td>
</tr>
</tbody>
</table>

OR, odd ratio; CI, confidence interval

Discussion

FSHR gene SNP

FSH and its receptor play a major role in the development of follicles and regulation of steroidogenesis in the ovary. The interaction of this hormone with its cell surface receptor initiates a chain of intracellular reactions characteristic of G-protein-coupled receptors, if any Structural changes occurred in this region that could lead to changes in the amino acid configuration of the FSH receptor gene, resulting in functional changes in the gene in which Some of it leads to enhance functionality of the receptor, while some reduce it.10 Many studies investigated about the main cause of amenorrhea and the impact of polymorphisms on the disease, but there is a little knowledge about it in Iraq so this study could help to seek for the main genetic cause of amenorrhea. Our results illustrate that significant differences in the distribution of FSHR genotypes between PA patients and control group. The proportions of Thr/Thr, Thr/Ale and Ale/Ale were 54.54%, 29.1% and 25.45% respectively in patient group and 12%, 25% and 3% correspondingly in control group, and there was a significant difference between the two. According to11 results showed that there was a difference in distribution between non ovulatory patients and normal-ovulatory controls was significant for polymorphism 6165 (P<0.05 for position307), with a distribution of (AA) and (AG), genotypes higher in patients than controls, that agree with our findings, also there were some studies that disagree with these results and failed to prove the significance of the SNP and disease.12

Despite the fact that multiple causes might increase susceptibility to primary amenorrhea, the results of this study showed that single nucleotide polymorphisms in FSHR gene might consider as one of the susceptible factors that result in amenorrhea. However, additional investigations are recommended to be directed on other ethnic populations to approve the results of this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References


Study Effect of Clarithromycin drug on Cryptosporidium Parvum and Efficiency of ELISA Technique in Diagnosis Comparison with Some Traditional Methods in vitro.

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Abstract

This research aimed to study effect of Clarithromycin drug on Cryptosporidium Parvum and efficiency of ELISA technique in diagnosis comparison with some traditional methods in vitro. Samples collected from four different sources water were 400 liters:100 liters tap water provider by Al-Kufa District River,100 liters house tank water,100 liters from sea of Al-Najaf and 100 liters stagnant water during period from October 2018 to April 2019.Examined samples by three laboratory methods: Modified Ziehl-Neelson stain, Flotation by saturated sugar solution method and ELISA to detecting from Cryptosporidium oocysts in the water as used Clarithromycin drug effective against C.parvum in vitro. Capability test was performed by impregnation of isolate on cell monolayers and determination the parasite counted after 48 h from the incubation at 37°C. Differ concentrations from Clarithromycin were 4, 8, 16 and 32 mg/L respectively compared with control group.

Results of the current study showed that oocysts were found in 182 from 400 liters 45.5% as showed that height oocysts in stagnant water then followed Sea of Al-Najaf were 55% and 45% respectively, height percentage of oocysts were in April and March were 69.09% and 66% while decrease percentage of infection in October was 25%. As showed study results presence significant differences in efficiency of ELISA technique was 83.33% at detection from oocysts compared with Flotation by saturated sugar solution and Modified Ziehl-Neelson stain methods were 66.66% and 61.11% respectively. Shown results this study that Clarithromycin drug in concentrations of 16 and 32 mg/L caused decrease in number oocysts was 17.3±3.5 and 15.4±3.9, sporozoites counts was 17.1±3.4 and 14.9±4.0 compared with control group was 20.5±2.8. As observed significance differences (P˂0.05) in the mean numbers of oocysts and sporozoites in three replicate plates after 48 h. These results proved that Clarithromycin have effectiveness in inhibition C.parvum in vitro.

Keywords: Cryptosporidiosis, Water, Oocysts, Sporozoites, Clarithromycin, in vitro.

Introduction

Cryptosporidiosis is protozoan disease occurred by Cryptosporidium spp from phylum Apicomplexa is unicellular organisms include number of pathogenic infect human and mammals by food and contaminated water (1). Oocysts transmitted in arduous environment where not killed by conventional disinfectants and chlorination which causes diarrhea, massive dehydration, malnutrition and weight loss lead to abomasal cryptosporidiosis (2). Oocysts resisted tough environmental condition for six months where can stay alive for twelve weeks at 10°C (3). Transmission riot by the fecal oral and involve drinking water, recreational water, person to person, animals to person and contribution of sexual pursuit may be locomotion through persons with human immunodeficiency HIV virus (4).

C.parvum infections diagnosed through knowledge number of infestation and non-invasive methods. The non-invasive methods, microscopic examination more common (5) or identification of these parasite is based on morphologic examination by using modified acid-fast...
staining method (6), important alternate the development of antigen parasite enzyme linked immune-sorbent assay (ELISA) these assay shown comparable sensitivity to experienced microscopic examinations, fairly simple to perform and do not require the observation of intact organisms (7).

Recently, observed there increase in number of infection with C.parvum correlating with increase in number of immune-compromised patients and resistance against drugs, this disease currently endemic in 88 countries world and showed diffusion increase in immunosuppressive conditions such as HIV/AIDS (8), Clarithromycin known as being semisynthetic antibiotic from macrolide derivative family (9). Also good distribution excellent activity against intracellular pathogens such as: Toxoplasma gondii (10), Leishmania donovani (11) and Cryptosporidium parvum (12) invitro as featuring effective, least side effects, low cost and easy administration. Clarithromycin, Azithromycin and Roxithromycin are effective in inhibition C.parvum growth at concentrations close to those achievable (13). The aim this research is study effect of Clarithromycin drug on oocysts and sporozoites in vitro and efficiency of ELISA technique in diagnosis comparison with some traditional methods.

Materials and Method

Water samples were collected during the period from October 2018 to April 2019 of Al-Najaf governorate. A total amount was 400 liters: 100 liters of tap water supplies by Al-Kufa District River, 100 liters of house tank water, 100 liters sea of Al-Najaf and 100 liters of stagnant water, put in sterile bottles which delivered to the laboratory of pharmacognosy and medicinal plants in pharmacy college/ kufa university.

Diagnosis parasite: 5 liters from water samples distributed in sterile test tubes placed in the centrifuged 1000g/10-15 min to get on sediment, taken 5 ml of sediment and added 15 ml of distilled water then aspirate during six layers of gauze for get rid of the suspended waste then centrifugation procedure was performed again 1000g/10 min. 10 ml add of saturated sugars solution to the sediment well mix (14). Taken drop from the upper layer of the tube by Pasteur pipette placed on glass clean slide left to dry in the air for 10 min, smear fixed by absolute methyl alcohol for 5 min and left to dry, added modified ziehl-neelson stain to the fixed smear and heating for 5 min by Bunsen burner until vapor appears and washed with tap water, the slide is immersed in 10% HCL for 10-15 sec and washed again with water then dye was stained with methylene blue for 2 min, wash the well slide with running water then air-dried, examined by microscope 40X and 100X magnification to investigate parasite oocysts by many criteria’s as size, shape, color stain and surface feature (15).

ELISA technique: Used in these technique particular oocysts antibodies where placed these antibodies in the pits for the plate ELISA method and these antibodies have ability to interact with oocysts find in samples then plate incubated for 1 h at 21°C, wash the plate by washing solution and add the conjugation solution containing antibodies to the parasite associated with the peroxidase enzyme then incubate and wash the plate again. Add chromogen tetramethylbenzidine is solution of the reactive substance which contained bottle inside size 25 ml with ELISA diagnostic components, if the water container on oocysts, conjugation solution remains linked with the pit and the enzyme converts chromogen from colorless to blue color composite where strength this color is proportional with number of oocysts in the examined sample, stopped the enzyme reaction after addition of phosphoric acid solution then measured optical density at the wavelength of 450 nm using optical spectrum (16).

Experiment study invitro: Oocysts isolated from the water by ELISA method was used throughout this study. Clarithromycin (Abbott, Italy) were dissolved in 50% methanol and 50% acetone to obtain 1 mg/ml of stock solution for perpetrated different concentrations where stored at –80°C in the dark. In these experiment indicated that concentrations of methanol, acetone were used in dilution of drugs did not inhibit the growth of C. parvum.

Oocysts Preparation: Occur by suspending portion of stock oocysts in bleach solution containing nine parts sterile deionized water and one part 0.55% sodium hypochlorite for 10 min, washed twice in sterile water, centrifuged and re-suspended in Dulbecco’s modified Eagles medium (DMEM) (Bio-Whittaker) then incubation in phosphate buffered saline PBS (Bio-Whittaker) containing penicillin G (2000 U/mL), streptomycin (2000 mg/L) and amphotericin-B (10 mg/L) for 4 h at 37°C. Excystation of sporozoites was achieved by incubating oocysts in PBS containing 0.25% trypsin (Sigma-Aldrich) and 0.75% sodium hypochlorite for 60 min at 37°C. Free sporozoites were pelleted by
centrifugation 500g/10 min, re-suspended in (DMEM), counted in haemocytometer for culture (17).

Cell Culture: Isolated 500 cells from human stomach carcinoma were maintained in 25cm2 tissue culture flasks. Medium consisted of (DMEM) with 10% fetal bovine serum (Bio-Whittaker), 4% L-glutamine (Bio-Whittaker), 1% non-essential amino acid, penicillin G (100 U / ml), streptomycin (100 mg/l) and amphotericin B 0.5 mg/l. Cells were lifted from the surface of flasks by using solution of 0.25% trypsin and 0.53 mM EDTA in phosphate buffered saline then quantitated using hemocytometer. 500 cells were plated onto 35 mm diameter tissue culture plates at concentration of 105 viable cells in total volume 5 ml, viability was assessed by trypan blue exclusion (18).

Infection of cells with C.parvum: Infection of the cell monolayer was started by adding 105 sporozoites in volume of 0.2 ml of medium. After incubation for 4 h at 37°C in 5% CO2 to allow attachment and penetration of sporozoites, monolayers washed with (DMEM) to remove noninvasive sporozoites, residual oocysts and no adherent epithelial cells. Infected cell cultures were keeping at 37°C in 5% CO2 throughout this study (19).

Antibiotics agents: Clarithromycin concentrations prepared were 4, 8, 16 and 32 mg/L respectively, Experiments were performed in triplicate the monolayers were incubated for 48 h at 37°C in 5% CO2. Following four washes in phosphate buffered saline to remove free oocysts and no adherent epithelial cells then fixed with 75% methanol and stained by Giemsa stain to estimate presence of sporozoites within cells to vision both intra and extracellular oocysts. The number of parasites was calculated by mean ± standard deviation of the mean of number of sporozoites and oocysts observed in three monolayers of each of ten isolates exposed to same concentration of drug by microscopic examination of 40 fields under 1000X magnification (20).

Statistical analysis: In these study association between presences of oocysts with various source of water depending to months this study and efficiency of the diagnostic methods used during the experiment by (P<0.05) (21).

Results

Table (1): Percentage of Infection with Cryptosporidium oocysts to different water sources in the Study.

<table>
<thead>
<tr>
<th>Source of water</th>
<th>No. of samples examined (liter)</th>
<th>NO. of Samples (+ve)</th>
<th>Percentage of Infection (+ve) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water provides by Al-Kufa river</td>
<td>100</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Tank / houses</td>
<td>100</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Sea of Al-Najaf</td>
<td>100</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Stagnant water</td>
<td>100</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>182</td>
<td>45.5</td>
</tr>
</tbody>
</table>

Table (2): Percentage of Infection with Cryptosporidium oocysts according to months the Study from October 2018 to April 2019

<table>
<thead>
<tr>
<th>Months</th>
<th>No. of samples examined</th>
<th>NO. of Samples (+ve)</th>
<th>Percentage of Infection (+ve) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>60</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>November</td>
<td>50</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>December</td>
<td>60</td>
<td>25</td>
<td>41.66</td>
</tr>
<tr>
<td>January</td>
<td>65</td>
<td>30</td>
<td>46.15</td>
</tr>
<tr>
<td>February</td>
<td>60</td>
<td>22</td>
<td>36.66</td>
</tr>
<tr>
<td>March</td>
<td>50</td>
<td>33</td>
<td>66</td>
</tr>
<tr>
<td>April</td>
<td>55</td>
<td>38</td>
<td>69.09</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>182</td>
<td>45.5</td>
</tr>
</tbody>
</table>
Table (3): Comparison between efficiency of ELISA technique in diagnosis with traditional methods

<table>
<thead>
<tr>
<th>Diagnosis methods</th>
<th>No. of samples examined</th>
<th>NO. of Samples (+ve)</th>
<th>Percentage (+ve) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flotation by Saturated sugar solution</td>
<td>90</td>
<td>55</td>
<td>61.11</td>
</tr>
<tr>
<td>Modified Ziehl-Neelson stain</td>
<td>90</td>
<td>60</td>
<td>66.66</td>
</tr>
<tr>
<td>ELISA</td>
<td>90</td>
<td>75</td>
<td>83.33</td>
</tr>
</tbody>
</table>

Table (4): Effect of clarithromycin drug on number of Cryptosporidium oocysts and sporozoites invitro.

| Mean number of C. parvum per 40 microscope fields with Clarithromycin Concentration (mg/ml) |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Samples examined                             | 4               | 8               | 16              | 32              |
| Control (+ve)                                 | 20.5±2.8        | 20.5±2.8        | 20.5±2.8        | 20.5±2.8        |
| Oocysts                                       | 19.8±3.1        | 19.5±3.3        | 17.3±3.5        | 15.4±3.9        |
| Sporozoites                                   | 20.4±2.8        | 19.7±3.1        | 17.1±3.4        | 14.9±4.0        |

Discussion

Cryptosporidiosis is one of the major diarrheal diseases caused by protozoan parasites and poses significant public health worldwide and poorly understood and many livestock farming industries as water consider of sources for zoonotic infections.

In the present study found oocysts 182 of 400 liters from water samples with total prevalence 45.5% this rate varies depending to water source with significant differences. Oocysts found about 40% in tap water provides by Al-Kufa river, as found 42% in tank houses water while oocysts appear 45%, 55% in sea of Al-Najaf and stagnant water samples respectively, this study agrees with (22) showed infection rate in the children at Al-Najaf city was 13.6% causes contamination drinking water contained on oocysts (23). As in Table1

As showed in the relation between oocysts presences and season appeared significant differences was recorded upper infection in April and March were 69.09% and 66% while decrease percentage of infection in October was 25%, this study agrees with (24) where recorded highest percentage in the spring and lowest winter, in Iraq which attributed to appropriate climatic conditions help on survival of oocysts in the environment and increased consumption of water contaminated. As in Table2

Results of the study current existence significant differences in efficiency of ELISA technique was 83.33% at detection from Cryptosporidium oocysts compared with Flotation by saturated sugar solution and Modified Ziehl-Neelson stain methods were 66.66% and 61.11% respectively, which may lead to examination of large number of samples, accuracy in reading results and shortening the duration of the diagnosis, this study agree with (25). As in Table3

Clarithromycin showed potent anthelmintic activity in vitro, crucial importance is whether the drug would penetrate into larger metacestode tissues in our in vitro experiments, by using metacestode material cut into small blocks; this possible barrier was not of concern (20). In current study showed that clarithromycin in concentrations of 16 and 32 mg/L caused decrease in number oocysts was 17.3±3.5 and 15.4±3.9, sporozoites counts was 17.1±3.4 and 14.9±4.0 compared with positive control group was 20.5±2.8 respectively, lead to significance differences in the mean numbers of oocysts and sporozoites in three replicate plates after 48 h. As in Table4
The mechanism by clarithromycin decreases the number of parasite attributed to ability inhibit protein synthesis by binding to the transpeptidation site of the larger ribosomal subunit thus is logical that these agents should also affect C.parvum by inhibiting protein synthesis (26).

There are research showed that this drug is effective against protozoa such as T. gondii, Cryptosporodium spp(27) and L. Major in vitro by action mechanism effects on microorganisms is carried out through reversibly connecting to 50S ribosomal subunits and inhibiting the protein synthesis (28).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict Of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Indirect Method for Determination of Paracetamol in Pure and Pharmaceutical Drugs using CFIA with Homemade Ayah 3S\textsubscript{BGR-X3-3D} Solar Cell Microphotometer Analyzer

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Abstract

A method is described for the determination of paracetamol (based on the formation of an orange-reddish color species by reduction of Fe(III) to Fe(II) by paracetamol and then reacted with 1,10-phenanthroline) in the range 0.001-0.09mMol.L\textsuperscript{-1} (r = 0.9801, r\textsuperscript{2} = 0.9606 , and R\textsuperscript{2} = 96.06) with a detection limit (LOD) 21.77ng/160µL(3SB)(S/N=3) and with RSD\% for 0.01 and 0.07mMol.L\textsuperscript{-1} less than 1\% (n=6). The method was applied successfully for determination of paracetamol in pharmaceuticals formulation. Analysis of drugs used the standard additions method via the individual t-test. The results showed a significant difference between the quoted value of each company with calculated t-test at 95% confidence(\(\alpha\)=0.05) from new method.

Key words: spectrophotometer, flow injection analysis, paracetamol

Introduction

Paracetamol (Acetaminophen, 4-Acetamidophenol, N-(4-hydroxyphenyl) acetamide), Tylenol), is now probably the most commonly used drug worldwide, available over the counter, used as an analgesic and antipyretic drug\textsuperscript{(1,2)}. It is preferred alternative analgesic-antipyretic to aspirin, particular in patients that could not be treat with non-steroidal anti-inflammatory drugs, such as people with pregnant or breastfeeding women, children under 12 years of age, bronchial asthma, salicylate-sensitized people, peptic ulcer disease , hemophilia. It is available in uncontaminated type as various trade-name preparations for oral use and organize combined in over 200 preparations with further drugs\textsuperscript{(3)}.

Some side effect may occur of paracetamol observed after higher doses (4g/24h), specially in the liver, so United States Food and Drug Administration (USFDA) produced it on the recorded elaborations, which will endure definite checking on the basis of data from the system on adverse reactions. The preparations containing paracetamol will be assess in term of introducing adverse skin reactions \textsuperscript{(4)}.

Several methods for determination of paracetamol have been reported such as LC\textsuperscript{(5)}, RP-HPLC\textsuperscript{(6)}, spectrophotometry\textsuperscript{(7)}, Chemiluminescence\textsuperscript{(8)}, Electrochemical detection\textsuperscript{(9-13)} and Merging zone-continuous flow injection technique\textsuperscript{(14)}. In the present study, (Ayah 3S\textsubscript{BGR-X3-3D} solar cell microphotometer analyzer\textsuperscript{(15)} was used) as a sensitive and simple method, for the determination of paracetamol via the recorded of the signal from the measurement of absorbance at 525nm green light emitted diode (LED) as a source.

Experimental

Chemicals

The following stock solutions were prepared

- Paracetamol solution, 0.05 Mol.L\textsuperscript{-1}(151.16g.Mol\textsuperscript{-1}, BDH,UK). Dissolve 1.8895g of C\textsubscript{8}H\textsubscript{9}NO\textsubscript{2} in distilled water in a 250mL volumetric flask.

- 1,10-phenanthroline, 0.1 Mol.L\textsuperscript{-1}(180.2g.Mol\textsuperscript{-1}, BDH,UK ). Dissolve 9.01g of C\textsubscript{12}H\textsubscript{8}N\textsubscript{2} in distilled water in a 500mL volumetric flask.

- Iron(III),0.2Mol.L\textsuperscript{-1} (278.0146g.Mol\textsuperscript{-1})
Dissolve a weight of 13.9007g FeSO$_4$.7H$_2$O after that the addition of 2-3 drops of concentrated H$_2$SO$_4$ followed by the addition of 80mL of H$_2$O$_2$.0.1Mol.L$^{-1}$ which is prepared and purified through passing on a cation exchanger Amberlite 120) warming the solution with constant stirring for the completion of oxidation of Fe(II) into (III), continuation of doing so until bubbles ceases for removing the excess of H$_2$O$_2$ followed by the addition 10-15mL of concentrated H$_2$SO$_4$ (in order to avoid the turbidity of the solution, during the process of oxidation of Fe(II) to Fe(III)) . The solution quantitatively transferred into a volumetric flask (250mL), the volume is completed with distilled water. 

**Apparatus**

Ayah 3S$_{BGR}$X3-3D solar cell microphotometer was used in this work as a homemade instrument that measures the absorbance of any different sample segment color which is capable of absorption in the visible region of the spectrum by uses three sources of irradiation : Blue (470nm), Green (525nm), and Red (635nm) with three solar cells as a detector at range of 450-1150nm. The instrument is composed of a block of brass having the dimensions of 20(width), 20(thickness) and 90mm(length). Flow cell with ID=2mm and OD=4mm that embedded inside a metal block. Nine source of light emitting diodes were used as an array arrangement act as emission sources at 100mm distance with three solar cell as a detector. The output of measure by solar cells was used without any further amplification of the transmitted light signal as shown in fig.1-A(X,Y-t potentiometric recorder,(Kompsono, Graph C-1032, Siemens, Germany, 1-500 volt, 1-500mV) ).

Fig.1-B shows the manifold system that uses flow system represent by peristaltic pump (An ISMATEC, Switzerland, type ISM796 peristaltic pump, two channels), six ports rotary injection valve (Six ports medium pressure (model,V-450)(upchurch, scientific INC) valve (Teflon) was used with a variables sample volumes) and (Homemade Y liquid junction point. made of poly methylmethacrylate (PMMA)).

![Figure 1:A- 3D-representation of the brass metal housing of the used LED$_s$, their location, flow cell, and the three solar cell; each collect light from one set(single colour) of LEDs](image-url)
B- Schematic diagram of flow system for the determination of paracetamol

Methodology

A manifold system used in this research consist of two line flow feed unit: first line supplies Fe(III) (0.03mMol.L\(^{-1}\) with flow rate 2.4mL.min\(^{-1}\)) which passes through injection valve carrying the paracetamol sample(0.03mMol.L\(^{-1}\) with sample volume 160µL) which lead to reduction of Fe(III) to Fe(II) then combined with the second line (1,10-phenanthroline, 0.3mMol.L\(^{-1}\) with flow rate 2.5mL.min\(^{-1}\)) at a liquid junction point (Y-junction) that will produce orange-reddish color which passes through delay reaction coil (32cm,0.25mL) to completed formation of colored species and then to Ayah 3S\(_{BGR}\)X3-3D to measure absorption process at green light emitted diode(LED) as a source. A proposed mechanism for the reaction occurred in the manifold system expressed in sketch number 1.

Sketch number 1: A proposed equation of reaction between Fe (III) and paracetamol with 1,10-phenanthroline

Results and Discussion

Chemical Variables

A spectroscopic study for colored species (orange-reddish color) was monitoring by a homemade Ayah 3S\(_{BGR}\)X3-3D solar cell CFIA microphotometer at three different super bright light diode (LED) as a source (blue 470nm, green 525 nm, and red 635 nm) using paracetamol(0.03mMol.L\(^{-1}\),130µL)- Fe(III) (0.01mMol.L\(^{-1}\))- 1,10-phenanthroline (0.07mMol.L\(^{-1}\)) system with flow rate at 2.3 and 2.4 mL.min\(^{-1}\) of the line no.1 (carrier stream) and line no.2 (reagent stream) respectively. Fig.2 shows the maximum response measured in mV was obtained using green light emitting diode so it used for the determination of paracetamol in next studies.

Effect of Fe(III) concentration

Fe(III) solutions of different concentrations were prepared (0.005-0.07mMol.L\(^{-1}\)) using physical parameters : flow rate 2.3 and 2.4 mL.min\(^{-1}\) for the Fe(III) stream and 1,10-phenanthroline(0.07mMol.L\(^{-1}\)) stream respectively , sample volume 130µL(0.03mMol.L\(^{-1}\) paracetamol) and open valve mode. The maximum response of the colored species obtained was noticed at 0.03mMol.L\(^{-1}\) of Fe(III) concentration (more than 0.03mMol.L\(^{-1}\) could due to restriction of the passage incident light due to. small. colored colloidal. precipitate.
which be formed in front of solar cells). So it was found that 0.03mMol.L\(^{-1}\) of Fe(III) was the most suitable.

**Effect of 1,10-phenanthroline concentration**

Solutions containing 0.03mMol.L\(^{-1}\) Fe(III) and 0.03mMol.L\(^{-1}\) paracetamol with different 1,10-phenanthroline concentrations(0.05-0.7mMol.L\(^{-1}\)) were prepared. It was noticed at more than 0.3mMol.L\(^{-1}\) a decrease in response which can be attributed to increase of the colored species density which work as, internal filter that really prevent the remaining light intensity, after absorption. process.so it was chosen 0.3mMol.L\(^{-1}\) of 1,10-phenanthroline concentration for optimum sensitivity and reproducibility response.

**Physical variables**

**Flow Rate**

Using chemical variation for : paracetamol(0.03mMol.L\(^{-1}\))- Fe(III)( 0.03mMol.L\(^{-1}\))-1,10-phenanthroline(0.3mMol.L\(^{-1}\)) system and physical parameters including : sample volume (130µL) and open valve mode with variation of flow rates (1.9-2.7) ,(1.9-2.7) mL.min\(^{-1}\) for Fe(III) line and 1,10-phenanthroline line respectively. A flow rate of 2.4 and 2.5 mL.min\(^{-1}\) for Fe(III) and 1,10-phenanthroline line respectively was chosen as better flow rate because it was noticed at flow, rate ranged 1.9-2.1mL.min\(^{-1}\) there is an increase in area of colored segment in front of detector (increase in \(\Delta t_B\) (base width) versus peak height and dilution factor) while at higher flow rate (>2.4 and 2.5 mL.min\(^{-1}\) for Fe(III) and 1,10-phenanthroline stream respectively) the response is not very high because departure of reactant from measuring cell before to completion of reduction Fe(III) to Fe(II) (decrease time in front of detector and \(\Delta t_B\))

**Sample Volume**

Different sample volume (50-200µL) with fixing all previous experimental parameters (chemical and physical parameters) with 525nm as a source for irradiation were used. The results shows that increase in sample loop, led to an increase in S/N and \(\Delta t_B\) (base width) up to 160µL (above 160µL, there were a broadening at the peak height and \(\Delta t_B\)) so as a comparison between \(\Delta t_B\) , peak height and sensitivity , 160 µL could be used as best sample volume for the determination of paracetamol in next studies.

**Purge Time**

The purge time (time for purging of the paracetamol segment from the six port injection valve) was carried out in this work(5-30sec). It was noticed from results obtained that open valve mode at 30 sec was chosen as most suitable purge time for the totally departure of colored species from the injection valve to obtain better response for determination of paracetamol.

**Effect of reaction coil length**

Variation coil length (0-57cm) was used for the study of effect the reaction delay coil (after Y-junction point) on S/N peak profile , \(\Delta t_B\) and concentration of paracetamol after dilution . It was noticed that best coil length for the homogenization and completion of the reduction Fe(III) to Fe(II) with suitable base width (\(\Delta t_B\) , decrease dilution effect and regular response was 32cm(0.25mL).

**Preparation of calibration graph**

Various concentrations (0.001-0.09mMol.L\(^{-1}\)) of paracetamol was prepared using the parameters achieved above. Each measurement was repeated three times. Regression analysis gave r (correlation coefficient ) 0.9801, r\(^2\) (coefficient of determination ) 0.9606 , and R\(^2\)% (linearity percentage ) 96.06 as tabulated in table 1.

**Limit of detection and repeatability**

Gradual dilution of low concentration in the calibration graph and depend on the linear equation(numeric value of slope) was used for the calculated of detection limit. The repeatability studied for using Ayah 3S\(_{BGR}\)X3-3D analyser by paracetamol-Fe(III)- 1,10-phenanthroline system at concentration 0.01 and 0.07mMol.L\(^{-1}\). All calculated obtained was tabulated in table 1.
## Table 1: Summary of calibration graph, detection limit and repeatability results for the determination of paracetamol using Ayah 3S<sub>BGR</sub>X3-3D solar cell CFI analyser

<table>
<thead>
<tr>
<th>Linear range of [paracetamol] mMol.L&lt;sup&gt;-1&lt;/sup&gt; (n=10)</th>
<th>Ŷ&lt;sub&gt;mv&lt;/sub&gt;=a±S&lt;sub&gt;a&lt;/sub&gt;t+b±S&lt;sub&gt;b&lt;/sub&gt;t[x] mMol.L&lt;sup&gt;-1&lt;/sup&gt; at confidence level 95%, n-2</th>
<th></th>
<th>Detection limit</th>
<th>Repeatability at 95% confidence level, n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001-0.09</td>
<td>0.9801 0.9606 96.06</td>
<td>2.306&lt;&lt; 13.96</td>
<td>(9x10&lt;sup&gt;-5&lt;/sup&gt;)</td>
<td>5.19 μg/160μL</td>
</tr>
</tbody>
</table>

### Application

Ayah 3S<sub>BGR</sub> X3-3D solar cell CFI analyser was used for the analysis of paracetamol using paracetamol- Fe(III)-1,10-phenanathroline system in the different drug manufactures (SDI-Iraq, Advance- United Kingdom, and Kernadol, Kern pharma-Espain). A series of solutions were prepared of each paracetamol drug (20mMol.L<sup>-1</sup>) (0.3023g) by transferring 0.01mL to each 10mL volumetric flask, followed by the addition of step wise volumes (0.0, 0.01, 0.02, 0.03, 0.04 mL from 10mMol.L<sup>-1</sup> standard solution) to obtain 0.0-0.04 mMol.L<sup>-1</sup>. Table 2 shows all results obtained of standard calibration graph, practically content of paracetamol in each sample of drug and efficiency of determination using developed method at confidence level 95%.

## Table 2: Summed up of standard addition results, practical found values and efficiency determination

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Commercial name and company country</th>
<th>95% confidence interval for the average weight W&lt;sub&gt;i&lt;/sub&gt;±1.96 σ&lt;sub&gt;n&lt;/sub&gt;/√n (g)</th>
<th>Sample weight equivalent to 0.3023g (0.02 MoL.L&lt;sup&gt;-1&lt;/sup&gt;) of the active ingredient (g)</th>
<th>Theoretical content of the active ingredient at 95% (mg)</th>
<th>Equation of standard addition at 95% for n-2 Ŷ&lt;sub&gt;mv&lt;/sub&gt;=a±S&lt;sub&gt;a&lt;/sub&gt;t+b±S&lt;sub&gt;b&lt;/sub&gt;t x R&lt;sup&gt;2&lt;/sup&gt; %</th>
<th>Practical content W&lt;sub&gt;i&lt;/sub&gt;±4.303σ&lt;sub&gt;n&lt;/sub&gt;/√n (mg) for (n=3) at 95%</th>
<th>Efficiency of determination (Rec. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SDI-Iraq</td>
<td>0.1213±0.0058</td>
<td>0.0733</td>
<td>500±23.91</td>
<td>501.8±52.22±233.02 [paracetamol]mMol.L&lt;sup&gt;-1&lt;/sup&gt; 0.9988, 0.9975, 99.75%</td>
<td>537.6±3.45</td>
<td>107.5</td>
</tr>
<tr>
<td>2</td>
<td>Advance United Kingdom</td>
<td>0.1482±0.0532</td>
<td>0.0896</td>
<td>500±179.5</td>
<td>328.0±140.80±17100±5748.19 [paracetamol]mMol.L&lt;sup&gt;-1&lt;/sup&gt; 0.9837, 0.9467, 96.76%</td>
<td>479.9±2.82</td>
<td>95.9</td>
</tr>
<tr>
<td>3</td>
<td>Kernadol, Kern pharma Espain</td>
<td>0.1023±0.0495</td>
<td>0.0619</td>
<td>500±241.94</td>
<td>668±103.00±31600±4205.39 [paracetamol] mMol.L&lt;sup&gt;-1&lt;/sup&gt; 0.9974, 0.9948, 99.48%</td>
<td>527.0±3.54</td>
<td>105.4</td>
</tr>
</tbody>
</table>
The standard addition method in this research work was put into individual t-test between x (mean) and quoted value, (500mg). There is a significant difference between claimed value of each company with t calculated at α=0.05 (95% confidence interval).

Conclusion

The method in this research work described for the determination of paracetamol by reduction of Fe(III) to Fe(II) and then combination with 1,10-phenanthroline to formation orange-reddish colored species. Homemade Ayah 3S BGR X3-3D solar cell CFI analyser was used for the measurement of absorbance at 525nm green light emitted diode (LED)as a source. The new technique was applied successfully. for the determination of sample (paracetamol) in three different drugs.

Acknowledgement: I would like to express my deepest gratitude to Prof. Dr. Issam M.A.Shakir Al-Hashimi for his appreciable advice, important comments, support and encouragement.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

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Expression and Conservation of *rol*-genes in Rue, *Ruta graveolens* L., Plants Regeneration from Hairy Roots

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Abstract

In this investigation hairy roots were induced on leave petioles of *Ruta graveolens* L. plants. These roots were white in color and negative geotropism. Paper electrophoresis of hairy roots extract resulted in the separation of black spots of agropine. These transformed roots were cured from *Agrobacterium rhizogenes*, some cultures of these roots were directly produced shoots, and other was orientated to produce callus. Subsequently this callus was capable to produce numerous regenerates. Both groups of shoots were rooted easily and successfully adapted to field conditions. Evidences of molecular biology assessment, including isolation and amplification(PCR) of DNA of each transformed tissues and plants coupled with DNA electrophoresis data proved the transfer of *rol*-genes, except *rol B*, in the regenerated plants.

Conclusion: The genetic modified *R. graveolens* plants possess unique characters due to the conservation of *rol*-genes in their genomes.

**Key words:** *Ruta graveolens*, *Agrobacterium rhizogenes* R1601, Agropine, PCR, *rol*-genes.

Introduction

A recent study reported that *Ruta graveolens* L. plants reacted easily in culture, acceptable to many *in vitro* techniques and possess high regeneration capability (¹). Also plants were regenerated from leaf segments derived callus (²) and from axillary shoot multiplication (³). It is well-known that this medicinal plant is rich with different industrial plant products, such as alkaloids, coumarine, flavonoids (⁴) and rutin (⁵). The present study was designed whether *Agrobacterium rhizogenes* R1601, the natural vector, is efficient to create genetically modified tissues and whether genetically engineered plants can produced from them. In such studies experiments involved to demonstrated the transfer of T-DNA, synthesis of agropine, isolation of genomic-DNA of plant, amplification plant DNA by PCR coupled with gel electrophoresis of the amplified DNA (⁶). The aim of this research is to specify and confirm which *rol*-gene(s) was transferred and expressed.

Materials and Method

Preparation of bacterial suspension.

Agropine type *Agrobacterium rhizogenes* R1601 harboring Kana<sup>Res</sup> and Carb<sup>Res</sup> genes, as genetic markers, on Ri-plasmid was supplied from Prof. E.G. Nester(Washington university, U.S.A.). This strain was grown in agar solidified APM medium (⁷), provided with 100 mg.L<sup>-1</sup> of each Kanamycin and carbencillin. Agrobacterial suspension was prepared using single colony used to inoculate 50 ml of liquid APM medium supplied with the same above antibiotics. Cultures were kept on rotary shaker 100 rpm for 72 h. Inoculum was harvested and the precipitated bacterial cells was resuspended in 10 ml liquid APM. Optical density was adjusted to 1.90 (⁸).

Direct injection of leaf petioles with *A. rhizogenes* R1601.

Leaves petioles of 3.0 cm length were excised from two years old Rue ( *Ruta graveolens* L.) plants, washed with water and surface sterilized through soaking in the sterilizing solution consist of 1:2 (v:v) of sodium hypochlorite NaOCl: water for 15 min.. Explants were washed thoroughly with sterilized water three times 5 min./each time (¹). The tip needle tip was immersed into the inoculum and directly inject the top ends of the sterilized petioles in 2-3 sites. Each inoculated petiole was
cultured in vertical position into 25 ml of agar solidified MSO medium contained in 100 ml glass vessel. Hairy roots developed on inoculated samples were excised and transferred to agar solidified MSO medium containing gradual conc. of Cefotaxime to eliminate the habitant agrobacterium, as previously reported\(^9\).

Detection of agropin in hairy roots by paper electrophoresis.

Samples of bacterial-free young hairy roots were cut from their cultures and grinded in eppendorf tubes in the presence of 100 µl 0.1N HCl. The mixture was centrifuged at 6000 rpm for 20 min., volumes of 30 µL of each clarified sample and of standard agropin were spotted on the chromatography paper as described\(^10\).

Plant regeneration from transformed tissues.

Cluster of young hairy roots were transferred to 20 ml of agar solidified MS medium. Friable callus of white-yellow color was developed. Subsequently this callus was differentiated producing number of shoots. In other cultures shoots were produced from the green-tips of hairy roots. Both types of shoots were excised and each shoot was plunged vertically in the rooting medium MS+0.5 mg.L\(^{-1}\) IBA\(^11^,12\). All shoots were readily rooted, and adapted using peat-moss medium.

Conservation of \textit{rol-genes} in genomic DNA of transformed plant.

This involved intensive laboratory work including:

\textbf{Isolation of DNA:} The extraction solution CTAB (Cetyltrimethyl Ammonium Bromide) was used for DNA isolation from transformed plant\(^13\). Purity and concentrations of the isolated DNA were determined at 260 and 280 ηm\(^14\).

\textbf{Gel electrophoresis of the amplified isolated DNA:} One gram of agarose (Vivantis LE grade Agrose, USA) was dissolved in 100 ml of IX TBE solution. The melted agarose at 40°C was carefully poured in the tray. Gel layer thickness was 8 mm and of dimension 8.5×10cm. After complete solidification of gel the plastic comb was removed carefully, and the layer was put in the tank (Electrophorasis MS MINI U.K.) in the presence of 250 ml of the extraction solution 1X TBE. The loading samples was each consisted of 8.0µL of DNA mixed with 2.0 µL of loading buffer. One of the terminal well was loaded with 3.0 µL of the ladder λ DNA 50 bp DNA, total size 1350pb. At the end of samples loading electrical current of 50 volt for 90 min. was pass through. Then, remove the layer and transfer to dish containing 500 ml of ethidium bromid stain solution of final conc. 0.5 mg.ml\(^{-1}\) for 40min.. Finally examine the layer by UV light of 300 ηm to visualize the separated bands.

\textbf{Polymerase Chain Reaction test:} To detect the putative transfer of \textit{rol-gene(s)}, from T-DNA and their integration into plant genomic DNA the specific primers mentioned below (Table 1) used.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
primers & Primers sequences & MW. (bp) & suppliers \\
& \((3' \quad 5')\) & & \\
\hline
Kana.\textsubscript{res+} & F: TAGTCTCTGTATCGCAACCGA \\
& R: TAGTCTCGTCTGCTAACAGA & 593 & Geneaid Biotech Ltd. \\
\hline
rol A & F: ATTAAGGTAAGACTTTGTAC \\
& R: CCTAAATTATGCGTGCACCGA & 450 & \\
\hline
rol B & F: GTTAAACCTTACAGTGAACACT \\
& R: TTCGATTTCGCTGACCTT & 850 & \\
\hline
rol C & F: TGTGTCGCTAGGAAGTCCGTA \\
& R: ACGTCTAAAGGTTAGCCGATT & 650 & \\
\hline
rol D & F: AGCGTTTCAACATTACCGCGTA \\
& R: GCTACCTTGTCGCTGAATT & 1000 & \\
\hline
\end{tabular}
\end{table}
The reaction mixture was prepared in premix tubes containing 5.0 µl of the mixture (Taq DNA polymerase + dNTPs + Tris-HCl + KCl + MgCl₂ buffer X10). To this added 1.0 µl of the specific essential primer of conc. 10 Becamol Microliter -1. Similar volume and conc. of the reverse primers and of the temple DNA at conc. 50 ng µl -1. Complete the final volume to 20 µl by addition 9.0 µl of distilled, deionized sterilized water. Tubes were inserted into the thermal polymer to begin the amplification reaction following the same conditions previously mentioned (15).

### Results

- Establishment of cured hairy roots cultures.

The response of petiole explant to direct injection by *A. rhizogenes* R1601 resulted in the formation of adventitious roots on both inoculated and non-inoculated sites (Table 2)

#### Table 2: Induction of hairy roots on leaf petiole explants of *R. graveolens* to direct injection by *A. rhizogenes* R1601.

<table>
<thead>
<tr>
<th>Explants</th>
<th>Mean No. of explants inoculated : Responded</th>
<th>Hairy roots Mean No. : length(cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated</td>
<td>16:9</td>
<td>2.75 : 1.5</td>
</tr>
<tr>
<td>Non-inoculated (control)</td>
<td>16 : 0</td>
<td>0 : 0</td>
</tr>
</tbody>
</table>

Hairy roots began to form as tiny bodies (Fig. 1: A), developed to white and fine hairy roots (Fig. 1: B). These hairy roots were excised as cluster or single root. They cultured on agar-solidified MS medium and were negatively geotropism as growing producing mass of these hairy roots. They grew rapidly on the surface of solid MSO medium (Fig. 1: C), whereas samples of excised normal roots failed to continuing their growth. Data indicated that gradual transfer of hairy roots culture three times on 100, 200 and 300 mg.l⁻¹ of cefotaxine led to the production of bacterial free culture of hairy roots (Fig 1: D). Overall these results considered the first signs of transformation of these roots.

![Fig. 1: Production of “agropine-positive” transformed hairy root of *R. graveolens* by *A. rhizogenes* R1601. (A): Formation of small bodies (arrowed) on inoculated petioles. (B): Development of single hairy roots (arrowed) from bodies in (A). (C): Cluster of hairy roots grew on the surface of agar-solidified MSO medium. (D): Development of hairy roots in (C) note their negative geotropism growth.](image-url)
Detection of the synthesized agropine.

Examination of electrophorctogram proved the separation of agropine that synthesized in these hairy roots. The black spots (approx. 2.0 cm diam.) were corresponded in positions and diam. to spots of standard agropine. This result represent an additional proof that this type of roots and their derived callus are genetically transformed by agropine type *A. rhizogenes* R1601 used in this study.

**Regeneration of plant from transformed tissues.**

The results revealed that culture of ‘agropine positive’ hairy roots of *Ruta graveolens* grown on solid hormone-free MS medium express their totipotency and producing number of shoots. Other cultures of hairy root were orientated to the spontaneous formation of callus, Subsequently, this type of callus acquired the green color and numerous shoots were differentiated.

The results indicate that shoots regeneration from bud developed on hairy root culture (Fig. 2. A), also regenerated directly from hairy roots (Fig. 2. B), and from hairy-root derived callus (Fig. 2. C,D). All types of shoots were readily rooted in agar-solidified MS medium supplemented with 0.5 mg L\(^{-1}\) IBA (Fig. 2. E). Plantlets were adapted and successfully acclimatized to field condition (Fig. 2. E). As they continuing growth clear morphological variations were found comprised the leave size, flower number, shape and flowering period.

**Fig. 2: Regeneration of genetically transformed *Ruta graveolens* L. from “agropine-positive” hairy roots induced by *Agrobacterium rhizogenes* 1601.**

(A): Shoot bud(arrowed) emergence hairy root grown on solid MSO medium start producing shoots (B): Direct shoot regeneration(arrowed) from hairy roots grown on MSO. (C): Spontaneous callus(arrowed) formation from hairy root grown on MSO. (D): Indirect shoot regeneration(arrowed) from callus grown on MSO. (E): Rooting of shoots(arrowed) in (A), in MS medium provided with 0.5 mg.L\(^{-1}\) IBA. (F): Transfer and acclimatized plants to field conditions.

**Concentrations and purity of DNA.**

Data showed that concentrations of DNA of transformed plants regenerated directly from transformed hairy roots and callus was exceed the conc. of DNA isolated from normal callus and plants (Table 3).
Table 3: Concentrations and purity of genomic DNA of *Ruta graveolens* L. plants transformed by *Agrobacterium rhizogenes* 1601.

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>Concentration (ng/µl)</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves of transformed plant</td>
<td>102.9</td>
<td>1.94</td>
</tr>
<tr>
<td>Leaves of normal plant</td>
<td>33.7</td>
<td>1.95</td>
</tr>
<tr>
<td>Roots of normal plant</td>
<td>82.3</td>
<td>1.87</td>
</tr>
</tbody>
</table>

Detection of *rol*-genes in genomic DNA of transformed *Ruta graveolens* plant.

Examination of electrophoresis gel layer of PCR products showed the separation of single band of amplified DNA of callus produced from transformed hairy roots. Its M.wt. was equal to the M.wt. of the specific primers for each of Kana \( \text{Res}^+ \) *rol A, rol B, rol C, and rol D* genes as single bands. The separation of these bands confirmed the presence of the responsible genes in cells genome of transformed hairy root, callus and plants regenerated from this type of callus. In the mean time the absence of such bands of amplified DNA isolated from non-transformed callus and plants donate an additional evidence in this aspects (Fig.3).

Fig. 3: Gel electrophoresis showed the presence of Kana \( \text{Res}^+ \), *rol A, rol B, rol C and rol D* genes in amplified genomic DNA isolated from leaves of genetically transformed *Ruta graveolens* L. by *Agrobacterium rhizogenes* R1601.

Lane (M): Lambda DNA.

Lane (1, 4): amplified DNA isolated from seedling leaves.

Lane (2, 5): amplified DNA isolated from leaves of genetically transform plant.

Lane (3, 6): amplified DNA isolated from seedling roots.

Those results strongly ensured that transformed hairy roots and callus conserved Kana \( \text{Res}^+ \) and *rol*-genes group except *rol B*. This indicate their transfer from the bacterial vector to genome of the recipient cells. The
expression of $Kana^{\text{Res}^+}$ gene enable hairy root derived callus to continue growth on MS medium contained with kanamycin. Whereas expression of $rol$-genes encouraged synthesis of agropine and the development of some variations on plant morphology.

**Discussion**

The production of genetically modified Rue, *Ruta graveolens* L., plant via *A. rhizogenes* 1601 mediated transformation probably explained the capability of this bacterial vector the rapid transformation of many plant species such as *Vigna radiate*\(^{(12)}\), tomato and potato\(^{(16)}\). In this study data proved the successful interaction between *A. rhizogenes* and petioles leaf, and the incidence of hairy roots pointed out the first sign of biotransformation\(^{(17)}\). During this stage acetosyringone compound release induce the transfer of T-DNA to plant cells\(^{(18)}\). Additionally, this compound stimulate T-DNA genes, of Ri-plasmids, to fuse with cell genome producing a genetically transformed clone\(^{(19)}\). This is supported the spontaneous induction of callus\(^{(20)}\) on hormone –free MS medium as occurred with *Solanum nigrum*\(^{(21)}\) and *Rubia akane* L.\(^{(22)}\) plants. Bacteria was eradicated by the use of cefotaxime antibiotic which recommended by several studies\(^{(15)}\). The expected explanation of agropine synthesis in hairy roots and its callus emphasized that $rol$ $D$ gene controlled it’s synthesis when *Agrobacterium*-plant functional interaction only take place\(^{(23)}\). In the present work production of genetically- transformed Rue plants from “agropine-positive” tissues is an expected status. At the same time it represented an advanced interested phenomenon. The expectation was not to obtain high transformation frequency (TF) as occurred with carrot\(^{(15)}\) plants.

**Acknowledgement:** It is a pleasure to acknowledge the staff members working in Biotechnology unit, Dept. of biology at Education College for Pure sciences, Univ. of Mosul for their assistance.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

**References**


Assessment of Bioactive Resin-Modified Glass Ionomer Restorative as a New CAD/CAM Material. Part I: Marginal Fitness Study

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Abstract

The objective of this in vitro study was to evaluate and compare the marginal fitness of monolithic crowns fabricated from a newly developed bioactive CAD/CAM resin block and reinforced resin CAD/CAM block pre- and post-cementation with adhesive and self-adhesive resin cements. Bioactive CAD/CAM block were fabricated from ACTIVA BioACTIVE-RESTORATIVE (Pulpdent Corporation, USA) using a clear rectangular Teflon mold. Thirty-two human maxillary first premolar teeth were prepared to receive full crowns then divided into two main groups of 16 teeth each according to the type of block used to fabricate the crowns: Group A: crowns fabricated from the bioactive resin block, Group B: crowns fabricated from reinforced composite block (BRILLIANT Crios, Coltene). Each group was then subdivided into two subgroups according to the type of resin cement used for cementation, Subgroups (A1, B1): RelyX Ultimate cement, Subgroups (A2, B2): ACTIVA BioACTIVE-cement. The prepared teeth were scanned using CEREC Omnicam digital intra-oral and the crowns were then designed using CEREC Premium software (version 4.4.4) and milled using CEREC MC XL milling unit. The marginal gap of each crown was measured before cementation at four points on each tooth surface using a digital microscope at a magnification of 230x. Each crown was then cemented on its respective tooth according to the manufacturers’ instructions of each cement, and the marginal gap was measured again at the same aforementioned points. The results of this study showed that the marginal gap of all groups are below the clinically acceptable limit. Meanwhile, the marginal gap of the crowns fabricated from both block types increased significantly after cementation with both types of cement. Pre-cementation, student’s t-test revealed that there is no statistically significant difference in the marginal gap of crowns fabricated from both block types (p > 0.05). Post-cementation, a statistically highly significant difference was seen between both block types with both types of cement (p < 0.01). From the results of this study, the newly developed bioactive resin block seems a promising material for CAD/CAM applications in terms of marginal fitness.

Keywords: resin block, ACTIVA BioACTIVE, BRILLIANT Crios, CAD/CAM, marginal gap.

Introduction

Marginal adaptation is a vital factor for long-term longevity and clinical success of dental restorations⁰¹,². Marginal discrepancies may lead to cement exposure to oral fluids, resulting in marginal microleakage and luting agent dissolution with percolation of bacteria, food and oral debris, potentially causing secondary caries, and periodontal disease³,⁴.

Numerous restorative materials for CAD/CAM systems differing in chemical structure and indications are now available on the dental market⁵. However, ceramic material has many disadvantages such as fragility, requirement of excess time for fabrication and abrasive effect. Additionally, low modulus of elasticity of ceramic material makes it unable to absorb the pressure of mastication. These disadvantages of ceramics have led to increased interest in resin composites CAD/CAM blocks⁶,⁷. Their exceptional machinability, edge stability and reduced brittleness relieve some of the drawbacks of ceramic CAD/CAM blocks.
Bioactive materials have been used in almost all fields of dentistry. However, bioactive materials have not yet been implied in the field of CAD/CAM materials. ACTIVA BioACTIVE-RESTORATIVE material is a resin-modified glass ionomer that delivers all the advantages of glass ionomers in a strong, resilient, resin matrix. As claimed by manufacturer, it chemically bonds to teeth, releases more calcium, phosphate and fluoride and is more bioactive than glass ionomers. Moreover, this material is claimed to be more durable and fracture resistant than resin composites. ACTIVA material contains a rubberized resin component that makes them tougher and more resistant to fracture and chipping than composites. It doesn’t contain Bisphenol A, Bis-GMA and BPAdervatives.

These positive characteristics of ACTIVA material encouraged the idea of developing a bioactive resin block for CAD/CAM applications that can release favorable ions and stimulate apatite formation in response to pH changes in the oral cavity.

The aim of this study was to assess the marginal fitness of crowns fabricated from the newly developed bioactive CAD/CAM blocks and compared with those fabricated from one of the commercially available resin blocks (BRILLIANT Crios) pre-and post-cementation with adhesive and self-adhesive resin cements.

**Materials and Method**

Bioactive resin blocks were fabricated from ACTIVA BioACTIVE-RESTORATIVE (Pulpdent Corporation, USA) using a clear rectangular Teflon mold. The internal dimensions of the Teflon mold size are in accordance with size 14 CAD/CAM block. The material was injected directly in the mold using disposable auto-mixing tips. A celluloid strip was then placed on the top surface of the mold and a glass slab was placed above. A 500 gm weight was placed above the glass slab to release air bubbles and remove excess material. A period of 30-seconds was waited to allow for the self-curing reaction to start. The mold was then placed in the chamber of UV light curing box (Vertex Eco Light Box, Netherlands, Holland) for 10-minutes. The block material was then separated from the mold by unscrewing of the base of the mold. A metal holder specially fabricated for this study was then fixed to the fabricated block.

Thirty-two sound human maxillary first premolar teeth extracted for orthodontic purposes were selected. Each tooth received tooth preparation for full crown in accordance with the guidelines of tooth preparation for full crown made from resin block. The prepared teeth were divided into two main groups of 16-teeth each according to the type of block used: Group A (study group): crowns fabricated from the bioactive resin block, Group B (control group): crowns fabricated from the reinforced composite block (BRILLIANT Crios, Coltene/ Whaledent AG, Switzerland). Each group was further subdivided into two subgroups of 8 teeth each according to the type of cement used for the cementation of the crowns: 

- **Subgroup 1**: crowns cemented with adhesive resin cement (RelyX Ultimate, 3M ESPE, USA),
- **Subgroup 2**: Crowns cemented with self-adhesive bioactive resin cement (ACTIVA BioACTIVE-Cement, Pulpdent Corporation, USA). The prepared teeth were scanned using CEREC Omnicam digital intra-oral scanner. Crowns were designed using Sirona inLab CAD SW 15.1 and milled using inLab MC XL machine. Each crown was then seated on its respective tooth under a standard static load of 5 Kg. The vertical marginal gap of each crown was then measured using a digital microscope at a magnification of 230x. Measurement was done at four points predetermined on each surface of specimen using image-J software. Sixteen measurements were obtained for each specimen and the mean of these measurements was taken which represented the pre-cementation marginal gap. Each crown was then cemented on its respective tooth with either type of cement after surface treatment of the internal surface of the crown and the tooth following the manufacturers’ instructions of the two types of cement. Crowns of both groups were sandblasted with 50 μm aluminum oxide. For group A, the internal surface was etched with phosphoric acid gel. For group B, ONE COAT 7 UNIVERSAL light-cured dental adhesive (Coltene/ Whaledent AG, Switzerland) was applied to the internal surface of the restoration according to the manufacturer’s instructions. Teeth of subgroups A1 and B1 (cemented with RelyX Ultimate cement) were etched with phosphoric acid gel for 15 seconds, rinsed and dried, then Single Bond Universal Adhesive (3M ESPE, USA) was applied according to the manufacturer’s instructions. On the other hand, teeth of subgroups A2 and B2 (cemented with ACTIVA cement) didn’t receive any surface treatment prior to cementation. The internal surface of each crown was loaded with either type of cement and each crown was seated on its respective tooth using a dental surveyor under a constant static load of 5 Kg, followed by light curing for 20 seconds per surface.
after removal of the excess cement. The specimen was then removed and kept on bench for one hour and then stored in distilled water for 24 hours (13). Marginal gap was then measured again at the same points used for pre-cementation measurement. The recorded measurements were then statistically analyzed using students’ t-test at level of significance of 0.05.

**Results**

The descriptive statistics of the vertical marginal gap in μm of the two groups and their subgroups pre- and post-cementation are shown in Table (1) and Figure (1).

**Table (1): Descriptive statistics of the marginal gap (in μm) of the different groups between blocks pre- and post-cementation.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-cementation</th>
<th>Post-cementation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cement Block</td>
<td>Min.</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>RelyX Ultimate</td>
<td></td>
</tr>
<tr>
<td>ACTIVA</td>
<td>39.130</td>
<td>60.861</td>
</tr>
<tr>
<td>Crios</td>
<td>33.330</td>
<td>55.260</td>
</tr>
<tr>
<td>ACTIVA</td>
<td>36.940</td>
<td>62.300</td>
</tr>
<tr>
<td>Crios</td>
<td>32.989</td>
<td>55.247</td>
</tr>
<tr>
<td>Total</td>
<td>ACTIVA</td>
<td>36.940</td>
</tr>
<tr>
<td>Crios</td>
<td>32.989</td>
<td>55.260</td>
</tr>
</tbody>
</table>

From this table and bar-chart, it can be seen the lowest mean of marginal gap was recorded by subgroup A2, in which crowns were fabricated from ACTIVA block and cemented with ACTIVA cement (49.718), while the highest mean of marginal gap was recorded by subgroup B1, in which crowns were fabricated from Crios block and cemented with RelyX Ultimate cement (85.925). On the other hand, there was an overall increase in the marginal gap of all groups post-cementation.

The comparisons between the marginal gaps of both groups pre-cementation and post-cementation using student’s t-test at a level of significance of 0.05 are shown in Tables (2) and (3), respectively.
Table (2): Student’s $t$-test for comparison of significance of the marginal gap of the two groups pre-cementation.

<table>
<thead>
<tr>
<th>Block type</th>
<th>Mean</th>
<th>SD</th>
<th>$t$</th>
<th>df</th>
<th>$p$  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>45.145</td>
<td>7.100</td>
<td>0.095</td>
<td>14</td>
<td>0.926 NS</td>
</tr>
<tr>
<td>B1</td>
<td>45.501</td>
<td>7.955</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>43.883</td>
<td>9.671</td>
<td>0.612</td>
<td>14</td>
<td>0.550 NS</td>
</tr>
<tr>
<td>B2</td>
<td>41.336</td>
<td>6.704</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTIVA</td>
<td>44.514</td>
<td>8.222</td>
<td>0.395</td>
<td>30</td>
<td>0.695 NS</td>
</tr>
<tr>
<td>Crios</td>
<td>43.419</td>
<td>7.425</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Student’s $t$-test for comparison of significance of the marginal gap of the two groups post-cementation.

<table>
<thead>
<tr>
<th>Cement</th>
<th>Block type</th>
<th>Mean</th>
<th>SD</th>
<th>$t$</th>
<th>df</th>
<th>$p$  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rely X Ultimate</td>
<td>A1</td>
<td>63.331</td>
<td>15.123</td>
<td>3.122</td>
<td>14</td>
<td>.008 HS</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>85.925</td>
<td>13.798</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTIVA</td>
<td>A2</td>
<td>49.718</td>
<td>7.708</td>
<td>3.962</td>
<td>14</td>
<td>.001 HS</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>75.890</td>
<td>17.021</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>ACTIVA</td>
<td>56.524</td>
<td>13.560</td>
<td>4.678</td>
<td>30</td>
<td>.000 HS</td>
</tr>
<tr>
<td></td>
<td>Crios</td>
<td>80.907</td>
<td>15.840</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From these two tables, it can be seen there was no statistically significant difference between the two block types pre-cementation ($P > 0.05$), while statistically highly significant differences were seen post-cementation ($P < 0.01$).

On the other hand, further comparison for the effect of type of cement on the marginal gap of two block types was done.

Table (4): Student’s $t$-test for comparison of significance of the effect of type of cement on the marginal gap of two block types.

<table>
<thead>
<tr>
<th>Block type</th>
<th>Cement</th>
<th>Mean</th>
<th>SD</th>
<th>$t$</th>
<th>df</th>
<th>$p$  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTIVA</td>
<td>A1</td>
<td>63.331</td>
<td>15.123</td>
<td>2.268</td>
<td>14</td>
<td>.040 S</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>49.718</td>
<td>7.708</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crios</td>
<td>B1</td>
<td>85.925</td>
<td>13.798</td>
<td>1.295</td>
<td>14</td>
<td>.216 NS</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>75.890</td>
<td>17.021</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The use of the ACTIVA cement resulted in significantly less mean marginal gap of crowns fabricated from the bioactive block than the RelyX Ultimate cement ($p < 0.05$).
Discussion

The vertical marginal gap measurement is the most frequently used method to quantify the accuracy of fit of the restorations (14). It is well-agreed among studies that marginal gaps less than 120 μm are considered clinically acceptable (15-17).

The results of this in vitro study showed that the marginal gaps of all groups pre- and post-cementation were below the clinically acceptable limit.

Before cementation, the statistically non-significant differences in the marginal gap between the two block types could be attributed to the standardized process in the fabrication of crowns (including standardized tooth preparation, scanning, designing, and milling), and the relative similarity in the basic composition of both block types as they belong to the same category of all-ceramic materials which is resin composite block. However, evaluating the marginal gap of crowns without luting them on their respective teeth is not reflective of clinical reality because the cement and cementation process play a relevant role in the final discrepancy achieved (18).

In this study, two types of resin cements that differ in their chemical composition and adhesive strategies (adhesive and self-adhesive) were selected.

After cementation, there was an increase in the marginal gaps of crowns fabricated from both block types and cemented with the two types of cements. This result is in agreement with other studies that found that the cementation procedure causes a significant increase in the vertical marginal discrepancy (18-20). This might be due to the hydraulic pressure developed during cementation that is going to push the cement upward.

In the designing software of CAD/CAM system, the marginal spacer was set “zero”, while the radial and occlusal spacer was set “100μm” starting 1mm above the finishing line. Therefore, when the crown carrying the cement is placed on the prepared tooth and pressure was applied, as the crown approaches the final position, there is no space for escapement of cement through the cervical marginal collar, resulting in great amount of luting cement to be accumulated on the occlusal surface of the prepared tooth that might interfere with proper seating of crown restoration, increasing the vertical marginal discrepancy (21).

The results of this study showed that the marginal gap of crowns fabricated from ACTIVA block and cemented with both types of cement were lower than those of crowns fabricated from Crios block with statistically highly significant differences. This could be attributed to that the cementation of restorations fabricated from Crios requires the application of one coat 7 universal adhesive to the inner surface of the restoration prior to cementation after sandblasting according to manufacturer’s recommendations, owing to the high degree of polymerization of these blocks. This forms an additional interfacial layer between the restoration and the tooth that might interfere with adaptation of the restoration.

Concerning the type of cement, ACTIVA BioACTIVE self-adhesive cement exhibited less increase in the marginal gap with both block types than the RelyX Ultimte adhesive cement. This may be due to the difference in the bonding strategy between the adhesive and self-adhesive cements. Cementation of indirect restorations with self-adhesive cements reduces the number of application steps as compared to adhesive cements that require multiple interfacial bonding layers that may compromise adaptability. Additionally, it has been found that light curing of the adhesive prior to placement of resin cement produces thicker adhesive film thickness that may interfere with the seating of the restorations (21).

Conclusions

1. Crowns fabricated from the bioactive resin blocks showed better marginal fitness post-cementation than those fabricated from Crios blocks regardless of the type of cement used.

2. The marginal gaps of crowns fabricated from both block types increased significantly post-cementation.

3. Crowns cemented with the self-adhesive cement showed better marginal fitness than those cemented with adhesive cement irrespective of the type of block material.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding
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Study Genotoxicity of Ciprofloxacin in white rats

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Abstract

The genotoxic and cytotoxic effects of ciprofloxacin drug on the bone marrow of white male rats were assessed by the Micronucleus test (MN) in polychromatic Erythrocytes (PCEs). The DNA damage was assessed by the Comet assay technique in bone marrow, liver and spleen. The white male rats were administered by oral gavage of single doses (93.5), (187) mg.kg\(^{-1}\) of body weight with twice a day for 7 consecutive days, while the negative control group was administered distilled water. The results showed a significant increase in the number of micronuclei corresponding to the duration of exposure, there was a change in the percentage of immature erythrocytes in bone marrow. This increased with an increase in treatment duration. As for the number of immature erythrocytes containing micronuclei, there was a significant increase corresponding to the treatment duration. When using the comet assay technique, the values of DNA damage increased in a dose-related manner.

Keywords : Ciprofloxacin, Comet Assay, Micronucleus.

Introduction

Ciprofloxacin (CFX) is a broad spectrum antibiotic belonging to the fluoroquinolone family \([1]\). It is regarded as the second generation of this family and has a wide spectrum and efficacy compared to other types within this family. Ciprofloxacin is considered the most commonly known antibiotic, used to treat many bacterial infections in the respiratory tract, skin, bones, joints, urinary tract infections and diarrhea \([2]\).

It is an antibiotic that kills bacteria by altering the efficiency and function of the DNA gyrase enzyme, which is responsible for bacterial DNA vas damage. This inhibition of the DNA gyrase efficiency causes rapid bacterial death \([3, 4]\).

Test design:

5 rats for MMC positive control, 5 rats for negative control distilled water, 5 rats for each treatment with doses of 93.5 and 187 mg.kg\(^{-1}\) of body weight.

Micronucleus Assay of immature erythrocytes in the male bone marrow of white male rats.

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B: polychromatic Erythrocyte (PCE) containing a micronuclei from the bone marrow of a rat of the group treated with ciprofloxacin drug (CFX) (May- Grunwald + Giemsa) X100

<table>
<thead>
<tr>
<th>Number of Micronuclei Mean % ± S.E</th>
<th>Number of polychromatic erythrocytes with Micronuclei Mean % ± S.E</th>
<th>Percentage of polychromatic erythrocytes PCE, (%)</th>
<th>No of animals</th>
<th>Treatment Dosage Mg.kg⁻¹ body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.800 ±0.860</td>
<td>4.400±0.400</td>
<td>9.84</td>
<td>5</td>
<td>Negative control</td>
</tr>
<tr>
<td>124.40 ±5.22**</td>
<td>81.20± 2.92 **</td>
<td>18.93</td>
<td>5</td>
<td>Mitomycin-c 1</td>
</tr>
<tr>
<td>21.40±1.50**</td>
<td>14.80±1.07**</td>
<td>11.13</td>
<td>5</td>
<td>Ciprofloxacin 93.5</td>
</tr>
<tr>
<td><strong>4.91±49.60</strong></td>
<td>32.60±2.91**</td>
<td>11.22</td>
<td>5</td>
<td>Ciprofloxacin 187</td>
</tr>
</tbody>
</table>

Table (1) shows the micronuclei in the bone marrow of white male rats after treatment with different doses of CFX, negative control and positive control (MMC)

* Significance at the probability level P≤ 0.05 (t-test)

** Significance at the probability level P≤ 0.01 (t-test)

This test was used in the current study to assess the genotoxicity and cytotoxicity of ciprofloxacin drug (CFX). The table shows an increase in the percentage of polychromatic erythrocytes (PCEs) in bone marrow of white rats at treatment with dose 93.5 mg.kg⁻¹ of body weight by 11.13%. Treatment with a dose of 187 mg.kg⁻¹ of body weight gave a slight increase by 11.22%, and these are results which show a little change from the negative control of 9.84. This indicates that the abovementioned drug may have a cytotoxicity. It also indicates to the effect of the mentioned two doses of the drug in stimulating the cells generating erythrocytes to increase division in order to produce more cells and replace lost cells as a result of the toxic effect of the drug in the treated animal. As for the positive control, it was the largest percentage compared to the negative control and treatment with a percentage at 18.93%. The results of the table showed that the arithmetic mean of micronuclei with the dose 187 mg.kg⁻¹ of body weight was (4.91 ± 49.60) which is of significance compared to negative control (0.860 ± 6.800) and the arithmetic mean with the dose 93.5 mg.kg⁻¹ of body weight was (21.40 ± 1.50) which is of significance compared to negative control 0.860 ± 6.800. This indicates that the drug may be genotoxic because it caused an increase in the frequency of micronuclei, and this increase is related to the increase in the concentration of the drug.

The results of the table showed that the arithmetic mean of polychromatic erythrocytes containing micronuclei when treated with the dose 187 mg.kg⁻¹ of body weight was of significance at (2.91 ± 32.60) while the treatment with the dose 93.5 mg.kg⁻¹ of body weight was of less significance with (14.80 ± 1.07) compared to negative control of (4.400 ± 0.400). This leads to the assumption of genotoxicity of the drug by its ability to develop micronuclei in polychromatic erythrocytes. These results are consistent with studies conducted to assess and measure the genotoxicity of the drug [7-9].

It is possible that the mechanism for the formation of micronuclei by ciprofloxacin drug in the bone marrow of treated rats is that the drug inhibits Topoisomerase II enzyme in the treated rats. Ciprofloxacin is known to be anti-bacterial by inhibiting DNA gyrase. And this enzyme is not present in eukaryotic cells but may be functionally and precipitatively connected to Topoisomerase II enzyme for eukaryotic cells [10]. The Topoisomerase II and DNA gyrase are essential for life, as they play a role in the formation of active chromatin synthesis, limiting strand twisting and dismantles of interlocking strands during DNA replication, and contributing to chromosome depletion during mitosis and chromosomal separation during the anaphase [11]. Inhibition of the Topoisomerase
II enzyme prolongs the metaphase and interferes with the dissociation of sister chromatids in the anaphase, but does not prevent the cell from that causing chromosomal abnormalities and indivisibility, thus leading to the formation of fine micronuclei \[12\]. The micronucleus test is widely used in many exploratory research to understand the potential underlying mechanism of genotoxicity. Its simplicity and applicability to a wide range of cell types inside and outside the body makes it a flexible tool for understanding the keys to genotoxicity and its effect on humans. The micronucleus test has shown to be one of the preferred methods for estimating chromosomal damage since it has been able to measure both total chromosomal loss or chromosomal fractions in a reliable manner because only micronuclei can be expressed in cells that have completed nuclear division. There is a hypothesis stating that micronuclei can have predictive value for cancer, as well as genetic mutations, and therefore replace chromosomal aberrations as a life indicator of cancer risk \[13\].

![Image](image.png)

**Figure (2) A:** Estimating the induction of DNA damage in bone marrow, spleen and liver cells of male white rats using the Comet assay estimation technique. **B:** shows the natural DNA of a cell from the negative control group (S.G) 10X **C:** shows abnormal DNA from the treatment group (CFX) (S.G) 10x

The figures show different levels of DNA damage in cells of both the negative control group and the Ciprofloxacin treatment group. The table clarifies the percentages of cells with damage, mean values, standard error in the bone marrow DNA of the white rats treated with the drug, and negative control and positive control (MMC)

**Table (2):** Percentage of cells with damage and levels of damage in the DNA of bone marrow of white rats treated with the drug, and negative control and positive control (MMC)

<table>
<thead>
<tr>
<th>Cells with damaged DNA Mean % ± S.E</th>
<th>Percentage of cells with damage</th>
<th>Total number of cells examined</th>
<th>No. of Animals</th>
<th>Treatment Dosage Mg.kg⁻¹ body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.510 ± 2.400</td>
<td>2.4</td>
<td>500</td>
<td>5</td>
<td>Negative control</td>
</tr>
<tr>
<td>3.39± 31.40**</td>
<td>31.4</td>
<td>500</td>
<td>5</td>
<td>Mitomycin-c 1</td>
</tr>
<tr>
<td>2.03± 13.20 **</td>
<td>13.4</td>
<td>500</td>
<td>5</td>
<td>Ciprofloxacin 93.5</td>
</tr>
<tr>
<td>19.80± 2.92 **</td>
<td>19.8</td>
<td>500</td>
<td>5</td>
<td>Ciprofloxacin 187</td>
</tr>
</tbody>
</table>

* Significance at the probability level P≤ 0.05 (t-test)

** Significance at the probability level P≤ 0.01 (t-test)
Table (3): Percentage of cells with damage, levels of damage in the spleen DNA of white rats treated with the drug, and negative control and positive control (MMC).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage Mg.kg⁻¹ body weight</th>
<th>No. of Animals</th>
<th>Total number of cells examined</th>
<th>Percentage of cells with damage</th>
<th>Mean % ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>2.4</td>
<td>0.510 ± 2.400</td>
</tr>
<tr>
<td>Mitomycin-c 1</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>33.2</td>
<td>2.08 ± 33.20**</td>
</tr>
<tr>
<td>Ciprofloxacin 93.5</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>14</td>
<td>2.51 ± 14.00 **</td>
</tr>
<tr>
<td>Ciprofloxacin 187</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>21.2</td>
<td>21.20 ± 2.58 **</td>
</tr>
</tbody>
</table>

* Significance at the probability level P≤ 0.05 (t-test)

** Significance at the probability level P≤ 0.01 (t-test)

Table (4): Percentage of cells with damage, levels of damage in the liver DNA of white rats treated with the drug, and negative control and positive control (MMC).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage Mg.kg⁻¹ body weight</th>
<th>No. of Animals</th>
<th>Total number of cells examined</th>
<th>Percentage of cells with damage</th>
<th>Mean % ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>3.6</td>
<td>0.510 ± 3.600</td>
</tr>
<tr>
<td>Mitomycin-c 1</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>35.8</td>
<td>6.23 ± 35.80**</td>
</tr>
<tr>
<td>Ciprofloxacin 93.5</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>16.2</td>
<td>16.20 ± 1.02 **</td>
</tr>
<tr>
<td>Ciprofloxacin 187</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>23.4</td>
<td>23.40 ± 2.54 **</td>
</tr>
</tbody>
</table>

* Significance at the probability level P≤ 0.05 (t-test)

** Significance at the probability level P≤ 0.01 (t-test)

Single Cell Gel Electrophoresis technique or Comet Assay technique is a direct method for identifying DNA damage in interphase cells. The tail represents the amount of damage done in DNA. This technique is particularly sensitive in the recognition of individual sequence fractures, and the locations of repair by cutting in single cells compared to conventional ways of identifying DNA damage. It is useful to show DNA damage in a single cell. It is a direct, sensitive, simple, fast, and effective technique used in studies of genotoxicology. It has been generally assumed that SCGE technique, which is conducted under alkaline conditions, recognizes the individual sequence fractions and the location of the alkalines in DNA [14]. The ability of DNA to migrate depends on the size and number of fractures in the molecule as a result of a particular factor [15].

The comet estimation technique requires high-level viability cells approximating 95%, and low levels of DNA damage were found in the negative control group during the experiment.

The results of Table (2) show the percentage of cells with comet tails that represent the affected DNA cells for both negative and positive control and treatment
groups with dose 93.5 and 187 mg.kg\(^{-1}\) body weight of ciprofloxacin in bone marrow cells. The results showed an increase in percentages of damaged DNA cells and the two groups treated with 187, 93.5 mg.kg\(^{-1}\) body weight respectively.

The results of the table also show mean values of DNA damage in bone marrow cells of white male rats. The results clarified that the treatment with the dose 187 mg.kg\(^{-1}\) of body weight was significant with (19.80 ± 2.92) compared with the negative control (0.510 ± 2.400), while the treatment with the dose 93.5 mg.kg\(^{-1}\) of body weight was significantly less than (2.03 ± 13.20) compared to the negative control of (0.510 ± 2.400).

The results of Table (3) show the percentage of cells with comet tails that represent the affected DNA cells of both negative and positive control and that treated with doses 93.5 and 187 mg.kg\(^{-1}\) of body weight with ciprofloxacin drug in spleen cells. The results show an increase in the percentage of damaged DNA cells for the positive control group (treated with MMC drug) and the two groups treated with the drug 187, 93.5 mg.kg\(^{-1}\) of body weight respectively.

The results of Table (4) show the mean values of DNA damage in the spleen cells of white male rats. The results clarify that the treatment with dose of 187 mg.kg\(^{-1}\) of body weight was significant with (21.20 ± 2.58) compared to negative control with (0.66 ± 3.20), while the dose treatment of 93.5 mg.kg\(^{-1}\) body weight was less significant and reached 2.51 ± 14.00 compared to negative control with (0.510 ± 3.600). These values are higher than those of DNA in bone marrow and spleen. This means that both the percentage of cells with comet tails and damage to the liver cell DNA are higher than in bone marrow and spleen cells. This may be due to the fact that the detoxification device is present in the liver which is a chromosomal device and is part of the defense system of the body. Most of the material is metabolized inside the liver, therefore, it converts it by introducing chemical changes so that it can be destroyed and get rid of its poisonous effect and highlighted in several operations as a useless metabolic material. This drug is partially metabolized in the liver by modifying the pepirazinel group into at least four metabolites. These metabolites, identified as diethylene ciprofloxacin, sulfo-ciprofloxacin, oxo-ciprofloxacin and cyprofloxacin N-acetyl, show a weaker microbiological activity than the initial form of the drug, but a similar or stronger activity for some other quinolones [16].

The results of the tables for MMC treatment were significant for liver cell DNA, and bone marrow and spleen cells DNA. Rjiba et al. have shown in their study that the effect of MMC drug was high [17]. The DNA damage caused by CFX drug may be due to its ability to release free radicals (void of oxygen) [18]. The oxygen free radicals attack the DNA causing mutations [19].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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References


Thirteen New Yeast Strains Isolated from Cancer Patients in Basrah-Iraq by ITS rDNA Sequencing

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¹Basrah University, College of Science, Ecology Department/Iraq, ²Southern Technical University, Basrah Technical institute, Health community department/Iraq, ³Basrah University, College of Science, Biology Department/Iraq

Abstract

Yeast isolates were grown on CHR OMagar Candida medium to detect different types of the yeast species, and then the unidentified isolates were diagnosed by using molecular analysis of ITS region. From a total of 54 yeast isolates, 37 isolates appeared as different species of the genus Candida, the rest of species belong to Hanseniaspora, Kazachstania, Kluyveromyces, Magnusiomyces, Pichia and Saccharomyces. 13 yeasts species in this study reported as new strains in Gen Bank, also species belong to C. pronicula, H. uvarum, K. exigua, K. marxianus, M. capitatus, Magnusiomyces sp., P. kudriavzevii, P. manshurica and S. bayanus x S. cerevisiae isolated for the first time in Iraq. We concluded that there are new emergent species of the yeasts inhabit the oral cavity of cancer patients undergoing chemotherapy, and the results showed that the molecular method provide a good approach for the identification.

Key words: yeasts, cancer patient, molecular identification.

Introduction

Certain fungi, notably some of Candida species, are inhabited the oral cavity as a commensals, but the alteration of mouth environment with certain virulence determinants lead to overgrowth of fungal species causing oral infections. The incidence of oral candidiasis has been reported to be ranging from 7-52% among patients with different types of malignancies (1,2).

Cancer patients undergoing chemotherapy treatment are prone to higher risk for fungal infections because of the host immunosuppression as the action of these drugs, the identification of the causative agent requires rapid and accurate methods to identify the pathogen for guiding appropriate therapy. Molecular identification of fungal pathogens often involves polymerase chain reaction (PCR) for ITS1-5.8S-ITS2 rDNA gene (3). The non-coding complex ITS region is evolved more rapidly and variable among different species within a genus, therefore it is appropriate for taxonomy and identification than the coding and conserved 5.8S rDNA gene. ITS region has typically been most useful for molecular systematics at the species and strains level. (4,5).

The present study was conducted to detection and discrimination of the yeasts at the strains level, isolated from oral cavity of cancer patients, by using molecular genetic analysis of ITS region.

Materials and Method

500 samples were obtained from the lining of the oral cavity of patients (195 males and 305 females) with different types of cancer, were submitted to chemotherapy at Al-Sader Teaching Hospital-Basrah, Iraq, in the period between December 2014 to February 2015.

The yeast isolates were identified by CHRO Magar Candida medium to distinguish between the different species by type and color of the colonies (6).

From the total isolates which identified by conventional methods, there were 54 isolates their identification still uncertain at the species level, so we used the molecular methods for the purpose of accurate identification.

According to (7), the DNA extraction and PCR amplification has been accomplished, to amplify the internal transcribed spacer regions (ITS) of ribosomal
DNA with a 19 base forward primer, ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and a 20 base reverse primer, ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The result of PCR technique yielded distinctive products size of approximately 400-850 bp. The purification and protocol of PCR products for 54 samples were carried at MACROGEN Company http://dna.macrogen.com. All the alignments were observed by Clustal Omega www.clustal.org/omega/ and identified using Blast program.

Results

The amplified ITS regions were appeared in all 54 yeast isolates accurately. The identification of gene sequences showed 15 yeast species belong to 7 genera (Table 1).

Table 1: Genus and species of the yeasts identified by ITS1- 5.8S-ITS2 rDNA gene sequencing

<table>
<thead>
<tr>
<th>No.</th>
<th>Genus</th>
<th>Species</th>
<th>Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida</td>
<td>Albicans</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Dubliniensis</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glabrata</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parapsilosis</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prunicola</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tropicalis</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Hanseniaspora</td>
<td>Uvarum</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Kazachstania</td>
<td>Exigua</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Kluyveromyces</td>
<td>Marxianus</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Magnusiomyces</td>
<td>sp.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Capitatus</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Pichia</td>
<td>Kudriavzevii</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Manshurica</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Saccharomyces</td>
<td>bayanus X S. cerevisiae</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Cerevisiae</td>
<td>4</td>
</tr>
</tbody>
</table>

Out of 54 different isolates identified by PCR sequencing there were 13 isolates reported and published as new strains in Gen Bank (NCBI) (Table 2).

Table 2: Comparison between conventional and molecular identification for the yeast isolates

<table>
<thead>
<tr>
<th>No.</th>
<th>No. of isolate</th>
<th>Species</th>
<th>Conventional identification</th>
<th>Molecular identification</th>
<th>Similarity %</th>
<th>Name of new strain</th>
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</table>
Table 2: Comparison between conventional and molecular identification for the yeast isolates

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<th>Yeast 2</th>
<th>Method 1</th>
<th>Method 2</th>
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<td>Saccharomyces cerevisiae</td>
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<td>Untreated</td>
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</tbody>
</table>
Discussion

The accurate and timely species identification is important for clinical management of patients because the yeast species have different antifungal susceptibilities, that is why the choice of the ITS1-5.8S-ITS2 rDNA gene sequencing for discrimination of the species is important (8,9,10).

The universal primers ITS1 and ITS4 were used for amplifying ITS1 and ITS2 regions in yeast species, these primers showed high efficiency appeared in the identification of 13 new yeast strains, some of them have been isolated and reported for the first time from the oral cavity of cancer patients such as C. prunicola, K. exigua, K. marxianus and P. manshurica that could be due to the changes of the mouth environment and/or influence of chemotherapy drugs which acts as immunosuppressive then alteration the lining of oral cavity that upset the healthy balance of the oral microorganisms allowing to fungal infection (11,12).

Also, to our knowledge, different uncommon yeasts were isolated as new record species from oral cavity for the first time in Iraq: C. pronicula, H. uvarum, K. exigua, K. marxianus , M. capitatus, Magnusiomyces sp., P. kudriavzevii, P. manshurica and S. bayanus x S. cerevisiae, this appearance could be due to the suppression of the host immune system as a result of the cancer disease, use of chemotherapy drugs which suppressed oral/mucosal immunity, salivary gland dysfunction and alteration in oral flora leading to change the quantity and quality of saliva that encourage infection by opportunistic fungi as well as lack of physical activity and a high carbohydrate diet (2,13,14).

This identification were agreed with previous studies of (1,9,15,16), which isolated different species of Candida based on ITS region sequencing. The present study showed similar results to those observed from (17), who reported six of Candida species from cancer patients.

H. uvarum is considered as opportunistic yeast and its occurrence in clinical isolates is unusual, but in this study, the appearance of this species in the oral cavity is considered the first recording as a result of malignancies and chemotherapy impact. It was first isolated in Spain by (18) from the oral cavity of a 70 year old women with lesions produced by the dentures, and they suggested that the source of infection was associated to handling and consumption of raw fish (19).

Although K. marxianus is less frequently in clinical samples but its prevalence has increased over the past decade. The anamorph form of this species (C. kefyr) is an emerging pathogen with hematological malignancies (11), while in current study the result was not consistent with that previous study because two strains of K. marxianus were isolated from the oral cavity of females with breast cancer.

C. prunicola, P. manshurica and S. cerevisiae were isolated from clinical samples by (20), but to our knowledge, the present study considered the first work to isolate them from the oral cavity of cancer patients and identification them through molecular methods.

In spite of the teleomorph species K. exigua is unlikely to be human pathogen because it did not grow at 37°C but in this study it was isolated for the first time from oral cavity. The fungal sexually reproduction in human body have a higher enzymatic activity and thus greater the virulence of the host tissues, that explains the increased appearance of teleomorph conditions in clinical samples (22).

P. kudriavzevii, which a teleomorph state of C. krusei, is abundant in the environment and mainly associated with food spoilage. Many studies have shown that P. kudriavzevii is the 5th most common cause of candidemia in immunocompromised patients (23). To our knowledge, this study consider the first work that isolated the teleomorph condition of C. krusei from oral cavity of cancer patients.

Conclusions

Many yeasts species showed a high similar phenotypic features, so molecular methods by using amplification of ITS1-5.8S-ITS2 rDNA gene provide a good alternative approach for the identification of pathogenic yeasts.
Acknowledgement: We are grateful to the staff at Al-Sader Teaching Hospital for their assistance us to collect oral samples.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References


Evaluation of Effectiveness of *Boswelliaserrata* (Kundur) Gum Extract on Some Biochemical Parameters in Male Rats Induced Chronic Renal Failure (CRF)

Ahmed Neema AL-Mosawy¹, Saatar Jasim Hatroosh¹
¹Department of Biology, College of Education for Pure Science, University of Karbala, Iraq

Abstract

**Objective:** The study was to test water extract of Kundur by treating chronic renal failure that induced by Cadmium chloride CdCl₂.

**Method:** CdCl₂ at a concentration of 5 mg /kg body wt./day of rat at chronic level with drinking water for six weeks as animal model. Measuring some predicate biomarkers functional common kidney was done serum creatinine and novel biomarker measured KIM-1 and NAGL, Cystatin C, demonstrate that a regular oral intake of CdCl₂ solution (in drinking water) for 45 days.

**Results:** There is significant increase of Serum Urea, creatinine, KIM-1, NAGL and Cystatin C, when compared with control group, also indicate there is significant increase in serum biomarkers for kidney injury. After the treatment with water extract of Kundur by different concentrations 10, 20, and 30 mg/kg all these Urea, Scr., KIM-1, NAGL and Cystatin C were significantly decrease when compared with CdCl₂ group, compared with control group and after the treatment the damages were not found or minimize. At this exposure of CdCl₂.

**Conclusion:** The biomarkers KIM-1, NAGL and Cystatin C may be good predictive indicator of chronic renal failure, plant extract may be having a highly effect for treating the renal failure and showed highly response to treatment.

**Keywords:** Kundur, renal failure, KIM-1, NAGL and Cystatin C

Introduction

Changes in renal function are one of the most common manifestations of severe illness. Their importance is reflected in the routine physiological and biochemical monitoring of kidney function via urine output measures and blood laboratory measurement in critically ill patients. Chronic kidney disease is associated with high morbidity and mortality. The kidney contains many metabolizing enzymes and plays a central role in metabolizing drugs and foreign compounds in the body. Different studies persist to discover new biomarkers that are identification the kidney disease, and understanding the intensity and development of renal failure when examination noninvasively in urine and blood. The biomarker includes Cystatin C, KIM-1, NGAL. KIM-1 is a type I transmembrane glycoprotein which is not found in normal kidneys. The elevation in releasing of this protein was found at highly levels on the proximal tubule cells specially on the apical membrane after nephrotoxic or ischemic injury, and in rats it is widely induced in the tubules after ischemic or toxic injury. KIM-1 is released into the circulation where kidney injury in rodents and humans with CRF.

Cystatin C is a 13 kDa cysteine protease inhibitor that is produced by cells throughout the body. Cystatin C is abundant in serum/plasma and is readily filtered at the glomerulus. Serum levels of cystatin C have been proposed as a useful marker to estimate glomerular filtration rate. Unlike creatinine, cystatin C is not secreted by the proximal tubule. Instead, filtered cystatin

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C is taken up through a megalin-dependent process and then completely catabolized in proximal tubule epithelial cells. Serum levels of cystatin C are much less likely to be affected by factors such as gender, metabolic status or disease states than are levels of creatinine. Cystatin C has recently received some attention as a potential biomarker of Cd nephrotoxicity those studies focused primarily on the measurement of serum levels of cystatin C as an indicator of glomerular function and they yielded equivocal and somewhat conflicting results. Most of these studies did not include data on the possible effects of Cd on the urinary excretion of cystatin C. Medicinal plants and their derived bioactive phytochemicals have been gaining recognition in the treatment of neurological diseases. Kundur is an oleo-gum resin of Kundur. Various pharmacologically active chemical constituents were isolated from Kundur. Kundur is exhibit antifungal. Anti-inflammatory, Nephroprotective Activity and Antimicrobial Activity and (polyuria).

Method

Al-Kundur gum was obtained from Al-Razi Center for Alternative Medicine, Baghdad, Iraq the gum plant was identified by the National Herb in Abu Ghraib, Baghdad. The gum powder was moistened with 1:5 water (50 g of powder was mixed with 250 ml of distilled water). A horizontal shaker was used for 30 minutes, the sample was left stationary, then filtered using filter paper 3 times. Centrifuge (3000 rpm) was then used for 15 minutes. Concentrate the extract using rotary evaporator, and dry at 45 °C in the oven. The water extract was used in the study of animals.

Experimental design and treatments: Thirty adult male Rattus norvegicus weighing 200-240 g were used in the present study. The animals were obtained from the animal house in the biology department of College of Science of Thiqar University and were housed in well-aerated cages at normal atmospheric temperature and normal 12-hour light/dark cycle. The animals had free access to water and a standard diet of known composition. All efforts were done to reduce the number and suffering of animals and all animal procedures were in accordance with the recommendations of the animal ethics committee of Karbala University. The experimental animals were divided into five groups as follows:

- **Group I** positive control group: Induced renal failure by cadmium chloride (15 animals) 5 animals per week.

- **Group III** Treatment group first (T1): group of animals induced renal failure and treated with a water extract of gum Kundur 10 mg / kg body weight and the number of 5 animals per week.

- **Group IV** Treatment group Second (T2): group of animals induced renal failure and treated with a water extract of gum Kundur 20 mg / kg body weight and the number of 5 animals per week.

- **Group V** Treatment group third (T3): group of animals induced renal failure and treated with a water extract of gum Kundur 30 mg / kg body weight and the number of 5 animals per week.

At the end of the experiment, rats were sacrificed under mild anesthesia and blood samples were obtained for serum preparation. Kidneys were rapidly excised and immediately perfused with ice-cold saline. Samples from the kidney were homogenized in cold phosphate buffered saline (10% w/v), centrifuged and clear homogenate was separated and stored at -20°C. studies parameter was measured according to the instruction of the Elabscience company.

Statistical Analysis

Statistical analysis was performed using SPSS (v.25). Results were expressed as mean ± standard deviation (SD) and all statistical comparisons were made by means of the one-way analysis of variance (ANOVA) test followed by Tukey’s test post hoc analysis. (P value less than 0.05 was considered significant).

Results

Serum Urea & Creatinine levels for male rat

The results of the development of experimental chronic renal failure in laboratory animals by cadmium chloride Cdcl2, shown in Table 1(&2), show that serum urea & creatinine levels in male rats in the form of mean ± SD mean, Had a significant difference at a probability level of P< 0.05 in the cadmium chloride dosage group compared to the control group. It was found that the treatment of animals induced in chronic renal failure with the water extract of bitter gum and the dose of
10, 20, and 30 mg/kg and at a rate of once a day for (first, second and third week) at a value of P≤ 0.05 levels of serum Urea & Creatinine compared to the cadmium chloride group, but the level of decline did not reach the level in the control group. It was also shown from the table that the dosage period had an effect on serum Urea & Creatinine levels for male rats. The difference was statistically significant at P≤ 0.05 after (first, second, and third week) of the dosage of water extract of Kundurgum after six weeks of chronic renal failure.

Serum levels of KIM-1, NAGL, and Cystatin C for male rats

Table 3,4,5 showed that the development of experimental chronic renal failure in experimental animals led to an increase in serum of KIM-1, NAGL, and Cystatin C levels respectively, in the treated group with cadmium chloride compared to the control group. It was found that treatment of chronic renal failure patients with water extract of myrrh gum and dose 10, 20, (P <0.05) at KIM-1, NAGL, and Cystatin C levels compared with the control group, showed a significant effect (P <0.05) on KIM-1, NAGL, and Cystatin C in the serum of male rats where the decrease was significant (P <0.05) after (first week, second and third) of the dosage of the water extract of the gum compared with six weeks after Chronic renal failure.

Table (1) Effect of water extract of Kunduron the level of Urea mg/dlin male rats’ serum compared to control Mean± Standard deviation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Control</th>
<th>Cdcl2</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>30 mg/kg</th>
<th>LSD groups</th>
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<tbody>
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<td></td>
</tr>
<tr>
<td></td>
<td>First week</td>
<td>23.08±3.05</td>
<td>97.51±1.73</td>
<td>77.65±1.72</td>
<td>50.80±5.01</td>
<td>47.94±9.11</td>
<td>6.16</td>
</tr>
<tr>
<td></td>
<td>Second week</td>
<td>26.56±0.88</td>
<td>95.54±2.02</td>
<td>74.16±2.26</td>
<td>51.57±1.74</td>
<td>45.44±3.35</td>
<td>2.73</td>
</tr>
<tr>
<td></td>
<td>Third week</td>
<td>22.10±0.63</td>
<td>96.65±1.84</td>
<td>72.07±0.93</td>
<td>50.85±4.40</td>
<td>38.78±1.46</td>
<td>2.83</td>
</tr>
<tr>
<td></td>
<td>Mean of time</td>
<td>23.91±2.63</td>
<td>96.57±1.92</td>
<td>74.63±2.87</td>
<td>51.07±3.70</td>
<td>44.05±6.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LSD time</td>
<td>2.31</td>
<td>2.31</td>
<td>2.14</td>
<td>4.93</td>
<td>7.03</td>
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<tr>
<td></td>
<td>P.value extraction</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Kundur</td>
<td>LSD extraction</td>
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<td>0.00</td>
<td>0.00</td>
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</table>

Table (2) Effect of water extract of Kunduron the level of Creatinine mg/dlin male rats’ serum compared to control Mean± Standard deviation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Control</th>
<th>Cdcl2</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>30 mg/kg</th>
<th>LSD groups</th>
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<tr>
<td></td>
<td>First week</td>
<td>0.70±0.13</td>
<td>3.82±0.41</td>
<td>3.57±0.64</td>
<td>3.06±0.56</td>
<td>2.74±0.36</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Second week</td>
<td>0.71±0.10</td>
<td>3.89±0.55</td>
<td>3.23±0.56</td>
<td>2.76±0.54</td>
<td>2.37±0.61</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Third week</td>
<td>0.73±0.10</td>
<td>4.13±0.19</td>
<td>2.87±0.47</td>
<td>2.54±0.69</td>
<td>2.06±0.37</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Mean of time</td>
<td>0.71±0.10</td>
<td>3.94±0.40</td>
<td>3.22±0.60</td>
<td>2.78±0.60</td>
<td>2.39±0.52</td>
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<tr>
<td></td>
<td>LSD time</td>
<td>0.14</td>
<td>0.51</td>
<td>0.7</td>
<td>0.74</td>
<td>0.57</td>
<td></td>
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<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Kundur</td>
<td>LSD extraction</td>
<td>0.45</td>
<td>0.00</td>
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Table (3) Effect of water extract of Kunduron the level of KIM-1 pg/ml in male rats’ serum compared to control Mean± Standard deviation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Mean± S.D.</th>
<th>Control</th>
<th>Cddc2</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>30 mg/kg</th>
<th>LSD groups</th>
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<tr>
<td></td>
<td>First week</td>
<td>113.60±10.24</td>
<td>806.20±51.84</td>
<td>433.20±95.67</td>
<td>345.20±54.86</td>
<td>310.00±72.40</td>
<td>78.78</td>
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</tr>
<tr>
<td></td>
<td>Second week</td>
<td>111.40±7.30</td>
<td>808.40±74.77</td>
<td>389.40±46.12</td>
<td>326.20±73.58</td>
<td>290.20±60.07</td>
<td>71.84</td>
<td></td>
</tr>
<tr>
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<td>Third week</td>
<td>108.80±5.17</td>
<td>812.80±23.32</td>
<td>342.80±85.20</td>
<td>281.60±63.07</td>
<td>255.20±85.46</td>
<td>76.64</td>
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</tr>
<tr>
<td></td>
<td>Mean of time</td>
<td>111.27±7.54</td>
<td>809.13±50.28</td>
<td>388.47±82.20</td>
<td>317.67±65.61</td>
<td>285.13±71.87</td>
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<tr>
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<td>LSD time</td>
<td>9.73</td>
<td>67.22</td>
<td>97.44</td>
<td>79.7</td>
<td>90.96</td>
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<tr>
<td>Kundur</td>
<td>P.value extraction</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td></td>
<td>LSD extraction</td>
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</table>

Table (4) Effect of water extract of Kunduron the level of NAGL pg/ml in male rats’ serum compared to control Mean± Standard deviation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Mean± S.D.</th>
<th>Control</th>
<th>Cddc2</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>30 mg/kg</th>
<th>LSD groups</th>
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<tr>
<td></td>
<td>First week</td>
<td>1100.6±180.4</td>
<td>1964.7±98.8</td>
<td>1415.6±49.1</td>
<td>1222.7±103.6</td>
<td>1182.2±58.1</td>
<td>134.45</td>
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<tr>
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<td>Second week</td>
<td>1039.9±110.8</td>
<td>1984.6±69.4</td>
<td>1396.8±38.0</td>
<td>1190.9±153.9</td>
<td>1171.6±113.0</td>
<td>129.99</td>
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<tr>
<td></td>
<td>Third week</td>
<td>1026.4±117.0</td>
<td>2046.6±134.7</td>
<td>1358.4±62.3</td>
<td>1178.6±15.9</td>
<td>1117.0±41.2</td>
<td>107.6</td>
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</tr>
<tr>
<td></td>
<td>Mean of time</td>
<td>1055.6±133.5</td>
<td>1998.7±103.2</td>
<td>1390.3±53.1</td>
<td>1197.4±101.4</td>
<td>1156.9±77.3</td>
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<tr>
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<td>LSD time</td>
<td>173.12</td>
<td>129.48</td>
<td>62.93</td>
<td>133.26</td>
<td>95.61</td>
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<td>Kundur</td>
<td>P.value extraction</td>
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<tr>
<td></td>
<td>LSD extraction</td>
<td>0.99</td>
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</table>

Table (5) Effect of water extract of Kunduron the level of Cystatin C pg/ml in male rats’ serum compared to control Mean± Standard deviation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Mean± S.D.</th>
<th>Control</th>
<th>Cddc2</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>30 mg/kg</th>
<th>LSD groups</th>
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<tr>
<td></td>
<td>First week</td>
<td>87.44±8.26</td>
<td>195.34±14.22</td>
<td>156.28±8.04</td>
<td>142.41±14.75</td>
<td>143.15±13.23</td>
<td>14.95</td>
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<tr>
<td></td>
<td>Second week</td>
<td>89.77±10.36</td>
<td>193.52±12.19</td>
<td>144.40±8.40</td>
<td>140.77±14.55</td>
<td>135.93±10.68</td>
<td>14.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third week</td>
<td>91.30±8.22</td>
<td>201.64±11.65</td>
<td>147.75±9.85</td>
<td>142.33±9.94</td>
<td>138.36±10.27</td>
<td>12.45</td>
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</tr>
<tr>
<td></td>
<td>Mean of time</td>
<td>89.50±8.49</td>
<td>196.83±12.33</td>
<td>149.48±9.65</td>
<td>141.84±12.31</td>
<td>139.15±11.06</td>
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<tr>
<td></td>
<td>LSD time</td>
<td>11.16</td>
<td>15.79</td>
<td>10.9</td>
<td>16.44</td>
<td>14.22</td>
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<tr>
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<td>P.value extraction</td>
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<tr>
<td></td>
<td>LSD extraction</td>
<td>0.82</td>
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</table>
Discussion

Urea assessment in rats’ groups of induced chronic renal failure; Cdcl2, Control, treated with water extract at doses 10, 20 and 30 mg/kg.

The renoprotective activity shown by Kundur and its MS (Methanol soluble) fraction against Gentamicininduced nephrotoxicity during current study, may be attributed to the chemical constituents of Kundur having antioxidative potential. During the experiment it is fully justified that Kundur possesses renoprotective effect and further studies are warranted to explore itsmechanism of action (12).

Creatinine assessment in rats’ groups of induced chronic renal failure; Cdcl2, Control, treated with water extract at doses 10, 20 and 30 mg/kg.

Our current findings indicate that exposure to cadmium chloride has led to kidney failure, which is the significant increase in Creatinine and Urea concentration in the groups treated with cadmium chloride in the first and second weeks compared to control groups. Creatinine concentration shown significantly elevated in adenine group by comparisonwith the control group, this our data agrees with (17) in rat, that found, significant increase in serum creatinine and urea concentration by comparison with control group. It could therefore be suggested that the phytochemical constituents of aqueous stem bark extract of *Boswellia papyrifera* such as alkaloids, tannins, flavonoids, saponins and cardiac glycosides may be responsible for the nephrocurative effect of the aqueous stem bark extract of the plant. They are super antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anti-cancer activity and protects against all stages of carcinogens.

Kidney injury molecule -1 concentration in serum of male rats induced chronic renal failure treated with the water extract 10, 20 and 30 mg/kg.

Explanation could be that the physiological stress of renal dysfunction, lead to non-renal cells, expressing KIM-1 to shed this molecule into the circulation. Another possibility is that with renal decline, KIM-1 is less efficiently scavenge from the circulation. Patients with the lowest eGFR have the highest plasma KIM-1 concentrations (18).

The concentration of KIM-1 after treatment with tomato powder at dose 10% and 20% from diet cause significant decrease when compared with adenine group. Tomatoes could supply almost 85% of the lycopene in the diet.

Lycopene contains a high level of antioxidant, to help preventing different kinds of oxidative damages in cell and tissues (17).

Cystatin C concentration in serum of male rats induced chronic renal failure treated with the water extract 10, 20 and 30 mg/kg.

Cystatin C is an emerging marker of both ischemic andnephrotoxic kidney injury. Serumlevels of cystatin C have been proposed as analternative to traditional markers such as BUN andcreatinine for the estimation of glomerular filtration rate (17). At the same time, increases inthe urinary excretion of cystatin C have been proposedas a sensitive indicator of proximal tubular injury. The fact that cystatin C is rapidly being accepted as a measure of renal function in the clinic makes it extremely important to understand the effectsthat specific nephrotoxic agents, such as Cd, have on serum and urinary levels of cystatin C.

Conclusion

From current study conclude that the biomarkers Urea, Creatinine, KIM-1, NAGL and Cystatin C may be good predictive indicator of renal failure, plant extract may be having a highly effect fortreating the renal failure and addition to biomarkers tests whichreveal the induced CRF groups showed high response to treatment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

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13. Rajendra CE, Kumar DH, Yeshoda SV, Nadaf MA, HanumanthrajuN.Comparative evaluation of antimicrobial activity of methanolic extract of curcuma longa along with Boswellia serrata. IJRPC 2013;3(3);534-536.


The effect of Toxoplasmosis on Hematological and Biochemical Parameters in Pregnant Women in Thi-Qar Province

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1Dept. of Medical Lab.Tech./ College of Health & Medical Technology/ southern Technical University/ Republic of Iraq, 2Dept. Biology/ College of Pure Science/ University of Basrah/ Iraq

Abstract

The aim of the present study was to detect the effect of infection with Toxoplasma gondii on hematological and biochemical parameters in pregnant women. Blood samples (103) were collected from emergency unit of Bent Al-Huda Hospital in Thi-Qar province during the period from October 2018 till March 2019. Samples gave agglutination results with latex test (79) were tested with ELISA test. Ten blood samples were collected from healthy pregnant women as control. Concentration of blood hemoglobin (Hb), total WBCs count, liver enzymes Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT), lipid profile (total Cholesterol and Triglycerides) were estimated during this study using different commercial Kits.

Keyword: T. gondii, GOT, GPT, WBCs, Cholesterol, Thi-Qar

Introduction

Toxoplasma gondii is one of the most common parasite of warm- blooded vertebrates including human with high prevalence. The parasite causing either acute or chronic infection(1).

About 40% of the pregnant women have Toxoplasmosis. The fetus is got congenital toxoplasmosis during mother pregnancy. Transplacental transmission usually takes in the course of an acute infection (2). Indirect diagnosis is obtained by serological and biochemical technique (3).

Toxoplasmosis induces several immunological changes in the body of infected women which are characterize by the production of immunoglobulin’s IgG,IgM and IgA (4,5).

The replication of the toxoplasma inside the parasitophorus vacuole need substantial amount of the specific lipids for membrane biogenesis (6). (7) found an association between toxoplasmosis with hepatomegaly and some abnormal liver function test.

Materials and Method

Blood samples (103) of abortive women with Toxoplasmosis aged 17-46 years (at third month of pregnancy) were collected from emergency unit of Bent-Al Huda Hospital, in Thi-Qar province during the period from October 2018 till March 2019. Five ml. of blood samples were collected from each abortive woman with different number of abortion (1-3) times. Ten blood samples were collected from healthy pregnant women had similar age of infected women, which considered as control group. Three ml. of blood placed in gel tube, centrifuged and then the collected blood were used in serological test (Latex and ELISA).

Each serum sample collected was tested with latex agglutination (Spinarect company from Spain).The result is consider positive if the agglutination appeared after the serum and latex solution added to each other on the slide.

Samples gave agglutination result (79) were tested with ELISA test Kit (forsight company in Germany) to detect the antibodies of T. gondii according to the manufacturers’ instruction.

Concentration of blood hemoglobin (Hb) and total WBC count were carried out. Liver function was evaluated by the estimation of GOT, GPT and AlP enzyme. Spectrophotometer was used at 520 nanometer.
Level of lipid profile test (Total Cholesterol and Triglyceride= TGS) were estimated during this study using commercial Kits. Statistical analysis of the result were done using t-test, P value was considered significant when it was P≤ 0.05.

**Results**

1- Prevalence of *Toxoplasma gondii* antibodies:

Table (1) shows the higher prevalence of *T. gondii* antibodies IgG (84.8%) and 15.2% of IgM antibodies with high significant differences P≤ 0.05.

**Table (1). Prevalence of *Toxoplasma gondii* samples of aborted women according to IgM +, IgG+ antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG+</td>
<td>67</td>
<td>84.8</td>
</tr>
<tr>
<td>IgM+</td>
<td>12</td>
<td>15.2</td>
</tr>
<tr>
<td>total</td>
<td>79</td>
<td></td>
</tr>
</tbody>
</table>

2- Blood parameters:

Table (2) shows frequency and percentage of blood groups of aborted pregnant women. High percentage 41.8% was found in group 0 while lowest percentage 10.1% was found in group AB.

**Table (2). Frequency and percentage of blood groups of aborted women**

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>24</td>
<td>30.4</td>
</tr>
<tr>
<td>AB</td>
<td>8</td>
<td>10.1</td>
</tr>
<tr>
<td>B</td>
<td>14</td>
<td>17.7</td>
</tr>
<tr>
<td>O</td>
<td>33</td>
<td>41.8</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (3) show the mean of WBCs (9.02) compared with (5.97) in control, while in Hb 10.21 and 11.44 respectively. Hb in women infected with *T. gondii* were increase significantly P≤ 0.05 compared with control group, while WBCs count decrease significantly compared with control groups.

**Table (3). Mean and standard deviation of blood parameter of infected aborted women and control groups**

<table>
<thead>
<tr>
<th>No.</th>
<th>WBC</th>
<th>Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Toxo +</td>
<td>79</td>
<td>9.02± 3.59</td>
</tr>
<tr>
<td>control</td>
<td>10</td>
<td>5.97±1.18</td>
</tr>
</tbody>
</table>

3- Lipid profile

Table (4) shows the comparsion of lipid profile (cholesterol and triglyceride) activities between the patients (aborted women) and control groups. The cholesterol in women with *T. gondii* were decreased significantly P≤0.05 compared with control group, the triglycerides decrease in aborted women patient but not significant.

**Table (4). Mean and standard deviation of lipid profile of infected aborted women and control groups**

<table>
<thead>
<tr>
<th>No.</th>
<th>cholesterol mean±SD</th>
<th>Triglycerides mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxo +</td>
<td>79</td>
<td>200.51± 47.59</td>
</tr>
<tr>
<td>control</td>
<td>10</td>
<td>162.1±40.87</td>
</tr>
</tbody>
</table>

4- Liver enzymes

Table (5) shows the mean of GOT 15.11 compared with 12.1 in control groups while in GPT 14.67 and 12.4 respectively. The GOT and GPT in women infected with *T. gondii* were decreased significantly P≤ 0.05 compared with control groups.

**Table (5). Mean and standard deviation of liver enzymes of infected aborted women and control groups**

<table>
<thead>
<tr>
<th>No.</th>
<th>GOT mean±SD</th>
<th>GPT mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxo +</td>
<td>79</td>
<td>15.11±3.74</td>
</tr>
<tr>
<td>control</td>
<td>10</td>
<td>12.1±2.42</td>
</tr>
</tbody>
</table>

Table (6) shows t- test and P values of infected aborted women with *T. gondii*. All values were
significant at P≤ 0.05 except Triglycerides.

Table (6). t- test value and P values of infected aborted women according to the above parameters

<table>
<thead>
<tr>
<th>parameters</th>
<th>t-value</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>2.21</td>
<td>87</td>
<td>0.03</td>
</tr>
<tr>
<td>WBCs</td>
<td>2.65</td>
<td>87</td>
<td>0.009</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1.42</td>
<td>87</td>
<td>0.159</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2.43</td>
<td>87</td>
<td>0.017</td>
</tr>
<tr>
<td>GOT</td>
<td>2.47</td>
<td>87</td>
<td>0.015</td>
</tr>
<tr>
<td>GPT</td>
<td>2.34</td>
<td>87</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Discussion

The results of the present study shows the higher prevalence of *T. gondii* antibodies IgG 84.8% and 15.2% of IgM with significant difference with P≤0.05. (8) showed that high prevalence 68.75% antibodies of IgG, while in IgM 31.25% with high significant difference.

Increasing in the rate of Hb of aborted infected women in the present study may be due to physiological and immunological status pregnant women infected with Toxoplasmosis, while the decreasing in WBCs might be due to the affected of these cells by the parasites which regarded as the most important factors controlling the cellular and humeral immunity response in the body of pregnant infected women (9,10).

The decrease in mean of cholesterol and Triglycerides in the present study compared with control might be due to the infection with parasites which make huge variation in lipid parameters. In general cholesterol play a main role in cellular membrane organization dynamics function and categorization(11). The parasites need cholesterol biosynthetic enzyme and must take it from its host (12).

The main function of the liver enzyme is the storage and movement of nutrition detoxication and metabolism of water and electrolytes. *T. gondii* causes significant and progressive changes in the liver owing to remarkable proliferation of organisms (3).

It was found from the present study that *T. gondii* affect the liver function which is cleared by decreasing GOT and GPT levels. (13) recorded an increase in GOT and GPT enzymes. No significant difference in the severity of the infected mice and control one was showed by (14).

The enzyme activities of GOT and GPT in the serum of aborted women indicating decrease in protein catabolism (1).

The present results show a decrease of both enzymes which reflecting the degree of damage to the liver function. Regulation of hepatocyte metabolism depend either on increasing anabolism or decreasing catabolism (15, 16).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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References


Paper Based Glucose Biosensor Depending on SPCE Modified with Hemoglobin and Silver Nanoparticles

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Abstract

In this study, new strategy intended for rapid glucose detection utilizing disposable glucose oxidase (GOD) paper disk integrated with screen-printed carbon electrode (SPCE) modified with hemoglobin (Hb) and silver nanoparticles (AgNPs). The GOD adsorbed on the surface of paper disk and after drying it placed on the surface of the modified SPCE and 16µL of glucose solution were added for the testing. Different parameters such as applied potential, concentration of enzyme, pH, size of paper disk, and the volume of buffer were optimized to improve the efficiency of this glucose biosensor.

Key Words: Hemoglobin, Silver Nanoparticles, Glucose Biosensor, SPCE

Introduction

Glucose monitoring is a requisite tool in clinical trials where glucose concentration is a definitive indicator in type 1 and type 2 diabetes mellitus and other diseases such as endocrine metabolic disorders. Most patients with diabetic need to test their blood sugar periodically. Thus, great interest have been aroused from industrials and marketers people to develop glucose sensor with more time-saving, much reliable, easy, cost-effective and can be utilized in home-based care (¹). Paper-based biosensor has drawn much interest in analytical and clinical chemistry due to its discerning properties; cheap, simplicity, presorted reagent and not required special user, which can be largely used in the diagnostic field. Moreover, GOD is widely used in practical applications for glucose analysis, where it catalyzes glucose oxidation according to the following equation

Where, the glucose level can monitored by the detection of H₂O₂ generation or O₂ consumption. Overwhelmingly, electrochemical glucose biosensor based on O₂ detection but the detection of H₂O₂ was found to be more sensitivity.

Several analytical techniques have been employed for H₂O₂ analysis, such as spectrometry, fluorimetry, titrimetry, chromatography and electrochemical technique. Among these techniques, because of their rapid response, low cost, selectivity, simplicity and high sensitivity; the electrochemical method have been extensively utilized for this purpose (²).

Recently, with the large development in nanomaterial’s science, SPEs became more utilized in smart and modern biosensor applications to avoid some common problems of classical solid, in addition to it is characterized with; low cost, disposable, convenience, flexibility in design, easy to chemical modification and reduction of sample volume required (³). Different modifiers have been added in to screen printing ink to progress the sensitivity and selectivity of SPEs such as enzymes, mediators and nanomaterials (⁴). Hemoglobin (Hb) has considered a suitable model for H₂O₂ biosensor because hemoglobin could act as peroxidase-like to reduce H₂O₂, commercially available with moderate cost and excellent stability and it has a well-known structure (⁵). Nanoparticles attracted specific interests in the field of enzyme immobilization, due to unique properties, such as high electrical conductivity, good chemical and thermal stability, and large surface area (⁶).

In this study, we developed highly sensitive and stable paper based glucose biosensor by of utilized the previously fabricated hydrogen peroxide biosensor based on Hb immobilized on SPCE modified with AgNPs, and integration with GOD paper disk. Where,
in this paper based biosensor, glucose concentration can be electrochemically detected by using 16µL of sample. In addition, the analytical efficiency of present biosensor was evaluated in terms of repeatability, stability, sensitivity, and reproducibility after optimization of applied potential, pH, concentration of enzyme, and volume of buffer as the important parameters for biosensor development.

**Experimental part**

**Apparatus and measurement**

A potentiostat/Bipotentiostat (type DY2300, Digi-Ivy, Austin, U.S.A) was used to carry out the cyclic voltammetry, linear sweep voltammetry and Amperometric measurements, a screen-printed carbon electrode (SPCE) from Digi –Ivy (USA) with three electrodes was employed, consists of carbon electrode as working and counter electrodes, while pseudo Ag/AgCl was acted as a reference electrode. All the electrochemical experiments were done at room temperature (25 °C). The pH of the buffers was carried out using a HANNA pH meter. In cyclic voltammetry (CV) and linear sweep voltammetry (LSV) measurement, 16µL of 0.1M PBS (pH 7) was added onto the paper disk, with this volume a good contact was obtained between the paper disk and the modified-SPCE.

**Reagents**

Silver nanoparticles (0.02 mg/mL, suspension in aqueous buffer, 10nm), and Nafion (5%) were purchased from Sigma-Aldrich. Hb, and H2O2 from Sigma (USA), chitosan (CS) from Shanghai Biochemical (China), Glucose oxidase (GOD, 40IU/mg) from Fluka. A stock solution of 0.2M Na2HPO4, NaH2PO4 was used to prepare phosphate buffer solution (PBS, 0.1M) at various volume ratios and the pH was adjusted with 0.1M phosphoric acid or sodium hydroxide. All chemicals were of analytical reagent grade, and were used without further purification. Double distilled water (DDW) was used in all experiments. The stock solutions were stored at 4°C for further analysis.

**Preparation of GOD paper disc**

Grade 1 Whitman filter paper was carefully cut into round sheet with about 1 cm diameter using a paper punch. Then, 6µL of GOD solution (180 IU/mL) was carefully spotted on the center of each paper disk and left to dry at 25°C. The paper disk was placed onto the surface of the modified-SPCE to completely cover the working, reference and counter electrodes. Furthermore, GOD and glucose solutions with different concentrations were prepared and diluted with PBS (pH7).

**Results and Discussion**

**Electrochemical investigation of paper-based analytical devices**

The CV of modified SPCE integrated with GOD-paper disk and blank paper disk were examined by swept the potential in window of -0.6 V to +0.3 V at scan rate 0.1Vs⁻¹ in PBS (pH 7) with and without 1mM glucose solution, as shown in Figure (1).

No change in the cathodic and anodic peak current of modified SPCE was observed when blank paper was used in the presence of 1 mM glucose solution, in comparison with CV in PBS (pH 7) without glucose solution. Which refers to no H2O2 was produced in the absence of GOD in the reaction medium; also, the same result was obtained when GOD-disk paper was used in the absence of glucose solution.

While, there was an increase in the cathodic peak current of modified SPCE when glucose solution was added to paper disk pre-loaded with GOD, which is due to oxidation process of glucose, where the produced H2O2 in this reaction can be detect by the present modified SPCE.

![Figure 1: Cyclic voltammogram of modified SPCE integrated with GOD-paper disk and blank paper disk in the presence and absence of 1 mM glucose solution.](image-url)

Direct electrochemistry behavior of GOD disk paper with modified SPCE was investigated in the detection of glucose level with different modified electrodes using LSV method in potential range from +0.3 to -0.6 V at scan rate of 0.1Vs⁻¹. As shown in Figure (2), no reduction peak was observed for GOD-disk on the bare-SPCE in the presence of 1mM glucose solution, while, a weak peak current at -0.33 V was observed in Hb-
SPCE with GOD-disk paper. The reduction peak current was increased by 4.2 times when AgNPs was used in the modification of the electrode (NF-Hb-AgNP-CS-SPCE). Thus, GOD-disk paper integrated with NF-Hb-AgNP-CS-SPCE has a good electrocatalytic activity for oxidation of glucose, attributed to the high conductivity and high surface area of electrode that provided by using silver nanoparticles in modification of SPCE. In addition, it was demonstrated that Hb has a good electrocatalytic activity towards H$_2$O$_2$ reduction.

When the amperometric response of the paper based analytical devices is typically affected by the enzyme loading (7). Therefore, the amperometric experiment was investigated in 1 mM glucose solution for 60 s at scan rate of 0.1Vs$^{-1}$ with several GOD-paper disks prepared by immobilized different concentration of enzyme 0.2 IU/disk - 1.2 IU/disk. The optimum sensitivity of biosensor was recorded with an enzyme concentration of 0.8 IU/disk. While, excess loading of the enzyme resulted unchanged in sensor response for glucose detection, which is identity with enzyme kinetics.

In this amperometric study, PBS (pH 7) used as a carrier solution of analyte as well as supporting electrolyte. The volume effect of PBS containing constant concentration of glucose solution 0.1 mM on the amperometric response of GOD paper-based glucose biosensor was investigated in scan rate of 0.1 Vs$^{-1}$. Where, a suitable PBS volume was found to be 16 µL. In this volume of buffer, the contacting between the three electrodes of the modified SPCE and the wetting GOD paper was the best.

The pH value of the target buffer is a vital factor for the sensitivity of glucose biosensor, where two enzymatic reactions interested in the response of GOD paper-based NF-Hb-AgNP-CS-SPCE to glucose solution. An optimum pH rang influence both the electrochemistry of Hb and the bioactivity of the GOD. The extreme pH may change the kinetics of glucose measurements as a result of confusing the redox state of glucose oxidase reaction (8). For this matter, the effect of pH on the amperometric response of paper-based glucose biosensor to 1 mM glucose solution was investigated in 0.1 M buffer solution between 3 to 8 pH. From Figure (3), it can be deduced that the optimum enzyme activity was found at (pH 7) and in pH value above or below that, there was clear drop in response of fabricated glucose biosensor that may be due to denaturation of enzyme in these pH value. Based on the above results, a concentration of GOD with 0.8 IU/disk, a potential of -0.33, 16 µL of PBS with pH 7 were selected to obtain the optimum sensing.

The characteristics of biosensor response

Amperometric method is very suitable method for determination of glucose concentration, since its utilization is characteristic with fast and simple. By employing the optimum experimental conditions as
described above, the calibration graph of the biosensor response to the concentration of glucose solution was shown in Figure (4). It can be seen that the present glucose biosensor enabled us to determine the glucose concentration from 1.2 mM to 5.2 mM, with regression equation

\[ Y = 0.9059X + 3.0631 \]

Where \( Y \) refer to the response (current) in \( \mu \text{A} \) and \( X \) refer to the concentration of glucose in mM, with a correlation coefficient of 0.9905.

The limit of detection for glucose was calculated as 0.6 mM (based on LOD =) and the sensitivity of biosensor was found to be 11.91 \( \mu \text{A.mM}^{-1}.\text{cm}^{-2} \). The LOD value obtained was below 0.86 mM in Rungsawang, et al. study (9) and 3.12 mM in Li, et al. study (10). Meanwhile, the sensitivity is higher than the previously reported glucose biosensor (11). In addition, the resulted linear detection range of glucose that obtained by GOD paper disk integrated with NF-Hb-AgNP-CS-SPCE was 1.2-5.2 mM was almost the same as in other glucose biosensor, such as up to 5.7 mM in GOD-Au electrode (12), up to 6mM in GOD-AuNPs-NF-GC (13).

![Graph showing calibration curve](image_url)

a substrate saturation was observed when the glucose concentration is over than (5.2 mM), showing the characteristics of Michaelis-Menten kinetic (typical enzymatic reaction kinetics). The value of the apparent Michaelis-Menten constant \( K_m \) was calculated to be (4.4 mM), this value was significantly lower than 35mM in GOD-AuNPs-PA (14). Which implying that the GOD in our experiment exhibits a remarkable affinity for glucose.

**Stability and reproducibility of glucose biosensor**

In this work, the reproducibility of the biosensor was monitored by using 10 GOD paper disks prepared independently for testing 2 mM glucose solution, R.S.D was calculated to be 3.53%, while the bio electrocatalytic signal reached 90% of the steady-state value in 15 s.

The stability of the newly prepared GOD paper disks was evaluated at optimum conditions, by monitoring the signal decrease with storage time. The response of GOD paper based NF-Hb-AgNP-CS-SPCE was decreased to 62% of initial response after (20 days) of storage at 4°C. Thus, in this work the designed GOD paper based glucose biosensor can detect the concentration of glucose in small volume of sample with high sensitivity, good stability and low cost technique.

**Selectivity of biosensor.**

Selectivity of the glucose biosensor is important feature for practical applications, where in the amperometric biosensor, the electroactive compounds have been a problem in testing the biological or industrial samples. Eight possible potential interference species that may be influences the amperometric response of the proposed glucose biosensor were studied. The paper-based glucose biosensor response for a fixed concentration of glucose 2 mM was compared to that obtained for 1 mM of the possible interfering compound in the presence and absence of 2 mM glucose solution. As can be seen in Table (1), the paper-based glucose biosensor does not give response to any one of the interference compound that studied in the absence of glucose solution (close to background), that due to the specificity of enzyme.

However, in the presence of 2mM glucose solution plus fixed amounts of interfering species 1mM, the amperometric response is changes lower than 7% with exception of cysteine, where the registered signal decreased 12.3% that may refer to its interaction with some amino acid resides in the glucose oxidase molecule. Hence, our fabricated paper-based glucose biosensor are very suitable for the selective glucose determination in biological or industrial samples.
Table (1): Study of the interferences caused by different compounds to the response of paper-based glucose biosensor.

<table>
<thead>
<tr>
<th>Sample without glucose</th>
<th>Response (µA)</th>
<th>Sample with glucose</th>
<th>Response (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>0.83</td>
<td>Cysteine-glucose</td>
<td>4.56</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.74</td>
<td>Fructose-glucose</td>
<td>5.1</td>
</tr>
<tr>
<td>Vit.C</td>
<td>0.81</td>
<td>Vit.C-glucose</td>
<td>5.05</td>
</tr>
<tr>
<td>Urea</td>
<td>0.45</td>
<td>Urea-glucose</td>
<td>5.2</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.67</td>
<td>Uric acid-glucose</td>
<td>4.96</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.71</td>
<td>Sucrose-glucose</td>
<td>4.86</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.86</td>
<td>Xylose-glucose</td>
<td>4.91</td>
</tr>
<tr>
<td>Ca++</td>
<td>0.64</td>
<td>Ca++</td>
<td>5.06</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.52</td>
<td>Mannose-glucose</td>
<td>5.12</td>
</tr>
<tr>
<td>Background (PBS, pH7)</td>
<td>0.35</td>
<td>(2mM) Glucose</td>
<td>5.20</td>
</tr>
</tbody>
</table>

(n=3) SD= standard deviation

Conclusion

The paper-based biosensor comprised a linear response range for glucose over 1.2 mM to 5.2 mM with detection limit of 0.6 mM at signal to noise ratio of 3. While, the sensitivity and the apparent Michaelis-Menten constant (k) were calculated and found to be 11.91 µA.mM⁻¹.cm⁻² and 4.4 mM. In addition, the stability, reproducibility, repeatability and selectivity of present glucose biosensor were monitored in this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

References


Evaluation of Noise Pollution Levels in Hospitals and its Effects on Staff Health in AL - Najaf City in Iraq

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Abstract
The aim of the study was to determine levels and sources of noise pollution in the hospitals and its effects on staff health in AL - Najaf city in Iraq. The study included measure sound levels in all wards in three hospitals (Al-sadr teaching hospital, Al-hakeem general hospital and Al-zahraa’ teaching hospital) using a digital sound level meter. The present work also examined distribution of symptoms caused by noise in employees of three hospitals and main sources of noise in hospitals by using prepared questionnaire through direct interview in the workplace. The results showed that the average measured A-weighted equivalent continuous level ($L_{Aeq}$) in all hospitals and in different shifts was more than recommended level (20 – 35 dB) of World Health Organization (WHO). The study revealed that the main sources of noise in inside the wards rooms of hospitals Al-Sadr Teaching Hospital, Al-Hakeem General Hospital and Al-Zahraa’ Teaching hospital were “staff conversation” (91%); “maneuver-patient treatment” (83%); “doors opening or closing” (77.5%); “mobile phone ringing and conversation” (76.5%). Moreover, it was observed that (32%) of respondents were headache by noise in hospitals. Moreover, the type of noise health effects that most complained among employees were vocal fatigue (31%), dizziness (30.5%), and feeling sick at the end of the workday (28.5%). This study concluded that the noise level in three hospitals was higher than the WHO rating; therefore, this problem causes raising the noise annoyance on of hospital staff.

Key words: Noise pollution, Hospitals and Staff health.

Introduction
Noise in hospitals has become a subject of growing concern in recent years, since it is often cited as a major complaint by patients in hospitals (¹, ²) reported that noise levels in hospitals averaging 72 dBA during the day and 60 dBA at night. Environmental noise present in hospitals all over the worlds is a common exertion and is recognized as a serious health hazard and not just as a nuisance (³).

Noise pollution has many health effects, e.g., increased blood pressure, noise-induced hearing loss, sleep disorders, annoyance and irritability (⁴). In addition; high noise can also leave adverse effects on work activity and events. It can also cause impaired sleep and behavior, increased gastric intestinal activity, heart rate, blood pressure, respiratory rate, and oxygen consumption (⁵).

Noise may elevate blood pressure, increase heart rate, stimulate the release of epinephrine (adrenaline), increase pain, and alter quality of sleep (⁶)(⁷).

Noise has been increasing over the years, specifically in large metropolitan areas. This increase is also perceived inside hospitals. Different noises originated from distinct sources, such as the operation of different devices and conversation among professional cause noise pollution in the hospital environment and this may affect the individual’s physical and emotional health (⁸).

In general, the problem of hospitals’ noise pollution is a global issue and because of its negative health impacts, it requires and deserves further attention (⁹) endeavors to prevent medical errors and risks stemming from the presence of patients and employees only in the hospital environment are very important in terms of patient and employee safety (¹⁰). In addition, working
performance is also affected by noise pollution. The aim of this study was to assess noise levels and sources and its effects on staff health of three hospitals in AL-Najaf city in Iraq.

**Materials and Method**

This study was carried out in three large hospitals: Al-Sadr Teaching Hospital, Al-Hakeem General Hospital and Al-Zahraa’ Teaching hospital in Al-Najaf AL-Ashraf city in Iraq from November 10, 2018, to January 12, 2019.

**Workplace noise assessment:**

The noise levels measurements were conducted using Noise measurements were recorded by using digital sound level meter model: UNI-T; UT352, China in three major hospitals Al-Sadr Teaching Hospital, Al-Hakeem General Hospital and Al-Zahraa’ Teaching hospital. In each hospital, several locations were chosen to measure various noise parameters at different time intervals.

Noise level assessments were performed at four time periods include morning time (09:00 am–12:00 am), afternoon time (12:00 am–04:00 pm), evening time (04:00 pm–06:00 pm), and night time (06:00 pm–09:00 pm).

In this study, noise level was recorded in every measurement in Decibel (dB): A-weighted equivalent continuous level (L_{Aeq}). In order to obtain a realistic recording of noise levels, neither the staff nor patients in the four institutions were aware of the recordings. The time for each measurement was 5 minutes, recording was repeated for two times in each location, and then the average reading was recorded.

**Questionnaire surveys:**

The questionnaire has been applied to 200 employees selected from three hospitals. Structured questionnaires were used to assess the staff response on noise pollution in all locations of the hospitals. All participants completed a researcher-made questionnaire. It included two parts:

First part: noise sources inside and outside the wards rooms in hospital: The second part: sources of noise pollution inside and outside the ward rooms such as (television and radio sets, mobile phone ringing and conversation, doors opening or closing, medical equipment, heating, ventilation, air-conditioning or cooling system, bed creak, maneuver-patient treatment, patients moaning or crying, staff conversation, conversation of patients visitors, wheelbarrows).

Second part: symptoms caused by noise on hospital employees: symptoms caused by exposure to noise pollution such as (headache, anxiety/jitters, dizziness/dizzy, annoyance, tension or irritability, discomfort, uncomfortable around loud noise, sleep alterations, experience difficulty sleeping, inability to sleep, inattention, low concentration, unable to concentrate in work, gastric changes, problem in speech, difficulty hearing what people say, tinnitus, ear ache, hearing deficiency, blocked ear, vocal fatigue, and feeling sick at the end of the workday).

**Statistical analysis:**

The data were statistically analyzed by using SPSS (statistical package for social sciences). The independent sample t-test, ANOVA (analysis of variance) . All values were expressed as mean ± Standard Error of Mean. P-value less than 0.05 and 0.01 were considered statistically significant.

**Results**

**Sound levels in three hospitals:**

The results recorded that the average A-weighted equivalent continuous sound levels (L_{Aeq}) in different locations of Al-sadr teaching hospital was (62.80 ± 2.14 dB). In addition, it can be observed that the average A-weighted equivalent continuous sound levels (L_{Aeq}) in different locations for Al-hakeem general hospital was (61.50 ± 2.12 dB). Also, The study found that the the average A-weighted equivalent continuous sound levels (L_{Aeq}) in all locations of hospital Al-zahraa’ teaching hospital was (61.64 ± 2.11 dB) (Table 1).
Table (1) Average A-weighted equivalent continuous sound levels ($L_{Aeq}$) in Al-sadr teaching hospital, Al-hakeem general hospital and Al-zahraa’ teaching hospital.

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>Sound levels (dB) ($L_{Aeq}$)</th>
<th>F test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-sadr teaching hospital</td>
<td>62.80 ± 2.14</td>
<td>0.45</td>
<td>0.64</td>
</tr>
<tr>
<td>Al-hakeem general hospital</td>
<td>61.50 ± 2.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-zahraa’ teaching hospital</td>
<td>61.64 ± 2.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: (NS) = Not significant.

In addition, statistical comparison of mean A-weighted equivalent continuous sound levels ($L_{Aeq}$) among different hospitals on the basis of time periods, the result observed that no significant elevation in mean ($L_{Aeq}$) of Al-sadr teaching hospital during morning, afternoon, evening comparison with Al-zahraa’ teaching hospital and Al-hakeem general hospital in same period time (Table 2).

Table (2) Comparison of mean A-weighted equivalent continuous sound levels ($L_{Aeq}$) in (dB) among various hospitals on the basis of time periods.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sound levels (dB) ($L_{Aeq}$)</th>
<th>F test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-sadr teaching hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-hakeem general hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-zahraa’ teaching hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>64.7±1.24</td>
<td>0.58</td>
<td>0.62    (NS)</td>
</tr>
<tr>
<td>Afternoon</td>
<td>64.31±2.45</td>
<td>0.29</td>
<td>0.82    (NS)</td>
</tr>
<tr>
<td>Evening</td>
<td>62.07±4.01</td>
<td>0.57</td>
<td>0.57    (NS)</td>
</tr>
<tr>
<td>Night</td>
<td>60.1±1.29</td>
<td>0.35</td>
<td>0.75    (NS)</td>
</tr>
</tbody>
</table>

NS= Not significant.

**Sources of Sound levels in hospitals**

The present study revealed that the main sources of noise in inside the wards rooms of hospitals Al-Sadr Teaching Hospital, Al-Hakeem General Hospital and Al-Zahraa’ Teaching hospital were “staff conversation” (91%); “maneuver-patient treatment” (83%); “doors opening or closing” (77.5%); “mobile phone ringing and conversation” (76.5%) (Figure 1). Moreover, it was observed that the “talking of visitors or patient’s family members in corridors” (94.5%) and “Staff conversation” (92%) were the major noise sources outside the wards rooms in hospitals Al-Sadr teaching hospital, Al-Hakeem general hospital and Al-Zahraa’ teaching hospital (Figure 2).
Figure (1) Sources of noise inside wards rooms in in three hospitals according to hospitals employees.

Figure (2) Sources of noise outside wards rooms in in three hospitals reported by hospitals employees.
Distribution of symptoms caused by noise in employees of three hospitals:

The study indicated that (32%) of respondents were headache by noise in hospitals (Figure 3). Moreover, the result also showed increment in percentages of vocal fatigue (31%), dizziness (30.5%), and feeling sick at the end of the workday (28.5%) in employees of three hospitals (Figure 3).

Discussion

Sound levels in hospitals:

The results recorded that the average A-weighted equivalent continuous sound levels ($L_{Aeq}$) in different locations of Al-sadr teaching hospital, Al-hakeem general hospital and Al-zahraa’ teaching hospital were (62.80 ± 2.14, 61.50 ± 2.12 and 61.64 ± 2.11 dB respectively). In the present study, the mean equivalent noise levels observed on all wards exceeded the recommended WHO guidelines for hospitals (11).

Eivazzadeh, M. et.al. (2017) found the higher levels of noise in a emergency ward (69.65 ± 1.68) were linked to a higher the working procedure of this ward compared to other wards; Moreover, the data recorded that the highest mean $L_{max}$ and $L_{min}$ (94.43 and 50.43, respectively) in the emergency ward. This might be due to the emergency care unit does not have a fixe time for treatment activities and clinical operations take place on demand all day (12).

In addition, the result observed that no significant elevation in mean ($L_{Aeq}$) of Al-sadr teaching hospital during morning, afternoon, evening comparison with Al-zahraa’ teaching hospital and Al-hakeem general hospital in the same period in the same period time. The findings agreed with results of previous study by (13) conducted a measurement of noise levels in Mosul medical city center teaching hospitals and concluded that the mean equivalent noise level (94.35 dBA) during morning time was higher than level (90.14 dBA) during afternoon. Furthermore, the study also recorded mean equivalent noise levels were higher on the weekdays (94.05 dBA) than on the weekend (88.57 dBA).

Pai, et.al. (2007) observed increase of noise level in the wards and locations was between 50.3 and 68.1 dB in Taiwan hospital which exceeded the suggested hospital ward sound level. The study also found The quietest units were the Surgical Intensive Care Unit and recovery rooms with a noise level lower than 50 dB during the night. Moreover, The higher noise levels were in the hall and pharmacy which were highly populated areas (14).

Sources of Sound levels in hospitals:

This study recorded that that the main sources of
noise in inside the wards rooms of hospitals Al-Sadr Teaching Hospital, Al-Hakeem General Hospital and Al-Zahraa’ Teaching hospital were “staff conversation” (91%); “maneuver-patient treatment” (83%); “doors opening or closing” (77.5%); “mobile phone ringing and conversation” (76.5%) . Similar results obtained by (15) who found that the daily average sound levels measured inside these hospitals during daytime were between 52.6 and 64.6 decibels. They also reported that most nursing staff members expressed that “talking of visitors or patient’s family members” is the major source of noise inside the wards, whereas “talking of visitors or patient’s family members” and “children playing” are the two major noise sources outside the wards.

Furthermore, the study showed that the “talking of visitors or patient’s family members in corridors” (94.5%) and “Staff conversation” (92%) were the major noise sources outside the wards rooms in hospitals Al-Sadr teaching hospital, Al-Hakeem general hospital and Al-Zahraa’ teaching hospital. Similar findings were recorded by Similar results obtained (16) indicated that many studies have shown that noise levels in most hospitals in USA are much higher. They pointed out two general sources of noise in hospitals. The first one includes paging systems, alarms, bedrails, telephones, staff voices, ice machines, pneumatic tubes, carts, and noises generated by roommates. The second source includes the surfaces of the floors, walls, and ceilings which usually are hard and reflect sound rather than absorb it.

Distribution of symptoms caused by noise in employees of three hospitals:

The results indicated that (32%) of respondents were headache by noise in hospitals (Tables 1, and 2). Moreover, the type of noise health effects that most complained among employees were vocal fatigue (31%), dizziness (30.5%), and feeling sick at the end of the workday (28.5%). This finding agreed with (17) who measured noise level in different environments of a public hospital and to analyze its effects on staff from reporting complaints. The study results showed that the minimum sound level 52.5 decibels in the intensive care unit (NICU) and a maximum sound level 85 decibels in the emergency room. They also observed staff feel discomfort to loud sounds (74.4%) and (35.5%) feel sick after hours due to stress caused by noise that is produced by multiple devices combined with the sounds of alarms, works, visits and schedules conversation between the hospital employees.

Montes-González, et.al (2019) reported that one of the main hospitals in the Extremadura region (Spain) is presented here to allow a global assessment of the acoustic impact of outdoor sound sources. Taking into account the results obtained, the noise impact on this hospital is primarily influenced by three sound sources: road traffic, cooling towers of the hospital and the emergency helicopter(18).

Fillary, et.al. (2015) exposure to excessive noise is associated with sleep disturbance, and symptoms such as dizziness, confusion, fatigue, high blood pressure, digestive problems, heart arrhythmias and neuropsychological disturbances. Moreover, noise is considered as the most common cause of discomfort, imposes stress and disrupts communication(19).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

Funding: Self-funding

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5. Gerhardt, K J, & Abrams, R M. Fetal exposures to sound and vibroacoustic stimulation. Journal of


Influence of Emotional Intelligence on Academic Achievement among Students at Secondary Schools in Babylon City

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¹University of Baghdad /College of Nursing, Iraq

Abstract

Objectives: The main aim of the study is to determine the influence of emotional intelligence on academic achievement among the students. A descriptive study is carried to assess the influence of emotional intelligence on academic achievement among Students at secondary schools in Babylon City. A systematic random sample of (586) students are selected throughout the use of probability approach. The study sample selected from (4) secondary schools, which divided into (2) schools for female and (2) schools for male. The selection of sample divided into (141) students for each of male school and (152) for each female school. The schools were randomly chosen by lottery in the purpose to select 4 schools out of 28 schools (15%) and 25% of the students in these schools, which was convenient to the investigator. A total of (650) secondary school students, they were met the study criteria and agreed to contribute in the study. The finding reveals that there is a significant influence of students’ emotional intelligence on academic achievement at p-value = 0.01.

Key Words: Emotional Intelligence, Academic Achievement, Students, and Secondary Schools.

Introduction

Emotional intelligence (EI) defined as “the ability to perceive accurately, appraise, and express emotion; the ability to access and/or generate feelings when they facilitate thought; the ability to understand emotion and emotional knowledge; and the ability to regulate emotions to promote emotional and intellectual growth”. EQ consists of four domains of abilities which are the perception, use, understanding and regulation of emotion. Emotional intelligence is claimed to affect various aspects of human performance, namely in physical and psychological health, social interaction and performance at school and in the workplace. Unlike Intelligent Quotient (IQ), emotional intelligence (EQ) is associated with career and personal life success, including success in the academy. Emotional intelligence (EI) is defined as the ability to monitor one’s own and other people’s emotions, to discriminate between different emotions and label them appropriately, and to use emotional information to guide thinking and behaviour and to manage and/or adjust emotions to adapt to environments or achieve one’s goals. Emotional intelligence comprises interpersonal and intrapersonal intelligence. Interpersonal intelligence is the outer intelligence one uses to understand and manage relationships with other people. This is important for developing qualities like empathy and building up effective relationships. Intrapersonal intelligence is the inner intelligence one uses to know and understand oneself which is important for self-awareness, self-regulation and self-motivation. It can be postulated that management of interpersonal and intrapersonal emotions is vital for an individual’s academic and professional success. Those with higher EI are more likely to understand, regulate and manage emotions better both in themselves and in others. Low and Nelson (2006) claimed that EQ is crucial to a student’s personal health and success. They claimed that students with emotional intelligence skills are better able to cope with demanding and complex college experience. When individuals are able to lead their life successfully in the academy, they can focus on their learning and perform academically. Students who follow the lessons by integrating emotional intelligence will show significantly higher increase in Math scores. They have been integrated the ten aspects of emotional intelligence in teaching Math namely emotional awareness, self-esteem, self-confidence, self-control, motivation, communication, cooperation, empathy, problem solving and joy. Integration of emotional intelligence significantly increases the student’s attitudes.
toward Mathematics as a whole. Students who accept the integration of emotional intelligence significantly increase confidence, enjoyment and motivation towards math\(^4\). Apart from its contribution to academic success, a person with high emotional intelligence is also said to function better as a worker citing reasons such as being able to be a team player, work under pressure, and contribute to organization’s productivity\(^5\). It is becoming more evident that employers nowadays are no longer looking for employees with good grades alone but they are more interested in applicants who can meet the demands of a working world and adapt to the forever changing climate and needs of their organization. This is especially true in profession that requires the employees to be highly emotionally intelligent. An increasing amount of research is being carried out to examine stress in workplace including in learning institutions involving educators such as teachers and lecturers. This is because teaching profession is recognized to be one of the most stressful professions\(^5\).

**Materials and Method**

A descriptive study is carried out to assess the influence of emotional intelligence on academic achievement among students at secondary schools in Babylon City. The study has been conducted at the following secondary school in Babylon City; Mariam Al-Athraa Secondary School, Al-Batool Secondary School, Beirut Secondary School, and Al-Sadah Secondary School (2018-2019). A systematic random sample of (586) students are selected throughout the use of probability approach. The study sample selects from (4) secondary schools, which divided into (2) schools for female and (2) schools for male. The selection of sample divided into (141) students for each of male school and (152) for each female school. Through review of the related literature and previous studies, the questionnaire is constructed as a mean of data collection. It was consisted of (2) major parts:

**Part I:**

The first part is concerned with student’s socio-demographic characteristics which include (gender, age, students’ sequence in the family, students’ brothers number, students’ sisters number, residency, fathers education, mothers education, monthly income, scholastic stage and academic achievements (average)).

**Part II:**

This part is concerned with student’s emotional intelligence. It consist of (41) items (always, often, sometimes, rarely, never). These items were rated according to the Likers’ scale; always (5); often (4); sometimes (3); rarely (2); never (1) for the positive items, and vice versa for the negative items. The score estimation for the study scale has been evaluated according to responses’ percentages of the items. Each always answered item was assigned a score of 5. often answered items were assigned a score of 4. Sometimes answered items were assigned a score of 3. Rarely answered items were assigned a score of 2 and those never answered were assigned a score of 1. Total scores were summed and ranged in percentages. Score was assessed in percentages of the mean depending on the Likert scale as follows:

- High score: 3.67-5, (> 74 %).
- Moderate score: 2.34-3.66, (47 – 73 %).
- Low score: 1- 2.33, (<47 %).

A pilot study is carried out on December 22\(^{th}\) 2018 to December 30\(^{th}\) 2018 and it is conducted on (64) students who are selected randomly from the the chosen secondary school in the Babylon City. Finally, the sample of the pilot study is excluded from the original study.

The validity of the questionnaire was adjudged using Cronbach’s coefficient alpha calculated to test the reliability and internal consistency of the responses obtained from the respondents.

The data is collected through the use of a developed questionnaire (Arabic version) and student’s self-administration as a mean for data collection.

**Results and Discussion**

The descriptive analysis of the sample shows that more than half of the sample was females (51.9%) while the males were (48.1%), the distribution of the sample according to their age; the finding reveals that most of students are between (15-17) years old (41.0%) followed by a lesser proportion for those who were at age between (12-14) with (33.4%) and (25.6%) were more than 18 years old. The findings reveal that about, (28.0%) of participants were the first child in the family while
(22.2%) as second one. On the other hand, (18.1%) of students have third ordinary in the family. Also (15%) reported as fourth child. Additionally, (15.4%) fifth and more. Distribution of students’ brothers number shows that (4.4%) of students haven’t brothers, (20.8%) of students have one brothers, (27.3%) of students have three brothers, (27.6%) of students have three brothers, (12.3%) of students have four brothers and (7.5%) of students have five and more brothers. The findings show that (10.6%) of students haven’t sisters, (21.8%) of students have one sister, (28.7) of students have three sisters, (17.4%) of students have three sisters, (10.2%) of students have four sisters and (11.3%) of students have five and more sisters. The majority of participants live in urban areas (53.9%) while (46.1%) of participants live in rural areas. About (1.4%) of students’ father not read and not write, (3.4%) of students’ father can read and write, (22.2%) of students’ father have finished the elementary school, (31.7%) of students’ father have finished the secondary school, (36.5%) of students’ father have finished the diploma or bachelor study and (4.8%) of students’ father have higher education, while (7.2%) of students’ mother not read and not write, (9.9%) of students’ mother can read and write, (32.8%) of students’ mother are elementary school graduates, (21.5%) of students’ mother are secondary school graduates, (22.5%) of students’ mother are diploma or bachelor graduates and (6.1%) of students’ mother with higher education. more than a half of participants have a sufficient monthly income (60.4%). the distribution students according to scholastic stage were (17.1%) selected for each of first and second stage followed by equal numbers of students percent for each of (third, fourth, and sixth stage) with (16.4%) ; while (16.7%) for fifth stage.

This table indicates that (9.2%) of the students are have low level of Emotional Intelligence, (45.7%) of them were showing moderate level, and just (45.1%) show high level of Emotional Intelligence.

Table 2: Assessment of Students’ Academic Achievement Level.

<table>
<thead>
<tr>
<th>Academic achievement</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failed</td>
<td>62</td>
<td>10.6</td>
</tr>
<tr>
<td>Accepted</td>
<td>256</td>
<td>43.7</td>
</tr>
<tr>
<td>Adequate</td>
<td>158</td>
<td>27.0</td>
</tr>
<tr>
<td>Good</td>
<td>75</td>
<td>12.8</td>
</tr>
<tr>
<td>Very good</td>
<td>26</td>
<td>4.4</td>
</tr>
<tr>
<td>Excellent</td>
<td>9</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>586</td>
<td>100.0</td>
</tr>
</tbody>
</table>

This table indicates that (10.6%) of the students are have failed level of academic achievement, (43.7%) of them were showing accepted level, (27.0%) of the students have adequate level of academic achievement, (12.8%) of the students have good level of academic achievement, (4.4%) of the students have very good level of academic achievement, and just (1.5%) show excellent level of academic achievement.

Table 3: Correlation between Students’ Emotional Intelligence and Academic Achievement.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Academic achievement</th>
<th>Emotional intelligence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Correlation</td>
<td>1</td>
<td>.817</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>--</td>
<td>.000**</td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>817*</td>
<td>1</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.000**</td>
<td>--</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
This table reveals that there is a significant influence of students’ emotional intelligence on academic achievement at p-value= 0.01.

The findings also reveal that there are no significant association between students’ emotional intelligence and their Gender, Age, Residency, and Scholastic Stage on the other hand there are significant association between students’ emotional intelligence and their Sequence in the Family, Brothers Number, Sisters Number, Fathers Education, Mothers Education, and Monthly Income at p-value=0.05. The descriptive analysis of the sample shows that more than half of the sample was females (51.9%) while the males were (48.1%). These results agree with findings of Mohzan et al (2013)\(^1\), who found that the female in the sample are more than males. The results in also show that most of students are between (15-17) years old (41.0%) followed by a lesser proportion for those who were at age (12-14) with (33.4%) and (25.6%) were more than 18 years old. These findings are supported by a study conducted by Ream & kurt (2010)\(^7\) who stated that more than half of his sample was about (15-17) years old. These findings are also supported by a study conducted by Cooper (2017)\(^8\) who stated that mean age of (16.1) years old. The results reveal that about, (28.0%) of participants were the first child in the family while (22.2%) as second one. On the other hand, (18.1%) of students have third ordinary in the family. Also (15%) reported as fourth child. Additionally, (15.4%) as fifth and more, this agrees with Rust (2014)\(^9\) who found that most of his participants were as first child in family. Majority of participants in study live in urban areas (53.9%) while (46.1%) of participants live in rural areas. The current finding is supported with findings of Fitch (2015)\(^10\) who reported that more than half of the sample was living in the urban areas. (1.4%) of students’ father not read and not write, (3.4%) of students’ father can read and write, (22.2%) of students’ father have finished the elementary school, (31.7%) of students’ father have finished the secondary school, (36.5%) of students’ father have finished the diploma or bachelor study and (4.8%) of students’ father with higher education. These findings agree with a study of Neo (2012)\(^11\) who stated in his study that most of student father with graduated education. The findings shows that (7.2%) of students’ mother not read and not write, (9.9%) of students’ mother can read and write, (32.8%) of students’ mother are elementary school graduates, (21.5%) of students’ mother are secondary school graduates, (22.5%) of students’ mother are diploma or bachelor graduates and (6.1%) of students’ mother with higher education. In Iraq, according to the researcher’s point of view, as a result of the payment of the so-called customs and traditions sometimes or the harsh conditions at other times and neglect always can lead to low level of mothers educations. Actually more than a half of participants have a sufficient monthly income (60.4%) followed by (28.7%) of participants also have a somewhat sufficient monthly income, While (10.9%) of participants have an insufficient monthly income. The current finding is also supported with findings of Wijekoon, (2017)\(^12\) who found that 50% of his sample with sufficient monthly income. Additionally support by Neves (2016)\(^13\) who found that more than half of his sample with sufficient monthly income (63.8%). The distribution students according to scholastic stage were (17.1%) selected for each of first and second stage followed by equal numbers of students percent for each of (third, fourth, and sixth stage) with (16.4%); while (16.7%) for fifth stage. The current finding is supported with findings of Fitch (2013)\(^10\) who reported that the distribution of student according to scholastic stage were almost equal. According the level of academic achievement the findings indicates that (10.6%) of the students are have failed level of academic achievement, (43.7%) of them were showing accepted level, (27.0%) of the students have adequate level of academic achievement, (12.8%) of the students have good level of academic achievement, (4.4%) of the students have very good level of academic achievement, and just (1.5%) show excellent level of academic achievement. These findings are supported by a study conducted by Neves (2016)\(^13\) which stated that (55%) of them were showing accepted level of achievement scores. There are no significant association between students’ emotional intelligence and Residency at p-value=0.01. This finding agreed with a study established by Goodwin (2016)\(^16\) who stated that there are no significant association between students’ emotional intelligence and Residency.

**Conclusion**

There is a significant influence of students’ emotional intelligence on academic achievement. There are no significant association between students’ emotional intelligence and their Gender, Age, Residency, and Scholastic Stage. There are significant association between students’ emotional intelligence and their Sequence in the Family, Brothers Number, Sisters Number, Fathers Education, Mothers Education, and Monthly Income.
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Baghdad/College of Nursing, Iraq and all experiments were carried out in accordance with approved guidelines.

References


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Evaluation of Biochemical Health Measurements to Predict Backpack Achievement (50m)

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Abstract

The tests and measurement is one of the most important scientific subjects that seek to achieve the requirements of research by linking to the delicate aspects that are objective through the results of measurements that carry the scientific side, and swimming on the back is very important in the field of sports and biochemical indicators scientifically shows us the amount of potential To predict the level of achievement of young swimmers indicate the amount of safety of different devices and reflect the positive side of the level of real achievement.

Key words: Biochemical Health Measurements, Predict Backpack.

Introduction

Measurements are tools that can be identified on many indicators through the analysis or quantification of the components that indicate the knowledge of the scientific facts and the amount of prediction between them during what is required of the performance of the athlete, which achieves development rates in the side that works by To create a scientific experience for trainers or employees within their specialties and thus we may reach the desired performance of the best in the movement of sports according to a scientific measure which is a direct goal in this aspect. The research in the field of tests and measurement in the field of sports proved qualitative changes in order to secure the requirements of athletic achievement and raise the physical and functional level of athletes by giving us a precise description and analysis of different responses to the current or cumulative variables that occur to different body cells when practicing sports activity. Physical or functional for athletic effectiveness There are characteristics imposed on the various body devices depending on the nature of performance Sports efficiency characterized by high speed and maximum strength in a short time will inevitably impose different responses and functional adaptations, each depending on the nature of the actor And swimming is one of the activities that are not limited to a particular age or age, considering that all ages are suitable for development, but this level and its speed varies from age to stage and from one stage to another due to physiological, physical and dynamic reasons and whenever these qualities and elements are available at a high level When individuals learn the easier and better, The importance of research is one of the field indicators in most of the research and in this research is a scientific evidence shows us the amount of biochemical potential of the swimmers on which the safety of different devices and reflected positively on the level of real achievement. And the main problem of this event is the lack of scientific research that dealt with the prediction of swimming in terms of some biochemical indicators, and the lack of observation measurements that can be a scientific indicator in this area, which examines the most accurate details of the level of achievement, in addition to that we find that most swimmers do not have The scientific idea of biochemical measurements, which is one of the factors important for this type of swimming or other, and also noted the weakness of the researchers to achieve the new figures when most young swimmers in the country, and the reasons that the researchers see is the lack of interest most of the trainers in the note These measurements as possible to be one of the factors causing the failure of to achieve the required level, all of these reasons prompted the researchers to study this subject so that in front of

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employees and athletes in this area of work under the results that will be obtained by the researchers, and the most important goals seen by the researchers.

1- Identify the level of completion of the swimming pool (50) m for young swimmers.

2 - Identification of the measurements of some biochemical indicators in the members of the research sample.

3 - The conclusion of predictive equations for some biochemical indicators of enzymes studied.

Research methodology and field procedures

Research Methodology:

That «research in all scientific fields resort to the selection of a curriculum that is appropriate to the problem, so the use of the descriptive method of surveying methodas this approach is an appropriate approach to the study of social phenomena as it provides data on the reality of these phenomena and the relationship between the causes and results and analysis, In which it would be useful to draw conclusions and recommendations thereon.»

Research community and design:

The researchers are interested in selecting the community and the sample they choose. Hence, the research community was identified. They are young people in the province of Thi-Qar (20) swimmers for the sports season (2018-2019). The researchers chose the research sample. (100%). The two researchers conducted homogeneity in the studied indicators in order to derive scientific equations for prediction as shown in Table (1)

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Measuring unit</th>
<th>Mean</th>
<th>standard deviation</th>
<th>Median</th>
<th>Skewness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skewness</td>
<td>مس</td>
<td>165.6</td>
<td>2.83</td>
<td>164.2</td>
<td>-0.82</td>
</tr>
<tr>
<td>Skewness</td>
<td>مس</td>
<td>60.1</td>
<td>3.09</td>
<td>59.50</td>
<td>0.66</td>
</tr>
<tr>
<td>Skewness</td>
<td>مس</td>
<td>2.95</td>
<td>0.11</td>
<td>3.001</td>
<td>0.23</td>
</tr>
<tr>
<td>Skewness</td>
<td>مس</td>
<td>61.3</td>
<td>8.90</td>
<td>64.5</td>
<td>1.39</td>
</tr>
<tr>
<td>Skewness</td>
<td>مس</td>
<td>362</td>
<td>57.0</td>
<td>377</td>
<td>0.13</td>
</tr>
<tr>
<td>Skewness</td>
<td>مس</td>
<td>44.3</td>
<td>3.91</td>
<td>42.1</td>
<td>1.68</td>
</tr>
</tbody>
</table>

Table (1). Shows the values of the computational circles, standard deviations and torsion coefficients of the research sample.

Field research procedures:

**Measurements and Tests Used:**

* - Measurement of biochemical indicators:

* Metabolic measurement (CPK) and (LDH) in the blood

Objective of the test: an enzymatic measurement (CPK) and (LDH) in the blood before and after the voltage.

Used equipments :

* A link to the area of the humerus - medical cotton, sterile materials - syringe (syringe).

A blood-free tube to prevent anticoagulation (EDTA) - to determine the level of serum (CPK) concentration in the blood - to determine the level of LDH concentration in the blood - the centrifuge.

Performance: The special test for the measurement of CPK and LDH enzymes in the blood was carried out at one stage. At the time of rest, the blood was withdrawn from the sample of the research sample in the morning and before any effort was made (in the case of rest) by calling the swimmer on the chair And extends one of his arms as shown in Figure (13), the analyst lends a compressive ligament in the humerus to limit the blood in the vein of the humerus then sterilize a small area of the vein and injects the syringe in the vein (the attachment area) and begins to withdraw blood by (5C) Which is sufficient quantity according to the instructions indicated with the book, after which the analyst pulls the syringe from the swimmer Sterilize the area and decompose the compressive ligament and then empty the blood in a plantub and leave the blood at room
temperature to clot and then place the blood sample in the centrifuge to extract the serum and then place the necessary material in its position in the Copas machine as shown in Figure 14.) By placing CPK and LDH materials in the space assigned to the Kopas device. We put the sample of the test through placing 50 ml of the special nematocyst syrup and placing it in the space assigned to it, The device then gives the device an order to do a CPK and HDL scan and then the device analyzes them until the results appear on the device screen Twenty minutes later.

Second Test: The completion of a swimming pool (50) meters:

* Purpose of the test: Measurement of the level of achievement of swimming on the back (50) m.

* Test instruments: swimming pool (50) m - stopwatch number (8) - whistle - registration form.

* Method of performance: The laboratory begins to perform the moment of hearing the whistle start and jump to the specified area and be in accordance with the movement of successive arms by the consensus of the feet and start from the jump to water in the opposite direction so that the back of the swimmer in the water, which consists of stages:

1 - Hate: Enter the swimming pool in the opposite direction with the movement of the arms outside and the shoulders are going out slightly.

2 - Tensile and push: The elbow must be attached and is higher than the shoulders, and the water tightens back and ends the movement before the arms bend slightly on the body to be complemented by a strong and fast movement of the sponsors back in the back and the arm must reach behind the shoulder level during tension.

There should also be a continuation of the end-of-tensile movement to the back-forward through the compatibility between the movement of the arms and legs, in which the hand faces both individually and sequentially when the two arms move forward. 1. Body position: The body should be extended in a straight horizontal position, hands down and slightly outwards, legs moving sequentially with the arms at the push, with a small part of the shoulder appearing above the surface of the water. The movement begins from the position of the adjacent extended men and the combs outstretched. The movement starts by pushing the articulation of the thighs and knees to the abdomen in a sequential manner, so that the angle between the thigh and trunk is approximately 55 because the angle is greater, affecting the flow of the body, which reduces the speed of movement in the water.

Calculation of grades: Calculates the time of the swimmer within a distance of (50) m.

Main experiment: The main experiment was conducted on 23/6/2018 after the completion of the exploratory experiments and the validation of the devices and tools. This experiment was conducted on the swimming pool of the Sumer Youth Forum in Dhi Qar Governorate. The test was applied to the research sample and with the help of cadre Auxiliary work where the time to test the swimming pool was measured (50) meters for the sample of the research. On the second day, biochemical measurements (LDH-CPK) were performed by the specialized physicians in Dhi Qar Governorate.

Statistical Methods: The researchers used SPSS ver20 to process data.

Presentation, analysis and discussion of research results:

<table>
<thead>
<tr>
<th>Coefficients*</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>T</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>41.983</td>
<td>4.381</td>
<td></td>
<td>11.18</td>
</tr>
<tr>
<td>CPK</td>
<td>0.169</td>
<td>0.071</td>
<td>0.490</td>
<td>2.38</td>
</tr>
</tbody>
</table>

a. Dependent Variable: Achievement

\[ Y = ax + b = 42.15 \]
Table (3) shows the results of the statistical treatment of the value of slope (b) of the variable (cpk), noting that the value of inclination (b) was significant through T test where the calculated T (2.38) at the level of significance (0.02) (19). The results showed that the value of the regression constant (a) was 42.15.

Table (3) Shows the results of the significant stability of variance analysis of variable (CPK).

ANOVA*

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>42.800</td>
<td>1</td>
<td>42.800</td>
<td>5.675</td>
<td>0.02b</td>
</tr>
<tr>
<td>Residual</td>
<td>135.750</td>
<td>18</td>
<td>7.542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>178.550</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: Achievement
b. Predictors: (Constant), CPK

d. Table (4) shows the results of the statistical treatment of the significance of the regression of the variable (CPK) and the completion of (50) meters on the back in the test (F), where there are significant differences between the two variables with the value of (F) calculated at (5.675) 0.02b) and at degree of freedom (19). This confirms the predictability of the variable (CPK). The researchers believe that the reason for this is that the exercise of physical activities varies from one activity to another and the body gets Atp. Since swimming is one of the high-effort activities, requires quick compensation to the energy, which leads to the speed of the processes of representation within the body and also this works at the speed of nerve impulses. The release of various control mechanisms leads to an increase in the work of more than a device such as myocardial nervous system and this leads to a group of rapid reactions to meet the body’s need of energy needed during physical exertion through these chemical reactions decomposes (CP) the muscle stock that is decomposed by phosphate during the cycle. One in the cell that generates the energy needed during physical exertion. The enzyme CPK is a group of transferable enzymes. It transfers the phosphate group to the receiving nitrogen group, also called the CK. This enzyme is excreted into the blood and is increased in case of injury or after exertion. Blood CPK is an energy-rich chemical that plays an important role during the effort. This enzyme helps release a large amount of energy and rebuild the ATP, which is the first energy back. ADP is regenerated into a system called ATP-CP, Energy in the physical activity of oxygen, which lasts for about 10 seconds[^1]. The enzyme CPK is important in the preparation of the interactions of energy production necessary for physical activity, so we see concentrated in the skeletal muscle and heart muscle, which is an important parts of sports movements and it is known that most muscle cells have phosphocrytin CPK is two or three times greater than ATP and it is transformed in a small fraction of a second into ATP for the purpose of continuing consecutive muscle contractions[^4]. Thus, CPK can be used for young swimmers. Based on the above, (CPK) And the level of achievement of young swimmers to detect the significance of the regression between the two variables.

Table (4) Shows the significance of the degree (T) and the equation of the prediction of achievement in terms of (HDL)

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>T</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>T</td>
</tr>
<tr>
<td>(Constant)</td>
<td>32.785</td>
<td>4.100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LHD</td>
<td>10.025</td>
<td>0.011</td>
<td>0.469</td>
<td>2.254</td>
</tr>
</tbody>
</table>

*a. Dependent Variable: Achievement

Y= ax+ b = 42.70
Table (5) shows the results of the value of the tendency (b) of the variable (LDH), noting that the value of inclination (b) was significant through T (T) calculated (2.254) at the level of (0.03) 19. The results showed that the value of the regression constant (a) was 42.70. Thus LDH was used for young swimmers. The simple regression of the LDH variable and the level of achievement of the young swimmers were analyzed to detect the regression between the two variables.

Table (5) The results of the mean stability of the variance analysis of LDH

<table>
<thead>
<tr>
<th>ANOVAa</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>39.308</td>
<td>1</td>
<td>39.308</td>
<td>5.081</td>
<td>0.03b</td>
</tr>
<tr>
<td>Residual</td>
<td>139.242</td>
<td>18</td>
<td>7.736</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>178.550</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: Achievement
b. Predictors: (Constant), LHD

Table (6) shows the results of the statistical treatment to show the significance of the regression between the variable (LDH) and the completion of (50) meters swimming on the back in the test (F), where there are significant differences between the two variables as the value (F) calculated at (5.081) (0.03b) and the degree of freedom (19) This confirms the possibility of predicting the variable (LDH), and researchers believe that this enzyme is the most important characteristic of the chemical reactions that get directly between athletes from others through access to energy sources needed during work, (ATP) by anaerobic glucose analysis through a series of reactions ending with the conversion of pyrofil from glucose to no Which leads to the production of energy needed during physical work, assuring that “the conversion of pyruvace to lactic acid in the case of O2 in the muscles working at the large muscle activity through the processes of biodegradation of pyrofearc to lactic by hydrogen”6, as the high level of enzyme Lactate dehydrogenase (LDH) after exertion in some sports practices, including swimming, is due to its vital role in the biochemical reactions of the anaerobic system 8. It is the system on which the players depend on performing the physical efforts associated with the pills that require high physical exertion. Is consistent with Atwell et al. 1991. At the same time, the continuation of a high-intensity controlled physical load for 60 s produces a significant increase in blood LDH immediately after performance. In addition, LDH stimulates the conversion reactions Pyrenees to Lactate, which is consistent with what he reached. “LDH works to stimulate interactions of conversion of pyrons into lactat 7-9.

Conclusion

Determination of equations to predict the level of achievement depending on some measurements of some biochemical indicators through: The prediction of the enzyme C.P.K according to the following equation: Y = ax + b = 42.15. Predicting the enzyme (LDH) according to the following equation Y = ax + b = 42.70. Safety of devices Function in the composition of energy through the results of prediction of enzyme (cpk). Improve the time period of energy production after fatigue through (ck) and the unity of calcium.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Physical Education and Sports Sciences / Thi- Qar University and all experiments were carried out in accordance with approved guidelines.

References


Road Traffic Fatalities in Babylon Province – Six Years Epidemiologic Study

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¹University of Babylon /Hammurabi College of Medicine, Iraq, ²Professor of Surgery, University of Babylon, Iraq, ³Babil Health Directorate, Iraq

Abstract

Road Traffic Accidents are the fifth leading cause of morbidity and mortality worldwide. In Iraq, traffic accidents are increasing continuously leading to more fatalities that rank Iraq as number four in the world. Objectives: To assess the epidemiological features of fatal Road Traffic Accidents in a time, place, person epidemiologic model and to identify the trend of Road Traffic mortalities in Babylon province during six years. This was a descriptive cross-sectional study included a analysis of the forensic medicine data presented in the records of forensic medicine department in Babylon Health directorate for the period 2010-2015. After the approval of the study protocol by the local health ethical committees, a structured questionnaire was used to collect data according to the descriptive epidemiologic model (person, place, time model). During the period 2010-2015 mortality rates were higher in males than females with male to female ratio of 3:1. Higher mortality rates reported in the year 2015 and the highest frequency of mortality was in the age group (16-35) years (34.9%) followed by the age group (6-15) years (14.7%) the lowest rate was among the age group (46-55) years, the highest mortalities reported among Wage earners (58.8%).

Key words: Road Traffic Fatalities, Babylon Province, Epidemiologic Study

Introduction

Globally, Road Traffic Injuries (RTIs) have increased in the last twenty years, this makes RTIs one of the main five causes of morbidity and major cause of fatality all over the world particularly in the low- and middle-income countries¹. The RTA is defined as any vehicle accident occurring on a public road or highway and includes accidents where the place of occurrence is unspecified². The growth in the global economy in the last century has changed many aspects of people’s lives including their use of various means of transportation, this leads to a corresponding increase in the number of automobiles usually resulting in an increase in RTAs and consequent injuries and deaths³,⁴. The overall global RTIs fatality rate estimated to be 19.5 per 100 000 population, middle-income countries have the highest annual road traffic fatality rates, at 20.1 per 100 000, while the rate in high-income countries is the lowest, at 8.7 per 100 000. There is a wide variety in road traffic death rates in different regions of the world; the highest rate was reported in African regions, 24.1 per 100 000 and lowest in the European Region 10.3 per 100 000. Also, there is a considerable disparity within each region⁵. In the Eastern Mediterranean Region, one of the major challenges to the region is the constantly increasing incidence of RTIs⁶. This region has one of the world’s highest traffic fatality rates, mostly due to a lack of simple measures to reduce RTIs⁷ and inadequate pre-hospital medical emergency systems⁸. The available data referred to different death rates due RTIs in some Arab and regional countries; moderate to high fatality rates reported in United Arab Emirates⁹, Saudi Arabia 10 and Kuwait 11. However, road traffic injury fatality rates range from 9 in Turkey to 44.75 per 100 000 population in Iraq¹². In Iraq, traffic accidents are increasing continuously leading to more fatalities¹³. According to the WHO data about Iraq, road traffic fatality rate is preceded only by Namibia, Swaziland and Malawi⁵,¹². It is worth mentioning that in 2011 the injury toll from RTIs is almost four times greater than that from acts of terrorism in Iraq¹⁴. According to the agreement between Iraq and WHO, the Ministry of Health and the Ministry of Interior with the full cooperation of the WHO, Iraq launches the Decade of Action for Road Safety 2011-2020 and pledges to reduce
the level of road traffic fatalities by 2020. Accidents, therefore, can be studied in terms of agent, host and environmental factors and epidemiologically classified into time, place and person distribution. Previous studies referred that majority of the victims are within the age 15 to 50 years, furthermore, children account for approximately 13% of fatalities. The main victims of RTIs are males. The global burden of morbidity is estimated that around 20 to 50 million individuals are being injured or disabled each year with considerable social and economic losses. One million and three hundred thousand deaths are reported globally each year. The World Health Organization stated that without efforts to prevent these accidents, it is predicted that 1.9 million person in the world die annually by 2020 more than 90% of deaths due to traffic injuries occur in low-and middle-income countries including Iraq. In 1990, road accidents ranked 9th in the list of the most important factors threatening the health of the community but it is predicted that by 2020 they will rank the third cause of mortality. Another bitter fact about these reports is that 50% of the killed are individuals with a role in the economic development of societies. The frequencies of road accidents are rising in developing countries and are higher compared to those in developed countries. According to World Health Organization, the average standardized annual death rate due to road traffic accidents in Iraq is 44 per 1000000 population) and this is ranking Iraq as number 4 in the world. Because pre hospital transit times are long, most trauma deaths in these countries occur during the pre-hospital phase; hence efforts to improve survival rates should focus on better care outside the hospital.

**Objectives of the study:**

- To assess the epidemiological features of fatal RTIs in a time place person model in Babylon province.
- To identify the epidemiological trend of RTI mortalities in the province.

**Methodology**

The study protocol was approved by the ethical committee in Hammurabi college of medicine University of Babylon. The acceptance of ethical committee of Babel health directorate was taken as well. This was a cross-sectional descriptive study depended on a retrospective analysis of RTIs mortalities that were recorded in the forensic medicine department for the years 2010 – 2014. The time needed to conduct this study from 1st of February to end of May 2016. Data for the five years were reviewed by the researchers. The data for each victim include, age, gender, occupation, marital status and residence of the victims, in addition to the place of accident, date of accident (in day and month) and the site of injury in the body. Statistical analysis was done using Spss version 17 to calculate the Chi square test to evaluate the significant of difference between variables, P<0.05 considered as the statistical significant level.

**Results and Discusion**

Table (1) shows the distribution of mortalities for the six years according to gender, higher number of mortalities was reported among males at each year and over the whole period; out of the 2340 mortalities, males constituted 1791 (76.5%) while females were 549 (23.5%) with a male to female ratio of (3:1).

<table>
<thead>
<tr>
<th>Year</th>
<th>Male</th>
<th>%</th>
<th>Female</th>
<th>%</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>268</td>
<td>(76.1)</td>
<td>84</td>
<td>(23.9)</td>
<td>352</td>
<td>(100)</td>
</tr>
<tr>
<td>2011</td>
<td>298</td>
<td>(75.3)</td>
<td>98</td>
<td>(24.7)</td>
<td>396</td>
<td>(100)</td>
</tr>
<tr>
<td>2012</td>
<td>350</td>
<td>(79.4)</td>
<td>91</td>
<td>(20.6)</td>
<td>441</td>
<td>(100)</td>
</tr>
<tr>
<td>2013</td>
<td>251</td>
<td>(75.1)</td>
<td>83</td>
<td>(24.9)</td>
<td>334</td>
<td>(100)</td>
</tr>
<tr>
<td>2014</td>
<td>239</td>
<td>(75.9)</td>
<td>76</td>
<td>(24.1)</td>
<td>315</td>
<td>(100)</td>
</tr>
<tr>
<td>2015</td>
<td>385</td>
<td>(76.6)</td>
<td>117</td>
<td>(23.4)</td>
<td>502</td>
<td>(100)</td>
</tr>
<tr>
<td>Total</td>
<td>1791</td>
<td>(76.5)</td>
<td>549</td>
<td>(23.5)</td>
<td>2340</td>
<td>(100)</td>
</tr>
</tbody>
</table>

Table (2) show the higher overall number of mortalities due to road traffic injuries was in the age group (26-35) years (17.8%), age group (16-25) years (17.1%) and the age (6-15) years (14.7%).
Table 2. Distribution of death according to age For the years (2010 – 2015) in Babylon province

<table>
<thead>
<tr>
<th>Age</th>
<th>No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5 Years</td>
<td>275</td>
<td>(11.7)</td>
</tr>
<tr>
<td>6-15 Years</td>
<td>344</td>
<td>(14.7)</td>
</tr>
<tr>
<td>16-25 Years</td>
<td>401</td>
<td>(17.1)</td>
</tr>
<tr>
<td>26-35 Years</td>
<td>417</td>
<td>(17.8)</td>
</tr>
<tr>
<td>36-45 Years</td>
<td>321</td>
<td>(13.7)</td>
</tr>
<tr>
<td>46-55 Years</td>
<td>167</td>
<td>(7.1)</td>
</tr>
<tr>
<td>56-65 Years</td>
<td>205</td>
<td>(8.7)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>210</td>
<td>(8.9)</td>
</tr>
<tr>
<td>Total</td>
<td>2340</td>
<td>100</td>
</tr>
</tbody>
</table>

Table (3) shows the total mortalities reported during the 6 years were higher Wage nearer, (58.8%), followed by employed (21.1%), students (14.1%).

Table 3. Distribution of RTA death according to the occupation of victims

<table>
<thead>
<tr>
<th>accusation</th>
<th>Total</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wage earner</td>
<td>1371</td>
<td>(58.8)</td>
</tr>
<tr>
<td>Employee</td>
<td>495</td>
<td>(21.1)</td>
</tr>
<tr>
<td>Student</td>
<td>333</td>
<td>(14.1)</td>
</tr>
<tr>
<td>Retired</td>
<td>94</td>
<td>(4)</td>
</tr>
<tr>
<td>Others</td>
<td>47</td>
<td>(2)</td>
</tr>
<tr>
<td>Total</td>
<td>2340</td>
<td>100</td>
</tr>
</tbody>
</table>

Table (4) demonstrates the total reported deaths due to all causes during the period 2010-2015 were 7527 deaths. Deaths due to Road Traffic Accidents account for 2340 deaths giving an overall Proportional Mortality ratio of 31.1%. Moreover the higher Proportional Mortality ratio, (48.6%), was reported in the year 2012, and the lower ratio of (21.1%) was reported in the year 2014.

Table 4. Proportional mortality ratios of (R.T.A) in Babylon for the years (2010_2015)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total deaths</th>
<th>R.T.A Deaths</th>
<th>(%) Mortality ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>1098</td>
<td>352</td>
<td>(32.1)</td>
</tr>
<tr>
<td>2011</td>
<td>1190</td>
<td>396</td>
<td>(33.3)</td>
</tr>
<tr>
<td>2012</td>
<td>908</td>
<td>441</td>
<td>(48.6)</td>
</tr>
<tr>
<td>2013</td>
<td>1287</td>
<td>334</td>
<td>(26)</td>
</tr>
<tr>
<td>2014</td>
<td>1492</td>
<td>315</td>
<td>(21.1)</td>
</tr>
<tr>
<td>2015</td>
<td>1552</td>
<td>502</td>
<td>(32.3)</td>
</tr>
<tr>
<td>Total</td>
<td>7527</td>
<td>2340</td>
<td>(31.1)</td>
</tr>
</tbody>
</table>
Table (5) Proportional mortality Death of road accidents in Babylon for the years (2010_2015)

<table>
<thead>
<tr>
<th>Year</th>
<th>Population</th>
<th>Death of road accidents</th>
<th>Ratio %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>1700000</td>
<td>352</td>
<td>(20.7)</td>
</tr>
<tr>
<td>2011</td>
<td>1800000</td>
<td>396</td>
<td>(22)</td>
</tr>
<tr>
<td>2012</td>
<td>1850000</td>
<td>441</td>
<td>(23.8)</td>
</tr>
<tr>
<td>2013</td>
<td>1900000</td>
<td>334</td>
<td>(17.5)</td>
</tr>
<tr>
<td>2014</td>
<td>1950000</td>
<td>315</td>
<td>(16.1)</td>
</tr>
<tr>
<td>2015</td>
<td>2000000</td>
<td>502</td>
<td>(25.1)</td>
</tr>
</tbody>
</table>

In Iraq, there are lacking in the reliable data on the incidence of RTIs, nonetheless, it is one of the public health priority of Iraq that will need to be investigated. Analysis of these data revealed that the total mortalities for six years period in Babylon province years were 2340 victims with male predominance this finding is similar to the finding reported by other study. On the other hand it had been noticed that the death numbers increased during the years 2011 and 2012 then declined in the next two years, this decline in the trend of mortalities might be attributed to the increase of the public and the drivers awareness about the traffic safety and the monitoring program applied according to the agreement between Iraq and WHO, where, Iraq launches the Decade of Action for Road Safety 2011-2020 and pledges to reduce the level of road traffic fatalities by 2020 towards achieving the objectives of the decade at the national and international levels, additionally, the traffic police in last three years enforced in some areas of Iraq the use of seatbelt, helmet, and having driving license particularly in the safe zones of Iraq. Despite this decline reported in the current study, the death numbers still higher than what were reported in neighboring countries such as Saudi Arabia, and Iran. This study showed that more than half of the victim’s lives in urban areas, higher proportion of victims were unemployed and the majority of deaths occurred on the main roads, which consistent with other studies. The high rate of deaths in the current study might be attributed to different factors such as no speed limit on the roads in urban areas and inside the cities, crowded roads, presence of different types of vehicles (two wheels, three wheels and four wheels vehicles), absence of road signs, additionally, other possible reasons for the higher RTI related fatalities such as insufficient government policies that promote cycling, walking and investing in public transport as it found in some countries in the region, lack of education about the safety measures, like seat belt, helmets and periodic safety check for the vehicles. The highest death rate was noticed in December, almost, similar finding was reported in previous Iraqi study, however, this might be due to cold or rainy weathers as in December. The current study found that head was the dominant site of injuries among victims, during the whole period which account for 93.7%, followed by lower limbs and upper limbs.

Conclusion

Most fatalities occurred among males, in their productive age group and among wage earners, the highest rates of deaths occur during December. Head and lower limbs were the commonest sites of injuries.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon/Hammurabi College of Medicine, Iraq and all experiments were carried out in accordance with approved guidelines.

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A Comparative Evaluation of Gutta-Percha and Sealer Removal of Rotary Niti File Retreatment Systems

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Abstract

Forty lower premolars with single root canals prepared with ProtaperNext files to size 25, and obturated with GP/sealer using lateral compaction. Teeth divided randomly into four groups (group n=10). Protaper universal retreatment kit (PUR), D-Race desobturation files (DRD), R-Endo retreatment kit (RE) and Hedstrom (H) files (control) were used to remove GP/sealer in each group. Removal effectiveness assessed by measuring the GP/sealer remnants in the roots after sectioning them into two halves. Stereomicroscope with a digital camera used to capture digital images. Images processed by ImageJ software to measure the percentage of GP/sealer remnants surface area in total, coronal, middle and apical areas of the canal. In the coronal area, PUR had significantly lower R% than RE and H groups, respectively (p<0.05). Also, DRD had significantly lower R% than RE (P<0.05). There was no significant difference between PUR and DRD (p>0.05), as well as no significant difference between RE and H groups(p>0.05). In the middle, apical and total root areas, Both PUR and DRD had significantly lower R% than RE and H groups, respectively (p<0.05). There was no significant difference between PUR and DRD (p>0.05). Also, there was no significant difference in R% between RE and H groups (p>0.05).

Keywords: DRace, Hedstrom, REndo, retreatment, Protaper

Introduction

Retreatment of endodontically treated teeth after the failure of the initial endodontic treatment is a common and a conservative procedure compared to tooth extraction and endodontic surgery. Retreatment procedure involves removal of the root canal filling materials such as gutta-percha and sealer which are the most frequent materials to be used for root canal filling. The success of retreatment is directly related to the effectiveness of any file system to remove the old filling materials, which may harbor bacteria and debris. 1 The techniques used for removal of gutta-percha from root canals include Hedstrom (H) instruments with/without chloroform, ultrasonics, lasers, heat carrying instruments, as well as NiTi rotary instruments. 2 Recent advances in NiTi rotary files systems used in removal of gutta-percha and sealer enable better cleaning, less time and less fatigue to patient and operator compared to the conventional stainless-steel files because of super elasticity, shape memory and resistance to torsional fracture, which enhancing their accessibility to distant parts of root canal and their removal effectiveness. 3-5 Many NiTi rotary systems introduced for gutta-percha/sealer removal such as Protaper universal retreatment (PUR) system (Dentsply Maillefer, Ballaigues, Switzerland), Re-Endo system (RE) (Micro-Mega, Besancon, France), D-Race desobturation system (DRD) (FKG Dentaire, La Chaux-de-Fonds, Switzerland). A modified guiding tip, triangular convex cross-section and progressive increase in tapers are the main characteristics of ProTaper universal retreatment files. This system consists of D1, D2 and D3 files which have sizes and tapers of 30/0.09, 25/0.08 and 20/0.07, respectively. D1 file has an active tip, D2 and D3 have non-active tips in order to reduce root canal retreatment mishaps. 6 D-Race retreatment files consist of DR1 and DR2 files with sizes and tapers of 30/0.10 and 25/0.04, respectively. Length of DR1 and DR2 are 15mm and 25mm, respectively. DR1 used in the straight and coronal section of the canal as it has
an active tip to penetrate the filling materials followed by DR2 which is used to reach the full length of the canal. R-Endo retreatment files consist of four files, Re, R1, R2, and R3 in addition to an optional finishing file Rs. Retreatment of root canals with R-Endo retreatment system start with coronal conditioning followed by repreparation of the canal using the mentioned sequence of files. Determining the effectiveness of different rotary NiTi systems in removing gutta-percha/sealer may help dentists to choose the best system that enables the greatest/fastest removal of gutta-percha/sealer. Previous studies had controversial findings regarding the best effective rotary NiTi system in removing obturated GP/sealer.

Materials and Method

Sample Preparation:

Forty extracted single canal teeth were selected for the study. To standardize the samples, all teeth were decoronated to obtain root segments of 12mm length, working length was established by subtracting 1 mm from the real root length determined by introducing a number 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until it was visible through the apical foramen. Root canal instrumentation was performed using Protaper next system (Dentsply Maillefer, Ballaigues, Switzerland), as recommended by the manufacturer, up to size 25 using a crown-down technique and a standardized amount of 2.5% NaOCl was used to irrigate the canal. then each root canal was obturated with gutta-percha (Protaper next cones, Dentsply Maillefer, Ballaigues, Switzerland) and Endoﬁll sealer (PD, Switzerland) using the lateral compaction technique. The temporary filling was used to seal the coronal orifice, then the teeth were stored for one week in 37°C at 100% humidity. The roots were randomly divided into four groups (n=10/group) according to each retreatment file system was used:

Group I

The X-smart endodontic motor (Densply Sirona, Ballaigues, Switzerland) alongside the Protaper universal retreatment (PUR) system (Dentsply Sirona, Ballaigues, Switzerland) were used to remove the root canal filling materials. The PUR system was employed using D1 (size 30, .09 taper) in the cervical and beginning of middle thirds of working length. D2 (size 25, .08 taper) was used to remove GP from the middle and apical thirds until the working length was reached. D1 and D2 were used only without using D3 ((size 20, .07 taper) files. Speed setting used to remove gutta-percha/sealer from the canals was 500-700 rpm, gutta-percha/sealer removal was considered complete when no more remnants were detected in the file.

Group II

The DRD system (FKG Dentaire, La Chaux-de-Fonds, Switzerland) was employed using DR1 (size 30, .10 taper) and DR2 (size 25, .04 taper) files to remove gutta-percha/sealer from the coronal and apical half areas, respectively, until the working length was reached. The DR1 and DR2 files were used at slowest speed dictated by the manufacturer (600 RPM) and the torque was adjusted at 1.5 Ncm. The verification of complete gutta-percha/sealer removal was as previously described.

Group III

Re-Endo system (Micro-Mega, Besancon, France) files (Rm, Re, R1, R2, R3) were used as per the manufacturer’s instructions. The Rm stainless steel manual file (17 mm, 25/.04 taper) was used first to its full length. It was followed by nickel-titanium rotary instruments Re (25/.12 taper) orifice opener, R1(25/.08 taper) till cervical third, R2 (25/.06 taper) till middle third, R3 (25/.04 taper) to full working length at a speed of 300 rpm and a torque of 1.2 N cm.

Group IV

H files were used to remove the obturating material, H-files of sizes 20, 25 were used in a circumferential quarter turn push-and-pull motion until WL was achieved. The retreatment procedure was considered to be complete when no material could be visualized in the canal or file flutes.

Gutta-percha/sealer remnant quantification

Roots were grooved longitudinally in a buccolingual direction, then split into two halves using a chisel. Canals walls were examined under the stereomicroscope; and imaged at 25x magnification using a digital camera (Nikon, Tokyo, Japan) stabilized at a fixed distance using a holder. The images were magnified 100% using a digital zoom tool. The images were analyzed with ImageJ software (version 19 windows). The canal surface was divided into coronal, middle and apical areas digitally. The percentage of the area of remnants GP/sealer (A) was calculated using the following equation:

\[ A = \frac{\text{area of the remnants GP/sealer} \times 100}{\text{area of the root canal at either coronal, middle, apical thirds}} \]
total area of the root canal and remnant GP/sealer were calculated by summing the data of the coronal, middle and apical thirds. SPSS 24 was used to analyse the data. Inferential statistics included Kruskal Wallis H test and Man-Whitney U test, the significance level was assumed at \( p \)-value less than 0.05.

**Results and Discussion**

Table (1) shows the mean of the percentage of the area of remnants GP/sealer (R%) in coronal, middle and apical areas of the root canals, also, it shows the mean of the percentage of the area of remnants GP/sealer in the total surface area of the root canals. The lowest R% in coronal, Middle, apical and total root areas achieved in PUR, DRD, DRD and DRD groups, respectively. In each one of the tested groups, there was no significant difference in R% between coronal, middle, apical and total root areas, except in the H file group, there were significantly lower R% in coronal compared to middle areas of the root canal (\( p < 0.05 \)). In addition, there were significant differences (\( p < 0.05 \)) in R% between groups in each of the levels (the coronal, middle, apical and total root areas), respectively. In the coronal area, PUR showed the lowest R% compared to other systems. PUR had significantly lower R% than RE and H groups, respectively (\( p < 0.05 \)). Also, DRD had significantly lower R% than RE (\( P < 0.05 \)). There was no significant difference between PUR and DRD (\( p > 0.05 \)), as well as no significant difference between RE and H groups (\( p > 0.05 \)). In the middle, apical and total root areas, Both PUR and DRD had significantly lower R% than RE and H groups, respectively (\( p < 0.05 \)). There was no significant difference between PUR and DRD (\( p > 0.05 \)). Also, there was no significant difference in R% between RE and H groups (\( p > 0.05 \)), as shown in Table (1).

**Table (1): Remnant GP/sealer area mean percentages \( \pm \) (SD) of all groups at coronal, middle, apical and total root areas.**

<table>
<thead>
<tr>
<th>Levels</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-Endo</td>
</tr>
<tr>
<td>Coronal area (Remnant % mean(\pm)(SD))</td>
<td>20.1 (\pm) (18.6) \textsuperscript{a,b}</td>
</tr>
<tr>
<td>Middle area (Remnant % mean(\pm)(SD))</td>
<td>34.2 (\pm) (32.6) \textsuperscript{a,b}</td>
</tr>
<tr>
<td>Apical area (Remnant % mean(\pm)(SD))</td>
<td>45.7 (\pm) (21.7) \textsuperscript{a,b}</td>
</tr>
<tr>
<td>Total root area (Remnant % mean(\pm)(SD))</td>
<td>28.9 (\pm) (21.3) \textsuperscript{a,b}</td>
</tr>
</tbody>
</table>

Identical small letters superscript in the same row represent significant differences between relevant groups.

Periapical inflammation and failure may be occurred postoperatively because of the inadequately prepared and obturated root canal systems that harbor bacteria and necrotic tissue that need to be eliminated therefore removing as much sealer and gutta-percha as possible in retreatment procedure seem necessary. This study aimed to assess and compare the effectiveness of PUR, RE, DRD and H files in removing gutta-percha and sealer in natural single root canals by measuring remnant GP/sealer surface area percentage. As reported by other authors, none of the techniques tested was able to irradiate all GP/sealer remnant from retreated root canals\(^4,11,12\)

To simplify the standardization of the specimens, premolars with straight roots and relatively wide canals were selected for this study. The use of a longitudinal split to obtain images to inspect the root canal walls was advocated by several authors.\(^9,10,12\) Longitudinal sectioning together with the use of the stereomicroscope has been shown to be more efficient for detecting the remaining root canal filling material compared with radiographic techniques.\(^13\)
In the present study, speed and torque used were adjusted according to the manufacturer’s instruction for all tested groups. However, in order to standardize the size of the final instrument used to remove GP/sealer in the canals, the files with size 25 tip in each system were the final instrument used to remove GP/sealer, therefore, D3 file of PUR was not used to remove GP/sealer. No solvent was used to help remove the root canal filling materials with the rotary NiTi systems, as they generally plasticize gutta-percha through the heat generated by friction. Also, the solvent was not used with H files in order to minimize the number of variables involved in the study. In addition, using a solvent would generate a thin film of gutta-percha on the root canal walls that would be difficult to identify and remove. Similarly, we did not use Gates Glidden burs with H files, which are known to be effective in removing GP/sealer in the coronal part of the root canals. Also, three-dimensional visualization of the root canal after retreatment was not used, which would provide superior quantification of the filling remnant and prevent remnant loss during the splitting procedure as reported by.  

The present study showed that root filling materials could not be completely removed from the root canal walls. This observation was in agreement with those of the previous studies on retreatment efficacy in which various retreatment technique and root filling materials were used. The results of the present study revealed that PUR and DRD showed the lowest R% compared to other groups. There were no significant differences between PUR and DRD (p>0.05) in R% at all levels (the coronal, middle, apical and total areas, respectively). These findings agree with that of Colaco and Pai 2015 and Ibrahim et al. 2018, they have reported that there are no significant differences between PUR and DRD regarding GP remnants. However, both PUR and DRD were more efficient than R-Endo and H files in removing GP/sealer by leaving less amount of remnant GP/sealer, this higher efficiency of PUR and DRD may be attributed to the design of the PUR and DRD files. PUR files have a convex triangular cross-section, a larger internal mass and area, and variable helical pitch and taper. Which result in effective removal of GP coronally in the canal. DRD files exhibit smooth surface because of a special electrochemical surface treatment, in addition to a triangular cross-section, which enhances removal efficiency of GP from the canal and superior sharpness.

Conclusion

The amount of GP/sealer remaining after root canal retreatment with PUR and DRD was not significantly different but showed better retreatment efficiency than Re-Endo and H files. However, because all experimented techniques showed remnants of filling materials on the canal walls, additional measures such as the combination of manual and rotary techniques can help completely remove GP during endodontic retreatment.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry, University of Baghdad, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Clinicopathological Study of Oral Giant Cell Fibroma

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Abstract

Giant Cell Fibroma (GCF) is a relatively rare oral mucosal lesion, so named due to the characteristic giant cells present within the fibrous stroma of the lesion, limited number of clinicopathological studies were performed in previously published literature. This study was performed to evaluate the clinicopathological features of Giant cell fibroma in a sample of Iraqi patients. Formalin-fixed paraffin-embedded sections from 22 giant cell fibroma in period between 2010 and 2018 were retrieved from the laboratory of oral pathology of Baghdad University/College of Dentistry. Clinical data and microscopic features were reviewed and analyzed according to the available surgical reports. The mean age of patients at the time of diagnosis was 29.68 years with slight female predilections (1.4:1), the gingiva is the most common site of occurrence (36.4%), the lesions were 2-10 mm in greatest dimension. The most frequent provisional diagnosis is fibroma (54.5%) and papilloma (27.3%). Histologically, the distinctive diagnostic feature is the presence of mono, bi or multinucleated large stellate giant cells with a mean of 48.59 which is most numerous in the lamina propria beneath the epithelium.

Key words: Clinicopathological, oral giant, cell fibroma

Introduction

The giant cell fibroma is an oral soft tissue lesion with distinctive clinicopathological features. It was first described as a separate entity among fibrous hyperplastic soft tissue lesions by Weathers and Callihan in 1974 when 108 out of 2000 fibrous hyperplasia specimens fulfilled the criteria for GCF 1 . Unlike the traumatic fibroma, it does not appear to be associated with chronic irritation, 2 . GCF is an asymptomatic nodular mass less than 1 cm frequently occur in the gingiva, the surface may be smooth, lobulated or papillary so the lesion takes on the clinical appearance of a papilloma, almost occur before age 30 years, with a slight female predilection 3 . Histologically, the surface is stratified squamous epithelium, covering loose or dense fibrous stroma with numerous large stellate mono-bi or multinucleated giant cells. With well-demarcated cytoplasm, dendritic processes and surrounded by an artificial space or separation of the collagen fibers from the cell boundaries.

Materials and methods

The study sample consisted of 22 GCF tissue blocks with their surgical reports retrieved from the files of the laboratory of oral pathology of Baghdad University/College of Dentistry, from 2010 to 2018, the available clinical data (age, gender, site, provisional diagnosis, size of the lesion) was reviewed and analyzed. All tissue sections were processed routinely for H and E stain and examined under a light microscope (Olympus CH3), The clinical features were assessed in relation to site, sex, size, and age. number of giant cells was calculated in each case in 5 fields of a tissue section by experienced pathologists and correlated to the clinical information. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Independent t-test and Analysis of Variance (ANOVA) (two-tailed) was used to compare the continuous variables accordingly. Pearson’s correlation test (r) was used to assess the correlation between continuous variables accordingly. Chi-square test was used to assess the association between provisional diagnosis and certain information. A level of P – value less than 0.05 was considered significant.
Results and Discussion

As showed in (table 1) The demographical and clinical data of 22 GCF cases revealed that the mean age was 29 years. A slight female predilection was recorded (59.1% versus 40.9%) with female to male ratio of 1.4:1. Gingiva is the most frequent site representing (36.4%), followed by tongue (27.3%), palate (18.2%), buccal mucosa (13.6%), and Lip (4.5%). The size of the lesion ranging from 2 to 12 mm with a mean of 4.8 mm and the largest lesion was seen in the posterior upper gingiva and buccal mucosa.

Oral Fibrous Hyperplasia(fibroma) is the most frequently missed provisional diagnosis (54.5%) followed by papilloma in (27.3%) which is mostly located on the tongue and palate of young aged patient, peripheral giant cell granuloma (P.G.C.G) and pyogenic granuloma in (9.1%), however, As showed in (table 2) statistically there is no significant association detected between provisional diagnosis and age, gender, and site as P value (0.121, 0.336,0.151) respectively.

The gross examination of the specimens of GCF (incisional and excisional biopsies) revealed white soft tissue lesions, the surface of the mass often appears papillary, the size of specimens ranged from 0.2 cm to 1.2 cm in diameter (figure 1) Microscopically sections showed a keratinized stratified squamous epithelium showing mild to moderate hyperplasia pseudo epitheliomatous hyperplasia was seen in 7 out of 22 cases with elongated and thick rete ridges, the underlying stroma consisted of dense collagenous fibrous tissue made of haphazardly arranged dense fiber bundles with mild to moderate amount of mixed inflammatory cells infiltrate (figure 2).

The most characteristic histological feature is the stellate shaped giant cells which are mono-bi or multinucleated with large hyperchromatic nuclei, these cells were mostly present in the subepithelial and interpapillary region and may have Short dendritic cytoplasmic processes and surrounded by an artifactual space or separation of the collagen fibers from the cell boundaries (Figure 3). The mean of the total number of giant cells in our study was 48.59 with 27.30 standard deviations. Statistically there is no significant association was detected between the number of giant cells and age, sex, and site of the lesion P value (0.8, 0.331, 0.077) respectively. The clinical features of GCF were described by five previous large-sample clinicopathological studies, GCF can occur at any age. The mean age of the patients was reported previously to be approximately 28 years in two studies (7,8), which was very close to the mean age of 29 years reported in the present study. The peak incidence is in the second decade of life, with about 60% of the lesions occurring in the first three decades. (1, 8,7) Our study found the highest incidence in the second and third decade, and approximately 60% of GCFs were in the first three decades. However, two previous studies have reported the highest incidence to be in the fourth decade of life. (9, 10) this discrepancy may be attributed to the asymptomatic nature of the lesion, genetic and racial differences. A slight female predominance for the occurrence was found in our study (59.1%) which is agreed with Four studies (1,7, 9,10) whereas disagreed with other studies that demonstrated no significant sex predilection. (8,11)

All previously mentioned studies stated that the most common location for GCF is the gingiva While The tongue is the second most common location followed by the buccal mucosa or palate that is in agreement with our study (1,7,9,10), the reported size of the lesion is 0.5-1 cm which is matching our results, however, the most accepted explanation to the largest size of lesion in the upper posterior gingival and buccal mucosal area is presence of trauma from occlusion in this site which lead to inflammation that may increasing the size. No GCF was diagnosed correctly at initial clinical presentation, GCF is misdiagnosed most often as fibroma, fibroepithelial polyp, or papilloma. (1,7,10) Comparable results were also found in the present study. The fibroma was reported in any location specially in buccal mucosa while the papilloma was frequently anticipated when the lesion appeared on the tongue or palate of the young and middle-aged patients because of the similarity in the shape, age, and site of these two lesions.

The histopathological features were in agreement with all previous literature; however, the presence of these peculiar giant cells and whether these cells were differentiated from a fibrous, histiocytic or neural lineage remains to be controversial. The pathogenesis of PEH Pseudoepitheliomatous hyperplasia (PEH) is still unclear, Pathologically, PEH arises due to the release of various cytokines produced by the tumor cells or inflammatory cells subsequently resulting in the proliferation of the overlying epithelium. 12 therefore, the presence of PEH in GCF may be associated with inflammation that presented mostly in traumatic areas inside the oral cavity specially gingiva and buccal mucosa, however, in our
study, in addition to the mentioned anticipated sites, the PEH was noticed in GCF from different sites such as palate, tongue, and lip.

**Conclusions**

Though the clinicopathological features of giant cell fibroma are similar to the conventional fibroma/fibroepithelial polyp there are discriminative histopathologic features for giant cell fibroma that still a controversial issue in pathological literature that need further clinicopathological, immunohistochemical and molecular verification. A high index of suspicion and appropriate investigative workup is necessary for separate lesions to achieve a suitable diagnosis and offer appropriate therapy.

**Table 1: Distribution of study patients by clinical information**

<table>
<thead>
<tr>
<th>Site of lesion</th>
<th>No</th>
<th>Percentage (%)</th>
<th>Sex</th>
<th>Age</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>&lt; 20 year</td>
</tr>
<tr>
<td>Gingiva</td>
<td>8</td>
<td>36.4</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Buccal Mucosa</td>
<td>3</td>
<td>13.6</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Palate</td>
<td>4</td>
<td>18.2</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Tongue</td>
<td>6</td>
<td>27.3</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Lip</td>
<td>1</td>
<td>4.5</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 2: Association between provisional diagnosis certain information**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Provisional Diagnosis</th>
<th>Total (%)</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fibroma n= 12</td>
<td>Papilloma n= 6</td>
<td>CGC n= 2</td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>2 (33.3)</td>
<td>2 (33.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>20 - 35</td>
<td>6 (75.0)</td>
<td>2 (25.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>4 (50.0)</td>
<td>2 (25.0)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4 (44.4)</td>
<td>4 (44.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (61.5)</td>
<td>2 (15.4)</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td>Site of lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gingiva</td>
<td>4 (50.0)</td>
<td>0 (0.0)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>Mucosa</td>
<td>3 (13.6)</td>
<td>0 (0.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Palate</td>
<td>3 (50.0)</td>
<td>3 (50.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tongue</td>
<td>1 (25.0)</td>
<td>3 (75.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lip</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Conclusion

Formalin-fixed paraffin-embedded sections from 22 giant cell fibroma in period between 2010 and 2018 were retrieved from the laboratory of oral pathology of Baghdad University/College of Dentistry. Clinical data and microscopic features were reviewed and analyzed according to the available surgical reports. The mean age of patients at the time of diagnosis was 29.68 years with slight female predilections (1.4:1), the gingiva is the most common site of occurrence (36.4%), the lesions were 2-10 mm in greatest dimension. The most frequent provisional diagnosis is fibroma (54.5%) and papilloma (27.3%). Histologically, the distinctive diagnostic feature is the presence of mono, bi or multinucleated large stellate giant cells with a mean of 48.59 which is most numerous in the lamina properia beneath the epithelium.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry University of Baghdad and all experiments were carried out in accordance with approved guidelines.

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Impact of Health Education Program on Health Status of Heart Failure Patients: A Quasi-Experimental Study

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Abstract

Heart failure, is a major cause of morbidity and mortality, especially in ageing people, it affects and kills a large number of people. Objective: The study aims to evaluate the effectiveness of an education program on the health status of patients suffering heart failure. The study design is quasi-experimental. The patients were allocated into either the experimental group (taking the program, n = 200) or the control group (not taking the program, n= 200). The extent of samples knowledge about the disease was tested at both the pre and post education stages. A structured teaching program for imparting knowledge on various aspects of nutritional status was developed based on an extensive review of the literature and expert opinion. The results of the study were elicited based on two statistical approaches, first, descriptive statistics and the second is an inferential statistical analysis. The shows that health status sub main domains at pre-period in case and control groups was non significant, while the health status sub main domains at post period in both groups were high significant. Regarding the study global main domains in both groups along pre-post period were high significant.

Keywords: Education, Heart failure, Mortality, Knowledge

Introduction

Heart failure (HF), is a state in which the cardiac muscle is unable to push enough blood to body. As the heart does not push enough blood to all the body, it cannot meet the metabolic demand which must require.¹ The improper blood supply to the body result in subsequent incompetent supply of oxygen to the body tissues, and symptoms such as fatigue or activity intolerance appear as well as developing shortness of breath.² Heart failure lead to a major public health problem in the world with a significant risk of the disease on population and the individual, this burden of disease can be measured in terms of mortality, increase readmission rate, as well as the healthcare costs. Frequent HF hospitalization is a burden on the healthcare system and adversely affect patients and state outcomes.³,⁴ The most common causes of heart failure are cardiomyopathy, hypertension, heart valve disease, coronary artery disease, congenital heart disease and alcohol consumption.⁵ The usual symptoms are shortness of breathing, frequent cough, swollen extremities, abdominal swelling, fatigue, dizziness and sudden death.⁶,⁷ Risk factors for heart failure patient may be divided into two classifications; compliant and non-compliant risk factors. Compliant risk factors are those that a person can correct it, including high serum cholesterol level, a diet high in saturated fat, obesity, physically inactive, hypertension, cigarette smoking, and alcohol consumption. And non-compliant risk factors are those that an individual can’t change, such as age, gender, ethnicity and heredity.⁸ Heart failure is usually managed with style of lives changing and medicines, eating habits changes to maintain an ideal weight, diminish salt intake, appropriate exercise, smoking cessation, reducing and alcohol consumption.⁹,¹⁰ Investigation has shown that approximately half patients who readmitted to the hospital could be stopped if HF patients were compliant to their management regimen, and meet symptom monitoring.¹¹ Clients, as well as their caretakers, level of knowledge, is significantly related to their level of adherence to recommended care regimens.¹²,¹³ Patients knowledge is an essential component for the treatment of their disease.¹⁴ Studies focus on the education of patients and their families to improve their knowledge of self-care and compliance.¹⁵,¹⁶ Also studies checking HF patients’ knowledge and the outcomes of patient education recorded that health literacy was associated with higher HF knowledge and
Methodology

Design and Sample

A quasi-experimental design was applied to achieve the goal of the study. Non-probability, purposive sample, with the use of pre-post test approach for both study and control group. A sample of (400) heart failure patient chosen among patients who attended to three main hospitals; Hawler teaching hospital, Rojhelat emergency hospital, and Rizgary teaching hospital. The samples were divided into two groups; (200) patient as a study group was exposed to the health education program and the other (200) patients are not exposed to the health educational program, considered as the control group with the same demographic characteristic for both groups.

The Educational Program

A structured teaching program for imparting knowledge and nutritional status of heart failure was developed by the authors. The content of the educational program was designed based on an extensive review of the literature and expert opinion. The program composed of a set of modules related to health status, these modules include physical status, psychological status, nutritional status, exercise status, social status, economical status, medication status, as well as smoking status. Models content was created and edited by the researchers. Before the nutritional program is finalized, it has been presented to a group of experts. Those experts were asked to review the education program as well as the instrument for their content, clarity, and adequacy. After the review, some items were excluded and some others are added after face to face discussion with experts and the instrument considered valid after taking all the comments and recommendations in considerations.

Procedure

After ensuring informed consent from the patients, they were given the pre-test questionnaire before the administration of the educational program. Each patient was given a serial number to be followed in the second assessment (post-test). After administration of the pre-test questionnaire, the patients were imparted with a education program by face-to-face interview with the primary author. The face-to-face interview lasted ‘30-35 minute-sessions’ with using booklets and short videotapes. As a reminder, each participant heart failure patient was provided with a copy of the education booklet prepared and designed by the primary author and reviewed by other authors. The content of the booklet was similar to that of the educational program and it summarized the most important points in the program. For preventing bias recall, one month later, patients were asked by telephone to complete the same questionnaire a second time (post-test).

Data Analysis

The statistical data analysis approaches were used in order to analyze and assess the results of the study under the application of the statistical package (SPSS) ver. (22.0):

1- Descriptive data analysis: Frequencies, percentages, Mean of the score (MS), Standard Deviation (SD), Relative Sufficiency (RS%), as well as scoring scales of two categories, such that (Yes, and NO)and are responding with integer numbers (1, and 0) respectively.

2- Inferential data analysis: Alpha Cronbach, The Independent-Samples t-test, Matched Paired-Samples t-test (MP t-test), Pearson’s correlation coefficient.

Results

Table 1 : Health status sub main domains at pre-period in case and control groups

<table>
<thead>
<tr>
<th>Grand Main Domains</th>
<th>Group</th>
<th>No.</th>
<th>GMS</th>
<th>SE</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.526</td>
<td>0.009</td>
<td>-0.222</td>
<td>0.824</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.529</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychological Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.453</td>
<td>0.016</td>
<td>0.209</td>
<td>0.834</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.449</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 1: Health status sub main domains at pre-period in case and control groups

<table>
<thead>
<tr>
<th>Grand Main Domains</th>
<th>Group</th>
<th>No.</th>
<th>GMS</th>
<th>SD</th>
<th>SE</th>
<th>t-test</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Physical Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.438</td>
<td>0.126</td>
<td>0.009</td>
<td>-6.16</td>
<td>0.000</td>
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<tr>
<td></td>
<td>Control</td>
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<td>0.511</td>
<td>0.111</td>
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<td>Psychological Status of Heart Failure</td>
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<td>200</td>
<td>0.429</td>
<td>0.209</td>
<td>0.015</td>
<td>-10.83</td>
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<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.455</td>
<td>0.221</td>
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<td>0.000</td>
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<td>Nutritional Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.385</td>
<td>0.104</td>
<td>0.007</td>
<td>-1.37</td>
<td>0.171</td>
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<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.399</td>
<td>0.117</td>
<td>0.008</td>
<td>0.090</td>
<td>0.926</td>
</tr>
<tr>
<td>Exercise Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.500</td>
<td>0.109</td>
<td>0.008</td>
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<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.504</td>
<td>0.120</td>
<td>0.008</td>
<td>-3.19</td>
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<td>Social Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.281</td>
<td>0.144</td>
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<td>200</td>
<td>0.300</td>
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<tr>
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<td>Case</td>
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<td>0.435</td>
<td>0.174</td>
<td>0.012</td>
<td>-0.641</td>
<td>0.522</td>
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<td>Control</td>
<td>200</td>
<td>0.434</td>
<td>0.186</td>
<td>0.013</td>
<td>0.007</td>
<td>0.926</td>
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<tr>
<td>Medication Status of Heart Failure Patient</td>
<td>Case</td>
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<td>0.127</td>
<td>0.131</td>
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<td>Smoking Status of Heart Failure Patient</td>
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<td>0.462</td>
<td>0.219</td>
<td>0.037</td>
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</tr>
<tr>
<td></td>
<td>Control</td>
<td>44</td>
<td>0.487</td>
<td>0.064</td>
<td>0.005</td>
<td>-1.085</td>
<td>0.000</td>
</tr>
</tbody>
</table>

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non-Sig. at P>0.05; Testing based on two independent t-test.

### Table 2: Health status sub main domains at post period in case and control groups

<table>
<thead>
<tr>
<th>Grand Main Domains</th>
<th>Group</th>
<th>No.</th>
<th>GMS</th>
<th>SD</th>
<th>SE</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.502</td>
<td>0.110</td>
<td>0.007</td>
<td>-1.208</td>
<td>0.228</td>
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<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.514</td>
<td>0.109</td>
<td>0.008</td>
<td>-0.641</td>
<td>0.522</td>
</tr>
<tr>
<td>Psychological Status of Heart Failure</td>
<td>Case</td>
<td>200</td>
<td>0.516</td>
<td>0.121</td>
<td>0.009</td>
<td>0.877</td>
<td>0.381</td>
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<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.524</td>
<td>0.117</td>
<td>0.008</td>
<td>0.007</td>
<td>0.926</td>
</tr>
<tr>
<td>Nutritional Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.319</td>
<td>0.152</td>
<td>0.011</td>
<td>0.877</td>
<td>0.381</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.307</td>
<td>0.132</td>
<td>0.009</td>
<td>0.877</td>
<td>0.381</td>
</tr>
<tr>
<td>Exercise Status of Heart Failure Patient</td>
<td>Case</td>
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<td>0.435</td>
<td>0.171</td>
<td>0.012</td>
<td>0.155</td>
<td>0.877</td>
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<td>0.433</td>
<td>0.187</td>
<td>0.013</td>
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<td>0.926</td>
</tr>
<tr>
<td>Social Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.207</td>
<td>0.204</td>
<td>0.014</td>
<td>2.276</td>
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<td>0.171</td>
<td>0.012</td>
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<td>0.926</td>
</tr>
<tr>
<td>Economical Status of Heart Failure Patient</td>
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<td>0.100</td>
<td>0.163</td>
<td>0.028</td>
<td>-8.835</td>
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<td>Control</td>
<td>200</td>
<td>0.520</td>
<td>0.257</td>
<td>0.039</td>
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<td>0.926</td>
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<tr>
<td>Medication Status of Heart Failure Patient</td>
<td>Case</td>
<td>200</td>
<td>0.489</td>
<td>0.069</td>
<td>0.005</td>
<td>-6.16</td>
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</tr>
<tr>
<td></td>
<td>Control</td>
<td>200</td>
<td>0.493</td>
<td>0.061</td>
<td>0.004</td>
<td>0.007</td>
<td>0.926</td>
</tr>
</tbody>
</table>

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non-Sig. at P>0.05; Testing based on two independent t-test.
### Table 3: Global main domains along pre-post periods in case-control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Global Main Domains</th>
<th>Period</th>
<th>No.</th>
<th>GMS</th>
<th>SD</th>
<th>SE</th>
<th>MP t-test</th>
<th>P-value</th>
<th>C.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Health Status related to Heart Failure</td>
<td>Pre</td>
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<td>0.489</td>
<td>0.069</td>
<td>0.005</td>
<td>22.39</td>
<td>0.000</td>
<td>HS</td>
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<tr>
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<td></td>
<td>Post</td>
<td>200</td>
<td>0.420</td>
<td>0.060</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>Health Status related to Heart Failure</td>
<td>Pre</td>
<td>200</td>
<td>0.493</td>
<td>0.061</td>
<td>0.004</td>
<td>3.42</td>
<td>0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>200</td>
<td>0.487</td>
<td>0.064</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(* HS: Highly Sig. at P<0.01; Testing based on Matched Paired t-test.

### Discussions

Respect to subjects of studied socio-demographic variables in table (1), results shows that socio-demographic variables in studied groups had recorded no significant differences at P>0.05, except in age groups, and levels of education, which represented significant different in at least at P<0.05, rather than most of studied group’s individuals distribution concerning age, and levels of education are very similar. For summarizing preceding results in table (2), and due to an overall assessments, it could be conclude that suggested educational program could be applicable for case group, since the absence of significant differences between the two groups regarding health status of people with heart failure. Regarding the descriptive statistics of case and control groups in light of post period in table (3) , the result shows that the two groups in the light of sub main domains are recorded highly significant differences at P<0.01, with exception of no significant differences between the two groups concerning “Psychological Status of Heart Failure Patients, Social Status of Heart Failure Patients, and Economical Status of Heart Failure Patients” at P>0.05. For summarizing preceding results, and due to an overall assessments, it could be conclude that the suggested educational program has had a significant impact in improvement their health status.

### Financial Disclosure:
There is no financial disclosure.

### Conflict of Interest:
None to declare.

### Ethical Clearance:
All experimental protocols were approved under the Department of Adult Nursing, College of Nursing, Hawler Medical University, Erbil, Iraq and all experiments were carried out in accordance with approved guidelines.

### References
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Mouse Hepatocellular Carcinoma Sensitivity to Cisplatin and Docetaxel and Analysis of Related Proteins

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¹ Biology Department - College of Education, Ibn alhaithum – Baghdad University,
² Iraqi Center for Cancer and Medical Genetic Research, Mustansiriyah University, Iraq

Abstract
Hepatocellular carcinoma (HCC) globally ranked fifth common cancer and the third-leading cause of death. This study aimed to characterize the new mouse hepatocellular carcinoma cell line (HCAM) for some of the most important proteins involved in cell cycle regulation P53, HER2/neu, and EGFR by immunocytochemistry. Also, to measure the sensitivity of the cells to some common chemotherapeutic agents such as cisplatin and docetaxel by the MTT cell viability assay. The findings of immunocytochemistry appeared that HCAM cells proven to express the p53 and EGFR positively when compared with the negative control. Furthermore, showing nuclear only low expression for the HER2/neu. For evaluation of the chemotherapeutic agent’s efficiency, the cells of hepatocellular carcinoma (HCAM) were treated for 72 hours using different concentrations for Cisplatin and Docetaxel. The IC50 values of docetaxel and cisplatin after 72 h exposure for HCAM was 12.82 and 10.74 respectively. Our in vitro results demonstrate that Docetaxel and Cisplatin are toxic to HCAM cell line in a concentration-dependent manner. In conclusion, our results showed positive expression of p53, EGFR, and weak HER2/neu. Also, HCAM cell line showing to be sensitive to docetaxel and cisplatin, which inhibit cell proliferation.

Key words: Hepatocellular carcinoma, cisplatin, docetaxel

Introduction
Over the past several years, tumor cell lines have occupied significant parts in cancer researches to demonstrate molecular characteristics and evolving new therapies together ¹. Drug experimentation in cell lines of cancer is typically some of the initial steps in drug development. It permits the entrance of potential drugs numbers before committing to large scale expensive in vivo experimental approaches ². Cancers can develop resistance to particular therapeutics. The most type of primary liver cancer is hepatocellular carcinoma, which is cancer easily acquiring resistance to drug therapy. Therefore, many active anticancer agents available, some are associated with high levels of toxicity. For example, docetaxel agent is beneficial as microtubule depolymerization inhibition, and it has shown a strong influence against cancer ³. Also, Cisplatin, cisplatinum, or (cis-diaminedichloroplatinum) is a recognized chemotherapeutic agent; it is active against cancers types, including cancer of germ cell, carcinomas, and sarcomas. Cisplatin method of action through to its capability to linking with the purine bases on the DNA; causing DNA damage, intervening with DNA repair mechanisms, and then inducing apoptosis of cancer cells ⁴. Immunocytochemistry (ICC) analysis is a beneficial tool not only for cell characterization but in some cases, also for cell lines authentication ⁵. The suppressor gene of the tumor (p53) is mutated mostly in 50% of tumors. The p53 ability for various biological roles can be returned to its ability to act as a sequence-specific transcription factor to the regulation of different targets expression, and thus to manage multiple cellular processes including DNA repair, cell cycle arrest and apoptosis ⁶. These proteins (EGFR) are found on the surface of some cells of cancer and normal cells types and mediate cell survival, invasion, proliferation, and angiogenesis ⁷. A

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type I tyrosine kinase receptor encodes by HER-2/neu proto-oncogene; Also, HER2/neu stimulates the factors that facilitating cell motility and induces cell division. The aim of the work to evaluate proteins expression and analyzed the cytotoxicity assay of docetaxel and cisplatin against the new hepatocellular carcinoma cell line (HCAM).

**Methodology**

**Cell line and Cell culture**

The cell line of (HCAM) Murine Hepatocellular Carcinoma Ahmed Majeed was supplied from Iraqi center of cancer, and medical genetics research (ICCMGR) and cultured in vitro in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS), 100 µg / ml for ampicillin and streptomycin. The suspension was then transferred to a culture flask for 37°C incubation.

**Immunocytochemistry**

The Cells of HCAM were grown in RPMI-1640 supplemented 10% FBS to a confluent monolayer on glass coated with coverslips in a six-well plate and sustained in a humidified incubator at 37°C with 5% CO2. Before fixation, monolayer cells were washed using PBS twice. With 4% cold acetone, the cells on a coverslip were fixed for 10 min, rinsed gently in PBS. Endogenous peroxidase activity was neutralized by incubation for 10 min in a solution consisting of 3% H2O2 blocking reagents for 45 min firstly and then primary antibodies selection (anti -Her neu2, anti-P53, and anti-EGFR) (Santa Cruz Biotechnology), for 2 hours at 4°C for detection of markers for hepatocellular carcinoma. The antibodies dilution was (1:50–100) according to the recommendations of manufacturers. The color operation was achieved with the chromogen diaminobenzidine (0.6 mg/ml) in a 0.02% H2O2 solution. Cells were examined with a Leica light microscope after counterstaining of nuclei with hematoxylin.

**Quantitative image test**

ICC images were used for quantitative analysis protocol for the hematoxylin – DAB staining slides were taken by Leica inverted microscope and camera (Leica Microsystems, Germany), three different staining zones of immunocytochemistry images of each slide were analyzed in this study. Firstly, un-mix the DAB by color de-convolution technique, the areas of hematoxylin stained were leaving a complimentary image. As we take three new images. The first image is the hematoxylin stain, the second one is the DAB image, and we quantify the DAB image. The number of pixels of a specific intensity value vs. their respective intensity was raised using “Fiji” version of Imagej from http://fiji.sc. The intensity numbers in the results window were converted to Optical Density (OD) numbers with the following formula:

\[ OD = \log (\text{max intensity}/\text{Mean intensity}), \text{where} \ \text{max intensity} = 255 \text{ for 8-bit images} \]

**Cytotoxicity assay**

**Reagents**

Cisplatin and Docetaxel were obtained from the pharmacy with a concentration of (cisplatin 50 µg/ml, Docetaxel 100 µg/ml) and stored at 4°C. The preparation of the stock solution was accomplished by dissolving the reagents in distilled water. Before each experiment, this solution was diluted in the free serum medium and immediately used in the tissue culture.

**Cell viability assess**

The examine of cell proliferation, cells were plated in 96-well plates (Sarstedt, Denmark) with 200 µl of cells in each well and incubated at 37°C. At monolayer, cells exposed with concentration of Cisplatin (50µg/ml), prepare series dilution (50, 25, 12.5, 6.25 and 3.125) µg / ml, and Docetaxel (100 µg/ml), prepare series dilution (100, 50, 25, 12.5 and 6.25) µg / ml which were diluted with RPMI-1640 free serum medium. Then 200µl of each dilution of chemotherapy described earlier were added to each well. The microplate was incubated at 37°C for 72 hours. After 72 hours of the exposure the viability of cells was assessed and treatment by removing the medium, adding 100 µl of 2 mg/ml solution of MTT and incubating for 2 hours at 37°C, and dissolving of insoluble formazan crystals in 150 µl of dimethyl sulfoxide (DMSO).

The optical density (OD) was measured at 490 nm of the wavelength by a microplate reader. The cell growth inhibition rate calculated using the following formula: \( (IR) = (A-B)/A\times100 \). Where A: mean of the optical density of untreated wells, and B: optical density of treated wells.

**Morphological study**

The chemotherapy exposed and non-exposed...
control hepatocellular cells were photographed at 72h and fixed in 4% paraformaldehyde in phosphate buffer (10 min) at room temperature. The cell was stained with hematoxylin and eosin (H&E) stain. The cells were examined using the Leica inverted microscope. The affected cells were recognized according to cytological features 15.

**Statistical analysis**

The data was displayed as mean ± standard deviation. For ICC experiment, n = 3 images were used. One-way (ANOVA) analysis of variance multiple comparisons were done to show variations between groups. The statistical analyses were done using (GraphPad Prism, version 6.07 for Windows, GraphPad Software, San Diego, CA, USA), and p < 0.05 as statistically significant.

**The Results**

**Immunocytochemistry assay**

The result of immunocytochemical staining for some biomarkers was shown in figure 1. The P53, EGFR, and Her-neu2 expression in the HCAM cell line of hepatocellular carcinoma were noticed using an anti-mouse IgG immunocytochemistry kit. The findings presented that HCAM were positive for EGFR, weakly for Her-neu2, and p53 proteins expression. Her-neu2 showed nuclear expression when compared with control cells. The control cells were exposed to the secondary antibody only, without primary antibody (negative control).

**Digital Image Scoring**

Images of ICC stained HCAM hepatocellular carcinoma cells were analyzed using ImageJ software. Figure-1E displays demonstrative pixel and zones intensity analysis of ICC images. The analysis showed that P53, EGFR, and Her-neu2 proteins were significantly expressed when compared to control, not stained cells using ANOVA one-way multiple comparison test.

**Morphological study**

Cytopathic effect of Docetaxel and Cisplatin on HCAM cell line was studied after 72 hours of exposure. Light microscope unstained and H&E stained images for control untreated cells were relatively uniform in size and shape (spindle) in monolayer culture with and without H&E stain (X20).

Figure (1): Immunocytochemistry stain of HCAM cell line. A) EGFR-positive staining of HCAM cancer cells (magnification 20×). B) P53-positive staining of HCAM cancer cells (magnification 20×). C) Her/neu2 weak positive nuclear expression of HCAM cancer cells (magnification 20×). D) Negative control of HCAM cancer cells (magnification 20×). E) Digital Image Scoring is showing significant proteins expression when stained with relative mAbs against the markers that analyzed using ImageJ program.

Figure-2: Bar chart for the mean values of growth inhibition rate and ic50 of HCAM cell line (GI%) induced by Docetaxel and Cisplatin after 72 h of exposure.

(A) The IC50 value of docetaxel for HCAM cells.

(B) The IC50 value of Cisplatin for HCAM cells.
(C) The effect of Docetaxel (100 µg /ml) for HCAM cells.

(D) The effect of Cisplatin (50 µg /ml) for HCAM cells.

**Figure (4): cytotoxicity effect of Docetaxel and Cisplatin on HCAM cell line after 72 hours.**

(A, a) Untreated cells, HCAM cells were relatively uniform in size and shape (spindle) in monolayer culture with and without H&E stain (X20).

(B, b) Effect of docetaxel 100 µg /ml, (C, c) Effect of Cisplatin 50 µg / ml.

The cells tended to grow in clusters and arranged irregularly, cells detached and appeared as oval forms with and without (H&E) stain (X20).

**Discussion**

The ICC study presented that most of the HCAM cells were positive for EGFR, HER2/neu, and P53 proteins. The p53, EGFR, and HER2/neu genes expression impact the management of cancer cells due these genes provide both prognosis and therapeutic information \(^{16}\). HCAM cells were positive for P53 and EGFR as nuclear and cytoplasmic expression while for HER2/neu was very weak nuclear expression only. Positive expression of the cytoplasm and nucleus suggests mutation in p53, HER2/neu, and EGFR because it has important role in cell cycle control and DNA repair \(^{17-19}\). Several epigenetic and genetic alterations involved in the molecular pathogenesis of HCC as a somatic mutation of the tumor suppressor gene (p53) \(^{20}\).

In addition, \(^{21}\) shown that HCC models in both human and mouse, EGFR are upregulated in macrophages of the liver, where it acts as a tumor-promoting function. HER2/neu expressed weakly in non-neoplastic normal epithelia \(^{22}\). Otherwise, some researchers showed that development and modulation of HER2/neu oncogene in hepatocellular carcinoma is a rare event \(^{23}\). In agreement with our findings of HER2/neu nuclear expression in our HCAM cell line, another researcher \(^{24}\) found that hepatocytes with altered metabolic and cell cycle may have nuclear HER2/neu expression.

In the current study, MTT viability test was used to evaluate the cytotoxic effect of docetaxel and cisplatin on the growth rate of the cell line (HCAM) in vitro. Docetaxel and cisplatin can inhibit the growth of HCAM cells in a concentration-dependent manner. Docetaxel enhances the assembly of tubulin into stable microtubules and prevents their assembly that leads to a reduction of free tubulin and cancer death \(^{25}\). Our results demonstrated that docetaxel showed a cytotoxic effect of HCAM growth rate with high concentration 100, 50, and 25 µg/ ml at 72 hours. Docetaxel with high concentration level, can effects on cell cycle kinetics, apoptosis, or increased ROS level. \(^{26}\) Our data presented that cisplatin significantly inhibited the growth of HCAM cell with concentration (50, 25 and 12.5 µg/ ml) at 72 hours, which is consistent with a previous study \(^{30}\) that found hepatocellular carcinoma treated with cisplatin significantly inhibited the growth rate and induced apoptosis.

**Conclusion**

Our results suggest that HCAM cell line express p53 and EGFR proteins in both nuclear and cytoplasm, but weak nuclear HER2/neu expression. Also, HCAM cell line is sensitive to cisplatin and docetaxel that induces a significant cytotoxic effect. HCAM is a new important mouse model that can be used for anti-hepatic cancer drug development.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Faculty of Dentistry, University of Babylon, Hillah city, Iraq and all experiments were carried out in accordance with approved guidelines.
References


24. Doring P, et al., [Nuclear Her2 expression in


Triple Negative Breast Tumors In Iraqi Women

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1Msc in Physiology-College of Pathological Lab. Techniques- Al-Bayan University, 2Msc in Human Anatomy/ Histology And Embryology- College of Pathological Lab. Techniques- Al-Bayan University, 3Msc in Histopathology- College of Pathological Lab. Techniques- Al-Bayan university, 4Msc in Histopathology- Ministry Of Health- Medical City- Oncology Center, Corresponding auther- Basim Mohammed Abdulmajeed- pathological lab. techniques college- Al-Bayan University

Abstract

Breast cancer is heterogeneous disease. From this; a special group of tumors, triple negative tumors, has a distinct interest from having its unique behaviors regarding biological, clinical, histological, hormone receptors status, Her2/neu expression, and therapeutic modalities. We made a study on Iraqi women, with triple negative tumors, focusing on some important parameters in this group of patients, comparing our results with the others (from the world). We conclude that most of these parameters are identical except the tumor grade, where we found that it is significantly low. Results from studies done before on Iraqi women, for the same purpose, showed some differences from ours. These differences include percentage of these tumors among other types of breast tumors (our results looked less), grade of tumors (our results showed medium grade), and lymph nodes metastasis which looked less in our study.

Key words: Breast cancer, Triple negative tumors, Tumor grade, Er, Pr , Her2/neu

Introduction

Obviously; the breast cancers are the most killing cancers in women. According to this fact, these cancers were studied thoroughly. From many studies on this subject, it was found that; breast cancer is a heterogeneous disease. Many types were mentioned according to morphologic, immunologic, and molecular studies. Immunohistochemical studies, namely hormone receptors (estrogen receptors ER, progesterone receptors PR, and others like ki 67 and Her2/neu) showed important prognostic parameters.1 These parameters were found to affect strongly the planed therapy for breast cancers (1,2) Recently it was found that, a specific group of breast tumors called triple negative tumors, (1,3) this term was used for those tumors with estrogen hormone receptor negative, progesterone hormone receptor negative, and Her2/neu negative. (1, 3) They found that this group has some specific manifestations regarding age of patient, histological grade, lymph nodes involvement, response to therapy, and survival time after taking therapy. (4,5,6,7,8,9,10) Some authors found that these tumors account for 10-17% of all breast carcinomas. (4, 5, 6, 7, 8, 11, 12) Many authors defined that they occur in relatively younger age group (< 50 years age) (4, 5, 6, 13) and even they are more prevalent in African American women. (7, 12, 13) Most of the studies agreed about the similarity between triple negative tumors and basal-like tumors. (1, 2, 3) Though not all basal-like tumors are triple negative tumors and vise versa (only 77% of cases classified by gene expression profiling as basal-like show a triple negative phenotype, while only 72% of cases of triple negative cancers exhibit a basal-like gene expression profile). (3, 14, 15) It was also found that the triple negative tumors are with high grade (4,8) just like basal like tumors. Most of triple negative tumors are of high grade invasive ductal carcinoma (9,10,16) like that of basal-like tumors. Though one study found that there is high prevalence of lymph nodes metastasis in triple negative tumors, (4) other studies did not find this result. (5, 8) Some found that; there is
no correlation between size of the tumor and presence of metastases in triple negative tumors. In Iraq; the breast cancer is important cause of death. This cancer is usually presented with high stage and grade and in relatively younger age group at diagnosis. It was noted (in comparative study) that; triple negative tumors in Iraqi patients are higher (about 3 folds) than English patients. In another study, done on Iraqi patients, it was found that triple negative tumors constituted about 15.6%.

Generally speaking; triple negative tumors is of high percentage in Iraqi women with breast cancers, and this indicates a bad prognostic sign, as shown before.

Methodology

A retrospective study was performed on 477 female patients with breast cancers who visited the Medical City-Oncology Center during years 2014 and 2015 for sake of cancer therapy. Patient’s data, from records of the oncology center, were collected to study different parameters like patient age at time of diagnosis, histopathology diagnosis, grade of tumors, lymph nodes involvement, hormone receptors (ER, PR) status, and Her2/neu expression. Those data were processed to define whether these tumors belong to triple negative group or not and a statistical study were done to detect behaviors of triple negative tumors in this sample of Iraqi patients. For all cases in this study; a histotechnique using paraffin embedding technique and a staining by routine Hematoxyline and Eosin (H&E) staining protocol for initial diagnosis were adopted. Then an immunohistochemistry technique was used according to College of American Pathologist (CAP) Breast Biomarkers template, especially for estrogen receptors (ER) and progesterone receptors (PR). Her2/neu study was done by immunohistochemical technique initially, while equivocal cases needed to be confirmed by fluorescence in situ hybridization (FISH) technique (a molecular cytogenic technique) according to American Society of Clinical Oncology (ASCO) Guideline Update.

### Protocols followed in histotechniques (paraffin embedding)

1. Sample receipt & identification
2. Labeling with numbering
3. Fixation
4. Dehydration
5. Clearing
6. Impregnation (infiltration)
7. Section cutting
8. Staining
9. Mounting

### Hematoxylin and eosin staining protocol

1. Deparafinization
2. Hydration
3. Nuclear staining (hematoxylin)
4. Cytoplasmic staining
5. Dehydration and clearing
6. Mounting
Immunohistochemistry (IHC) protocol (LSAB method)

1. Tissue preparation
2. Inactivation
3. Antigen retrieval
4. Blocking
5. Primary antibody incubation
6. Secondary antibody incubation
7. Staining

Reporting Results of Estrogen Receptor (ER) and Progesterone Receptor (PgR) Testing (according to CAP)

<table>
<thead>
<tr>
<th>Results</th>
<th>Criteria</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Immunoreactive tumor cells present (≥1%)</td>
<td>The percentage of immunoreactive cells may be determined by visual estimation or quantitation. Quantitation can be provided by reporting the percentage of positive cells or by a scoring system, such as the Allred score or H score.</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt;1% immunoreactive tumor cells present</td>
<td></td>
</tr>
</tbody>
</table>

Reporting Results of HER2 Testing by Immunohistochemistry (IHC) according to (CAP)

<table>
<thead>
<tr>
<th>Results</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (Score 0)</td>
<td>No staining observed or Incomplete, faint/barely perceptible membrane staining in ≤10% of invasive tumor cells</td>
</tr>
<tr>
<td>Negative (Score 1+)</td>
<td>Incomplete, faint/barely perceptible membrane staining in &gt;10% of invasive tumor cells</td>
</tr>
<tr>
<td>Equivocal (Score 2+)</td>
<td>Incomplete and/or weak to moderate circumferential membrane staining in &gt;10% of invasive tumor cells or Complete, intense, circumferential membrane staining in ≤10% of invasive tumor cells</td>
</tr>
<tr>
<td>Positive (Score 3+)</td>
<td>Complete, intense, circumferential membrane staining in &gt;10% of invasive tumor cells</td>
</tr>
</tbody>
</table>

Reporting Results of HER2 Testing by In Situ Hybridization

<table>
<thead>
<tr>
<th>Result</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (not amplified)</td>
<td>Average HER2 copy number &lt;4.0 signals/cell</td>
</tr>
<tr>
<td>Equivocal</td>
<td>Average HER2 copy number ≥4.0 and &lt;6.0 signals/cell</td>
</tr>
<tr>
<td>Positive (amplified)</td>
<td>Average HER2 copy number ≥6.0 signals/cell</td>
</tr>
</tbody>
</table>
Statistical studies for those parameters, mentioned above, were done by using Chi square depending on P-value (p< 0.01 is regarded as significant).

**Results**

At the end of this study and according to statistical data, we had the following results.

On studying the parameters (ER, PR, and Her2/neu) of breast tumors of those 477 patients, we found that 46 women were with triple negative tumors, and this number constitutes about 10.4% of all patients in the study.

The average age of the women, with triple negative tumors, was about 49.4 years (32-67). This means that most of those patients are below the age of 50 years (table no. 1), and this average is significantly lower than that of all patients (P<0.01).

<table>
<thead>
<tr>
<th>Total no. of all patients</th>
<th>Av. age (years)</th>
<th>Range</th>
<th>No. of patients with triple negative tumors (%)</th>
<th>Av. age (years)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>477</td>
<td>57</td>
<td>28-83</td>
<td>46 (10.4)</td>
<td>49.4</td>
<td>32-67</td>
</tr>
</tbody>
</table>

On studying the tumor grade of triple negative tumors, we found that most tumors (76%) are of grade II (table no. 2). This result showed no important difference from all patients (P>0.01).

<table>
<thead>
<tr>
<th>No.</th>
<th>Grade</th>
<th>Total no.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>35</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>46</td>
<td>100</td>
</tr>
</tbody>
</table>

In our study, we found nearly all triple negative tumors were of invasive ductal carcinoma, not otherwise specified. 2 cases were with additional intraductal carcinoma in situ of high grade.

Regarding the lymph nodes involvement, we found that only 13 cases (28.3%) were with lymph nodes involvement (table no. 3). This result is not significantly different from all patients in this study (P>0.01).

<table>
<thead>
<tr>
<th>No.</th>
<th>Lymph node metastasis</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>present</td>
<td>13</td>
<td>28.3</td>
</tr>
<tr>
<td>2</td>
<td>Absent</td>
<td>33</td>
<td>71.7</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>46</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Discussion**

The results of this study were compared to other studies in the world and in Iraq, mentioned in the introduction.

The number of Iraqi patients, in this study, was 477, so those results can give us information about Iraqi women with breast cancers, but they are not necessarily represent all Iraqi women with breast cancer.

We found that triple negative tumors group in this study constitutes about 10.4% (46 patients). This finding looked similar to other studies in the world. (4, 5, 6, 7, 11, 12, 13) But this result is different significantly (P<0.01) from
previous results on Iraqi women (17, 19).

Average age of women with triple negative tumors in this study was 49.4 (< 50). This finding looked similar to other findings in the world (4, 5, 6, 13).

Most triple negative tumors in this study (76%) were of grade II, and others (24%) were with high grade (III) and no case with low grade (I). This finding looks different from the belief that triple negative tumors are usually with high grade (III). (3, 4, 8) Though some authors found that 10% of these tumors were with low grade. In Iraq, one study on triple negative tumors defined high grade histology. 17

The histological diagnosis of all cases in this study showed invasive ductal carcinoma with no specific histology. These findings are the same to what others, in the world, said, (9, 10, 16) and even in Iraq. 17

13 cases (28.3%) from the triple negative tumors in this study were presented with lymph nodes metastasis. This finding is highly different from one study 4 (p<0.0001), while it looked similar to others, (5, 8) with no significant difference (P>0.5). While one study from Iraq 17 showed significant difference from our result (P<0.001).

Conclusion

At the end of this study, we conclude that the triple negative tumors group, in this study, has nearly the same findings and behaviors to those seen in the worlds, except the grade of tumors. And there were some differences seen from other previous Iraqi results.

Conflict of Interest: Authors declare that they have no competing interest.

Source of Funding: Self funding

Ethical Clearance: All data collected has been approved by the manager of laboratory department-oncology center of medical city. No patient name or any personal data being collected.

References


Seroprevalence of Toxoplasma gondii (IgM,IgG) among Aborted Women in Some Karbala Hospital by using ELISA Technique and Microscopic Examination.

Bahaa Alaa Farhan
Department of Basic Medical Sciences /College of nursing /University of wraith Al-anbiya’a, Iraq

Abstract
In this study, 100 pregnant women experiencing unnatural birth cycle go to the Karbala Maternity and Children Hospital from October/2018 to April/2019. These women submit to a history and physical examination. Blood tests from pregnant ladies were tried for IgG and IgM. Antibodies and suicide Mo graphic information’s were gathered. Their normal age was 29 years. The investigation demonstrated that enemy of Toxoplasma IgG, IgM, and by and large seropositivity of all antibodies were 42.85% and 57.14% separately. There was noteworthy impact of age on extent rate. The most noteworthy disease rates were found at 20-25 age gathering, while the least contamination rate found at 36-40 age gatherings. The all out predominance was more in Second Trimester of pregnancy (12.22%). IgG was increasingly amassed in Second Trimester (13.33%) than IgM (11.11%). No noteworthy distinction was found among residency and the commonness of Toxoplasma gondii antibodies in both rustic and urban territories. Watchwords: Toxoplasma gondii Infection, Toxoplasmosis. Using a microscopic sample of blood samples, 14 positive blood samples from 100 were found and the infection was identified by its characteristic characteristics.

Key words: Toxoplasma gondii, IgM, IgG, Aborted Women

Introduction
Toxoplasma gondii that causes the malady toxoplasmosis is a commit intracellular parasitic protozoan. Serological investigations assess that up to 33% of the worldwide populace has been presented to and might be constantly tainted with T. gondii, in spite of the fact that disease rates contrast essentially among nations. Transmission of toxoplasma gondii happens generally by the ingestion of debased crude/half-cooked meat with tissue blisters, just as nourishment borne route through the ingestion of tainted vegetable/water with oocysts. The European Food Safety Authority (EFSA) has archived toxoplasmosis as parasitic zoonosis with the most elevated human rate. Analysts consider the eating of half-cooked contaminated meat as the greatest hazard and suspected ovine meat, to be a noteworthy hazard factor for human disease. Protein connected immunosorbent measure (ELISA), otherwise called a chemical immunoassay (EIA), is a biochemical strategy utilized by and large in immunology to distinguish the nearness of a neutralizer or an antigen in an example. In straightforward terms, in ELISA, an obscure measure of antigen is attached to a surface of wells, and after that explicit counter acting agent is connected over the surface so it can tie to the antigen. This counter acting agent is connected to a compound by utilize conjugated (catalyst Ab), and in the last stage a substance is included that the protein, and this can change over to some perceptible flag, most ordinarily a shading change in a synthetic substrate. The location of explicit IgM and IgG antibodies has been utilized serological marker for diagnosing ongoing toxoplasmosis. The reason for this examination was to decide the rate of Toxoplasmosis in pregnant ladies in karbala city from October 2018 to April 2019.

Materials
In this examination 100 blood test of pregnant ladies experiencing premature deliveries were gathered from patients who go to the Karbala Maternity and Children Hospital from October/2018 to April/2019. These ladies submit to a history and physical examination to ponder event of T. gondii.. The period of patients extended between (19-50) years of age. Five ml blood tests were
gathered by vein cut from every single contemplated lady subsequent to cleaning the skin with 70% liquor. Blood tests were gathered in plastic cylinders and left to cluster for around 30 min. at room temperature, at that point they were centrifuged for five minutes at 3000 rpm and isolated sera were moved into different cylinders. The gathered sera were put away at -20°C until utilized for the required test in the Central Public Health Laboratory/Karbala; where the ELISA test has been finished. For assurance of T.gondii antibodies (IgM and IgG), we utilized a third-age of compound immunoassay packs for T.gondii IgM and IgG ELISA units/BioCheck-USA.

Parasitological examination

One drop of blood placed at the end of one slide and by the other slide in angle of forty five touched the drop of blood by the end of the slanted slide so the blood run the space beneath it. The slanted slide was drawn quickly and the blood was pulled behind as previously mentioned (Zweygarth et al., 2002). The blood smear was died in air, fixed by absolute methyl alcohol and was stained with Giemsa 10% for 20 minutes in neutral phosphate buffer saline then was washed and dried. The slide was then allowed to dry prior to microscopic examination at 100x magnification.

Results

From 100 abortion women 21 (21%) serum sample were positive for anti- Toxoplasma antibodies and this positive results were diagnosed as 9 (42.85%) for anti IgM antibody and 12 (57.14%) for anti IgG antibody from 100 abortion women 21 (21%) serum sample were positive for anti- Toxoplasma antibodies and this positive results were diagnosed as 9 (42.85%) for anti IgM antibody and 12 (57.14%) for anti IgG antibody, microscopic sample of blood samples, 14 positive blood samples from 100 were found and the infection was identified by its characteristic characteristics. Table (1) showed IgG and IgM anti-toxoplasmosis percentage in patients distributed over different age groups; the highest percentage of acute infected patients was (18.91%) in the age group (20-25) years old, while the lowest percent was (5.55%) in the age group (36-40) years old while the highest occurrence of chronic infection were (11.76%) at the age groups (26-35) years.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>No. Sex</th>
<th>IgM-positive (No.)</th>
<th>IgM-positive (%)</th>
<th>IgG-positive (No.)</th>
<th>IgG-positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20&gt;</td>
<td>13</td>
<td>......</td>
<td>......</td>
<td>......</td>
<td>......</td>
</tr>
<tr>
<td>20-25</td>
<td>37</td>
<td>6</td>
<td>16.21%</td>
<td>8</td>
<td>21.62%</td>
</tr>
<tr>
<td>26-35</td>
<td>21</td>
<td>2</td>
<td>9.52%</td>
<td>3</td>
<td>14.28%</td>
</tr>
<tr>
<td>36-40</td>
<td>18</td>
<td>1</td>
<td>5.55%</td>
<td>1</td>
<td>5.55%</td>
</tr>
<tr>
<td>41&lt;</td>
<td>11</td>
<td>......</td>
<td>......</td>
<td>......</td>
<td>......</td>
</tr>
<tr>
<td>total</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (1): Anti Toxoplasma Antibodies and Percentage in Miscarriages Women According to The Age Groups by ELISA Test:

Table (2) Toxoplasmosis and Residency: The high ratio occurred in rural patients (12.72%) depending on IgM and (4.44%) in urban. on the other hand, results showed for IgG antibodies (14.54%) in rural, whereas they were (8.88%) in urban patients as revealed in table (4):

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>No. of individuals</th>
<th>IgM- Positive No.(%)</th>
<th>IgG- Positive No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
<td>55</td>
<td>7 (12.72%)</td>
<td>8 (14.54%)</td>
</tr>
<tr>
<td>Urban</td>
<td>45</td>
<td>2 (4.44%)</td>
<td>4 (8.88%)</td>
</tr>
<tr>
<td>total</td>
<td>100</td>
<td>9 (9%)</td>
<td>12 (12%)</td>
</tr>
</tbody>
</table>
Table (3) revealed IgG and IgM anti-toxoplasmosis percentage in patients distributed over three trimesters of pregnancy.

**Table (3): Distribution of Positive Samples for Toxoplasma According to Trimesters of Pregnancy:**

<table>
<thead>
<tr>
<th>Trimesters of Pregnancy</th>
<th>Total No</th>
<th>IgM- positive (No.)</th>
<th>IgM- positive (%)</th>
<th>IgG- positive (No.)</th>
<th>IgG- positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Trimester</td>
<td>43</td>
<td>3</td>
<td>6.97%</td>
<td>3</td>
<td>6.97%</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>45</td>
<td>5</td>
<td>11.11%</td>
<td>6</td>
<td>13.33%</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>12</td>
<td>1</td>
<td>8.33%</td>
<td>3</td>
<td>25%</td>
</tr>
<tr>
<td>total</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-value. <0.05

---

**FIGURE 1.** Giemsa stained thin blood smear showing Toxoplasma infection bradyzoit

**FIGURE 2.** Giemsa stained thin blood smear showing Toxoplasma infection tacyzoit

**FIGURE 3.** Giemsa stained thin blood smear showing Toxoplasma infection tacyzoit
The commonness of T. gondii contamination in the present investigation was (21%) in Karbala area and ; the most noteworthy level of intense tainted patients was (18.91%) in the age gathering (20-25) years of age, while the least percent was (5.55%) in the age gathering (36-40) years of age while the most elevated event of unending disease were (11.76%) at the age gatherings (26-35) years. There were likenesses and distinction between the recorded outcomes and numerous others from various area around Iraqi region. In AL-Mosul, 6 announced 69.2% seropositive for this illness in similar patients gatherings, while 49.85% were certain by 9 in Duhok, and 60.21%, by 1 and by 8 80.6% in Baghdad. 17 in Babylon who detailed 43% positive outcome. Such contrasts were normal as the malady event relied upon the ecological conditions what straightforwardly influences on the survival and spread of this parasite. Other examination likes 7 alludes to the nearness of more than one hazard factor may impact the event of toxoplasmosis as the clean conditions and propensities for individuals. In Arab nations, for example, Egypt, El Deeb, et al. (2012) has revealed that (67.5%) are seropositive for T. gondii and in Saudi Arabia are (38 %) 4 and (30.8%) are seropositive in Qatar (Marawan, 2010). In different zones of the world, the present outcomes are about like what announced in Nigeria, 46% in Tanzania and 47% in France (rustic region) 12. Be that as it may, they are higher than what in joined kingdom, (22%) and in South Korea, (43%) as detailed via Carl (2006). This high commonness of the sickness might be because of the high number of hazard factors and numerous springswells of disease which incorporate the half-cooked meat sufficed with growths, eating unwashed vegetables or crude meat ingestion of sporulated oocyst in soil (for example amid planting eating). such finding might be because of distinction in the immunological status of ladies under examination at the time of tests accumulation and this concurs with 14. The percent of affiliation antibodies is 13 (7.14%) from the seropositive, while it is 15 (12 %) by 7 . The investigation of 5 in Egypt alludes to the percent of IgM (27.3 %) and (36.4 %) for IgG counter acting agent. While this examination appears (42.85%) for against IgM and (57.14%) for hostile to IgG antibodies. It has seen that altogether more prominent frequency of premature birth happened in patients with high immune response titters of IgM counter acting agent. This outcome is upheld by 13. The nearness of against T. gondii (IgG) antibodies in the example of serum isn’t adequate to initiate the way that the patient has been contaminated 15, while the nonappearance of IgG early or before pregnancy permits distinguishing proof of ladies in danger for getting the disease 18. In any case, IgG class of antibodies is commonly useful in the analysis of intensely displaying sicknesses on the grounds that these antibodies take 1-9 weeks to create 13 and proceeds for a considerable length of time or years. The high level of contamination is in the second trimester of pregnancy 11.11 % and 13.33 % separately. No noteworthy impact of trimesters on the frequencies of toxoplasmosis is showed up amid the ongoing examination. Regardless of the quantity of patients from provincial territories is higher yet it is a piece of the aggregate; country ladies are sure for hostile to Toxoplasma counter acting agent and this outcome might be because of the way that numerous ladies in rustic locales live in low close to home cleanliness, poor sterile, insufficient legitimate treatment and low instructive dimension about hazard factors, such lead to transmission of T. gondii. The job of wellbeing instruction is considered by 16 as a fundamental factor in diminishing the event of this contamination. The urban regions in Babylon region demonstrate a blend of various financial classes. Such a large number of ladies in downtown area live in poor sterile and low financial state as or even not exactly a significant number of country regions incorporated into the present investigation; this brought about huge likenesses in the quantity of tainted prematurely ended ladies. This outcome is reliable with 2 in Tikrit, who has discovered that the seropositivity is higher in country zones (36.36%) than focus ones (32.05%), 19 in Kirkuk who found that the seropositivity is higher among pregnant ladies from rustic zones (50.0%) than those in focus regions (33.5%). This might be ascribed to the nearness of indoor felines, or even the stray one in the rustic regions more than in the city, which is considered as a last host for T. gondii and a primary hotspot for spreading the irresistible oocyst in the dirt, water, vegetables and natural products utilized for every day expending. Be that as it may, it isn’t steady with an investigation in Ninewah by 3, who has discovered a higher pervasiveness among the city inhabitant (38%) than the country one (29%), likewise in Osorno (Chili) by 23. who has discovered a slight higher commonness in the urban territories than the rustic ones.

**Conclusion**

The investigation demonstrated that enemy of Toxoplasma IgG, IgM, and by and large seropositivity of all antibodies were 42.85% and 57.14% separately. There was noteworthy impact of age on extent rate. The
most noteworthy disease rates were found at 20-25 age gathering, while the least contamination rate found at 36-40 age gatherings. The all out predominance was more in Second Trimester of pregnancy (12.22%). IgG was increasingly amassed in Second Trimester (13.33%) than IgM (11.11%). No noteworthy distinction was found among residency and the commonness of Toxoplasma gondii antibodies in both rustic and urban territories. Watchwords: Toxoplasma gondii Infection, Toxoplasmosis. Using a microscopic sample of blood samples, 14 positive blood samples from 100 were found and the infection was identified by its characteristic characteristics.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Basic Medical Sciences /College of nursing /University of wraith Al-anbiya’a, Iraq and all experiments were carried out in accordance with approved guidelines.

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Estimation of the Serum Concentration Levels of Ferritin and Vitamin D for Hypothyroid Patients

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2Babylon Technical Institute, Al-Furat Al-Awsat Technical University, 51015, Babylon, Iraq.

Abstract

The paper investigates the serum concentration levels and the association between the levels of ferritin or vitamin D with thyroid dysfunction in Hypothyroid Patients. 90 participants were involved in this study; 47 healthy people (controls) (18 males and 29 females) and 43 hypothyroid patients (16 males and 27 females). Both groups have a mean age (age ± standard error) and body mass index (BMI) (BMI ± standard error) of 34.36. ± 1.19 years and 27.55± 0.52 kg/m2, respectively. The obtained results indicated that the age of participants played an ignorable role in this investigation (P ˃0.05), while significant differences were noticed between the patients and controls (P <0.05) in terms of BMI, 25(OH) vit D, ferritin, TSH, T3 and T4. There was also a significant difference (P<0.05) between male and female patients in terms of ferritin, TSH, T3 and T4 in comparison with the controls.

Keywords: Hypothyroid; vitamin D; ferritin; TSH; BMI.

Introduction

Thyroid hormones are very essential and have various actions, for instance, they maintain normal growth and regulate metabolism. Therefore, hypothyroid patients usually suffer from fatigue and cold, and easily gain weight due to their altered metabolism 1. There are two thyroid hormones in the human body, Thyroxin (T4) and triiodothyroxine (T3); these hormones are produced in the thyroid gland 2. The latter converts the iodine in food, in a series of reactions, into thyroid hormones. Although a disorder of the thyroid hormones, which is also known as hypothyroidism, can be easily diagnosed and treated, late diagnosis and consequently late treatment could cause adverse effects, such as slowed metabolism, and changes in the levels of serum ferritin and vitamin D 3. Previous studies have confirmed that hypothyroidism is directly related to the concentration of iodine, as the ability of the thyroid gland to produce the required amount of T3 and T4 is limited by the concentration of iodine in the body, where excess or lack of this element could result in disorder of thyroid hormones 4. Hypothyroidism is mainly diagnosed by investigating the inverse relationship between TSH and T4 and T3, where it is expected that a normal person will have a low level of T4 or T3 and a high level of TSH 5. It is estimated that hypothyroidism occurs in from 3.8% to 4.6% of the world’s population 6. However, some studies have indicated that the occurrence of hypothyroidism varies according to the area of the study and ages of the studied people, where it could reach 8.4% 7. Moreover, the clinical indicators of hypothyroidism are highly influenced by different factors, such as the duration and the deficiency level of the thyroid hormones. Generally, hypothyroidism gives a set of associated symptoms such as tiredness, cold, weight increase and dryness of skin, which could be used to diagnose this disease 8. Thyroperoxidase enzyme, which is a thyroid hormone, plays an important role in the synthesisation of thyroid hormones 9. Recent studies have demonstrated that the synthesisation process of the thyroperoxidase enzyme requires a certain amount of iron, which explains the association between the disorder of thyroid hormones and the ferritin level 10. For example, both Takamatsu, Majima 11 and Sachdeva, Singh 10 have demonstrated that the thyroid profile is highly influenced by the ferritin.

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levels. It is noteworthy to mention that ferritin is an iron storage protein, with a diameter ca 10–12 nm; it is essential for physiologic and pathologic activities (12-14).

**Materials and Method**

The current study involved 90 participants; these participants were divided into two groups: healthy (controls) and hypothyroid patient groups. The hypothyroid patient group consists of 16 Males (37.21%) and 27 Females (62.79%), while the healthy people group consists of 18 Males (38.30%) and 29 Females (61.70%). The members of the control group were not suffering from any chronic diseases, and had no history of thyroid disease, nor were they on any medication that may interfere with the obtained results. They were living in Babylon Governorate, Iraq. They were examined at the laboratories of Marajan hospital and at Ibn al-Nafis specialist laboratory during the period of study (September 2017 to January 2018). The mean of their ages was 34.36 ± 1.19 years (mean ± S.E), and the mean of their body mass index (BMI) was 27.55± 0.52 kg/m2 (mean ± S.E). The BMI was calculated using the formula BMI= weight (kg)/ height2 (m)2. Vitamin D (25-hydroxyvitamin D), ferritin, T3, T4 and TSH levels were measured using the ELFA (Enzyme Linked Fluorescent Assay, Model: bioMérieux). It is noteworthy to highlight that the normal limit for vitamin D is ranging between 30 and 100 ng/ml. while the limits of ferritin are: male: 20-434 ng/ml, cyclic women: 20-159 ng/ml, menopausal women: 20-278 ng/ml. Finally, limits for serum T3, T4 and TSH thyroid dysfunction patients (hypothyroid) are: T3 between 0.9 to 2.33nmol/l, hyperthyroid <0.15, hypothyroid T4 between 60-and120 nmol/l, TSH thyroid 0.25->7.0. It is noteworthy to highlight that the results were analysed using SPSS package (version 18).

**Results and Discussion**

The obtained results showed that there was no relationship between the age of participants and the thyroid disorder, while the rest of the studied parameters showed a significant relationships (P<0.05). For example, it has been found that the values of BMI, 25(OH) vit D, ferritin, T3, T4 and TSH in the patients were 23.93± 0.39, 32.01±2.76, 33.16 ±3.02, 2.26 ± 0.18, 3.60 ± 0.12 and 19.04 ± 0.66, respectively. In contrast, the values of these parameters in the healthy people (controls) were 31.48 ± 0.56, 18.29 ± 1.54, 24.46±1.90, 11.82 ± 0.56, 1.18 ± 0.07 and 9.20 ± 0.18, respectively, as shown in Table 1. These differences indicate a significant association between these parameters and the thyroid disorder. Table 2 shows that the sex of the participants did not cause any a significant differences in the Age (years), BMI (kg/M2) and in the levels 25(OH) vit D (ng/ml). However, a significant variation was observed in the levels of ferritin(ng/ml), TSH(µu/ml), T3 (nmol/L)and T4(nmol/L) in male and female patients in comparison with the controls.
Table 1: Comparison between hypothyroid patients and control subjects for both sex.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (Mean ±S.E)</th>
<th>Patients (Mean ±S.E)</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n=18)</td>
<td>Female (n=29)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>2.88±32.16</td>
<td>1.69±31.58</td>
<td>2.93±32.93</td>
</tr>
<tr>
<td></td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
</tr>
<tr>
<td></td>
<td>0.79±24.21</td>
<td>0.41±23.77</td>
<td>1.03±29.18</td>
</tr>
<tr>
<td></td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
</tr>
<tr>
<td>BMI (Kg/M2)</td>
<td>0.79±24.21</td>
<td>0.41±23.77</td>
<td>1.03±29.18</td>
</tr>
<tr>
<td></td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
<td>The studied cases indicated that the concentration of this vitamin has a strong correlation with some diseases, such as thyroid dysfunction.</td>
</tr>
<tr>
<td>25(OH) vit D (ng/ml)</td>
<td>1.81±53.96</td>
<td>1.32±18.38</td>
<td>2.52±28.05</td>
</tr>
</tbody>
</table>
In the hypothyroid patients the linear regression analysis showed there was a significant negative correlation between TSH(µu/ml) and 25(OH) vit D(ng/ml) (r = -0.41, P=0.007), as shown in Figure 1, and between TSH(µu/ml) and ferritin (ng/ml) (r = - 0.35, P= 0.02), as shown in Figure 2.
Figure 1: The linear regression analysis of the thyroid-stimulating hormone (TSH) (µu/ml) with 25(OH) vit D (ng/ml) for hypothyroid patients.

Figure 2: The linear regression analysis of the thyroid-stimulating hormone (TSH) (µu/ml) with ferritin (ng/ml) for hypothyroid patients.

Discussion

The outcomes indicated a significant correlation between the level of vitamin D and the thyroid disorder. A decrease in the average of vitamin D was noticed as the average of TSH increased. This relationship could be related to the increase in bone turnover in hyperthyroid patients that increases the calcium level, which in turn alters the synthetisation of both parathyroid hormone and vitamin D\(^{19}\).

The outcomes of the current study agree with those of\(^ {20}\), which indicated that deficiency of vitamin D is responsible for low thyroid hormones. Mackawy, Al-Ayed\(^ {21}\) found two facts, firstly the level of serum 25(OH) vit D in hypothyroid patients is less than its level in healthy people. Secondly, the authors noticed a clear correlation between serum 25(OH) vit D and TSH,\(^ {1}\), which agrees with the results of Shilpa, Mishra\(^ {22}\) that showed a clear reduction in the level of vitamin D in hypothyroid patients (≤ 20 ng/ml). Additionally, the literature showed that treatment of the vitamin D deficiency in thyroid patients has enhanced thyroid functions\(^ {23}\). The literature also indicated an inverse relationship between the levels of vitamin D and TSH\(^ {24,25}\).

Conclusion

The results showed that hypothyroidism, represented by the high level of TSH, was associated with high BMI and low level of 25(OH) vit D, ferritin, TSH, T3 and T4, and age does not play a significant role.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kirkuk University and all experiments were carried out in accordance with approved guidelines.

References

Impact of Nutritional Milk for Lactating Mothers upon Efficiency of Breast Feeding for Iraqi Mothers at Maternity Hospital in Baghdad City

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Abstract

Objective: This study conducted to find out: The impact of nutritional milk for lactating mothers upon efficiency of breast feeding 2. The impact of nutritional milk for lactating mothers upon the number of breastfeeding 3. The impact of nutritional milk for lactating mothers upon the babies’ weight. This study conducted at AL-Elwaya Maternity Hospital in Baghdad City. The study started in January 2019 to March 2019. The study sample included (100) lactating mothers that feed their newborns by breast feeding only despite of the method of delivery (normal vaginal delivery & caesarian section) this sample divided in two groups the first one is the (study group) consist of (50) lactating mothers that consumption nutritional milk for three weeks and the other (control group) consist of (50) lactating mothers that not consumption nutritional milk and then comparison between the two groups to detecting the impact of nutritional milk on the efficiency of breast feeding. Results showed that the nutritional milk have impact upon the efficiency of breast feeding for the lactating mothers who were consumption the nutritional milk for at least three weeks and have impact upon their babies’ weight and growth.

Keywords: Nutritional milk, Lactating mothers, Breast feeding, Maternity hospital.

Introduction

Most studies regarding the lactation have focused on the quality and quantity of breastfeeding or on the benefits of the breast milk on infants. Far fewer studies have targeted the effects of lactation on short- or long-term maternal health. The breastfed infant doubles its weight in the first 4 to 6 months after birth and has additional energy demands beyond the gains in energy stores associated with growth. The metabolic adjustments that redirect nutrient use from maternal needs to milk synthesis and secretion involve nearly every maternal organ system1. Breast feeding results in a number of physiological adaptations which exert direct effects on maternal health. Nutrient needs during lactation depend primarily on the amount and composition of milk produced and on the mother’s initial nutrient needs and nutritional status. Among women exclusively breastfeeding their infants, the energy demands of lactation exceed pre-pregnancy demands by approximately 640 kcal/day during the first 6 months postpartum compared with 300 kcal/day during the last two trimesters of pregnancy (NRC, 1989). In contrast, the demand for some nutrients, such as iron, is considerably less during lactation than during pregnancy2. Breast milk is the best food for newborns and infants. The nutritional stores of a lactating woman may be more or less depleted as a result of the pregnancy and the loss of blood during childbirth. Lactation raises nutrient needs, mainly because of the loss of nutrients, first through colostrum and then through breast milk. Breast milk volume varies widely. The nutrients present in this milk come from the diet of the mother or from her nutrient reserves. The conversion of nutrients in food to nutrients in breast milk is not complete. To have good nutritional status the breastfeeding woman has to increase nutrient intake. Human breast milk has a fairly constant composition, and is only selectively affected by the diet of the mother. The fat content of breast milk varies somewhat. The carbohydrate, protein, fat, calcium and iron contents do not change much, even if the mother is short of these in her diet3.
Methodology

Experimental design was carried out throughout the present study with the application of a pre-test and post-test for the study group. The study was conducted at AL-Alwaya Maternity Hospital which is located at the center of Baghdad city, in Al-Rasafa sector. Non-probability (purposive sample), the study sample consists of (50) lactating mothers who were selected from Hospital, the study group was consumption nutritional milk for at least three weeks, nutritional milky that used in this study was (naktalia mama) is drink specifically formulated to meet the needs of mothers wishing to get pregnant, and those who are already pregnant or breastfeeding that supported with vitamins and essential minerals such as (calcium carbonate, potassium phosphate, magnesium carbonate, ferrous lactate, zinc sulphate, manges sulphate, cupper sulphate, sodium selenite, potassium iodide, Fer/iron) Fish oil, soya lecithin, antioxidant, fructose, oligofructose vitamins (C, E, PP, D3, B8, B5, A, B6, K1, B9, B1) and the control group consist of (50) lactating mothers who were do not consumption the nutritional milk; the criteria of this sample was lactating mothers in reproductive age, with different educational levels, who were feeding their babies with breastfeeding only. Data for such assessment was collected from the maternity hospital through the presence of the researcher in maternity hospital and her contact with lactating mother during one month period from 12 January 2019 through 12 March 2019. The criteria of this sample were Lactating mothers, Feeding their baby with breast feeding only and they delivered with normal vaginal delivery or C/S. The exclusion criteria Mothers who were feeding their baby by mixed or bottle feeding and Lactating mothers who were not persisting in consumption the nutritional milk for at least 3 weeks.

Results and Discussion

Table (1) Distributions of Mothers’ Reproductive History

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<tr>
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<td>Number of Para (Birth)</td>
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<td>16</td>
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<tr>
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</tr>
<tr>
<td></td>
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<td>Number of Gravida (pregnancy)</td>
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<tr>
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f: Frequency, %: Percentage
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<th>Control Group (N=50)</th>
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<td>Amount of Enough Breast Milk</td>
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<td>Taking Herbals Supplement to Increase Milk Production</td>
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<td>Allergy toward Foods</td>
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<td>Eating more than Three Meals per Day</td>
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<td>14</td>
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<td>11</td>
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<tr>
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<td>Normal</td>
<td>25</td>
<td>25</td>
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<td>15</td>
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f: Frequency, %: Percentage
### Table (3) Distribution of Babies’ Demographic Characteristics

<table>
<thead>
<tr>
<th>List</th>
<th>Characteristics</th>
<th>Study Group (N=50)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Age</td>
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<td></td>
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<tr>
<td></td>
<td>Newborn</td>
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<td>50</td>
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<tr>
<td></td>
<td>Infant</td>
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<td>2</td>
<td>Weight at Delivery</td>
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<tr>
<td></td>
<td>&lt; 250 gram</td>
<td>9</td>
<td>16</td>
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<td></td>
<td>250 – 300 gram</td>
<td>32</td>
<td>22</td>
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<td>300 &lt; gram</td>
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<td>12</td>
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<tr>
<td>3</td>
<td>Height</td>
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<tr>
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<tr>
<td></td>
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<td>50</td>
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<td></td>
<td>50 &lt; cm</td>
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</tr>
<tr>
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<td>Type of Feeding for Previous Baby</td>
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<td></td>
<td>Breast feeding</td>
<td>42</td>
<td>31</td>
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<td>Bottle feeding</td>
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f: Frequency, %: Percentage, cm: Centimeter

### Table (4) Breast Feeding Efficiency (before and after) Consumption of Nutritional Milk

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<td>f</td>
<td>%</td>
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<td>%</td>
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<td>1</td>
<td>Number of Feeding/Day</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 6</td>
<td>28</td>
<td>56</td>
<td>1</td>
<td>2</td>
<td>40</td>
<td>80</td>
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<tr>
<td></td>
<td>6</td>
<td>22</td>
<td>44</td>
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<td>50</td>
<td>8</td>
<td>16</td>
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<td>6 &lt;</td>
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<td>Number of Defecation / Day</td>
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<td>Observing Signs of Growth</td>
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<td>50</td>
<td>100</td>
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</tbody>
</table>

f: Frequency, %: Percentage

The highest percentage among study group is present a mothers with age group of 30-39 years old (36%), while 60% among the control group are mothers with age group of 20-29 years old. Regarding occupation, most of mothers in both group; study and control are housewife (98% and 94%); and also fathers are free job in both group (study=74% and control= 60%). The socio-economic status indicates a moderate among the subjects...
in the study group (68%) and in the control group (64%). 82% of mothers in both groups; the study and control are living in urban areas. Previous studies have found that factors such as socioeconomic and employment status, maternal and child characteristics, maternal/family intention and health care management contributed to breastfeeding initiation and/or duration. Among those factors, the effect of maternal age on breastfeeding practice is one of the most interesting research targets. This is because in the past 20 years, increasing maternal age at first childbirth in most developed countries has been observed (4,5,6). The present findings that both older maternal age of ≥ 35 and primipara were negative factors of success of EBF initiation (7,8). The findings reveal that half of mothers in the study group are got 2-4 pregnancy (50%), the mothers in the control group are having pregnancy 2-4 and more then 5 (34%). 56% of mothers in the study group and 44% in the control group are associated with 2-4 gravida. Regarding number of abortion, the highest percentages are showing no previous abortion (study=60% and control=82%), but 38% in the study group and 18% in the control group showing they have 1-4 abortion. More than half of mothers are confirmed they have cesarean section as type of delivery (study=62% and control= 60%). Most of mothers in both groups; the study and control showing that their gestational age were 27-40 weeks (100%). In previous study, 20–30% of mothers who did not initiate EBF at the time of discharge eventually turned out to have successful initiation at one month. Dewey et al. (2003) indicated that lactation difficulties during the first week postpartum are not uncommon, even among women who are highly motivated to breastfeed exclusively and receive good lactation guidance; and that early lactation success is strongly influenced by parity, but may also be affected by potentially modifiable factors such as labor medications; therefore, all breastfeeding mother-infant pairs should be evaluated at 72 to 96 hours’ postpartum (9,10). 84% of mothers in the study group and 72% in the control group are not taking a herbal supplements for increasing their milk and the remaining were used. Most of mothers in both groups have negative history of allergy toward foods (study= 96% and control= 90%). 54% of mothers in the study group are eating less than three meals per day while more than half of mothers in the control group are eating more than three meals per day (60%). Those mothers in the study group reveal that they not take vitamins supplements (78%) and only 22% taking vitamins; the mothers in the control group taking those vitamins (52%). More than two third of mothers in the study group and control group are showing a difficulties in breast feeding (72% and 74%). Half of sample in both groups showing that they have normal nipple (50%); and the remaining show a protruded nipple (30% and 28%). Maternal diabetes was a strong predictor of diabetes in children. Maternal preeclampsia was associated with increased risk for early and later onset of diabetes. 11 The World Health Organization recommends early initiation of breastfeeding (within the first hour after birth) and exclusive breastfeeding (no water, other fluids or foods) for 6 months 12. Breastfeeding reduces their risk of type 2 diabetes. This could be due to improving glucose hormones 13. Reduces their risk of developing hypertension and cardiovascular diseases (14,15). Helps them to lose weight, especially while their infants are being exclusively breastfed. 16.

Conclusion

The study showed that the nutritional milk have impact upon efficiency of breastfeeding. The study showed that nutritional milk have impact upon the number of breastfeeding. The study showed that the babies’ weight of lactating mothers who were consumption nutritional milk have increasing in their weight.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, University of Baghdad and all experiments were carried out in accordance with approved guidelines.

References

2. Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation: Meeting Maternal Nutrient Needs During Lactation, Washington, Copyright © 1991 by the National Academy of Sciences
3. Ares Segura S, Arena Ansótegui J, Diaz-Gómez NM. en representación del Comité de Lactancia Materna de la Asociación Española de Pediatría:


Risk of Osteoporosis in Patients with Crohn’s Disease Not Respond to Infliximab Therapy Undergoing Surgical Intervention

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Abstract

Osteoporosis is one of the most common complications of Crohn’s disease (CD). Proper history with blood samples were collected from (30) healthy control group, (30) Crohn’s disease patients have been respond to biological therapy (infliximab IFX) (response group) and (30) CD patients with (non-response group) to biological therapy undergoing surgical intervention for the estimation of some biochemical parameters. This study demonstrate a significant decrease in FGF-23 (fibroblast growth factor 23) and vitamin D Levels between (non-response group) and control group (p< 0.01). Similary, parathyroid hormone (PTH) Levels were increased significantly (p< 0.01) in (non-response) group. Moreover the calcium (Ca) and phosphours (P) decreased significantly (p< 0.01) in same group. While in CD patients that have been treated with infliximab only vitamin D and FGF 23 decreased significantly (p< 0.01) compared with control group. Levels of Serum FGF23 are considerably lower in CD patients, It is independent pattern was not affected by type of treatment (medical or surgical). Hypovitaminosis common finding in CD patient’s. Although CD is not disease of vit D deficiency but it clearly is a disease whose pathogenesis seems closely related to vit D Level.

Keyword: Osteoporosis, Crohn’s disease, Infliximab, Vitamin D

Introduction

Inflammatory bowel disease (IBD) includes two lasting intestinal disorders, which are: CD and Ulcerative colitis (UC) ¹. CD is a chronic inflammatory disorder that could involve any part of alimentary tract from mouth to anus ². These disorders were first described by Dr. Burril Crohn’s and his team in 1932 ³. Although its aetiopathogenesis is still not clear, it has been well recognized that CD is one of the complicated disorders which result from interaction of environmental, microbial, and genetic factors.⁴ Patient that have Crohn’s disease are under increased risks of the metabolic bone disease, which includes osteoporosis and osteopenia ⁵. They seem to be one of the most important complication since osteoporosis has been reported in (30%-50%) of patient with CD which may be a silent disease until a fracture occurs ⁶. BMD (Bone mineral density) is less in patients that have CD ⁷. The mechanism which is underlying the lower BMD hasn’t been clearly understood ⁸. None-the-less, various risk factor including increasing age ⁹ disturbances of calcium homeostasis, malabsorption and vitamin D, smoking and the actual inflammatory process by releasing cytokines that interact with bone metabolism ¹⁰. Malabsorption and the deficiency of vitamin D which results from the involvement of the small intestinal of disease and or surgical resections are usually found in patients that have CD ¹¹. Which is why, the deficiency of vitamin D may result in hypocalcemia in patients that have Crohn’s disease with short bowel syndrome, also hypocalcemia might be resulting from deficient parathyroid hormone which stimulates bone resorption resulting in bone loss ¹². On the other hand, regulation of the phosphate in bone homeostasis hasn’t been entirely explained, regulation of the phosphate includes interaction of a complicated kidney–intestine–bone parathyroid gland hormonal axis that remains insufficiently comprehended. A lately discovered phosphatonia, FGF23 disclosed now. Pathways in mineral metabolism pathophysiology ¹³. FGF23 is a new phosphaturic hormone which is
mainly generated by osteoblasts/osteocytes in bones, and with lower levels in other types of tissue, they target the kidneys for regulating vitamin D metabolism and phosphate homeostasis. Infliximab anti-body directed against TNF (tumor necrosis factor alpha), has been the first TNF antagonist which has been approved for patient that have CD. It was an important advance in the treatment of the disease due to their proven efficacy in inducing and maintaining clinical symptoms remission. 74% of all CD patients will require surgery. Surgical management typically with intestinal resection remains considered as a significant modality of treatment because of disease symptoms nature and complication namely strictures and perforation obstruction, starts so hard to be sufficiently controlled.

**Aim of study**: Aim of this study are characterize serum PTH, Ca, P, vit D , and FGF 23 levels at Crohn’s disease patient’s compared with controls , and to identify correlates of changes in these parameters levels following therapy.

**Material and Methods:**

**Study subject**

This research has been approved by the Ethics committee, department of chemistry, college of science, Mustansiriyyah University, Bagdad, Iraq. and the Iraqi Ministry of Health approved this work as well. The blood samples were taken after informed consent of participant were recruited from Gastroenterology and Hepatology teaching hospital at Bagdad Medical city, while the healthy group were volunteers. All the patients were diagnosed by senior doctors specialist in gastroenterology field, (60) sixty unrelated Iraqi Crohn’s disease patient’s divided in to two groups according to response to biological therapy (infliximab) the first group (30) patients was respond to infliximab according to classical regimen (loading dose 5mg/kg at week 0,2,and 6 followed by repeated infusion of 5mg/kg every 8 weeks) and (30) patients not respond to infliximab undergoing surgical intervention as well as (30) unrelated healthy person termed as control group without any systemic disease. All the patients and control aged between 18 and 64 years. As well as the two groups of patient’s and control were measured by electronic balance and measuring body mass index (BMI). Five milliliters of venous blood was obtained from patients and control group by 5 ml disposable syringe (without tourniquet) drained into get plain tubes and left in room temperature (25°C) for 15 minutes, Then it was centrifuged at 2000 xg for 10 minutes in order to collect sera. Sera aliquots were placed in eppendorf tubes and stored at -40C° until used.

**Biochemical analysis**

The human FGF-23- was measured in the sera for the all patient and control group using the double sandwich (ELISA) kit according to manufactures instruction (Cat No.MBS 263043, My bio source / USA). PTH was measured using the protocol of ELISA kit (Cat No.MBS2505074,My bio source /USA). And vitamin D was measured using the competitive (ELISA) kit principle (Cat No.MBS 2503525 , My bio source / USA). While Calcium and phosphorus determined by automatically performed by the Dimension ® Clinical chemistry system (SIEMENS, Germany).

**Statistical analysis**

The statistical analysis system SAS program has been utilized to compare between control and two CD patients groups ( response and non-response to biological therapy) in study parameters. (Analysis of variation-ANOVA) was used to compare between means (P value of 0.05 and 0.01 has been considered to be statistically significant).

**Result and Discussion**

Mean ± SD value of age and BMI were recorded from all subscribers as shown in table 1. Results of this study shown in table 2 there were a non-significant differences in mean age between all age of the studied group at (p<0.05). Age at the onset of CD was in the range between early childhood to beyond 70 years of age. Although the peak incidence of diseases between ages 20 to 39, about 25% of patients with Crohn’s is present before 20 years of age—among children with Crohn’s disease, 4% present before 5 years of age. 21. a second peak is recognized between ages 50-70. Age definitions of early and elderly onset of Crohn’s in literature are different and are usually dependent on local clinical practice (such as age of referring from pediatric to adult Crohn’s disease care), and that makes comparisons between population complicated. In addition to that, heterogeneity in the approaches of data collection and case ascertainment (such as, diagnostic criteria, access to diagnostic procedures) – and as a result, differences in the capability of capturing each case of Crohn’s disease in the population- might result in bias in any of those
comparisons also shown that CD patients have a significant BMI decrease (P<0.01) from (26.743±2.699) Kg/m² to (22.816±2.025) Kg/m² in (non-response group) patients need surgical intervention compared with control healthy group this could be represented with malabsorption, decreased dietary intake, metabolic disturbances like increased expenditure of energy, increased substrate oxidation rates, and decreased respiratory quotient, could as well be one of the reasons that cause patient’s protein, glucose, and fat loss. To some extent, medical therapy including corticosteroid, mesalamine, TNF- alpha antagonists, and azathioprine, could enhance the BMI of CD patients.

The laboratory examination data in the three groups are shown in table( 2),also table (3) illustrated the statistics comparison of different biochemical analysis among the studied groups. Serum parathyroid hormone, calcium and phosphate were not significantly charged in patient response to infliximab group compared with healthy control group. The FGF23 and vitamin D significantly decreased (P>0.01) between the same groups. A research in the animal model TNF- mediated bone erosion have showed that treating mice with anti-TNF factor combined with osteoprotegerin or PTH results in repairing local bone erosions. There for the treatment with infliximab or adalimumab have an advantageous impact on bone metabolism in patients that have CD.

Abreu, etal. have reported that patients that have Crohn’s disease have appropriately increased Levels of active hormonal form of vitamin D 1,25 (OH)₂D. Those Level are inversely correlated to BMD probably due to the fact that 1,25 (OH)₂D mobilizes the stores of skeletal calcium, the mechanism for increasing 1,25(OH)₂ D is via increased 1-α hydroxylase expression by activated lamina propria macrophage, that some other way in which IFX has a positive impact on bone metabolism is via inhibiting macrophage activation in gut and decreased expression of 1,α-hydroxylase on the other hand vitamin D is essential for absorbing calcium in small bowel over time, vitamin D deficient generates hypocalcemia and consequently a secondary increase in the PTH Level also the regulation of calcium and phosphate metabolism, as physiological correlation between the levels of its main regulators PTH and vitamin D.

While there is significantly increase (p>0.01) in PTH levels and significantly decrease (p>0.01) in Levels of Ca, P and vitamin D in(non-response group) patients that surgical treated compared to control group in this study parathyroid hormone is released from parathyroid cells as a response to low extra cellular concentration of free calcium. Secondary hyperparathyroidism has been detected in more than 1/3 of patients that have Crohn’s disease who have operated on with small bowel resection, increased levels of PTH were related to increased bone turnover and decreased BMD preferentially in cortical bone.

Table 1. Statistical analysis of Age and BMI, measurements distributed among patients of Crohn’s disease (response and non-response) and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>SE</th>
<th>95% C.I. for Mean</th>
<th>Min.</th>
<th>Max.</th>
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<td></td>
<td></td>
<td></td>
<td>L.b.</td>
<td>U.b.</td>
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<tr>
<td>Age (Years)</td>
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<td>A</td>
<td>40.551±10.715</td>
<td>1.989</td>
<td>36.475</td>
<td>44.627</td>
<td>19.00</td>
</tr>
<tr>
<td>B</td>
<td>37.700±11.262</td>
<td>2.056</td>
<td>33.494</td>
<td>41.905</td>
<td>18.00</td>
</tr>
<tr>
<td>C</td>
<td>38.500±9.846</td>
<td>1.797</td>
<td>33.823</td>
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<td>18.00</td>
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<td>BMI (Kg/m²)</td>
<td></td>
<td></td>
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<td>A</td>
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<td>25.020</td>
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<td>22.816±2.025</td>
<td>0.369</td>
<td>22.059</td>
<td>21.572</td>
<td>17.20</td>
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</table>

Table 2. Statistical analysis of PTH, Ca, P, FGF 23 , and VitD parameters distributed among patients of Crohn’s disease (response and non-response) groups and control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Mean±SD</th>
<th>SE</th>
<th>95% C.I. for Mean</th>
<th>Min.</th>
<th>Max.</th>
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</thead>
<tbody>
<tr>
<td>PTH (pg/ml)</td>
<td>A</td>
<td>84.892±11.613</td>
<td>1.156</td>
<td>79.074-87.909</td>
<td>59.41</td>
<td>103.50</td>
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<tr>
<td></td>
<td>B</td>
<td>86.182±8.337</td>
<td>1.522</td>
<td>83.069-89.295</td>
<td>67.82</td>
<td>111.42</td>
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<tr>
<td></td>
<td>C</td>
<td>123.724±37.926</td>
<td>6.924</td>
<td>109.561-137.886</td>
<td>79.31</td>
<td>216.42</td>
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<tr>
<td>Ca (mg/dL)</td>
<td>A</td>
<td>8.133±0.709</td>
<td>0.131</td>
<td>7.863-8.403</td>
<td>6.56</td>
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<tr>
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<td>B</td>
<td>7.663±0.641</td>
<td>0.117</td>
<td>7.423-7.902</td>
<td>6.73</td>
<td>9.84</td>
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<tr>
<td></td>
<td>C</td>
<td>6.568±2.021</td>
<td>0.369</td>
<td>4.813-6.323</td>
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<td>8.71</td>
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<tr>
<td>P (mg/dL)</td>
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<td>3.648±0.695</td>
<td>0.129</td>
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<td>3.521±0.523</td>
<td>0.095</td>
<td>3.325-3.716</td>
<td>2.60</td>
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<td>1.858-2.512</td>
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<td>FGF (pg/ml)</td>
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<td>1047.189±27.113</td>
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<td>1006.20</td>
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<td>747.423-799.788</td>
<td>640.31</td>
<td>932.40</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>744.064±50.337</td>
<td>9.190</td>
<td>725.267-762.860</td>
<td>680.52</td>
<td>830.30</td>
</tr>
<tr>
<td>VitD (pg/ml)</td>
<td>A</td>
<td>29.983±12.677</td>
<td>2.354</td>
<td>25.161-34.805</td>
<td>4.61</td>
<td>55.40</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.187±1.047</td>
<td>0.191</td>
<td>1.796-2.578</td>
<td>0.71</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.1169±0.994</td>
<td>0.181</td>
<td>0.797-1.540</td>
<td>0.01</td>
<td>4.82</td>
</tr>
</tbody>
</table>


Table 3: Multiple comparison significant (ANOVA) for parameter between the different groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PTH (pg/ml)</th>
<th>Ca (mg/dL)</th>
<th>P (mg/dL)</th>
<th>FGF (pg/ml)</th>
<th>VitD (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A &amp; C</td>
<td>0.0012 **</td>
<td>0.001 **</td>
<td>0.001 **</td>
<td>0.001 **</td>
<td>0.001 **</td>
</tr>
<tr>
<td>B &amp; C</td>
<td>0.002 **</td>
<td>0.002 **</td>
<td>0.003 *</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>A &amp; B</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.002 **</td>
<td>0.001 **</td>
</tr>
</tbody>
</table>

**Conclusion**

The conclusions that have been drawn in this paper have stated the fact that Levels of serum FGF23 are considerably lower in patients that have Crohn’s disease, it is an independent pattern not affected by type of treatment (medical or surgical). Also hypovitaminosis D was common finding in CD patients Although Crohn’s is not a condition of the deficiency of vitamin D but it
is clearly a disease whose pathogenesis seems closely related to the Level of vitamin D. in addition to that, patients that have CD subjected to surgical intervention are under a risk to develop low Bone mineral density and secondary hyperparathyroidism.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science, Mosul University, Iraq and all experiments were carried out in accordance with approved guidelines.

References


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Bioactivity of Gutta flow II versus Modified Gutta Percha based Silicon Endodontic Sealers by Nanobioactive Fillers

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2Baghdad University/ College of Dentistry- Dept. of Restorative and Esthetic Dentistry

Abstract

The success of endodontic therapy is relied on radicular system cleaning, shaping, elimination of microorganisms, and three dimensional filling of the radicular complex. This study was conducted to develop and assess new root canal sealer incorporating nano-sized bioactive glass into Gutta Flow II. The following concentration was used depend on a pilot study included adding (3%) of 45S5 bioactive glass into the Gutta Flow II. These materials were tested through assessment bioactivity. bioactivity test was undertaken after immersion of the tested samples into PBS for three days, seven days, fourteen days, and twenty eight days using FTIR too. study was found that it’s peaks was appear at level 800-1000 cm⁻¹. The results showed that GFII group revealed no peak at the 910 cm⁻¹, while, while BG3% revealed 179.85 pixel height, in conclusion the newly developed sealers exhibited apparent apatite and apatite precursor forming ability significantly with BG 3% while GuttaFlow II sealer showed no apatite layer forming ability.

Key words: bioactivity, chemical analysis, 45S5 bioactive glass

Introduction

Although gutta-percha based silicon sealers like Gutta Flow II is characterized by many advantages like simplicity of handling, setting expansion that may improve its sealing ability, in addition to certain extent of anti-microbial activity due to the effect of silver particles which were added as fillers 1 but generally gutta percha still has shortcomings, involving the inability to re-enforce the root canal treated tooth 2. Furthermore, the ability of gutta-percha to provide hermetic seal is still controversial as it does not capable to bond with root dentine 3. Specifically, Gutta Flow II still has considerable cytotoxicity, insufficient antibacterial activity, no bioactivity 4. Calcium silicate–based materials are classified as bioactive substances due to their ability to stimulate hard tissue remineralization and regeneration in both the dental pulp and bone 5. Silicone-based endodontic sealers showed relatively good biological features 6. The silicone-based sealer GuttaFlow II (Coltene Whaledent, GmBH + Co KG) constitutes a blend of polydimethylsiloxane and gutta-percha powder with nanometer-sized silver particles incorporated as a preservative. GuttaFlow II, a development of its predecessor GuttaFlow, is also a cold thixotropic flowable system. Both GuttaFlow and GuttaFlow2 are silicone-based root canal sealants that have variable forms of the silver particles used 7. A novel formula of polydimethylsiloxane with gutta-percha powder joined with calcium silicate particles was introduced in late 2015 and named GuttaFlow Bioseal (Coltene/Whaledent AG, Altstatten, Switzerland). In the present study nano-sized bioactive fillers of 3% were incorporated and distributed mechanically into gutta flow II sealer in order to develop bioactivity. Nano-sized 45S5 bioactive glass were chosen to be used in this study to lower the percentage of the incorporated powder in order to keep the physical characteristics of the original sealer regarding solubility and flow ability. In addition, to keep the newly developed material clinically applicable.

Materials and Method

Sample grouping:

Group 1 (control group): ten samples were prepared from Gutta flow II sealer.

Group 2: ten samples were prepared from Gutta flow II incorporated with nano sized 45S5 bioactive glass particles of optimum percent by weight according
to the optimization results of the pilot study.

**Preparation of bioactive glass containing Gutta Flow II 3%:**

The sample was prepared by mixing 0.3 mg of 45S5 bioactive glass with 7.7 mg of gutta flow II base. The pistons of both base and catalyst were separated by diamond disc that mounted on engine driven straight hand piece at their connected base. The gutta flow II was extruded on sterile screw cup, then 0.3mg was removed from the base to be replaced by 0.3 mg of bioactive glass. The mixing procedure was accomplished by adding the 0.3 bioactive glass carefully, and the mixture was mixed well by using disposable plastic spatula which was installed into INGCO cordless drill till complete homogenization of the mixture. The mixing time was 2 minutes and the speed was 750 R.P.M. Then the newly mixed material was reloaded in the base tube of the syringe carefully by using plastic spatula. The base piston was re-inserted into the base tube and pushed down to the level of the catalyst tube and rejoined at their base by using adhesive liquid.

**Assessment of apatite forming ability (bioactivity) using Fourier Transform**

**Infrared Spectroscopy**

Disk samples were impregnated into phosphate buffered saline solution (PBS) to mimic tissue fluid immersion and examined at 3, 7, 14, 28 days post immersion. At each period, samples were displaced from storage solution. The pH of the saline solution was usually checked by pH meter to keep its pH at 7.2. 0.1 mg of each disc was taken from its surface and tested by FTIR according to the manufacturers instructions to detect the development of carbonated hydroxyapatite layer at each sample surface.

**Results and Discussion**

**Chemical analysis using FTIR**

According to the chemical structure of the gutta percha figure (5) and its other included materials gutta flow II, the FTIR spectra range was 400 – 4000 cm⁻¹. The spectra of 50 Nano meter sized particles of 45S5 was 910 cm⁻¹, in which the primary peaks in correspond to the Si-O vibrational modes of the bonds in the glass network, the stretching vibration at 1090 cm⁻¹ and the rocking vibration at 450 cm⁻¹. The broad peaks in the 750-950 cm⁻¹ region correspond to Si-O-Si vibrational modes associated with the Ca⁺² and Na⁺ ions in the glass network as shown in the figures (2). The chemical groups in the FTIR spectrum of HA are PO₄³⁻, OH¹⁻, CO₃²⁻, as well as HPO₄⁻² that are characteristic of non- stoichiometric HA. PO₄³⁻ group forms intensive IR absorption bands at 1000 –1100 cm⁻¹. CO₃²⁻ intensive peaks between 1460 and 1530 cm⁻¹ which represent HA as shown in the figure (2).

![Fig (1) shows FTIR chart of GF II which appears no peak at 910 cm⁻¹ level](image1)

![Fig (2) shows FTIR chart BG3% which appears a peak of 63.95 pixel at 910 cm⁻¹ level](image2)

**Bioactivity test:**

According to the structure of the carbonated layer figure (4) the FTIR study was found that it’s peaks was appear at level 800-1000 cm⁻¹

According to the results of the present study, GFII showed no development of carbonated hydroxyapatite layer

The peak of carbonated material start to appear in the BG 3% group at the third day with obvious increment of it’s length figures (3).

Apparently BG 3% group reach to the maximum concentration of the carbonated HA layer which was indicated by increase the length of the peak at day 28,
Fig (3) shows FTIR chart of BG3% after 14 days which appears a peak of carbonated hydroxyapatite at 900 cm$^{-1}$ level.

Fig (4) shows FTIR chart of BG3% after 28 days which appears a peak of carbonated hydroxyapatite at 900 cm$^{-1}$ level.

Discussion

Throughout the obturation step of endodontic remedy, periradicular tissues may contact the root canal sealents via extrusion through the apical foramen. When sealers are in intimate contact with the periapical tissues for extended periods of time, their breakdown toxic products may hamper the periapical healing process by inhibiting the proliferative capability of the periradicular cell population. Therefore, apart from good physical and chemical characteristics, endodontic sealers should be biologically compatible. Several methods have been recommended for filling of root canals. Use of gutta-percha, a semi-solid root filling material, in combination with sealer is the most commonly performed method of root filling. Low dose of bio-active material is needed because the nano-particles allowed more surface area that may provide better activity, this may prevent adverse effect on the sealer flow characteristics. Incorporation of nano-

...
sized bioactive materials enhance bioactivity of already available endodontic sealer this is agreed with 13.

Particulate size has been proved to have effect on biomineralization; nanoparticles showed better performance than microparticles in induction of biomineralization 14. Paying attention to this fact, there might be still potential for enhancement of the performance of bioactive features.

there is no specific information on the osteogenic and cementogenic potential of calcium silicate-based sealers on mesenchymal progenitor cells through out the repair of periodontal ligament and relative surrounding tissue, and the molecular mechanisms underlying the osteogenesis and cementogenesis induced by various dental substances are unclear 15. This report is disagree with the present study. While

16 stated that calcium silicate-based sealers are new root canal sealers that have demonstrated apatite forming ability. This studies are agree with the present study.

Biomineralization and formation of hydroxyapatite (HA) begin with release of ions, especially calcium ions from the inorganic calcium-silicate particles and continue with formation of Si-OH groups at the material’s surface. The Si-OH groups on the surface function as ideal site for nucleation of HA. HA, i.e. Ca10(PO4)6(OH)2 precipitates first as an amorphous layer with the stoichiometric ratio Ca/P 1.67 and later crystallized into carbonated hydroxyapatite (CHA). HA precipitation is favored by an increase in the pH of the surrounding solution. The increase of the pH of the interfacial solution to around 7.9 and above around the dissolving BG has been shown. The combination of adequate physical charateristics containing silicone-based sealer and bio-active bioactive glass-ceramic (BG) particles is a prospective option to conventional root canal sealers. Silicone-based endodontic sealers also showed advantages against mineral trioxide aggregate (MTA) cements in manipulation and clinical application. This study explores in detail the potential of GB, a new endodontic sealer containing BG particles to induce biomineralization. The aim was to demonstrate apatite forming ability, the influence of the BGC on the surface structure and examine ion dissolution patterns of the endodontic sealer GB more widely that is currently available in the scientific literature. Most of the data in similar studies are now limited to concern only calcium ion release. In addition, change of pH in the surrounding media. This is agreed with 17.

BGs gradually dissolve when exposed to aqueous media. The degree of dissolution depends on the ratio of the surface area of the glass in contact with a certain volume of solution. However, the Ca/P ratio levelled off rather slow. This is agree with 18. The rather weak intensities of the HA reflections are indicative of a thin HA layer, which also results in strong interfering signals from the crystalline filler materials inside the underlying matrix 17. Biomineralization requires excess of mineral forming ions to be present in the solution and in this case, the ions originated from the dissolution of BG particles and from the SBF. In this study, the carbonated HA was measured by using FTIR analysis. Although Ca ion release is strongly related to bioactivity most of the studies in current scientific literature ignores the release of other ions which also have biological interest. Different BG compositions used as bioactive fillers in composites are known to have different dissolution behaviours. The newly developed materials showed low number of BG particles in the PDMS matrix, but despite of that, biomineralization, and more importantly increase of pH to the bacteriostatic level was seen. Therefore, it can be assumed that the sealer may have beneficial effects also in vivo conditions where ratio of surface area/volume favors the emerging local alkalinity. This is agreed with 17. FTIR has been intensively used in the study of the surface reaction of bioactive materials after immersion in simulated body fluid (SBF) solution 19 and after implantation 20. Both diffuse reflectance and specular reflectance can be used for monitoring the formation of the HCA layer at the surface of bioactive materials. The HCA layer is characterized by the P=O bending vibration peaks at 560 and 604 cm⁻¹ and the P=O asymmetric stretching vibration bands between 1000 and 1150 cm⁻¹ 21. The peaks corresponding to the bending vibration are most used to discriminate between HCA and bioactive material since the P=O stretching band is superimposed on the Si=O stretching band corresponding to bioactive glasses while the absorption band corresponding to Si-O bending is between 400 and 500 cm⁻¹ 21, at lower frequencies compared with P=O bending in HCA. The magnitude of HCA peaks at 560 and 605 cm⁻¹ increases relative to the foam Si-O peaks at 450 cm⁻¹ as the immersion time is longer. This indicates that the HCA layer is thicker for the samples soaked in simulated body fluid (SBF) solution for longer times. However, the signal-to-noise ratio of the reflection spectra is poor due to the porous surface,
600 interferograms being needed to obtain the spectra. Therefore, the easiest application of FTIR is the study of bioactive powders with a KBr diffuse reflectance device. In this case, the signal is much stronger and the preparation time is less. Carbonate peak at 875 cm$^{-1}$ developed at 0.005 g ml$^{-1}$ and increased in intensity with prolonging the time of immersion into SBF. In particular, the band around 500 cm$^{-1}$ can be related to the longitudinal optical (LO) Si–O–Si vibration, indicating a strong distortion of the SiO4 tetrahedra at high content of glass network modifiers (i.e. Ca$^{2+}$ and/or Na$^+$) 22. The next band at 600 cm$^{-1}$ can be attributed to either the P–O bending vibrations of PO4$^{3-}$ groups present in the amorphous phosphate, or pseudolattice vibrations of the PO4 tetrahedra with at least one bridging oxygen 23.

The bands in the 732–805 cm$^{-1}$ range were attributed in the literature to the Si–O–Si bending mode. The band in the 1000–1050 cm$^{-1}$ range could be related to stretching vibrations of the Si–O–P 24. A higher intensity of the bands at around 930 and 854 cm$^{-1}$ for the 45S5 glass indicates an increased degree of depolymerization of the silicate network. The FTIR spectra of the bioactive glass confirm the beginning of crystallization, while the band at around 930 cm$^{-1}$ becomes more intense and sharper, indicating the crystallization of the major Na$_2$Ca$_3$Si$_3$O$_9$ phase (combeite). Additionally, a weak band at 696 cm$^{-1}$ can be ascribed not only to Si–O–Si stretching mode, but also to P–O bending. This is agreed with 26.

**Conclusion**

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of dentistry- Dept. of Oral diagnosis, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


13. Waltimo T, et al. Antimicrobial effect of


Assessment Self-Care of Patients’ Undergoing Hemodialysis with end Stage Renal Disease

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Abstract

Objective: this study designed to assess self-care of patients’ undergoing hemodialysis with end stage renal disease and to determine out association between self-care of patients with end stage renal disease and their socio-demographic characteristics. Descriptive cross-sectional design is adopted in the recent study to perform the early stated objects. Started the study from (October 1st, 2016 until May, 6th, 2017). A Non-Probability (Accidental Sample) of (61) ESRD patients, those who visit dialysis centers of Al-Sadder Medical City in Al-Najaf Al-Ashraf., are included in the study sample. Analyzed data by descriptive analysis contains (Frequencies & Percentages) and statistical figures (Pie Charts) and Pearson’s Correlation Coefficients. The outcome of the present study that the self-care activities are interdependent and there is a positive relationship between the patients’ self-care with their age, income and marital status. While there is a non-significant difference between self-care activities and their clinical data in all items except there number of admission to emergency is a high significant difference. The study concludes that most patients with ESRD need moderate assistance in performing in dietary, exercise and other activities.

Keywords: assessment, self-care, patient, End Stage Renal Disease (ESRD).

Introduction

End-stage renal disease (ESRD) is irreversible and a progressive chronic disease reasons a main health crisis worldwide. The occurrence of ESRD is rising by 7% every year ¹. It is a chronic, life-threatening condition has been emerged as a main public health problem around the world (², ³). ESRD is a growing public health problem, given the raising prevalence international. It is estimated that by 2020 the number of patients ESRD will increase by 60% when compared with the number of patients recorded in 2005. Data from 150 countries showed that over 3 million patients were treated for ESRD worldwide by the end of 2012 ⁴. Hemodialysis is the process used to removal fluid and waste products from the body when the kidneys are notable to doing. The object is to maintain the life and well-being of the patient until the renal function is repaired. Mechanism of the dialysis working on the principles of the diffusion of solutes and ultrafiltration of fluid transit a semi-permeable membrane. There are three types of dialysis i.e. hemodialysis (primary), peritoneal dialysis, hemofiltration, as well as there are two secondary types of dialysis which are hemodiafiltration, and the intestinal dialysis ⁵. Self-care defined as an active participation in own health care, it is about learning what to do to take care of on self and when to decide to ask for help; it is an achievement, and measures persons get to enhance their health and well-being, to prevent and reduce the likelihood of disease, and to restore health after injury or illness. According to Orem (1998) self-care theory is founded on the philosophy that “all patients wish to care for themselves” if the patients are allowed to perform their own self-care requisites to the best of their ability, it will recompense more rapidly and holistically. The theoretical frame-work of Orem’s self-care theory was taken as a base for carrying out the current study aiming at assessing self-care practices of patients’ on maintenance hemodialysis at one of teaching hospital ⁶.
Methodology

Design of the Study

Descriptive cross-sectional design is adopted in the current study to achieve the early stated objectives. The study started from October 1st, 2016 until May 6th, 2017. A Non-Probability (Accidental Sample) of (61) ESRD patients, those who visit dialysis centers of Al-Sadder Medical City in Al-Najaf Al-Ashraf, are included in the study sample.

An assessment tool is adopted and developed by the researcher to assess of self-care activities for patients’ with ESRD. The final study instrument consists of three parts:

- **Part 1: Demographic Data:**
  
  This part consists of (7) items, which involve, age, gender, level of education, monthly income, residence, marital status, occupation status.

- **Part 2: Clinical Data:**
  
  This part consists of (6) items: (number of admission to emergency, previous hospitalization/day, problems begin since: years, hemodialysis begin since: years, number hours hemodialysis sessions/week, heart disease, diabetes mellitus, hypertension and respiratory disease)

- **Part 3: Self Care:**
  
  This part of the questionnaire comprised of (4) domains: (dietary, exercise, psychological and general care)

Collected the data by applying of the developed questionnaire with aid of structured interview technique with the subjects as they were individually interviewed. The study subjects are interviewed in a similar way. The interview technique spends about 20-25 minutes for each subject.

Statistical Analysis

The data were analyzed by using statistical methods to evaluate the study result:

- **Descriptive Data Analysis:** This approach includes the following measurements: A- Frequencies and Percentages. B-Mean, Mean of scores (MS) C- through using Split Half.

- **Inferential data analysis:** include Pearson’s Correlation Coefficients to determine the reliability of questionnaire (Internal consistency).

Ethical consideration:

This is one of the most essential principles before collecting the data, to keep the patient’s morals and self-esteem. The researchers achieved this agreement from the ethical committee at the Faculty of Nursing / University of Kufa. The researchers promised to keep the patient’s information confidential, and use these data for this study only then he explained the purpose of this study to each participant without affecting the routine visiting and care. In addition to above the researcher told each participant that this is an involuntary work, and they can leave any time even the interview process is not completed.

Results

Table (1): Distribution of the study sample according to their demographic data

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Rating and intervals</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/ years</td>
<td>&lt;= 29</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>30 - 39</td>
<td>13</td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td>40 - 49</td>
<td>10</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>50 - 59</td>
<td>8</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>60 +</td>
<td>16</td>
<td>26.2</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>39</td>
<td>63.9</td>
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<tr>
<td></td>
<td>Female</td>
<td>22</td>
<td>36.1</td>
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</table>
### Levels of education

<table>
<thead>
<tr>
<th>Education Level</th>
<th>Frequency</th>
<th>Percent</th>
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<tr>
<td>Unable to read and write</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Able to read and write</td>
<td>15</td>
<td>24.6</td>
</tr>
<tr>
<td>Primary school grad.</td>
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<td>23</td>
</tr>
<tr>
<td>Intermediate school grad.</td>
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<td>13.1</td>
</tr>
<tr>
<td>Secondary school grad.</td>
<td>5</td>
<td>8.2</td>
</tr>
<tr>
<td>Institute graduated</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>College graduated</td>
<td>3</td>
<td>4.9</td>
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</table>

### Income

<table>
<thead>
<tr>
<th>Income Type</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
<td>Sufficient</td>
<td>22</td>
<td>36.1</td>
</tr>
<tr>
<td>Sufficient To What Limit</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Insufficient</td>
<td>28</td>
<td>45.9</td>
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</table>

### Residency

<table>
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<tr>
<th>Residency Type</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
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<td>Rural</td>
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<td>41</td>
</tr>
<tr>
<td>Urban</td>
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<td>59</td>
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</table>

### Marital status

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<thead>
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<th>Marital Status</th>
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<th>Percent</th>
</tr>
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<tr>
<td>Single</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Married</td>
<td>49</td>
<td>80.3</td>
</tr>
<tr>
<td>Widowed</td>
<td>1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

### Occupation

<table>
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<th>Occupation Type</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
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<td>4</td>
<td>6.6</td>
</tr>
<tr>
<td>Housewife</td>
<td>18</td>
<td>29.5</td>
</tr>
<tr>
<td>Employee</td>
<td>10</td>
<td>16.4</td>
</tr>
<tr>
<td>Jobless</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Free job</td>
<td>15</td>
<td>24.6</td>
</tr>
</tbody>
</table>

Table (1) This table shows that (26.2%) of the study subjects age within (29 years old and more), the study results revealed that the majority (63.9%) are males, In addition, the study results present that (24.6%) of the sample are able to read and write, while (23%) are primary school graduated. additionally, the study results reveals that (45.9%) of the sample are present insufficient monthly income. Also (59%) are living in urban residential area. Concerning the subjects marital status, (80.3%) of the study sample are married. In regards to occupational status (29.5%) are housewives.

Table (2): Statistical distribution of the study sample according to their clinical data

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Rating and intervals</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
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<tr>
<td>Number of admission to emergency</td>
<td>&lt;= 0</td>
<td>15</td>
<td>24.6</td>
</tr>
<tr>
<td></td>
<td>1 - 5</td>
<td>34</td>
<td>55.7</td>
</tr>
<tr>
<td></td>
<td>6+</td>
<td>12</td>
<td>19.7</td>
</tr>
<tr>
<td>Previous hospitalization/ day</td>
<td>&lt;= 0</td>
<td>23</td>
<td>37.7</td>
</tr>
<tr>
<td></td>
<td>1 - 5</td>
<td>26</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>6+</td>
<td>12</td>
<td>19.7</td>
</tr>
<tr>
<td>Problems begin since: years</td>
<td>&lt;= 5</td>
<td>49</td>
<td>80.3</td>
</tr>
<tr>
<td></td>
<td>6- 10</td>
<td>7</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>11+</td>
<td>5</td>
<td>8.2</td>
</tr>
<tr>
<td>Hemodialysis begin since: years</td>
<td>&lt;= 3</td>
<td>56</td>
<td>93.3</td>
</tr>
<tr>
<td></td>
<td>4- 6</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>7+</td>
<td>2</td>
<td>3.3</td>
</tr>
</tbody>
</table>
This table shows that the number of admission to emergency, the highest percentage (55.7%), the study results show that (42.6%) of the study sample are Previous hospitalization, the study results show that (80.3%) of the study sample are Problems begin showing <= 5years, (93.3%) showing <= 3.00 years, Hemodialysis begin, the study results present that (91.8%) of the sample are hemodialysis sessions above 5 years, (85.2%) are no suffering from heart disease, (62.3%) are no suffering from diabetes mellitus, (70.5%) are the suffering from hypertension, (86.9%) are no suffering from respiratory disease.

**Table (3): Assessment self-care of patients’ end stage renal disease**

<table>
<thead>
<tr>
<th>Self-care domains</th>
<th>Levels</th>
<th>Frequency</th>
<th>Percent</th>
<th>M.S.</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary</td>
<td>Independent</td>
<td>28</td>
<td>45.9</td>
<td>2.2</td>
<td>Interdependent</td>
</tr>
<tr>
<td></td>
<td>Interdependent</td>
<td>31</td>
<td>50.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dependent</td>
<td>2</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>Independent</td>
<td>9</td>
<td>14.8</td>
<td>1.7</td>
<td>Interdependent</td>
</tr>
<tr>
<td></td>
<td>Interdependent</td>
<td>30</td>
<td>49.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dependent</td>
<td>22</td>
<td>36.1</td>
<td></td>
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</tr>
<tr>
<td>Psychological</td>
<td>Independent</td>
<td>28</td>
<td>45.9</td>
<td>2.3</td>
<td>Interdependent</td>
</tr>
<tr>
<td></td>
<td>Interdependent</td>
<td>32</td>
<td>52.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dependent</td>
<td>1</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General Care</td>
<td>Independent</td>
<td>41</td>
<td>67.2</td>
<td>2.5</td>
<td>Independent</td>
</tr>
<tr>
<td></td>
<td>Interdependent</td>
<td>20</td>
<td>32.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table shows that the patients’ responses are interdependent at dietary, exercise and psychological domain. Independent at the general care domains.
Table (4): Overall Assessment self-care of patients’ end stage renal disease.

<table>
<thead>
<tr>
<th>Levels</th>
<th>Frequency</th>
<th>Percent</th>
<th>M.S.</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent</td>
<td>24</td>
<td>39.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interdependent</td>
<td>36</td>
<td>59</td>
<td>2.2</td>
<td>Interdependent</td>
</tr>
<tr>
<td>Dependent</td>
<td>1</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
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</table>

Table (4) This table shows that overall patients’ self-care are Interdependent

![Self-care](image)

Figure (1) Overall Assessment for Patients’ Self care

Table (5): Relationship between the patients’ self-care and their demographic and clinical data

<table>
<thead>
<tr>
<th>Demographic and clinical data</th>
<th>Pearson Correlation</th>
<th>Sig. (2-tailed) P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age / years</td>
<td>-.343</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HS</td>
</tr>
<tr>
<td>Gender</td>
<td>0.068</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Levels of education</td>
<td>-.064</td>
<td>0.625</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Income</td>
<td>-.271</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Residency</td>
<td>-.114</td>
<td>0.382</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Marital status</td>
<td>-.275</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Occupation</td>
<td>-.121</td>
<td>0.354</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>
Cont.. Table (5): Relationship between the patients’ self-care and their demographic and clinical data

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of admission to emergency</td>
<td>.306</td>
<td>0.017 HS</td>
</tr>
<tr>
<td>Previous hospitalization/ day</td>
<td>-.059</td>
<td>0.654 NS</td>
</tr>
<tr>
<td>Problems begin since: years</td>
<td>-.071</td>
<td>0.584 NS</td>
</tr>
<tr>
<td>Hemodialysis begin since: years</td>
<td>0.033</td>
<td>0.805 NS</td>
</tr>
<tr>
<td>Number hours hemodialysis sessions/ week</td>
<td>0.056</td>
<td>0.667 NS</td>
</tr>
<tr>
<td>Heart disease</td>
<td>0.17</td>
<td>0.191 NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.186</td>
<td>0.151 NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.15</td>
<td>0.25 NS</td>
</tr>
<tr>
<td>Respiratory dis.</td>
<td>0.203</td>
<td>0.116 NS</td>
</tr>
</tbody>
</table>

NS: Non-Sig. at P>0.05, S: Sig. at P<0.05, HS: high significant at p-value less than 0.01.

The table reveals that there is a highly significant association among the patients (self-care activities) and their (age and emergency), at p-value < 0.01 and significant in relation with (income and marital status) at p-value <0.05. While there is a non-significant association with remaining demographic and clinical data.

This study is the effort to investigate Personal care for dialysis Patients in Najaf Governorate Iraq in general. That the majority of the study subjects (26.2%) are within the age group (more than 60 years). This finding comes along with 7. Whose results indicated that the thirty five percentage were age group of 60 and over years. Concerning to the study subjects gender, the results indicate, that the higher percent of the study sample are males. This result comes along with 8, 9. Concerning educational levels, the higher percentage (24.6%) are able to read and write. This result is in agreement with other studies Al-Garni, (2006) and Anees, et al. (2011) in their studies found that the majority of the study subjects are able to read and write 10, 11. Most of the sample results indicate that (45.9%) of the study sample are insufficient monthly income. This come is consistent with another study of Mahmoud and AbdElaziz, (2015) and Halle, et al. (2015) reveals in their studies that most of the study sample hasn’t enough monthly income 12, 13. Regarding residency, the current study results show that most of the sample (59%) is live who at urban area. This result in agreement with Crews, et al. (2014) they indicated that the majority (98.4%) patients of ESRD is living in urban area. Concerning to marital status, majority of subjects (80.3%) are married 14. Table (2) shows that the majority of the study subjects (55.7%) are admitted to the emergency department for 1-5 days annually. While the number of hospitalization days, the study results indicates that the majority of the subjects (42.6%) are hospitalized for 1-5 days annually 19. In addition to that and in regarding to the period when the renal problems appear, the results show that the (80.3) are equal or less than (5 years). While for the hemodialysis duration, about (91.8%) from the study subjects were meeting dialysis for more than 5 hrs weekly. This finding agrees with the result 20. In addition to the problem, the higher percentage (85.2%) of study results had no heart disease then diabetes mellitus (62.3%) which represent the highest percentage of other included problem followed by hypertension (70.5%) and (86.9%) are no suffering respiratory disease. This result is supported by 21, 22. The study findings indicate that the overall assessments for patients’ self-care activities
are interdependent. It shows that the sample responses to the following self-care activities are interdependent which mean they need some assistance in performing these activities, while the sample self-care activities were independent in general care domain which means they do not need any assistance. These results agree with 6. According to the study results indicate that the high-significant relationship between the patients self-care activities and their (age and emergency), and significant in relation with (income and marital status) while there is a non-significant relationship with other demographic and clinical data. Present study is in agreement with (10, 23).

Conclusions

All the patients with ESRD are completely independent in General Care themselves in their daily self-care activities and Most patients with ESRD need moderate assistance in performing in dietary, exercise and psychological in their daily self-care activities.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Nursing, University of Kufa and all experiments were carried out in accordance with approved guidelines.

References

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Hypertension in Relation with Smoking in Azadi Teaching Hospital at Kirkuk City

Saad S. Mustafa¹, Taghlub H. Ryhan², Ali A. Ismail³


Abstract

Background: smoking and hypertension are two common health problems effecting body organs and increasing morbidity and mortality.

Aim of Study: determining the relationship between smoking and hypertension, the affect of the amount and duration of smoking on the severity of hypertension and complications of smoking among hypertensive patient.

Patient and Method: the study is cross sectional, in which 200 hypertensive patient were collected in Azadi Teaching Hospital in Kirkuk. There blood pressure was measured and the patients were asked about smoking habits, and if they have complication of hypertension.

Results: hypertension is more common among male, married, illiterate and primary educated, intermediate socioeconomic state, physically inactive and more in urban than rural area. Uncontrolled rate of hypertension is high in both smokers and non smokers and this rate increase with increase with increasing duration of smoking. Heart and cerebrovascular complications of hypertension is very high in smokers compared with non smokers. P-value less than 0.05 regard as significant relationship.

Conclusion: very low control rate of hypertension in both smokers and non smokers. We recommended public health education via mass media regarding the side effect of smoking combined with hypertension and it’s complications.

Keywords: Smoking, Hypertension, Blood pressure, Tobacco

Introduction

Elevated blood pressure and tobacco smoking are, respectively, the first and second leading causes of preventable mortality worldwide(1). Hence, from both a population perspective and a patient perspective, the joint occurrence of tobacco smoking and elevated blood pressure has enormous health consequences. Worldwide, the absolute number of tobacco smokers has increased because of population growth, despite a decrease in the age-adjusted prevalence of smoking(2). In the United States, the estimated number of tobacco smokers has dropped, as a result of tobacco-control efforts, from 45.1 million in 2005 to 36.5 million smokers in 2017 (3) . However, while use of tobacco products has decreased, use of electronic cigarettes (e-cigarettes) has increased substantially in the United States, along with concerns about their potential health risks(4). Tobacco smoking and e-cigarette use may raise blood pressure and accelerate atherothrombotic processes though a variety of potential mechanisms, including deleterious effects on endothelial function, inflammation, lipids, and thrombosis(5) . The effects of smoking on blood pressure and hypertension are discussed in this topic. Hypertension control and smoking cessation to prevent cardiovascular disease and the approach to smoking cessation are discussed elsewhere.

The effects of tobacco smoking on blood pressure are complex, with evidence that tobacco smoking increases blood pressure acutely and increases the risk of renovascular, malignant, and masked hypertension. Passive (or secondhand) smoke exposure also appears to raise blood pressure(6).
The aims of the study:

Clarify the relationship between smoking and Hypertension.

Identify the relationship between the amount and duration of smoking and the severity (Stage) of hypertension in comparing with non-smokers.

Identify the cardiovascular complications of smoking in hypertension patients.

Patients and methods:

The design of the study: The design of the study was cross-sectional study. The place of study was the center of Azadi Teaching Hospital in Kirkuk city.

The duration of the study: The duration of the study was extended from March 2018 till September 2018.

Sample/population: Patients were visiting Azadi Teaching for diagnosis, treatment and follow-up purposes. The sample size was 200, all patients were hypertensive with two criteria of diagnosis: First one any patient with blood pressure more than 140/90 mm Hg in two different occasions (each 10 minutes apart); The second criterion was any patient on antihypertensive drug even with normal blood pressure. Information was also taken from patients about smoking habits, to determine the relationship between smoking and severity (stage) of hypertension and the effect of smoking on hypertension complication like cardiovascular and cerebrovascular complications.

The systolic and diastolic blood pressure was measured by a random zero mercury type sphygmomanometer on both arms (each five minutes apart). The mean value of the two readings was calculated. The blood pressure measuring was after asking patients to rest for 30 minutes, and if the patient was a smoker, to stop smoking for 30 minutes before measuring blood pressure.

The questionnaire: The questionnaire was designed to contain the following major questions that meet with the aims of the study:

Sociodemographic questions: -

Sociodemographic questions, which contains the name of the patients, age, sex, marital status, physical activity, residence, educational level, and economic state.

Questions about history of medical diseases:- Questions about history of medical diseases to determine the secondary causes of hypertension, and to exclude the other risk factors for the major complications of hypertension. These questions include asking about diabetes mellitus, ischemic heart disease, drug history, bronchial asthma, cerebrovascular accidents, chronic obstructive pulmonary disease, and surgical history.

Questions about the complications of hypertension:- Questions about the complications of hypertension including ischemic heart disease, left ventricular hypertrophy, atrial fibrillation protein urea, renal failure, stroke, and retinopathy.

Questions about smoking: - Questions about smoking, including the number of smoking per day and the duration of smoking.

The questionnaire also contained the measuring of vital signs: - The questionnaire also contained the measuring of vital signs including systolic and diastolic blood pressure, heart rate, and respiratory rate.

Statistical methods: - The sample was chosen by simple random sampling, the data was analyzed with the using statistical package for social sciences (SPSS Ver.23) program, standard methods were used to obtain summary statistics such as means, prevalence and other measures.

Ethical consideration: - All the studied individuals were asked whether they were interested in the study or not. All agreed voluntarily to participate.

Results

In this study we explored 200 hypertensive cases, the mean age was 55, the range of the age was from 32 – 88, the high portion of age from 43 – 64 year with a percentage of 64% male more than female, married more than single, the urban area more than rural, not educated more than educated as it is illustrated in (Table 1), with high proportion of intermediate economical state and low physical activity.

Smoking and hypertension: From total 200 cases, 143 was non-smoker in a percentage of 71.5%, and 57 was smokers in a percentage of 28.5%. The relationship between smokers habit and different stages of hypertension is showed in (Table 2).
The effect of duration of smoking on stages of hypertension: In this relationship we notice that the uncontrolled systolic BP group are in the duration of 10 – 19 years, while the larger number of controlled diastolic BP group in the duration of less than 10 years, and this adverse relationship between duration and hypertension can be clarify by the effect of another factors like physical inactivity or inappropriate pharmacological treatment as showed in (Table 3).

The effect of the amount of smoking on stages of hypertension: The uncontrolled hypertensive cases increase with the amount of smoking with larger number in those who smoke more than 20 cigarette per day as in (Table 4).

The effect of smoking on heart and cerebrovascular complications in hypertensive patients in relationship with non-smokers: In both (Table 5) and (Table 6), it is very clear that both heart and cerebrovascular complications increase in smoker patients. The percentage of heart complications in smokers to non-smokers are 47% to 0.6%, also the same percentage regarding the cerebrovascular complications.

### Table 1. Socio-demographic characteristics.

<table>
<thead>
<tr>
<th>%</th>
<th>N</th>
<th>Variables</th>
</tr>
</thead>
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<td>32 – 42</td>
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<td>33.5</td>
<td>67</td>
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<td>61</td>
<td>54 – 64</td>
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<tr>
<td>15.5</td>
<td>31</td>
<td>65 – 75</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>76 – 88</td>
</tr>
<tr>
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<td></td>
<td></td>
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<tr>
<td>52.5</td>
<td>105</td>
<td>Male</td>
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<td>47.5</td>
<td>95</td>
<td>Female</td>
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<td>Marital status</td>
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<td></td>
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<td>24.5</td>
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<td>Single</td>
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<td>75.5</td>
<td>151</td>
<td>Married</td>
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<td>Residence</td>
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<td>85.5</td>
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<td>Urban</td>
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<td>14.5</td>
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<td>Rural</td>
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<td>66</td>
<td>132</td>
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<td>Economical state</td>
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<td>28</td>
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<td>Low</td>
</tr>
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<td>63</td>
<td>126</td>
<td>Intermediate</td>
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<td>9</td>
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<td>High</td>
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<tr>
<td>63</td>
<td>126</td>
<td>Inactive</td>
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<td>32.5</td>
<td>65</td>
<td>Mild activity</td>
</tr>
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<td>4.5</td>
<td>9</td>
<td>Regular activity</td>
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</table>
Table 2. Smoking and hypertension

<table>
<thead>
<tr>
<th>Systolic BP (mm Hg)</th>
<th>Total N (%)</th>
<th>&gt; 179 N (% )</th>
<th>160 – 179 N (% )</th>
<th>140 – 159 N (% )</th>
<th>120 – 139 N (% )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>143</td>
<td>92 (64)</td>
<td>41 (29)</td>
<td>10 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>26 (45.7)</td>
<td>11 (19.2)</td>
<td>5 (8.7)</td>
<td>15 (26.3)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 109</td>
<td>100 - 109</td>
<td>90 - 99</td>
</tr>
<tr>
<td></td>
<td>143</td>
<td>19 (13.2)</td>
<td>84 (58.7)</td>
<td>40 (28)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>7 (12.2)</td>
<td>22 (38.5)</td>
<td>13 (22.8)</td>
<td>15 (26.3)</td>
</tr>
</tbody>
</table>

P - Value = 0.001

Table 3. Duration of smoking and hypertension.

<table>
<thead>
<tr>
<th>Systolic BP (mm Hg)</th>
<th>Total N (%)</th>
<th>&gt; 179 N (% )</th>
<th>160 – 179 N (% )</th>
<th>140 – 159 N (% )</th>
<th>120 – 139 N (% )</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 (26.3)</td>
<td>2 (13.3)</td>
<td>2 (13.3)</td>
<td>1 (6.6)</td>
<td>10 (66.6)</td>
<td>&lt; 10 years</td>
</tr>
<tr>
<td></td>
<td>33 (58)</td>
<td>18 (54.5)</td>
<td>8 (24)</td>
<td>3 (9)</td>
<td>4 (12)</td>
<td>10 – 19 years</td>
</tr>
<tr>
<td></td>
<td>9 (15.7%)</td>
<td>6 (67)</td>
<td>1 (11)</td>
<td>1 (11)</td>
<td>1 (11)</td>
<td>≤ 20 years</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diastolic BP (mm Hg)</th>
<th>Total N (%)</th>
<th>&gt; 109 N (% )</th>
<th>100 – 109 N (% )</th>
<th>90 – 99 N (% )</th>
<th>80 – 89 N (% )</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 (26.3)</td>
<td>3 (20)</td>
<td>4 (26.6)</td>
<td>0 (0)</td>
<td>8 (53.4)</td>
<td>&lt; 10 years</td>
</tr>
<tr>
<td></td>
<td>33 (58)</td>
<td>12 (37)</td>
<td>10 (30)</td>
<td>5 (15)</td>
<td>6 (18)</td>
<td>10 – 19 years</td>
</tr>
<tr>
<td></td>
<td>9 (15.7%)</td>
<td>1 (11)</td>
<td>7 (78)</td>
<td>0 (0)</td>
<td>1 (11)</td>
<td>≤ 20 years</td>
</tr>
</tbody>
</table>
Table 4. Number of smoking and hypertension.

<table>
<thead>
<tr>
<th>Systolic BP (mm Hg)</th>
<th>Number of cigarettes / day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N (%)</td>
<td>&gt; 179 N (%)</td>
</tr>
<tr>
<td>7 (12.2)</td>
<td>1 (14.2)</td>
</tr>
<tr>
<td>15 (26.3)</td>
<td>7 (46.6)</td>
</tr>
<tr>
<td>35 (61.5)</td>
<td>18 (51.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diastolic BP (mm Hg)</th>
<th>Number of cigarettes / day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N (%)</td>
<td>&gt; 109 N (%)</td>
</tr>
<tr>
<td>7 (12.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>15 (26.3)</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>35 (61.5)</td>
<td>7 (20)</td>
</tr>
</tbody>
</table>

P - Value = 0.001

Table 5. Smoking and heart complications

<table>
<thead>
<tr>
<th>% of Complications</th>
<th>Total N (%)</th>
<th>Heart complications</th>
<th>No complications</th>
<th>Smoker’s habit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MI N (%)</td>
<td>Angina N (%)</td>
<td>134 (94)</td>
</tr>
<tr>
<td>0.6%</td>
<td>143 (71.5)</td>
<td>3 (2)</td>
<td>6 (4)</td>
<td></td>
</tr>
<tr>
<td>47%</td>
<td>57 (28.5)</td>
<td>3 (5.2)</td>
<td>24 (42)</td>
<td>30 (52.8)</td>
</tr>
</tbody>
</table>

P – Value = 0.001

Table 6. Smoking and cerebrovascular complications.

<table>
<thead>
<tr>
<th>% of complications</th>
<th>Total N (%)</th>
<th>Cerebrovascular complications</th>
<th>No complications</th>
<th>Smoking habit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hypertensive encephalopathy N (%)</td>
<td>Infarction N (%)</td>
<td>TIA N (%)</td>
</tr>
<tr>
<td>0.6%</td>
<td>143 (71.5)</td>
<td>3 (2)</td>
<td>4 (3)</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>47%</td>
<td>57 (28.5)</td>
<td>2 (3.7)</td>
<td>5 (8.7)</td>
<td>20 (35)</td>
</tr>
</tbody>
</table>

P – Value = 0.001
Discussion

This study clarified several findings in the relationship between smoking and hypertension, the effect of smoking on the complications of hypertension, and the relationship of sociodemographic characteristic and hypertension. All these facts are important in controlling of hypertension and reducing the complications of hypertension in relationship with smoking.

In the study the main age group of patients are between 43 – 64 years with a percentage of 64%, the mean age is 55, the proportion of male is higher than female with a percentage of 53%, these results are similar to those in USA(7) that hypertension prevalence increase with age and more in male due to reduced vascular compliance that the prevalence of hypertension increases greatly with age, changing from 15.2% of 18 – 24 years – olds to 60.2% of 65 to 74 years.

The study showed that all 143 non-smoker were poorly controlled hypertension, i.e., all of them with blood pressure more than 140 / 90 mmHg, while within 57 smoker patients there are 15 cases with control blood pressure. This poor controlled blood pressure among non-smokers may related to other factors like physical inactivity, obesity, stress, inappropriate treatment.

The amount and duration of smoking affect adversely on the controlling of blood pressure, the study clarified that the uncontrolled rate for those patients smokes more than 20 cigarette / day is 76% for systolic and 91% for diastolic blood pressure, and similar uncontrolled rate for those smoking for more than 20 years with a percentage of about 88% for both systolic and diastolic blood pressure. This result is similar to that study in Vietnam(8) that smoking rises blood pressure acutely and the level return to baseline in about 15 minutes. And if a person smokes more than 30 cigarettes / day this can elevate the blood pressure continuously(8).

The study clarified that the cardiac complications of hypertension (including angina and myocardial infarction), and cerebrovascular complications (mainly transient ischemic attack and cerebral infarction) are very high in smokers with a percentage of 47% in comparing with very low percentage of 0.6% in non-smokers.

Conclusions

The major conclusions of the study are: Poor blood pressure control present in the majority of the patients with a percentage of 93% the uncontrolling blood pressure was mainly in males, married, illiterate and primary educated, low physical activity persons, intermediate socioeconomic state and more in urban than rural areas. High blood pressure was in both smokers and non-smokers. The heart and cerebrovascular complications of the hypertension increase in smokers compared with non-smokers.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kirkuk Health Directory, Iraq and all experiments were carried out in accordance with approved guidelines.

References

Determination of Some Volatile Organic Compounds in the water produced at Al-Ahdab oilfield in the Governorate of Wasit, Iraq using Headspace SPE-GC-FID

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¹AL-Mustansiriya University, College of Science, Chemistry department. ²The University of Baghdad, College of Education for Pure Sciences/ Ibn Al-Haitham (Correspondant author:

Abstract

The study was conducted over the period of Oct 2018 to Apr 2019 and is aimed for the detection and estimation of four hazardous Volatile Organic Compounds VOC (benzene, toluene, ethylbenzene, and xylene) so-called (BTEX) in samples collected from the produced water in the Al-Ahdab oil field in Iraq also to track their availability in the important natural water sources around the field. These compounds pose a risk to human health as well as environment. To avoid the laborious and tiresome conventional extraction methods, water samples were collected and concentrated using solid-phase extraction technique (SPE) which is a robust and cost-effective method of sample extraction with minimal exposure and handling of solvents and then to be analyzed via a gas chromatograph with a flame ionization detector (GC-FID). All of the collected samples were found contaminated with the BTEX compounds by unacceptable limits exceeding the recommended percentages. BTEX compounds were also found in the aquatic samples outside the field. Standard deviation (SD) was ranging from 0.01-0.73. The purpose of this study was to track and monitor the BTEX concentrations in the water produced by the Al-Ahdab oilfield and compare it with the BTEX levels in the water sources near the oilfield. Which is very important to control the impact of the discharged waters on the environment.

Keywords: BTEX, Al-Ahdab oil field, Al-Dalmaj Marsh, General Downstream Canal (GDC), Main Outfall Drain (MOD)

Introduction

Most oil fields often yield petroleum from underground reservoirs accompanied by what is called produced water, which is brine or water that necessitates disposal. Treatment of this water is of great importance to make it safe to be discharged to the environment (1,2). The produced water³(PW) usually contains different hazardous materials especially high concentrations of dissolved BTEX organic compounds, i.e. (benzene, toluene, ethylbenzene, and xylene)⁴. BTEX compounds are harmful to plants and toxic for other livings. Direct contact with benzene including inhalation may affect the central nervous system of the human body. However, prolonged exposure to it consequentialy leads to alteration in hemopoietin tissue appears as anemia and leukopenia⁵-⁷. Furthermore, high lipophilicity of BTEX made it possible to leak to the brain and causing brain damage, cardiac depression, dizziness, and nausea. While moderate exposure affects the function of kidney and liver⁵-⁷. When benzene and other BTEX compounds discharged to the environment they can leak to groundwater toxify it with concentrations much higher than those usually detected in surface water due to evaporation suppression⁶. There are different analytical procedures to measure the availability and concentration of BTEX in water⁸-¹⁰ and in air¹¹-¹³. However, a pretreatment is often necessary for water specimen to separate the components of interest from sample matrices. Solid Phase Extraction (SPE) become a common technique for the analysis of water samples, as it can retain volatile and semi-volatile compounds from the aqueous medium. It is environmentally benign as it requires minimal volumes of solvents and doesn’t require any special or complicated equipment¹⁴. The General Downstream Canal¹⁵ (GDC) [Appendix-2] or the Main Outfall Drain¹⁶ (MOD), known before the year of 2003 as Saddam River or the Third River. With more than a 565 Km long¹⁷, it is upstreaming from Baghdad.
north and downstream into the Shatt Al-Arab in Basrah. It was an old project since the 1950s then re-implemented into the 1990s. It is the main source of water for the marshes in southern Iraq\(^1\). The Al-Ahdab oilfield is located in the Al-Kut governorate and takes the required quantities of water from the MOD close to wetlands of Al-Dalmaj marsh\(^2\). As well as the Al-Ahdab oilfield discharges the produced water to the MOD. Any contaminations discharged to the MOD will reach and toxifying the natural environment and the aquatic life of the wetlands. Thus, it is very vital to regularly examine the water quality released to MOD\(^3,4\). The produced water samples were collected directly pipelines and reservoirs from the Al-Ahdab oil field then extracted using SPE cartridges for later analysis with GC-FID for the determination of BTEX toxic compounds those may go with the drainage to reach the water resources affecting the environment. The presence of such harmful compounds in both water produced from the oil fields and water sources close to the fields must be examined and supervised regularly to control it and to prevent any bad consequences on humans and biodiversity around the area.

**Experimental**

**Chemicals and materials:**

- Gas chromatograph, Shimadzu GC-2014 (Kyoto, Japan) equipped with a flame ionization detector FID.
- Certified standards of BTEX (benzene, toluene, ethylbenzene, and p-xylene) were purchased from ChemService at a concentration of 2000 μg/mL in methanol (West Chest, PA, USA).
- Intermediate solutions were prepared by diluting the standard mixture at concentrations of 1 and 20μg/ml in methanol, Hydrochloric Acid (HCl) 6N(BDH).
- Solid Phase Extraction Tubes (SPEC-R31930B), SiliaPrep SPE Cartridges CleanENVI (Silicycle inc, Canada).

**Locations of the collection of PW samples:**

PW water samples were collected in 1-liter bottles from three sites within the Al-Ahdab oilfield and five sites on the Main Drain river outside the field in Wasit province, Iraq. The sample bottles were sealed with Teflon then transported on the same day to the laboratory under refrigerated conditions.

In the laboratory, samples were preserved at a temperature of 4° C, and then were filtered through a 0.45μm Millipore filter and diluted for the analysis of BTEX. Figure (1) showing the location of the Ahdab oilfields in Iraq. The Al-Ahdab oilfields campus dimensions are about 10 Km long by 3 Km. The oilfield area is located among three Secondary Surface Drain canals (SSD) and a Main Surface Drain canal (MSD) those dispense directly into the MOD which flows toward the southern marshes. While table (1) specifies the spatial coordinates of the sample collection sites.

![Figure 1. The location of the Al-Ahdab oil fields mediated among SSD(s) and MSD those discharge directly into the MOD\(^2\).](image-url)
Table 1: Spatial coordinates of the produced water samples classified as per their stage of process and site of collection

<table>
<thead>
<tr>
<th>Sample Group</th>
<th>Description</th>
<th>PW treatment stage</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water produced within the Al-Ahdab oil field</td>
<td>PW before treatment</td>
<td>32°49′ 14.65 N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PW after treatment</td>
<td>32°49′ 22.46 N</td>
</tr>
<tr>
<td>2</td>
<td>PW surplus in evaporation ponds</td>
<td></td>
<td>32°48′ 82.77 N</td>
</tr>
<tr>
<td>3</td>
<td>Just before Al-Ahdab Injection water supply line from MOD</td>
<td></td>
<td>32°44′ 96.28 N</td>
</tr>
<tr>
<td>4</td>
<td>Water collected outside the oil field from MOD</td>
<td>Just before Al-Ahdab Injection water supply line from MOD</td>
<td>32°40′ 41.36 N</td>
</tr>
<tr>
<td>5</td>
<td>After Al-Ahdab Injection water supply line from MOD</td>
<td>After Al-Ahdab Injection water supply line from MOD</td>
<td>32°39′ 94.95 N</td>
</tr>
<tr>
<td>6</td>
<td>From Al-Ahdab Injection water supply ponds</td>
<td>After Al-Ahdab Injection water supply ponds</td>
<td>32°38′ 90.78 N</td>
</tr>
</tbody>
</table>

Sample injection parameters GC/FID conditions:

A cold trap (cry focus) cooled with a flow of liquid nitrogen (LN2), mounted prior to the separating column RTX-624,20m,0.18mm,1μm for the purpose of re-concentration of the VOCs.

The aqueous samples were extracted and concentrated (5ml) then placed into 20ml headspace vials. The injected volume was set to 1 ml (the split ratio was 5:1 and the linear velocity was set to 45 cm/sec. The GC oven temperature started from 50 °C for 1 min, gradient temperature, ramping 35 °C/min up to 280 °C. The water samples were purged with helium (99.995% purity) at a flow rate of 40 ml/min for 11 min at 26 °C. The volatiles was thermally desorbed and transferred to the GC which attached to a Flame Ionization Detector (FID) via a heated transfer line. After desorption, the trap was cleaned by heating it up to 200 °C for 8 min.

The analysis was adapted according to the EPA method 624 for the measurement of VOCs in water. The usage of an (Elite 624 capillary column 30m x 0.25mm, 1.40μm) as a separating phase with such narrow bore column enables the reduction of the analysis time (fast GC) nonetheless maintaining the chromatographic resolution successfully.

The vaporized samples were injected from the headspace to the column quite slowly as per the normally small split ratio (5:1) implemented in favor of sensitivity. However, the application of fast GC approach in combination with the slow headspace sampling has compensated the lateness in analysis duration.

Results and Discussion

One liter of each PW aqueous sample was extracted and retained on SPE cartridges then retrieved by desorption from the sorbent bed of the SPE cartridge by 1 ml of dichloromethane. Which increased the limit of detection and sensitivity of the method by concentrating the samples 1000 times. The resultant extract has been placed in a 10 ml headspace vial (5 replicate vials for each sample). Direct injection in headspace HS mode was performed at the temperature of 280 °C in programmed temperature vaporization (PTV) injector for a GC-FID.

The BETX levels in water samples were expressed in the unit of μg/ml. The limit of detection (LOD) for benzene has turned out to be 0.001 µg/ml. Figure (2) exhibited a GC-FID chromatogram of a standard mixture of BTEX at the concentration of (1 μg/ml) of each component. The total chromatographic runtime was less than 8 minutes. While table (2) listed common chromatographic values for the analysis of (1 μg/ml) standard BTEX samples.
Figure 2. An HS–GC–FID chromatogram for the standard solution of BTEX at the concentration of (1 μg/ml) for each component.

Table 2. Tabulation of the chromatographic values worked out for the analysis of standard BTEX samples in the concentration of (1 μg/ml) for each component where; retention time in minutes, (K') Capacity factor, (α) Separation factor, (Rs) Resolution, and (N) the Number of Theoretical Plates.

<table>
<thead>
<tr>
<th>Concentration (1 μg/ml)</th>
<th>Peak area</th>
<th>K'</th>
<th>α</th>
<th>Rs</th>
<th>N</th>
<th>Recovery</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>3.26</td>
<td>109773</td>
<td>9.867</td>
<td>..</td>
<td>..</td>
<td>3214.397</td>
<td>97.3</td>
</tr>
<tr>
<td>Toluene</td>
<td>3.94</td>
<td>116824</td>
<td>12.13</td>
<td>1.21</td>
<td>3.24</td>
<td>2425.563</td>
<td>99.2</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>5.95</td>
<td>146627</td>
<td>18.83</td>
<td>1.51</td>
<td>3.65</td>
<td>3211.111</td>
<td>98.8</td>
</tr>
<tr>
<td>Xylene</td>
<td>6.96</td>
<td>156049</td>
<td>22.2</td>
<td>1.17</td>
<td>3.06</td>
<td>4610.741</td>
<td>100.1</td>
</tr>
</tbody>
</table>

The collected samples from inside the field were classified into three main groups; The first group (Group 1) represents the PW aliquot before treatment, (Group 2) represents the PW from the stage after treatment, and (Group 3) representing the PW collected from the evaporation ponds as shown in Table (3).

Table 3. The average concentrations of BTEX found in aquatic samples collected from the site of the Al-Ahdab oil field. Analyses were taken place using HS-SPME-GC-FID

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample concentration in ( μg/ml )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td></td>
<td>Min    Max  Mean  SD   Min  Max  Mean  SD   Min  Max  Mean  SD</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.88 - 1.63 1.26 0.26</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.23 - 2.31 1.29 0.73</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>0.14 - 1.52 0.83 0.40</td>
</tr>
<tr>
<td>Xylene</td>
<td>1.39 - 1.85 1.62 0.16</td>
</tr>
</tbody>
</table>
While those samples collected from outside the oilfield were represented into five main sets (Group 4 to 8); directly from MOD before Injection water supply line, from Al-Ahbad Injection water supply line from MOD, from less than a kilometer next to Al-Ahbad Injection water supply line, from Al-Ahbad Injection water supply ponds, and after it respectively as listed in Table (4) below. The data listed in the table concluded, there was no contamination with BTEX for the water samples taken from Al-Ahbad Injection water supply line from MOD (group 5) and directly from MOD before Injection water supply line (group 4). While the water samples collected from less than a kilometer next to Al-Ahbad Injection water supply line (group 6) as well as those collected from Al-Ahbad Injection water supply ponds and after it (groups 7 and 8) respectively have shown some contamination with BTEX compounds which confirm our suspicions that the oilfield may get rid off some untreated PW directly to the MOD which may pollute to the marshes in the southern area of Iraq those fed from MOD steam and the serious impact on the environment and biology.

**Table 4. The average concentrations of BTEX found in aquatic samples collected from MOD. The BTEX levels in samples of the groups (4, and 5) were undetectable. Analyses were taken place using HS-SPME-GC-FID**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample concentration in (μg/ml)</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min Max Mean SD</td>
<td>Min Max Mean SD</td>
<td>Min Max Mean SD</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>u.d-0.135 0.0675 0.045</td>
<td>u.d-0.102 0.051 0.034</td>
<td>u.d-0.321 0.1605 0.106</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>u.d-0.109 0.0545 0.036</td>
<td>u.d-0.201 0.1005 0.067</td>
<td>u.d-0.132 0.066 0.044</td>
<td></td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>u.d-0.114 0.057 0.038</td>
<td>u.d-0.322 0.161 0.107</td>
<td>0.121-0.322 0.222 0.047</td>
<td></td>
</tr>
<tr>
<td>Xylene</td>
<td>0.232-0.504 0.368 0.064</td>
<td>0.289-0.566 0.428 0.048</td>
<td>0.521-0.948 0.735 0.101</td>
<td></td>
</tr>
</tbody>
</table>

Average concentrations of BTEX were found within the range of μg/ml. These findings point out the are of the same order of magnitude as those reported by other authors, such as in the effluent of produced water from platforms of two different petroleum production units located in the Campos Basin, State of Rio de Janeiro, Brazil (283–1855 mol/ L for benzene, 87.04–2224 mol/L for toluene, 16.77–1220 mol/ L for ethylbenzene and 67.35–5969 mol/ L for xylenes)\(^2^6^)\) and in Norway total BTEX of 8000 μg/L was measured\(^2^7^)\).

**Conclusion**

BTEX pollutants were successfully collected from water samples inside and around Al-Ahbad Oilfield using the environmentally friendly technique of SPE. The extracted water samples were accurately quantized by GC-FID. Reliability and performance of the method of determination were validated by working out the detection and quantification limits, precision and accuracy. Concentration levels ranged from 0.21 and 1.62 μg/mL for BTEX in the PW samples collected from inside Al Ahbad oilfield.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the AL-Mustansiriyah University, College of Science, chemistry department and all
experiments were carried out in accordance with approved guidelines.

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Histological Changes in Mice (Balb/c) Induced by Newcastle Virus in Digestive System

Abbas Abed Sharhan1, Ahmed Obaid Hussain2, Ameer Jawad Hadi3, Ruqya jaafar baqer3
1College of biotechnology, Al-Qasim Green University, Iraq, 2College of biotechnology, Al-Qasim Green University, Iraq, 3Babylon Dental Specialize Center, Iraq

Abstract
Many domestic and Newcastle disease is a contagious bird disease affecting wild avian species, it may be transmissible to humans. Detection of Newcastle virus confirmed by rapid test technique (Immunochromatography). ten sample (10%) out of 100 faeces sample collected from chickens were positive. Twelve mice used in this study devided into two groups, first group consist of six mice induced with 0.2 ml from one positive sample of Newcastle virus suspension to evaluate some histopathological changes caused by Newcastle virus in small intestine and stomach of mice, second group induced with 0.2 ml from phosphate buffer saline only. The results revealed that histopathological changes in small intestine (duodenum) induced by using 0.2 ml from the positive Newcastle virus suspension to complete necrosis and fragmentatin in the villi. Jejunum of mice treated with this concentration of Newcastle virus has partial necrosis for villi with filtration of villus core while ilium of mice treated with same concentration has wide spread of villus necrosis. Stomach of treated mice has oedema in submucosa layer and partial necrosis in gastric glands.

Key words: Histological changes, Newcastle virus, digestive system

Introduction
Newcastle disease is caused by avian paramyxovirus serotype-1 (APMV-1), which is also disease virus (NDV). It is a highly contagious viral diseases that affects domesticated and wild bird species throughout the world as well as NDV is a human pathogen and the most common sign of infection in humans is conjunctivitis that develops within hours of NDV exposure to the eye (1,2). However, disease host species and in different geographical locations. NDV is classified in the genus Avulavirus within subfamily Paramyxovirinae, family Paramyxoviridae and order Mononegavirales. This enveloped virus has a negative sense non-segmented, single stranded RNA genome has 15198 nucleotides in length. The genome encodes six structural and two non-structural proteins. Based on the fusion (F) gene strains are classified into lineages or genotypes; however the discrepancies between the two classification systems are nominal. NDV is spread primarily through direct contact between healthy birds and the bodily discharges of infected birds. The disease is transmitted through infected birds’ droppings and secretions from the nose, mouth, and eyes. Clinical manifestation or severity of the ND depends largely upon the isolates involved in disease outbreak. Based upon pathogenicity, these strains are commonly categorized into velogenic (mesogenic and lentogenic types). The varying level of pathogenicity is attributed to amino acid sequence motif present in protease cleavage site of the precursor F protein.

Materials and Method
Samples collection:
A total of 100 faeces samples were collected from chicken suffering from clinical signs and symptoms of severe greenish watery diarrhea. Detection of Newcastle virus performed by rapid test (Immunochromatography) supplied from Biochek company – USA. The positive samples for Newcastle by rapid test were diluted with phosphate buffer saline or normal saline and stored at -20 °C in freeze. One positive samples of Newcastle was further used for the experimental study on laboratory animals (mice) for evaluation the effects of Newcastle disease on histologicalsections of these
Experimental study: A total of 12 males mice species Balb/c have aged two months and weight 100-120 g divided into two groups. The first group consists of six mice injected orally with 0.2 ml of Newcastle virus suspension for one positive sample. The other control group received 0.2 ml of sterile phosphate buffer saline (PBS) according to methods (12,13). After 4-6 days, clinical signs were recorded in infected animals, whereas observed Experimental mice were sacrificed after anesthetization by chloroform and open abdomen cavity by medical scissors. Tissue from small intestine, stomach, liver were collected from the experimentally infected mice and placed in formalin 10% for histopathological examination in later. Histological sections and staining were prepared according to methods described by 14. The histopathological changes were observed by Dr. Nemah H. Al-jabori/college of medicine / Babylon university under the magnification power 10X and 40X of light microscope.

Results

Histological changes observed in stomach and small intestine which infected with 0.2 ml from Newcastle virus suspension, these changes shown in figure 1,3,5,7. Figure (1) Stomach of mice treated with 0.2 ml from Newcastle virus suspension shows edema in submucosa layer and partial necrosis in gastric gland. The results in figure (3) small intestine of mice (duodenum) treated with 0.2 ml concentration from virus Newcastle suspension revealed to complete necrosis and fragmentation of the villi. Figure (5) small intestine of mice (jejunum) treated with 0.2 ml concentration from Newcastle virus suspension revealed to partial necrosis of villi with filtration of villi core of lymphocytes. Ilium of mice treated with 0.2 ml concentration of virus suspension indicated to widespread villus necrosis.

While the figures 2,4,6,8 revealed to stomach, small intestine (duodenum), small intestine (jejenum) and small intestine (illium) respectively for control mice treated with...
Figure (5) : jejenum of control mice. The slide shows the normal histology of the duodenum with normal villi core. H&E 20X.

Discussion

The authors consider lesions of Newcastle virus most prominent duodenum jejunum and ileum. Even in birds showing neurological signs prior to death, little evidence is found nervous system. Lesions are usually present in the respiratory tract when clinical signs indicate involve men 15. The results of present study about effects of Newcastle virus on stomach and small intestine of mice which experimentally infected with this virus revealed to histopathological changes within stomach and small intestine including duodenum, jejunum and ileum in the mice infected with 0.2ml from Newcastle virus suspension. These histopathological changes were observed in figure 3,5 were indicated histopathological changes in small intestine including duodenum, jejunum and ileum of mice treated 0.2ml viral suspension. Other reports mentioned the occurrence of villi atrophy and atrophy with blunting of the villus tips in the ileum and jejunum of pigs infected with rotavirus 13. Similar findings were reported by 16 which mentioned the severe necrotic and degenerative lesions of the villous enterocytes in piglets. Stomach of mice treated with 0.2ml from Newcastle virus suspension had macroscopic and microscopic changes in the stomach, histopathological changes in stomach and small intestine of mice which treated with Newcastle virus suspension due to most replication for newcastle virus in small intestine. Causes of histopathological changes in small intestine of mice due to enterovirus replicates predominantly in the cytoplasm of differentiated small intestinal villous epithelial cells and thus the virus induces changes that are restricted to the small intestine and these viruses induced epithelium cells destruction and inflammatory response to these parts of intestine 21, 22.

Conclusion

In Conclusion the Newcastle virus suspension caused clear histological changes in stomach and small intestine of mice (Balb/c).

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of biotechnology, Al-Qasim Green University, Iraq and all experiments were carried out in accordance with approved guidelines.

References


The Effects of Newcastle virus Histologically Suspension on Some Organs of White Mice (Balb/c)

Alaa Abd Alzahraa¹, Ahmed Obaid Hossain¹, Abbas Abd Sherhan¹

¹College of biotechnology, Al-Qasim Green University, Iraq

Abstract

Newcastle disease is a contagious bird disease affecting wild avian species and many domestic, it may be transmissible to humans. Detection of Newcastle virus confirmed by rapid test technique (Immunochromatography). Ten sample (10%) out of 100 faeces sample collected from chickens were positive. Twelve mice used in this study divided into two groups, first group consist of six mice induced with 0.2 ml from one positive sample of Newcastle virus suspension to evaluate some histopathological changes caused by Newcastle virus in small intestine and stomach of mice, second group induced with 0.2 ml from phosphate buffer saline only. The results revealed that histopathological changes in liver induced by using 0.2 ml from the positive Newcastle virus suspension as hyperpalsia in kupfer cells. Lung of mice treated with this concentration of Newcastle virus has partial pneumonia and thicking the intra alveolar walls. While brain of mice treated with same concentration suffering from increase in number of glial cells (gliosis) and apoptosis in brain cells.

Key words: Newcastle virus, histologically suspension

Introduction

Newcastle disease is caused by avian paramyxovirus serotype-1 (APMV-1), which is also disease virus (NDV). It is a highly contagious viral diseases that affects domesticated and wild bird species throughout the world as well as NDV is a human pathogen and the most common sign of infection in humans is conjunctivitis that develops within hours of NDV exposure to the eye (1,2). However, disease host species and in different geographical locations. NDV is classified in the genus Avulavirus within subfamily Paramyxovirinae, family Paramyxoviridae and order Mononegavirales. This enveloped virus has a negative sense non-segmented, single stranded RNA genome has 15198 nucleotides in length. The genome encodes six structural and two non-structural proteins. Based on the fusion (F) gene strains are classified into lineages or genotypes; however the discrepancies between the two classification systems are nominal (5,6,7). NDV is spread primarily through direct contact between healthy birds and the bodily discharges of infected birds. The disease is transmitted through infected birds’ droppings and secretions from the nose, mouth, and eyes. Clinical manifestation or severity of the ND depends largely upon the isolates involved in disease outbreak. Based upon pathogenicity, these strains are commonly categorized into velogenic(mesogenic and lentogenic types). The varying level of pathogenicity is attributed to amino acid sequence motif present in protease cleavage site of the precursor F protein (10,11).

Materials and Method

1-Samples collection:
A total of 100 faeces samples were collected from chicken suffering from clinical signs and symptoms of severe greenish watery diarrhea. Detection of Newcastle virus performed by rapid test (Immunochromatography) supplied from Biochek company -USA. The positive samples for Newcastle by rapid test were diluted with phosphate buffer saline or normal saline and stored at -20°C in freeze. One positive samples of Newcastle was further used for the experimental study on laboratory animals (mice) for evaluation the effects of Newcastle disease on histological sections of these mice.

2-Experimental study: A total of 12 males mice species Balb/c have aged two month and weight 100-120 g divided into two groups, the first group consist of six mice injected orally with 0.2 ml of Newcastle virus suspension for one positive sample. The other as
control group was received 0.2 ml of sterile phosphate buffer saline (PBS) according to methods of (12,13) after 4-6 days clinical signs were recorded in infected animals. were observed Experimental mice were sacrificed after anesthetization by chloroform and open abdomen cavity by medical scissors, tissue from small intestine, stomach, liver were collected from the experimentally infected mice and placed in formalin 10% for histopathological examination in later. Histological sections and staining were prepared according to methods described by (14). The histopathological changes were observed by Dr. Nemah H. AL-jabori/college of medicine / Babylon university under the magnification power 10X and 40 X of light microscope.

Results

Histological changes of current study observed in liver, lung and brain of mice infected with 0.2ml from newcasstle virus suspension, these changes shown in figure1,3,5, while figures 2,4,6 represented control group of mice treated with 0.2ml phosphate buffer saline. In this results figure (1) represented the liver of mice treated with 0.2ml from Newcasstle virus suspension shows hyperpalsia in kufper cells. The results in figure (3) indicated to lungs of mice treated with 0.2ml concentration from virus Newcasstle suspension revealed to partial pneumonia and thicking the intra alveolar walls. While the figure (5) revealed to the brain of mice treated with 0.2ml concentration from newcasstle virus suspension revealed to increase in number of glial cells (gliosis) and apoptosis in brain cells. Finally the figures 2,4,6 revealed to liver, lung and brain respectively for control mice treated with phosphate buffer saline. No histological changes observed in liver, lung and brain of control mice group

Discussion

The authors consider lesions of Newcasstle virus most prominent duodenum jejunum and ileum. Even in birds showing neurological signs prior to death, little evidence is found nervous system. Lesions are usually present in the respiratory tract when clinical
signs indicate involvement\textsuperscript{15}. The results of present study about effects of Newcastle virus suspension on liver, lung, and brain of mice which experimentally infected revealed to histopathological changes within liver, lung and brain of mice infected with 0.2ml from Newcastle virus suspension. These histopathological changes were observed in figure 1,3,5. Figure (1) showed histological changes in liver represented in hyperpalsia in kupfer cells. This results similar to recent studies mentioned that Newcastle virus outbreaks in a poultry facility in Japan was characterized, among other lesions, by hepatic necrosis\textsuperscript{16}.

The results of lung mice infected with same concentration of this virus revealed to partial pneumonia and thicking the intra alveolar walls. This results accept with other reported mentioned that some Newcastle virus strains have been shown experimentally to cause moderate lesions in the respiratory system, these changes were obtained only through aerosolization or use of very high viral titers direct air sac instillation of the virus\textsuperscript{(17,18)}. While the results of lung mice infected with same concentration from Newcastle virus suspension recorded increase in number of glial cells (gliosis) and apoptosis in brain cells. This results agreement with some studies mentioned finding Interactions between the Newcastle disease virus and mouse tissues\textsuperscript{(19,20)}. Causes of histopathological changes in liver, lung and brain of infected mice perhaps due to virulence of Newcastle virus and rapid replication effect on three systems are digestive system (e.g: liver), respiratory system (e.g: lung) and nervous system (e.g: brain)\textsuperscript{(21,22,23)}.

**Conclusion**

The Newcastle virus suspension caused clear histological changes in liver, lung and brain of mice (Balb/c).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of biotechnology, Al-Qasim Green University, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

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Molecular identification of *Ascaridia columbae* in the Local Healthy Pigeon (*Columbia livia domestica*, Gmelin, 1780) in Karbala Province

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Abstract

The current study was conducted to investigate dissemination of gastrointestinal Ascariasis in local pigeons (*Columba livia domestica*) living in Kerbala province. Forty domestic pigeons with different ages were purchased from different markets. Each bird was examined for intestinal parasites. Out of 40 birds examined, the prevalence of gastrointestinal Ascariasis in examined birds were (12.5%), Ascaris sp. was detected in 100% of intestinal contents using macroscopic and microscopic examination assays, molecular diagnosis from bird belly, with the emphasis on primer designed specificity it used in our study.

**Key words:** Ascaridia columbae, pigeon, primer design

Introduction

The rock pigeon (*Columba livia domestica*) is subspecies of the rock pigeon (*Columba livia*) which is a free-living in environment and live side by side with human and other animal species in the environment. The rock pigeon (*Columba livia*) belong to the order Columbiformes and spread widely in all the world. Columbiform pigeons were infect with *Ascaridia columbae* and causes significant infection. Untile now only ~25,000 species have been detected although bilateral animals with an estimate of 1–10 million species. The gastrointestinal helminth (*ascaridia spp*) that infect chickens, pigeons and parrots. Pigeons may be consider reservoirs for many of helminth infections of veterinary importance especially *ascaridia spp* to other avian. *A scaridia spp* infestations have a major role and effect direct or indirect on health status of birds lead to economic losses in the production of poultry meat and eggs. *A scaridia spp* are one of the most common gastrointestinal helminth problems of pigeons. They harbor *Ascaridia columbae* and *Ascaridia galli*. The infected pigeons suffer from various clinical signs which differ from loss of weight, growth, egg production, elementary canal disturbance and gastrointestinal blockage with increase mortality rates. The aim of the present study to investigate prevalence of gastrointestinal helminth in healthy local pigeons in Kerbala province.

Materials and Method

Ethics statement

This experiment was approved by Veterinary medicine Animal Care and Animal Ethics Committees, Faculty of veterinary Medicine, University of kerbala, Iraq.

Study areas and Parasitological analysis

Forty local domestic pigeons (*Columbia livia domestica*) with dubious clinical emaciated signs which include (inability to fly properly, Sitting too still, Drooping wings or slouched and Head listing to one side) were admitted to the Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Kerbala University. In this current study, several suspected Ascaridia eggs were examined in the sample stool of each emaciated bird, then was directed to the veterinary parasitological Laboratory, Faculty of veterinary Medicine, University of kerbala Iraq, from February till the end of April 2019. After the stool was mingled with 0.85 % physiological salt solution in order to refine it from several separate
particles and all pigeon were stool examined according to the. The infected birds were isolated and killed by neck dislocation in order to isolate the infected intestines from the belly of the birds, later on, the intestine was opened longitudinally from esophagus to cloaca. The intestinal contents were examined macroscopically for the presence of adult nematodes or other parasites. Microscopical examinations were included direct smear, sedimentation and floatation methods and the ultra structures for both male and female. A scaridia spp. that were presence in various places of intestine were collected and put it into Petri dishes then transferred in labeled bottles contain tap water. Amplification of specific DNA sequences by PCR and restriction endonuclease analysis of PCR products have been used for identification and characterization of ascariasis. The aim of this study was to determine the prevalence of pigeon ascariasis and to design a specific primer selection of identified Ascaridia columbae in kerbala province.

**DNA extraction:**

Adult Ascaridia columbae were collected from luminal content in the pigeons were harvested, single worm was harvested using a procedure carried out according to. The DNA of worm obtaining was extracted by genesig Easy DNA/RNA Extraction Kit (Southampton, UK) according to the manufacturer company.

**Primer designing:**

Dual nucleotides primers were designed according to sequencing that obtained from NCBI website(https://www.ncbi.nlm.nih.gov), PerlPrimer was used in the design of primers and calculates primer melting temperature (http://perlprimer.sourceforge.net). Table 1.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’ direction) Cytochrome oxidase 1 Cox1</th>
<th>G-C content</th>
<th>Melting temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forword</td>
<td>TGGTGGTTTAGGTGTTGACTG</td>
<td>40%</td>
<td>54</td>
</tr>
<tr>
<td>Reverse</td>
<td>CCAACAAACAAAAGGCAACATT</td>
<td>41%</td>
<td>55</td>
</tr>
</tbody>
</table>

The primers were manufactured by (Alpha DNA, Montreal, QC, Canada). PCR amplification was carried out with a thermal cycler (Analytik Jena’s Biometra thermal cycler, Germany.) and by using a premix PCR kit (AccuPower® PCR PreMix, Korea) and by following the instructions of the manufacturer instruments, PCR reaction assay were formed with a dual primers designed on the basis of the published nucleotide sequence of the Ascaridia columbae complete Cytochrome oxidase 1 Cox1 gene (length, 1,563 bp; GenBank database accession JX624729 and NC_021643.1). Briefly, A product of mixing PCR consisted of 5 μl of a sample containing DNA was added to each premix well, 7 μM each primer, and double-distilled water to a final volume of 25 μl. PCR optimizations were denatured at 94°C for 5 min. thirty five cycles were run under the following processes: DNA denaturation at 92°C for 3 minutes, annealing temperature at 52°C for 1 min, and extension process at 72°C for 1 min. After the final cycle, the reactions were terminated by an final extension at 72°C for 7 min. The PCR products were analyzed by Safe-Red™-agarose gel electrophoresis.

**Results and Discussion**

Ascariasis can probably affect any mammal. Its occurrence is sporadic and worldwide. Birds, particularly pigeons, often carry the agent in their intestinal contents and contribute to its reservoir (figure 1). They are rarely affected clinically, and then mostly on mucous surfaces, there were no clear signs of the birds unless they were weak, shivering emaciated and ruffled feathers, The internal organs are not clear due to the multiplication of worms forming almost like a cover enriched with roots of tree that cause intestinal blockage (figure 1). Ascariasis most frequently affects the mucous surfaces on which the worm is abnormally found, possibly the digestive tract of pigeon; it typically remains confined to areas of villus epithelium due to blunting of the villi.

Pigeon is often associated with ascariasis (through eggs) or intensive exposure. The infestation of pigeon
has an important role in causing significantly reduce due to the economic losses in the eggs production. Also, they may have essentially harmful or lethal effects on birds infections, especially small birds (small squares), causing growth delay, intervention in healthy development, and making older birds susceptible to secondary infection.

Microscopically and macroscopically methods were used to determine the difference between males and females, the male’s posterior end is curved ventrally and has a bluntly pointed tail with copulatory spicules which help the worm to dialate the vulva and can also work as a canal for the spermatozoa (figure 2). There was no apparent difference in parasite anterior end, so, this ends are more slender than the posterior ends. Males have pineal, spices or spine like extensions nears its posterior opening whereas females lack these structure.

A female sediment from intestinal contents is mixed with an equal amount of ddH₂O on a slide and a cover slip is added. Microscopically, the internal organisms appear as bright circular lacunae in a dark field, containing the eggs in their centers.

Avian Ascariasis affects chickens, turkeys, pigeons, and other birds. It resembles ascariasis of humans involving the anterior digestive tract. In the young, it can be a blocking digestive disease and cause considerable mortality.

We searched the Cytochrome oxidase 1 Cox1 site, on the Ascaridia columbae genome Acession number (JX624729) using NCBI Nucleotide BLAST for complete sequences (http://www.ncbi.nlm.nih.gov/BLAST/) limited by the Entrez query ‘Ascaridia columbae’. among data obtaining, the study was selected the target gene containing the Cytochrome oxidase 1 in the Ascaridia columbae, and also tested the specificity of the 1563 bp sequence of the target, the selected 263 bp Cytochrome oxidase 1 and the 22 and 20 bp for forward and reverse primer regions, respectively, at both ends among the target region using NCBI Nucleotide BLAST figure 3.

In PCR-based parasite identification, PCR-product amplified was also be shortly and appropriately detected. As a general, the amplify DNA sequences can be determined by agarose gel electrophoresis after Polymerase chain Reaction assay amplification. Numerous identification systems for specified of parasite investigation such as Ascaridia columbae based on the combination of PCR and gel electrophoresis have been already progressing and commercialized.

The primer design and aligned method was based on a number of available sequencing that upload on the the NIH genetic sequence database, unfortunately, in our current study, we found only two available
GenBank sequence database for Ascaridia columbae mitochondrion, complete genome which included two accession number (JX624729 and NC_021643.1) They belong to researchers who record that accession number (NC_021643.1) has not yet been subject to final NCBI review and confirmed this reference sequence is identical to JX624729.

Our results was used gel electrophoresis is an easy method of PCR products, according to our knowledge, this primers can used to differentiate between specific PCR amplification products and non-specific ones (figure 3), In our current study, we have not observed any convergence and alignment of the nucleotides sequences used between the Ascaridia columbae wit other ascariasis except for some animals or the parasite far from an pigeon infestation, Therefore, we can say that this primer is considered as a specific diagnosis of the this strain.

Figure 3: Align of Ascaridia columbae with more sequences with other prokaryotic and eukaryotic cells using BLAST

Conclusion

In conclusion our study was demonstrated the rapid and specific detection of PCR DNA amplified from an Ascaridia columbae genome using a specific primer sequences.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of pathology and poultry diseases, College of veterinary medicine, University of Al_ Qadisiyah, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Effect of Addition of Composite Polyamide Micro Particles and Silicone Dioxide NanoParticle on Some Mechanical Properties of Room Temperature Vulcanized Maxillofacial Silicone Elastomer Before and after Artificial Aging

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Abstract

Background: The most common reason for re-making a maxillofacial prosthesis is the degradation of the mechanical properties of the silicone.

Aim of this study: To assess some mechanical properties of VST-50F maxillofacial silicone reinforced with a composite of silicon dioxide nanoparticle and polyamide-6 microparticle before and after artificial aging.

Material and Method: Preparing 240 samples tested for tear strength, tensile strength and elongation percentage, hardness, and roughness before and after aging. The Silicon dioxide was added in concentrations of 1% by weight and Polyamide-6 in the concentration of 0.25% and 0.5% by weight to the VST-50F RTV maxillofacial silicone. The one-way ANOVA and post hoc tests were used for inferential statistics.

Results: The one-way ANOVA showed a highly significant difference between all tested groups. The effect of the addition of composite fillers showed an increase in tear strength, hardness, and surface roughness but a decrease in tensile and percentage of elongation. However, the effect of artificial aging showed increased in tear strength, Percentage of elongation, and surface roughness, but a decrease in tensile strength and hardness.

Conclusion: Addition of composite of fillers into the silicone elastomer allowed enhancement of some mechanical properties. The composite of different types of filler reinforcement improves the anti-aging properties of silicone and maintain some of the mechanical properties to enhance the service life.

Keywords: Composite, silicone dioxide, polyamide, Maxillofacial silicone, Mechanical properties, artificial aging.

Introduction

The distortion in appearance and function could be restored with a maxillofacial prosthesis that reproduces the natural features of the lost tissues from acquired, developmental, and congenital head and neck defects. The first treatment of choice is plastic surgery. However, when a medical procedure is ill-advised, because of undesirable conditions, restoration with maxillofacial prosthesis could be an alternative method for improving patient’s appearance and confidence and encouraging their resumption to society. Rahman et al. after systematic review for various researches concluded that there was no ideal maxillofacial material that could resist different aging conditions. Liu et al. concluded that merging two types of micro-particles in specific percentages could give a maxillofacial prosthesis closer to ideal properties. On the other hand, Nano- and micro-filler mixture is a practical approach to improving the strength and the features of material.

This study assessed some mechanical properties of VST-50F maxillofacial silicone reinforced by a composite of silicon dioxide nanoparticle and...
Materials and Method

The materials used in this study were the VST 50F RTV silicone (Factor II Inc., USA), SiO2 (Sky Spring, Inc. USA) and PA-6 (Changfeng Chemical Co., Ltd. China).

The SiO2 was added in 1% by weight and PA-6 in the 0.25% and 0.5% by weight to the VST-50F silicone.

A total of 240 samples had been prepared and separated into four groups according to the performed tests, so each test included 60 samples and each test group further subdivided into six sub-groups (Control, Control/aged, 0.5PA+1SiO2, 0.5PA+1SiO2/aged group, 0.25PA+1SiO2, 0.25PA+1SiO2/aged) with ten samples for each sub-division.

The shapes and dimensions of the molds for the test samples were designed utilizing AutoCAD 2013 and fabricated with CNC machine into which the silicone poured. The two templates had dimensions of 30 cm length x 20 cm width x 2.2 ± 0.05 mm thickness for tear and tensile strength test and 6 ± 0.05 mm thickness for hardness and roughness tests.

The fabrication of the samples for each of the experimental groups started by weighing the fillers in the mixing bowl then the silicone base added and mixed for 10 min. The mixing was conducted under vacuum to eliminate air entrapment in the mix for the first three minutes. The vacuum was switched off to prevent the suction of the nanoparticles and then it was switch on for the last 7 min. The vacuum pressure was -28 inch Hg, and the speed of the mechanical mixer was 140±10 RPM. Before adding the catalyst, the mixture was left for 2 minutes to cool — the catalyst mixed with the base mixture for 5 minutes ± 5 seconds.

Pouring of the material was carried out under a temperature of 23 ±2°C, and the humidity around 50±10% 6. The homogeneous mixture was dispensed carefully into a plastic-syringe of 60 mL (Figure 1). The acrylic mold placed over a dental vibrator working at a low vibration and the mixture poured in an excess amount from the disposable syringe. The cover was placed over the poured material in the matrix, starting from one end part of the cover by resting the margin and lifting the margin of the other end part. Then the lid was gently lowered slowly and carefully onto the matrix to allow for the escape of the excess material out of the mold. After that, a load of one kilogram placed over the middle part of the mold’s cover, and the cover secured in place with nuts and G-clamps, then the weight was removed (Figure 2). The mold was fixed firmly until the samples hardened 1.

All the samples organized, and the tests performed according to ISO 23529 6 specifications.

The following four tests were performed to assess the various mechanical properties of the VST-50F maxillofacial silicone:

1. Tear strength test: According to ISO 34-1 specifications, the samples had been prepared and tested by the universal testing machine clamps (Laryee Technology Co., Ltd., China). The tear strength sample was the type C tear sample.

2. Tensile strength and elongation percentage tests: The testing procedure accomplished according to ISO 37 8 specifications with dumbbell-shaped.

3. Hardness Test: According to ISO 48-4 9 specification, the testing procedure accomplished. The samples marked with five points, one at the center and the others 6mm away at each direction around the center point. Shore A hardness durometer mounted on the mechanical stand used for measurement. The device was forced firmly by hand clamp on a mechanical stand over the surface of the sample for 1 second with a 1Kg load according to specification, and the mean of 5 reading recorded 9.
4. Surface Roughness Test: All samples prepared according to ISO 48-4 specifications. Portable digital roughness tester with (0.001μm) accuracy was used to conduct a surface roughness test. The device mounted on a stand and adjusted in a way so that the stylus just contacted the sample surface in 3 various places so that three measures obtained for each sample and their mean value represented the roughness value 10.

SEM (AIS2300C, Angstrom Advanced Inc, USA) scanning the dispersion of the composite fillers within the silicone matrix.

SPSS software v23.0 used for data analyzes for the descriptive and inferential statistics with a confidence interval of 95%.

Results

ANOVA test showed that a highly significant difference between all tested groups.

The results showed no significant increase in the tear strength after fillers reinforcement.

The effect of artificial aging for 150 hours showed that the tear strength of the control group decreased significantly, but for the reinforced silicone increased. It was a highly significant difference for 0.5PA+1SiO2 and a non-significant difference for the 0.25PA+1SiO2 group in comparison to the control group.

The results showed a non-significant decrease in the tensile strength after fillers reinforcement.

The effect of artificial aging for 150 hours showed that the tensile strength decreased highly significantly for the control group and the reinforced silicone. It was a highly significant difference for 0.5PA+1SiO2 and 0.25PA+1SiO2 groups in comparison to control group before aging and the non-significant difference in contrast to the control group after aging.

The results showed a significant decrease in the percentage of elongation after 0.5PA+1SiO2 fillers reinforcement and a highly significant decrease after the 0.25PA+1SiO2 fillers reinforcement.

The effect of artificial aging for 150 hours showed that the percentage of elongation of the control group decreased non-significantly, but for the reinforced silicone increased highly significantly. It was a non-significant difference for both percentages in comparison to the control group before and after aging.

The results showed a highly significant increase in the hardness after filler reinforcement.

The effect of artificial aging for 150 hours showed that the hardness of the control group increased non-significantly, but the reinforced silicone decreased highly significantly. It was a non-significant difference for both percentages in comparison to the control group before and after aging.

The results showed a highly significant increase in the surface roughness after 0.5PA+1SiO2 fillers reinforcement and non-significantly increased after the 0.25PA+1SiO2 fillers reinforcement.

The effect of artificial aging for 150 hours showed that the surface roughness increased non-significantly for the control and the reinforced silicone groups. It was highly significantly different for 0.5PA+1SiO2 group in comparison to the control group before and after aging.
and also highly significantly different for the 0.25PA+1SiO2 group, in contrast to control before aging, but it was only significantly different in comparison to control after aging (Table 1 and 2).

The SEM results showed a good dispersion of the Nano-fillers within the silicone matrix (Figure 3),

**Table (1) Mean values of conducted tests before and after artificial aging**

<table>
<thead>
<tr>
<th>Tests Groups</th>
<th>Tear strength (N/mm)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation percentage (%)</th>
<th>Shore A hardness (IU)</th>
<th>Surface roughness(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.0056</td>
<td>6.2284</td>
<td>596.6517</td>
<td>27.9160</td>
<td>0.3607</td>
</tr>
<tr>
<td>Control /aged</td>
<td>24.7655</td>
<td>5.0542</td>
<td>594.6524</td>
<td>28.5500</td>
<td>0.4347</td>
</tr>
<tr>
<td>0.5PA+1SiO2</td>
<td>28.1010</td>
<td>5.6591</td>
<td>571.9662</td>
<td>30.8200</td>
<td>0.5195</td>
</tr>
<tr>
<td>0.5PA+1SiO2 /aged</td>
<td>29.8892</td>
<td>4.9269</td>
<td>595.8612</td>
<td>28.2400</td>
<td>0.5871</td>
</tr>
<tr>
<td>0.25PA+1SiO2</td>
<td>27.2575</td>
<td>5.7993</td>
<td>569.4999</td>
<td>31.0500</td>
<td>0.4575</td>
</tr>
<tr>
<td>0.25PA+1SiO2 /aged</td>
<td>28.5915</td>
<td>4.9768</td>
<td>595.6062</td>
<td>28.3900</td>
<td>0.5491</td>
</tr>
</tbody>
</table>

*(Table 2) Post-hoc Tukey HSD for all tests except Games-Howell for elongation test*

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Tear</th>
<th>Tensile</th>
<th>Elongation</th>
<th>hardness</th>
<th>roughness</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>Control /aged</td>
<td>0.050</td>
<td>0.000</td>
<td>1.000</td>
<td>0.752</td>
<td>0.368</td>
</tr>
<tr>
<td></td>
<td>0.5PA+1SiO2</td>
<td>0.699</td>
<td>0.066</td>
<td>0.016</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.5PA+1SiO2 /aged</td>
<td>0.005</td>
<td>0.000</td>
<td>1.000</td>
<td>0.982</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>0.25PA+1SiO2</td>
<td>0.999</td>
<td>0.283</td>
<td>0.008</td>
<td>0.000</td>
<td>0.118</td>
</tr>
<tr>
<td></td>
<td>0.25PA+1SiO2 /aged</td>
<td>0.306</td>
<td>0.000</td>
<td>1.000</td>
<td>0.911</td>
<td>0.000</td>
</tr>
<tr>
<td>Control /aged</td>
<td>0.5PA+1SiO2</td>
<td>.001</td>
<td>0.043</td>
<td>0.026</td>
<td>0.000</td>
<td>0.226</td>
</tr>
<tr>
<td></td>
<td>0.5PA+1SiO2 /aged</td>
<td>.000</td>
<td>0.988</td>
<td>1.000</td>
<td>0.985</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>0.25PA+1SiO2</td>
<td>0.021</td>
<td>0.006</td>
<td>0.013</td>
<td>0.000</td>
<td>0.990</td>
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<tr>
<td></td>
<td>0.25PA+1SiO2 /aged</td>
<td>0.000</td>
<td>0.999</td>
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<tr>
<td>0.5PA+1SiO2</td>
<td>0.5PA+1SiO2</td>
<td>0.189</td>
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<td>0.004</td>
<td>0.000</td>
<td>0.470</td>
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<tr>
<td></td>
<td>0.25PA+1SiO2</td>
<td>0.874</td>
<td>0.981</td>
<td>0.977</td>
<td>0.996</td>
<td>0.565</td>
</tr>
<tr>
<td></td>
<td>0.25PA+1SiO2 /aged</td>
<td>0.987</td>
<td>0.015</td>
<td>0.008</td>
<td>0.000</td>
<td>0.968</td>
</tr>
<tr>
<td>0.5PA+1SiO2 /aged</td>
<td>0.25PA+1SiO2</td>
<td>.012</td>
<td>0.001</td>
<td>0.002</td>
<td>0.000</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>0.25PA+1SiO2 /aged</td>
<td>0.530</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.911</td>
</tr>
<tr>
<td>0.25PA+1SiO2</td>
<td>0.25PA+1SiO2</td>
<td>0.499</td>
<td>0.002</td>
<td>0.004</td>
<td>0.000</td>
<td>0.158</td>
</tr>
</tbody>
</table>

*P-value≤0.05=significant,P-value≤0.01=highly significant.
Figure 3: SEM image of VST-50F silicone elastomer. A: Before addition, B: after addition 0.5PA/1SiO2 C: after addition 0.25PA/1SiO2.

Discussion

Many types of research showed that most of the commercially present materials had not satisfied all of the required, ideal properties and the highest chance of early deterioration was the major limitation of silicone material that may exhibit reduced tear strength, somewhat fitting margin, discoloration of material and modified texture. It clarifies the constant increase in the number of researches to develop enhanced maxillofacial silicone material, by changing the formulation of previous materials or by reinforcement with various types of fillers in different percentages \(11\). Silica particles can adsorb polydimethylsiloxane chains to its surface and form strong hydrogen bonds between its surface hydroxyl group and the oxygen in the polymer chain. These bonds increase polymer chains resistance to rupturing when subjected to tearing forces due to high shear strength between the nanofiller and the polymer chains \(12\). Also, the PA-6 could form multiple hydrogen bonds among adjacent elements because of the high polarity natural of fillers due to the amide (-CO-NH-) groups. It is leading to an alteration in the whole density and more tear resistance of the polymer due to the formation of fillers with a three-dimensional network in the polymer matrix. On the other hand, rubber can scatter stress-energy close the apex of the growing cracks and thus increasing the strength in elastomers. So, this may explain the increase in tear strength after SiO2 and PA-6 filler reinforcement \(13\). The hardness increase might be referred to the difference in the crosslink density with filler contents; the filler particles may cause a reduced in the distance among the crosslinks of the polymeric matrix thus the reduction of the softness of the material.
contributed to the polymer and filler interaction. As mentioned early, the SiO2 nanoparticles increase the cross-linking network that leads to improve the overall stiffness of the polymer and increase its resistance to tearing, but the higher cross-linker tightened the net to upper limit so reducing the flexibility and deteriorating tensile properties obtaining extremely brittle samples. The decrease in elongation could also be clarified by the reduction in the space between the fillers aspect ratio and contents. Therefore the elongation at fracture was decreased by the relatively reduced distance of rubber molecules. On the other hand, the cross-linking was reversible for PA-6 particles with temperature and UV light inside the Weather-Ometer chamber and could result in a polymer with altered physical and mechanical properties. The decrease in cross-linking leads to a reduction in tensile strength and an increase in the percentage of elongation. It may be because of chain scission that results from the generation of free radicals which react with oxygen leading to the formation of polymer oxy- and peroxy radicals via the enhanced chemical reaction (photo-oxidation). It influences the distribution of molecular weight and reducing the density of the network structure by breaking the bonds, commonly C-H bonds, between two diverse chains or within the same main chain due to strength value of material reduced.

The particles of the nanofiller (SiO2) may be associated strongly with the polymeric chains even after severe condition. If these particles were detached, an increase in the porosity of the polymer and reduction in the hardness would be expected. So the surface roughness increase results from the formation of microcracks and pits on the surface level of material.

Conclusions

Within the limitations of the study, the following were drawn: The addition of various types of fillers in appropriate proportions to silicone elastomer could enhance some of the mechanical properties. The composite of Nano-SiO2 and micro PA-6 fillers tend to increase in tear strength, hardness and surface roughness but a decrease in tensile and percentage of elongation. The composite of different types of fillers reinforcement is promising to improve the anti-aging properties of silicone and maintain some of the mechanical properties to enhance the service life.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry/ University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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The Elements of the Crime of Copyright Infringement on the Internet

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Abstract

With the creation of the human mind, the intellectual production of such machine created by the Creator began to appear in various forms starting from drawing and engraving on stones, the manufacture of clothing from plants leaves and animal skins, and making fishing rods and eventually to all cognitive sciences known by the world today and the evolution reached in all fields and aspects. Intellectual property rights are among the most important forms of human rights related to his intellectual production in the scientific, literary, artistic, and all forms of intellectual production in the different aspects of human life. It is clear to all that this production has its material and moral fruits. The intellectual property rights have become one of the most important forms of material rights that entitles its holder to dispose them, give them up and invest them. This right can be defined from the researcher point of view as a set of ideas that are written and issued by the human mind, and that can be accessed and identified just as tangible material objects. It includes all rights resulting from the intellectual activities of man in the literary, artistic, scientific, industrial, commercial and similar fields.

Keywords: Crime, Elements, internet

Introduction

Through this research, we address a number of points by study and interpretation: What works are covered by criminal protection ¹, in terms of definition, conditions to be provided, and the elements of the work. The corpus delicti of the Crime of copyright infringement on the internet. Criminal Punishment for such crime in the Light of Iraqi Legislation ². The research was conducted on four themes, in the first of which the researcher addressed the works covered by the legal protection in three sections. Section one introduces the definition of works, section two addresses conditions to be provided in the work covered by legal protection and section three provides the elements of the works subject to the crime in question. The second theme handles the physical corpus delicti of the crime of copyright infringement on the internet. It is introduced in three sections: the first of which is concerned with the criminal activity, the second of which deals with the criminal result, the third of which provides for the causal relationship ³. The third theme handles the moral corpus delicti of the crime of copyright infringement on the internet. It is introduced in two sections: the first of which is concerned with premeditation in the crime of copyright infringement on the internet, the second of which deals with the motive of the crime of copyright infringement on the internet ⁴-⁶.

Methodology

Conditions to be provided in the work subject to such crime

The author copyright related to his work, due to all of the above mentioned reasons, may fall as a victim of counterfeiting and illegal imitation, so the right holder (the author) incurs physical and moral damage. Thus, the legislator must intervene to the extent necessary for providing the legal protection sufficient for the author and guarantee his rights with regard to his work in order to hive him reassurance and thus give more room for creativity and intellectual production.

The elements of the works subject to the crime

Neither jurisprudence nor law, nor even the judiciary system, interferes in the development of certain elements that are required to establish the literary, social, scientific, artistic or cultural value of the digital work, so that it can be considered a subject of crime. In other words,
the purpose of the work is not significant in determining whether or not legal protection should be available.

The elements to be available in the work so as to be covered by legal protection are two elements that are:

1. Creation: The legal jurisprudence considers innovation to be an essential element for the protection of intellectual property. The French jurisprudence considers innovation the essence of originality and seriousness, so that the work reflects the personal nature of the work author, which appears in the stage of creation and expression during the creation and production of the electronic work. This is evident by its definition of innovation as (the personal imprint placed by the author on his work). The Egyptian jurisprudence defines innovation as (the work being characterized by originality in terms of construction or expression). The Iraqi jurisprudence defines innovation as “the emergence of the personal effort of the author of the idea, regardless of its literary or material value 6. This does not differ if work is created by the author, in whole or in part, through different elements being collected and arranged in a particular form that reflects the character of the author through the production of the work covered by legal protection).

2. Material physical form: An abstract idea is not enough for providing legal protection, even if it is innovative and new, since this idea must have a tangible physical existence. Therefore, the digital works covered by legal protection should have a tangible physical existence that commensurate with the prevailing trend in jurisprudence and legal legislation of the expression of material physical existence. The latter limits range of the legal protection of digital works to a large extent, especially those that take the form of public performance such as Quranic recitation, musical melody or poetic poems

**Section (1): Criminal Activity**

The most prominent element of the crime corpus delicti in general is the first material notice of the crime existence to the outside tangible world, and as far as the crime of copyright infringement on the internet is concerned, the criminal activity here means the act of infringement of the author literary and financial copyright, either by the imitation of the work wholly (total reproduction) or in part as when some of the ideas contained in the work that belong to the author are attributed to another person, who is usually the perpetrator of the imitation crime.

Imitation within the framework of intellectual property rights is all the forms of action against these rights, and since the criminal legislator 7 has avoided setting the definition of imitation along with mentioning the acts that are considered, the legal jurisprudence defined it as making something new less valuable than the original one or similar there to with the aim to benefit from the value of the difference between the two things.

**Results and Discussion**

It is the second element of the crime corpus delicti, since the criminal conduct entails consequential effect, and this effect is the criminal result. There is no doubt that such an effect is required for the legal establishment of the crime, since a crime can never be established unless there is a criminal conduct and consequences arising there from. Therefore, the extent of such legal effect conformity to that effect specified in the legal model of crime shall be considered. The effect of the criminal conduct in the infringement of copyright on the Internet crime represents an infringement of the interests and rights protected by law. This is the conventional legal concept of the criminal result as an element of the crime corpus delicti. The right of the legal author related to his work falls within the intellectual property rights. It is also the general right of the human right to preserve his intellectual life away from the intervention of others and grant him the right to lead this life as he pleases within the limits of the law. The criminal result in accordance with this concept is a legal idea based on the moral damage affecting the interest or right protected by the law in its text. According to this legal idea, the crime may have occurred even if the criminal conduct did not lead to material damage at the time of such conduct occurrence because the normal course of events foresees that such damage will inevitably occur in the future, and this possibility of the damage incurred by the interest protected by law is the criminal result. The legal provision that criminalizes certain conduct has been established only for the wisdom of the legislator 8, and the latter has not established the legal provision merely to avoid tangible material damage, but rather to seek punishment for the same criminal conduct. The mere fact that the criminal conduct leads to the criminal result, even if it does not occur, is sufficient to punish those whoever commits such conduct. This applies to the crime of infringement of the author legal rights on the Internet, since the perpetrator violates the intellectual
property right of the victim, protected by the law, without the need to investigate the external material effects resulting from such conduct. Such crime is a risk crime that are sometimes called formal offences. The causal relationship means the association that links the criminal conduct to the criminal result is one of the elements of the crime corpus delicti and is considered the link between the two other elements (the criminal activity and the criminal result). The issue of causation and its significance is clearly manifested when other factors interfere with the criminal activity of the perpetrator, contributing to the criminal result. The latter have already been given a general description as the violation of the victim rights through unlawful means. The question how is this convenient as far as the crime of copyright infringement on the internet is concerned? If other factors interfere with the criminal activity of the perpetrator, contributing to the criminal result, does this negate the causal relationship, and therefore one of the elements of the crime corpus delicti is missing or this does not affect the existence of the causal relationship, which is proved along with the rest of the crime elements to form together the corpus delicti of the crime? The establishment of a criterion for proving the causal relationship between the criminal conduct and the criminal result is subject to the discretion of the competent court and its experience and precedents that enable it to give precedence to one of the criteria of proving the causal relationship and to judge its availability as an element of the crime corpus delicti or not. This did not prevent the criminal legislator in other criminal legislation from establishing a specific criterion for determining the availability of the causal relationship or not. The Iraqi judiciary has different positions in the cases considered between the period between the old Baghdad penal code and until after the issuance of the Penal Code in force no. 111 of the year 1969, since before the issuance of the current Penal Code and because the Baghdad Penal Code lacks any text that determines a criterion for establishing the causal relationship, the judiciary went to follow the theory of direct cause, influenced by the trend of the French judiciary and jurisprudence and some of the Egyptian judiciary decisions. However, the Iraqi judiciary has committed itself to apply the causal relationship criterion adopted by the theory of equivalence of conditions as stipulated in article (29) of the Iraqi Penal Code after its promulgation. One of the decisions issued by the Court of Cassation in Iraq, clearly demonstrate such trend in its this decision, stating that: the kidnap attempt, apart from assault, is a crime in itself and is indeed an act that comes in contravention of law, which caused the victim extreme agitation, and since she was suffering from heart disease, this has resulted in her death. The penal code held the perpetrator accountable for the crime, offence that did not result from his criminal conduct but he is responsible for that offence if, together with his criminal conduct, some other prior, contemporaneous or subsequent cause, even though he was unaware of it, played a part in its commission. Whereas the expert doctor mandated in the case has stated that the psychological agitation or irritability makes the patient heart unable to carry out its task and leads to the acceleration of death, so the court decided to confirm the decision issued in the case, convicting the defendants and sentencing them according to such conclusion.

The mental element of the Crime of copyright infringement on the internet

The mental element is the second pillar of the crime, and it means that the intention of the perpetrator goes to committing the criminal activity and the violation of the law either intentionally or inadvertently. The mental element of the crime has two forms: the first of which is the so-called criminal intent (the vicious will) in which the perpetrator has directed his or her will to committing the criminal conduct aiming to reach the criminal result consequent thereto. The mental element of the crime cornerstone on which the criminal legislator and the judiciary rely in ensuring legal justice and achieving the social purposes of the penalty, since the legal nature of the criminal liability and the penalty sufficient there for are determined based on such element.

Conclusions

The author shall be given two types of rights on his electronic work that are the literary and financial rights, and one of them is likely to over weigh the other considering the different nature of the beneficiary whether the author during his life or his heirs or successors after his death. The crime of infringing legal copyright as other crimes has its corpus delicti and mental element. Committing such crime entails the imposition of original and consequential penalties, and the crime of the author copyright infringement on the internet is deemed as a misdemeanour. Most of the
comparative legislations included controls that clearly describe the outstanding work of the author in the field of arts, literature or other cognitive sciences so that it can be considered as a work covered by legal protection. The author’s copyright is not subject to prescription, but this does not necessarily mean accepting the notion of permanence or eternity. If we accept that the permanent right is not subject to prescription, which means that the right holder or his successor has the right to defend his right before the infringer at any time, and the infringer may not use prescription as a basis for pleading the lapse of the copyright of the author or his successor. However, this idea is different from the permanence, which is intended to protect the right of the author related to his work that applies on an eternal basis and is not limited to any period of time, even if such period lasts for long.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Law, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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Effect of Iron Oxide Nanopaticlal on The FSH, LH and Testesteron Hormones in The offspring of Albino Rats

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Abstract

The study was carried out to investigate the effect of iron oxide nanoparticle(NP) on FSH,LH and Testosterone hormones in the offspring of albino rats. The study included twenty (20) offspring divided into two groups, treated and control group. The results of the hormonal study showed the existence of significant increase (P≤ 0.05) in the mean levels of FSH,LH, Testosterone of offspring treated groups compared with controls.

Keywords: Iron Oxide Nanopartical, FSH, Testesteron Hormones

Introduction

Nanotechnology can be defined as a branch of engineering and science, technology dealing with nanoparticles ultrafine objects in a range between (1 – 100) nm in dimension, the shape and size of these nanomaterial’s can be controlled by finding its way in a wide range of applications 1. Nanoparticles (NPs) are at the forefront of rapid development in nanotechnology, their exclusive size-dependent properties make these materials indispensable and superior in many areas of human activities 2. Being the most current transition metal in the Earth’s crust, iron stands as the backbone of current infrastructure in comparison to group elements such as cobalt, nickel, gold, and platinum are some what neglected 3. Iron and oxygen chemically combine to form iron oxides (compounds), and there are ~16 identified iron oxides, in nature iron (III) oxide is found in the form of rust 4. Generally, iron oxides are prevalent, widely used as they have an imperative role in many biological and geological processes, they are also extensively used by humans, e.g. as iron ores in thermite, catalysts, durable pigments (coatings, paints, and colored concretes) and hemoglobin 5. The three most common forms of iron oxides in nature are magnetite (Fe3O4), maghemite (γ-Fe2O3), and hematite (α-Fe2O3) 6. Hematite is extensively used in gas sensors, catalysts and photocatalysts, magnetism and electrochemical capacitor 7,8,9. Iron oxides with different particle sizes and morphologies have been obtained by a variety of physical and chemical approaches such as chemical precipitation, solvo thermal, pulsed layer ablation, electro-spinning, hydrothermal and sol-gel methods 10,11. Since NPs are incorporated more and more in a variety of consumer products, it is likely that the general public may be exposed to NP-containing products such as personal care products, food and food packaging materials, textiles and medicine 12,13. To develop such insights toxic properties of NPs should be identified and dose-response relationships established, despite the fact that there are a number of publications concerning undesirable effects of NPs various gaps still exist in the knowledge on the intrinsic hazards of NPs the potential human exposures to NPs and the relationship between exposure and adverse health effects (risks) of NPs 14,15,16. Existing information on potential adverse health effects is mainly based on animal experiments although in vitro studies with cell lines have recently added to the insight in potential hazards of NPs, these particles have been shown for example to induce cytotoxic, genotoxic, inflammatory and oxidative stress responses in different mammalian cell lines 17,18,19

Material and Method

Animals:

Thirty six healthy adult female albino rats weight (225±10gm), age (10-12)weeks were purchased from Iraqi Center for Drug Research/ Baghdad. All these animals were housed during the period of experiment in the animal house unit in science college of Babylon University, under controlled temperature (21 ± 1°C)
and constant light-dark schedule (12 hours light and 12 hours dark cycle), food and water were available. The pregnant rats administrated orally (150 mg/kg) of B.W. iron oxide NPs by gavage tube for 21 days of pregnancy until they give birth, they offspring left for tow months until they became adult and then killed, the serum was taken from the offspring for hormonal study.

**Experimental Design:**

Twenty offspring were randomly divided into two groups.

**Group 1:** Control group (n=10).

**Group 2:** Treated group (n=10).

**Blood Samples Collection and Serum Preparation**

To perform the blood biochemical tests, blood sampling was collected from adult offspring (male and female). The blood samples were centrifuged and the serum of samples were separated at 3000 RPM for 10 minutes. The concentrations of LH, FSH and Testosterone of serum specimens were measured according to Cusabio Elisa kit.

**Statistical analysis:**

All data were subjected to a one-way analysis of variance (ANOVA) to determine the level of significance between control and the treated groups. The significance was tested by finding LSD. Data are reported as mean ± standard error (±SE).

**Results**

**Follicles Stimulating Hormone (FSH) level**

The results of our study showed that FSH levels increased significantly (P≤0.05) in the offspring (females and males) (1.581±0.017b, 1.309±0.009b) respectively as compared with control groups (0.814±0.011a, 0.809±0.008a) (Table 1-1).

**Luteinizing Hormone (LH) level**

The results of study revealed significant increased (P≤0.05) in the level of LH in the females of treated groups (1.3608±0.440b) compared with control (0.825±0.220a) while males had non-significant change in the treated groups (1.004±0.1003b) compared with control groups (0.832±0.121b) (Table 1-1).

**Testosterone Hormone level**

The results of our study showed no significant change in the level of testosterone in the offspring of both females and males groups (2.081±2.885a, 0.826±0.005a) respectively compared with control groups (0.510±0.014a, 0.517±0.006a) (Table 1-1).

**Table (1): Effect of iron oxide nanoparticle on LH, FSH and Testosterone level in the offspring of Albino rats.**

<table>
<thead>
<tr>
<th>Hormones (pg/ml)</th>
<th>FSH</th>
<th>LH</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Mean ± S. D</td>
<td>Mean ± S. D</td>
<td>Mean ± S. D</td>
</tr>
<tr>
<td>Females Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control female</td>
<td>0.814±0.011a</td>
<td>0.825±0.2202a</td>
<td>0.5108±0.014a</td>
</tr>
<tr>
<td>Treated female</td>
<td>1.5815±0.017b</td>
<td>1.3608±0.440b</td>
<td>2.0818±2.885b</td>
</tr>
<tr>
<td>Sig. level (P≤0.05)</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>males Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control male</td>
<td>0.8095±0.0089a</td>
<td>0.832±0.1219a</td>
<td>0.517±0.006a</td>
</tr>
<tr>
<td>Treated male</td>
<td>1.3093±0.009b</td>
<td>1.004±0.1003b</td>
<td>0.826±0.0058b</td>
</tr>
<tr>
<td>Sig. level (P≤0.05)</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

*different symbols mean significant differences (P≤0.05).*
Discussion

The present study results showed that there were significant increases (P≤ 0.05) in the levels of FSH, LH and testosterone in offspring. NPs may effect hormone secretion in two ways: 1. NPs pass through the blood-brain barrier into the hypothalamus and secretory cells of the pituitary altering the secretion of GnRH, LH and FSH, thus undermining the normal positive and negative feedback of the hypothalamic-pituitary-gonadal axis and affecting the normal secretion of ovarian estrogen and progesterone. 2. NPs enter the ovaries through circulation and accumulate in theca cells and granulose cells, which effects steroidogenesis. Some in vitro studies showed that some NPs could be swallowed by granulose cells resulting in changes in the secretion of hormones and dysplasia of the ovum, another studies showing that NPs can enter both thecal cells and granulose cells and affect their normal function particularly relating to their key role in hormone secretion. Before ovulation, androgens and androstenedione secreted by theca cells diffuse into granule cells and are transformed into steroid hormones, during this process NPs can directly affect the secretion of sex hormones by destroying these secretory cells in the ovaries. In vivo studies have shown that long-term (90 consecutive days) exposure to titanium dioxide NPs (TiO2 NPs) in female mice results in an imbalance of sex hormones and mineral element distribution leading to a reduction in pregnancy rate and oxidative stress and disruption of ovarian gene expression. Moreover, in vivo experiments in rats showed that silver NPs could get transferred from the mother to offspring through the placenta and breast milk. The neurohormones such as GnRH, follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by the hypothalamus and pituitary play crucial roles in positive and negative feedback regulation during oogenesis, NPs may indirectly effect oogenesis and ovarian health by disturbing the balance of these sex hormones. In which the treated female groups showed significant differences in this parameter comparing to the related control. Those results agree with who found that iron oxide nanoparticle treatment led to increases in the levels of the serum LH in female mice. In contrast to study used copper nanoparticle revealed decrease in the level of sex hormones FSH, LH and testosterone in male rats. In this study, iron oxide nanoparticle treated groups in female and male rats showed significant increases in production of reproductive hormones as compared to control. Another study showed significant increases in the levels of LH and FSH in males of Wister rats using gold nanoparticles. Alterations in testosterone levels leading to impaired male reproductive function that have been investigated in numerous in vivo studies that observed inhalation rats to nanoparticle-rich diesel exhaust (NRDE-NPs) significantly increased testosterone, these findings suggest that the increased levels of testosterone due to the disrupted balance between androgen-metabolizing and testosterone biosynthetic-enzymes. Another attempting to clarify the mechanism responsible for the rise in testosterone levels, research group confirmed that increased testosterone biosynthesis could be attributed to an increase in the mRNA expression of StAR and cytochrome P450 side-chain cleavage (P450scc) an enzyme responsible for the conversion of transported cholesterol to pregnenolone in Leydig cells. Another study investigated the effect of silver nanoparticles consumption instead of drinking water animals from each group were selected and the levels of sex hormones were evaluated also and the level of testosterone increased with “testicle hyperactivity.”

In our study Fe2O3-NPs might have interfered with hypothalomo-hypophyseal-testicular axis as a potential endocrine disruptor contributing to the increased serum testosterone levels as reported by. As evidenced from our results the iron content in the serum and testes revealed that Fe2O3-NPs accumulated in both, therefore the Fe2O3-NPs crossed and entered the blood tested barrier as reported in mice by.

Conclusion

The study included twenty (20) offspring divided into two groups, treated and control group. The results of the hormonal study showed the existence of significant increase (P≤ 0.05) in the mean levels of FSH, LH, and Testosterone of offspring treated groups compared with controls.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Collage of Science, Babylon University, Iraq and all experiments were carried out in accordance with approved guidelines.

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Genetic variability of the Mite *Varroa destructor* Isolated from Honey Bees in Iraq and Some Middle Eastern Countries

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Abstract

This study was conducted to isolate and diagnose different isolates of *Varroa* parasite collected from different regions of Iraq, Iran, Turkey, Syria, Egypt, and Jordan. The different *Varroa* isolates were identified using the polymerase chain reaction (PCR) technique to amplify the mitochondrial cytochrome oxidase gene I (mtCOI) to determine the nucleotide sequences generated from these isolates. These nucleotide sequences were analyzed to identify the similarities and differences in the amplified gene regions. The results obtained from the nucleotide base-sequence analysis indicated that all isolates of *Varroa* parasite collected in this study were *Varroa destructor*. Furthermore, the results showed differences in the nucleotide sequences resulting from the amplification of the target mtCOI by PCR. The isolates from Egypt (No.12) and from Syria (No.14) were the most genetically different among the other isolates in this study (17 samples). By the phylogenetic tree analysis, isolates of parasites numbered with 12 and 14 appeared in separate clades from the other clades of isolates under study. Besides, these isolates (No.12 and No.14) showed differences to other isolates of the same parasite formerly recorded at NCBI. The *V. destructor* isolate of Egypt (12) and Syria (No.14) showed a genetic similarity of 99%.

Key words: Honey bees, *Varroa destructor*, Apiculture in Iraq, Polymerase chain reaction.

Introduction

Bees have an important role in the environment by pollinating both wildflowers and many agricultural crops as they forage for nectar and pollen, in addition to producing beeswax and honey. Like all other living organisms, honey bees are subject to many pests and pathogens that harmfully affect their behavior and production. Among the most important of these pests is Varroa (Varroa destructor), which invaded Iraq for the first time in 1985 and was officially registered as an epidemic pest to honey bees (Apis mellifera L.) in 1987, known then as Varroa jacobsoni (M.H.E.S.R, 1987, 12). The registry was modified afterwards to Varroa destructor according to 16. The Varroa parasite is affected by geographical location and natural climatic conditions, especially temperature, humidity, and others. This may lead to genetic mutations that contribute to causing morphological, physiological and behavioral changes allowing for their continual reproduction (1,7,10,13,17). According to observations by beekeepers, there are new species of Varroa as confirmed by the Iraqi Ministry of Agriculture could be because of imported bees infected with Varroa parasite. 16.

Materials and methods:

Sample Collection

Seventeen samples of *Varroa* parasites were collected directly from the infected bee colonies of ten living parasites from adult *Varroa* females for each sample. They were placed in a separate tube of ethyl alcohol (95%), brought to the Department of Plant Protection/ Faculty of Agriculture/ University of Kerbala, and kept at 4°C until the DNA was extracted.
The samples were collected from different regions of south, central and northern Iraq, as well as samples of bees imported into Iraq. Other samples were obtained from some countries of the Middle East, including neighboring countries (Iran, Turkey, and Syria), where bees can enter Iraq naturally by swarming or male travelling (Table 1 and Fig. 1). The other method of transmission is the beehive trade, often from three countries (Iran, Turkey, and Egypt), while a few bees enter from Jordan, often bee queens, often in informal ways.

Table (1) Sites and coordinates Varroa sampling for molecular diagnosis.

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Country</th>
<th>Place</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Sample date</th>
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<tr>
<td>1</td>
<td>Iraq</td>
<td>Dohuk</td>
<td>37°07'54.0&quot;N</td>
<td>42°42'13.5&quot;E</td>
<td>14/04/2018</td>
</tr>
<tr>
<td>2</td>
<td>Iraq</td>
<td>Najaf</td>
<td>32°05'07.0&quot;N</td>
<td>44°34'39.8&quot;E</td>
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<tr>
<td>3</td>
<td>Iraq</td>
<td>Sulaimanya (Egyptian product)</td>
<td>35°34'55.1&quot;N</td>
<td>45°16'09.4&quot;E</td>
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<tr>
<td>4</td>
<td>Turkey</td>
<td>Sharnaq</td>
<td>37°15'08.8&quot;N</td>
<td>42°23'36.3&quot;E</td>
<td>03/04/2018</td>
</tr>
<tr>
<td>5</td>
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<td>Karbala</td>
<td>32°35'09.7&quot;N</td>
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<td>25/02/2018</td>
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<td>Muthanna</td>
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<td>30°44'38.2&quot;N</td>
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<td>8</td>
<td>Iraq</td>
<td>Diwaniya</td>
<td>32°03'21.7&quot;N</td>
<td>45°08'50.7&quot;E</td>
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<tr>
<td>9</td>
<td>Iran</td>
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<td>30°43'30.3&quot;N</td>
<td>48°27'00.4&quot;E</td>
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</tr>
<tr>
<td>10</td>
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<td>Erbil</td>
<td>36°06'58.0&quot;N</td>
<td>44°00'55.0&quot;E</td>
<td>15/03/2018</td>
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<td>Jordan</td>
<td>The Jordan Valley</td>
<td>31°59'42.3&quot;N</td>
<td>35°35'09.2&quot;E</td>
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</tr>
<tr>
<td>12</td>
<td>Egypt</td>
<td>Kafr El Sheikh</td>
<td>31°13'03.6&quot;N</td>
<td>30°33'17.8&quot;E</td>
<td>13/12/2017</td>
</tr>
<tr>
<td>13</td>
<td>Iraq</td>
<td>Najaf (Iran product)</td>
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<td>44°28'13.1&quot;E</td>
<td>20/01/2018</td>
</tr>
<tr>
<td>14</td>
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<td>Rif Dimashq</td>
<td>33°28'11.5&quot;N</td>
<td>36°21'44.9&quot;E</td>
<td>13/12/2017</td>
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<td>Dhi Qar</td>
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<td>46°17'33.7&quot;E</td>
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<td>16</td>
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<td>Wasit</td>
<td>33°03'12.3&quot;N</td>
<td>44°35'52.5&quot;E</td>
<td>02/02/2018</td>
</tr>
<tr>
<td>17</td>
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<td>Kirkuk</td>
<td>35°34'33.6&quot;N</td>
<td>44°21'52.6&quot;E</td>
<td>15/03/2018</td>
</tr>
</tbody>
</table>

Figure (1) Map shows sites of Varroa Sampling, Source: The researcher by GIS software, ArcMap 10.5
Genomic DNA Extraction and PCR Amplification of mitochondrial cytochrome oxidase I (mtCOI) in Varroa genome

To extract DNA from the Varroa parasite, a DNA extraction kit (Favorgen, Cat No. FAPGK 001) was used following the steps recommended by the manufacturer. PCR was performed using the Maxime PCR PreMix (i-Taq) kit (Cat. No. 25026) with the primer pair (CO1-F: TTGATTTTTGTCATCCAGAAGT and CO1-R: TCCAATGCCTAATCTGCCATATTA) to amplify the mitochondrial cytochrome oxidase I (mtCOI) gene.

The target region of Varroa genome was amplified for all samples to be diagnosed using the following PCR steps and conditions: Initial DNA denaturation for 5 minutes at 94°C, followed by 35 cycles each consisting of final denaturation for 30 seconds at 94°C, primer annealing for 30 seconds at 58°C and then an initial extension of the amplified PCR product for 1 min at 72°C with the completion of PCR steps by one cycle of the final extension at 72°C for five minutes. PCR-amplified products were electrophoretically separated on a 1% agarose gel for 140 min at 80 V, 400 mA and visualized with ethidium bromide staining under UV illumination and images were captured using Vilber Lourmat, Taiwan gel documentation system.

Nucleotide sequence analysis of DNA of Varroa Samples

To identify the nucleotide sequences of the PCR-amplified products of the various Varroa samples, the PCR products were sent to the Korean Macrogen company with both forward and reverse primers (CO1-F/R) used in DNA amplification to determine the species of Varroa parasite in the sample collection sites, and the degree of similarity and difference in the sequences of the nucleotide sequences of the amplified DNA of Varroa. The nucleotide sequences of the DNA product was processed into the database available at the National Biotechnology Information Center (NCBI) using the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was constructed using the MEGA6 program, using the Neighbor-joining method.

Results and Discussion

Molecular diagnosis of Varroa Isolates

The results of DNA extraction from Varroa isolates and their processing (PCR) showed the possibility of replicating the PCR-amplified products and the expected size (approximately 880 nitrogen bases) using the forward and reverse buffer (mtCOI_F/R) (Fig.2).

Figure (2) PCR-amplified products, using the mtCOI_F / mtCOI_R (PCR) pair of Varroa isolates from Duhok (1), Najaf (2), Sulaymaniyah (imported bees) (3), Turkey (4), Karbala (5), Al Muthanna (6), Basrah (7), Diwaniyah (8), Iran (9), Erbil (10), Jordan (11), Egypt (12), Najaf (imported bees) (13), Syria (14), Thiqar (15), Wassit (16), and Kirkuk (17). M= 1Kbp DNA ladder marker. NC: Control treatment (without adding DNA to the other PCR components). The results of the nucleotide sequence analysis of the amplified DNA products for Varroa isolates using BLAST showed that all isolates of the current study belong to V. destructor. The isolates from Egypt (No.12) and Syria (No.14) gave the most different in the nucleotide sequences with 99% similar with the other isolates belonging to the same parasite isolated in this study from different regions in some Middle Eastern countries (Table 2 and Fig. 3).
Figure (3) Similarity and difference in nucleotide sequence alignments of PCR-amplified products of isolates from Duhok (No. 1), Egypt (No. 12), and Syria (No. 14)
The results of the nucleotide sequence analysis for *Varroa* isolates from Egypt (No.12) and other isolates recorded at NCBI indicated that there is a difference in the nucleotide sequences, wherein the genetic similarity ranges between 86-99%. The Neighbor-joining tree analysis showed the isolate (No.12) was appeared in a separate clades from other isolates previously recorded at NCBI (Figure 5).

The results also of the nucleotide sequence analysis of the PCR-amplified mtCOI gene of *Varroa* isolates from Syria (No. 14) showed the presence of a clear genetic variation in the nucleotide sequences with the nucleotide sequences of the other isolates previously recorded at NCBI giving a similarity of 99%. The Neighbor-joining tree analysis showed that the isolate No.14 was appeared in a separate clade from the other clades of the same parasite previously registered in NCBI (Figure 6).

In the current study, PCR was used to diagnose 17 different isolates of the *Varroa* parasite collected from different parts of Iraq and some Middle Eastern countries by targeting the mitochondrial cytochrome oxidase I (mtCOI). PCR has been used efficiently in the diagnosis of many organisms; microorganisms (such as fungi) and insects (*Bemisia tabaci*) to avoid diagnostic problems based on morphological characters. Although phenotypic diagnosis is useful in categorizing the organisms under study into smaller groups before starting other methods of diagnosis, there are many problems associated with the phenotypic diagnosis of organisms, including the need of the diagnosis conductor to the high experience, especially for similar fungal species as well as the need for long time and substantial efforts. There are also other factors that may affect these morphological characters, including the type and nature of growth, moisture and light that can affect some of the morphological characteristics of the insect. Several researchers have also used the difference
between the mtCOI-based sequence to diagnose Varroa isolates from different regions of the world using PCR technology (6,8,22,23).

**Conclusion**

The results obtained from the nucleotide base-sequence analysis indicated that all isolates of Varroa parasite collected in this study were Varroa destructor. Furthermore, the results showed differences in the nucleotide sequences resulting from the amplification of the target mtCOI by PCR. The isolates from Egypt (No.12) and from Syria (No.14) were the most genetically different among the other isolates in this study (17 samples). By the phylogenetic tree analysis, isolates of parasites numbered with 12 and 14 appeared in separate clades from the other clades of isolates under study.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Plant Protection/ Faculty of Agriculture/ University of Kerbaand all experiments were carried out in accordance with approved guideline.

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Effect of Preoperative Breathing Exercise on Postoperative Patients’ Lung Functions

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Abstract

Pulmonary complications after surgery are a major cause of morbidity and mortality and therefore are a source area of concern in both developed and developing countries. Presently, surgical patients didn’t acquire education about deep breathing activities that were adequate and many of them only received some education post operation. Aim of the study: To investigate the Effect of Preoperative Breathing Exercise on Postoperative patients’ Lung Functions. A quantitative quasi-Experimental, Pre-Test and Post-Test design. A non-probability (purposive sample) of (60) patients divided into two equal groups distributed as the case and control groups (30) patients for the case group are exposed to the breathing exercise program, Pursed-lip method, and (30) patients without exposed to the breathing exercise program categories as control group. The study have been carried out in Al-Diwaniyah teaching hospital, This study conducted from 22nd of October 2018 to 25th of June 2019. There were statistically significant difference between case and control groups, patients in the case group had improvement in all parameters of lung functions than control group (P < .05) in the two periods of measurement post-operatively. The program show obvious difference between the lung function for both study and control groups during the post-test.

Keywords: Preoperative, Breathing Exercise, Postoperative, patients’ Lung Functions.

Introduction

Impairment in lungs functions after surgery are one of important postoperative complications, strength of respiratory muscles could be decreased and that one reason lead to lung function change 1.

During surgery, using of general anesthetics can affect the central regulation of breathing by changing the neural drive to respiratory muscles such as the diaphragm and because of this, the respiratory function cannot be separated from anesthesia 2. The components that can alter the pulmonary function contain unconsciousness, ventilation (whether its mechanical or spontaneous), a patient’s position, anesthetics drugs, that were used during the anesthetic process on the respiratory muscles 3. General anesthesia can alter mucociliary function, promoting retention of secretions, causing bronchoconstriction, decreased surfactant production, inhibition of alveolar macrophage activity. Moreover, anesthesia may cause atelectasis instantaneously in majority of the patients as an outcome of chest wall deformation, decreased inspiratory muscle tone, and reduced functional residual capacity (FRC) which may considerably influence gas exchange 4.

The effect of these factors on pulmonary function are appear in the early postoperative period. Lung function, measured as vital capacity (VC) and forced expiratory volume in one second (FEV1), is usually decreased by 35-60%, and a 6-13% Decreased lung function can linger for four months. In the first days following an operation, a decreased lung function can impact impaired gas exchange, and the strength of the respiratory muscle will also decrease during the first days after surgery 5. This may be explained by insufficient diaphragmatic breathing or by the respiratory fatigue that some patients experience during the post-operative period 6. The complications that related to anesthesia, tissue damage, immobilization, inhibition of cough, and incision of abdominal muscles which results in postoperative pain, major causes to decreased the lung volumes and limited airway clearance, which can lead to Post-operative pulmonary complications(PPC) 7-9.

Methodology

Study Design:
A quantitative quasi-Experimental, Pre-Test and Post-Test design have been carried out to study the effect of preoperative breathing exercise on postoperative patients’ lung functions, in Al-Diwaniyah teaching hospital, This study conducted from 22nd of October 2018 to 25th of June 2019.

Setting of the Study:

The study is conducted in Al-Diwaniyah City, Al-Diwaniyah Health Directorate, Al-Diwaniyah teaching hospital, Surgical Wards. This hospital is a governmental, and the largest general hospital, and presents different of the medical therapeutic services in Al-Diwaniyah city.

Study Sample:

A non-probability (purposive sample) of (70) patients undergoing abdominal surgery. (10) patients of the sample for pilot study and (60) patients of the sample is divided into two equal groups distributed as the case and control groups (30) patients for the case group are exposed to the breathing exercise program, Pursed-lip method, and (30) patients without exposed to the breathing exercise program categories as control group.

Criteria for Including the Sample:

1. Adult patients age from 20 years and above.
2. Patients undergoing abdominal surgery under general anesthesia.
3. Stable condition as reported by the physician.
4. Patients with normal respiratory and cardiac functions.

Validity of the Instrument:

The face validity of the study instruments are determined by a panel of (16) experts, have experience more than ten years, from different specialties from nursing faculties.

Reliability of Instrument:

In order obtains the reliability of the study instrument five patients undergoing abdominal surgery are selected from Surgical wards for the purpose of testing the instrument their lungs capacities (FEV1, FVC, FEV%) was measured in preoperative period by used two spirometers at one time, one of them is used in the present study and the other used at Al-Diwaniyah teaching hospital. A comparison between the results of them, The result indicate, spirometers which used in present study is a reliable instrument to measure the purpose of the study.

Through developed questionnaire for Arabic version, demographic and clinical data were collected. While physiological data (Lungs volume) are collected by using the pulmonary function test machine (Portable Diagnostic Spirometer, Mir, Italy) through preoperative and postoperative assessment. During preoperative phase, the assessment was obtained ( before one day of surgery for case group and, in the same day for control group), while in the post-operative phase, the assessment was done at 8th hours and 24th hours after surgery based on the previous scientific references such as Regarding case group the assessment of the lungs volumes applied before and after the application of the program. For the control group, the assessment was performed only without the application of the program. The data collection process has been performed from 11th January to 14th March 2019.

The data have been collected through the use of a constructed questionnaire tool. The data analysis was done through statistical package of social science (SPSS) version (25). The methods of analysis of data include; Descriptive Data Analysis and Inferential Data Analysis
### Results

#### Table (1) Clinical Data of Case and Control Groups  n= 60

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>Rating And Intervals</th>
<th>Case group</th>
<th></th>
<th>Control group</th>
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</tr>
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<td></td>
<td></td>
<td>Freq.</td>
<td>%</td>
<td>Freq.</td>
<td>%</td>
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<td>8</td>
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<td>Duration of smoking (years)</td>
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<td>16.7</td>
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<td>30 and more</td>
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<td>100</td>
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<td>10</td>
<td>1</td>
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<td>1</td>
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<td>100</td>
<td>13</td>
<td>100</td>
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<td>2</td>
<td>6.7</td>
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<td>40</td>
<td>8</td>
<td>26.7</td>
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<tr>
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<td>Hernia</td>
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<td>10</td>
<td>8</td>
<td>26.7</td>
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<tr>
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<td>Gastric surgery</td>
<td>4</td>
<td>16.7</td>
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<td>10</td>
</tr>
<tr>
<td></td>
<td>Others</td>
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<td>33.3</td>
<td>9</td>
<td>30</td>
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<td>80</td>
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<td></td>
<td>&gt;1:30</td>
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<td>33.3</td>
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<td>20</td>
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<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>
Table (1) represent the clinical data of the case and control groups. Regarding body mass index (BMI) the table shows most of the sample in both groups are overweight (46.7%) in control group while (43.3%) in case group. In smoking status condition the table shows the majority in control group (76.7%) is nonsmoker and (70%) in the case group. Regarding type of smoking in both groups are cigarette smokers with duration of smoking (66.6%) with period of (10-19) years for control group and (57.1%) for case group who spend more than 30 years in smoking. Regarding chronic disease the study results presents that chronic disease (60%) of case group with chronic disease and (66.7%) in control group with chronic disease(50%) of both group with hypertension. Regarding previous surgery (50%) in case group have previous surgery (20%) of them with Caesarian surgery and about control group (56.7%) have previous surgery (16.7%) of them with Caesarian surgery. Regarding type of present surgery the majority of the case group (40%) have cholecystectomy surgery, and (26.7) of control group have share cholecystectomy and hernia surgery. Concerning duration of surgery (80%) in control group is more than 1:30 hours while the case group is (66.7%).

Table (2) Differences Between the Lungs Volumes for the Case and Control Group Through Pre-Operative Periods n=60

<table>
<thead>
<tr>
<th>Lungs volumes</th>
<th>Pairs</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>T-value</th>
<th>d.f,</th>
<th>p-value</th>
</tr>
</thead>
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<td>FEV1</td>
<td>Case</td>
<td>3.3173</td>
<td>30</td>
<td>0.81448</td>
<td>0.311</td>
<td>58</td>
<td>0.757</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.2493</td>
<td>30</td>
<td>0.87842</td>
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<td></td>
<td>NS</td>
</tr>
<tr>
<td>FVC</td>
<td>Case</td>
<td>3.9960</td>
<td>30</td>
<td>1.02234</td>
<td>0.149</td>
<td>58</td>
<td>0.882</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.9577</td>
<td>30</td>
<td>0.97663</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>FEV1%</td>
<td>Case</td>
<td>81.9163</td>
<td>30</td>
<td>4.12529</td>
<td>0.618</td>
<td>58</td>
<td>0.539</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80.9903</td>
<td>30</td>
<td>7.09822</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Table (2) shows that is non-significant difference between the levels of all the lungs’ volumes between the case and control group at the pre-test in pre-operative period.

Table (3) Differences Between two Periods of Measurements of Lungs Volumes for Case and Control
Table (3) shows there are statically differences between studied groups according level of lung volume, P-value were less than 0.006) in two period of measurement post-operatively).

Table (4) Correlation between Lungs Volume and Socio-Demographic and Clinical Data of Case Group in Post-test 2 (after24 hour) N= 30

Table (4) shows there are a strong negative correlation between patient’s lungs volumes (FEV1, FVC,) and age (r=-0.895;0.001) (- 0.619; 0.001) respectively, FEV1 and body mass index (r = 0.722; 0.041), as well as a significant correlation among other studied parameters.

<table>
<thead>
<tr>
<th>Rating and Intervals</th>
<th>lungs volumes</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>FEV1</td>
<td>-0.895</td>
<td>0.001 HS</td>
</tr>
<tr>
<td></td>
<td>FVC</td>
<td>-0.619</td>
<td>0.002 HS</td>
</tr>
<tr>
<td></td>
<td>FEV%</td>
<td>-0.416</td>
<td>0.003 HS</td>
</tr>
<tr>
<td>Body mass index</td>
<td>FEV1</td>
<td>-0.722</td>
<td>0.041 S</td>
</tr>
<tr>
<td></td>
<td>FVC</td>
<td>-0.426</td>
<td>0.016 HS</td>
</tr>
<tr>
<td></td>
<td>FEV%</td>
<td>-0.399</td>
<td>0.001 HS</td>
</tr>
</tbody>
</table>

Discussion

Regarding Table (1) there was a high percentage of patients at age groups (50-59) years old, and the majority of study samples were male. This could be agreeable with their study have the same range age group, and the gender was also mainly male. The present results show
that most patients are primary school graduate, many people might live with number of social and economic barriers to stressful and conflict environment, this can prevent them for achieving higher educational levels, this result could be supported by 12 majority of their study are primary school graduate were (78.5%). It is show that there is non-significant difference between both groups in the baseline pulmonary functions parameters mentioned in the pre-operative period. This may be due to both groups were had resemble characteristic and functionally comparable to each other. The present result is similar to a study conducted by (13,14) are similar with the result of present study, and the pulmonary function test parameters did not differ between two groups before operation. After the application of the breathing exercise pursed lip methods through the present study, the results indicated that there is an improvement in the case group lungs volumes compared with those patients in the control group. The result shows that there is a significant difference between groups. Patients in the study group who demonstrate breathing exercise had a higher pulmonary function in all parameters than in control group at P- value less than 0.05 in all the post-operative periods. The applied method is an effective way to improve the post-operative patients’ lung function. The results of this study agree with the observations of 15, they concluded that the pre-operative deep breathing exercise improves the pulmonary functions, with significantly improved for lungs parameters such as FVC, FEV1 at P-value = 0.003, and P- value less than 0.001 respectively). The study shows that there is a relationship between the patient’s lung volume with their age and body mass index at p-value less than 0.05 after 24 hours postoperatively, while the other results indicate there is no relationship between patients lungs volumes and other demographic and clinical data at p-value more than 0.05. Pulmonary function may decrease and affected by age. The rate of respiratory and blood circulation are increases in childhood and become in a maximum level at age group between (20–30) years, then decline again in the older age. About high body mass index there are some reasons for decline the lung function. Firstly, the diaphragm position in the thoracic cavity is elevated obviously when individual increase weight. Secondly, it appears that fat accumulation on the chest wall will impede the movement of thoracic cage by a direct resistance or the abnormal function of intercostal muscle (16,17).

**Conclusion**

There were no differences between lung function in both case and control groups in the pretest. There is an improvement in the postoperative patients’ lungs function in study group after exposure to an educational breathing exercise program. The program show obvious difference between the lung function for both study and control groups during the post-test. Control group does not present any modification in their lung function post-operatively. In addition, there is relation between study groups and demographical data in age and body mass index only.

**Conclusion**

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of Kufa. Faculty of Nursing, Iraq and all experiments were carried out in accordance with approved guidelines.

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Glomerular Diseases: Systemic Lupus Erythematosus is Most Common Finding: A Cross-Sectional Study

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Abstract

The objective of this study was to assess the connection amongst’s immunological and histopathological findings of renal biopsy acquired from patients with glomerular diseases. This cross sectional study was done in Al-Kafîl private hospital and included 110 patients who were diagnosed by nephrologist as having glomerulonephritis as per clinical introduction, physical examination and laboratory findings of biopsy. The age scope of patients enlisted in the present investigation was between 2.8 to 72 years. The study included 67 male patients (60.9%) and 43 female patients (39.1%). The examination began at January 2016 and reached out through January 2017. At the point when clinical introduction and the entire workup of examination were at long last joined, with exceptional accentuation on histopathology and immunofluorescent tiny examination, the accompanying classification was gotten. Cases with glomerular association optional to foundational lupus erythematosis represented 27 out of 110 (24.5%). Consequently lupus nephritis is the most incessant reason for glomerulonephritis observed in the present examination. As indicated by the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) grouping of lupus nephritis, the most incessant compose was ISN/RPS class IV (A/C) represented 14 case (12.7%), trailed by ISN/RPS class III (A/C) (8 case, 7.3%)

Key words: Glomerular disorders, SLE, immunoflourescent, Iraq

Introduction

The kidney in spite of the fact that being moderately little size in examination with add up to body weight plays out a great deal of capacities that keep human body at ideal physiological conditions. These capacities incorporate water and electrolyte adjust, corrosive base direction, endocrine capacities and discharge of waste items, for example, urea and creatinine 12. Infection influencing the kidney may include the veins, glomeruli and tubulointerstitial compartments. Glomerular sicknesses represent a huge extent of dreariness and mortality in the populace 11. Glomerular sicknesses are regularly because of of some type of immunological affront. Immunological bases for glomerular damage can be expected do humeral or cell invulnerable reaction 7. Humeral resistant reaction includes a counter acting agent that is independent from anyone else poisonous to certain segment of the glomerulus, for example, Good Pasteur disorder in which the immunizer is coordinated against glomerular cellar layer 15. Then again humeral reaction might be because of affidavit of insusceptible edifices that are either framed in situ or effectively flowing in blood, for example, glomerular damage found in relationship with foundational lupus erythematosis (SLE) 13. Likewise, humeral insusceptible reaction might be because of enactment of elective supplement pathway 4. The established introduction of glomerular sickness is as proteinuria and/or hematuria 5. The time of beginning is exceptionally factor and is amazingly wide so glomerular ailment might be found in youngsters, immature, youthful grown-up and elderly people. The
particular order of the sort of glomerular damage is basic for both treatment system portion and guess of the sickness to be cleared up. For distinct conclusion of glomerular ailment, renal biopsy ought to be performed. Three principle steps take after renal biopsy got for suspected glomerular ailments; these are normal and extraordinary histological stains, electron minute examination and immunofluorescent tiny examination. The innovation of tissue immunofluorescent strategy, by Coons and Kaplan in 1950 and its application on renal biopsy by Mellors in 1955, constrained the requirement for electron microscopy in symptomatic renal biopsy. Immunofluorescence allows the recognizable proof of the guilty party immunoglobulin (IgG, M and An) and furthermore the included supplement segment (C3 and C1q) and furthermore allow the distinguishing proof of safe testimony whether membranous or mesanginal. In Iraq there is extremely set number of concentrates that managed this subject and the greater part of studies took a set number of cases and was constrained to a solitary or various glomerular issue. The vast majority of these examinations featured the histological adjustment as well as clinical angles. The absence of an Iraqi immunologic investigation that arrangements with extensive example and different sorts of glomerular infections defended the conduction of the streams think about.

The Aim of this study was to assess the connection amongst’s immunological and histopathological discoveries of renal biopsy acquired from patients with glomerular infection.

Patients and method

This cross sectional examination was done in Al-Kafif private doctor’s facility and included 110 patients who were analyzed by nephrologist as having glomerulonephritis as per clinical introduction, physical examination and discoveries of research center examinations. The age scope of patients enlisted in the present investigation was between 2.8 to 72 years. The example included 67 male patients (60.9%) and 43 female patients (39.1%). The examination began at January 2016 and reached out through January 2017. The procedure of patients’ determination begun at the nephrology meeting room. Any patient having the accompanying highlights was incorporated into the present investigation:

- Patients with regular picture of nephritic disorder
  - Massive proteinuria, summed up edema, hypoalbuminemia, hyper-lipidemia and lipiduria.
- Patients with regular highlights of nephritic disorder
  - Hematuria, hypertension, azotemia and oliguria
- Patients with highlights that are not suggestive of glomerular association; in any case, urinalysis of them indicated critical proteinuria as well as glomerular hematuria
- Patients with intense or constant disappointment with extra clinical highlights suggestive of glomerular malady

Any patient with highlights that are not suggestive of glomerular sickness and have no proof of huge proteinuria and/or glomerular heamturia was barred from the investigation. Routine histological appraisal, immunoflourescent ponder were the fundamental examination to which all patients were subjected, adjacent to hematological, serological, biochemical examinations and urinalysis.

Results

Demographic characteristics of the study sample

The present study included 110 patients with glomerulonephritis, 67 male patients (60.9%) and 43 female patients (39.1%) with a male to female ratio of 1.56:1. Mean age of patients enrolled in the current study was 31.28 ±13.83 years and it ranged from 2.8 to 72 years, as shown in table 4.1. According to 10 years age intervals patients were distributed as following: 2 patients less than 10 (1.8%), 21 patients from 11.20 (19.1%), 39 patients from 21.30 (35.5%), 23 patients from 31.40 (20.9%), 13 patients from 41.50 (11.8 %), 6 patients from 51.60 (5.5%), 5 patients from 61.70 (4.5%) and a single patient older than 71 (0.9%), as shown in figure 1.
Mean duration of disease is $1.88 \pm 1.66$ years and it ranged from 2 weeks to 7 years. Patients with disease duration of less than one year accounted for 34 (30.9%), patients with 1 to less than 2 years accounted for 19 (17.3%), patients with disease duration of 2 to less than 3 years accounted for 17 (15.5%), patients with disease duration of 3 to less than 4 years accounted for 22 (20%), patients with disease duration of 4 to less than 5 years accounted for 9 (8.2%), patients with disease duration of 5 to less than 6 years accounted for 7 (6.4%), patients with disease duration of 6 to 7 years accounted for 2 (1.8%).

**Lupus nephritis**

Cases with glomerular involvement secondary to systemic lupus erythematosus accounted for 27 out of 110 (24.5%). Hence lupus nephritis is the most frequent cause of glomerulonephritis observed in the present study. According to the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification of lupus nephritis, the most frequent type was ISN/RPS class IV (A/C) accounted for 14 case (12.7%), followed by ISN/RPS class III (A/C) (8 case, 7.3%) and lastly by ISN/RPS class III (A) (5 cases, 4.5%). Immunofluorescent study is shown in figure 2 and 3 in which there was mild diffuse segmental granular mesangial deposition of IgG and mild diffuse segmental granular mesangial deposition of IgM.

**Discussion**

In this investigation Cases with glomerular association optional to foundational lupus erythematosis were the most regular and represented 27 out of 110 (24.5%); the most incessant write was ISN/RPS class IV (A/C) represented 14 case (12.7%), trailed by ISN/RPS class III (A/C) (8 case, 7.3%) and ultimately by ISN/RPS class III (A) (5 cases, 4.5%). In one Iraqi investigation, it was demonstrated that 25 (45.5%) cases were because of lupus nephritis and in another Iraqi examination just two cases (3.4%) satisfied the clinical, serological and histopathological criteria of lupus nephritis. We concur with that lupus nephritis is the most incessant reason for glomerulonephritis in Iraq and can’t help contradicting who depicted a predetermined number.
for lupus nephritis in his investigation. In another examination in India, 3 assessed the clinicopathologic parts of crescentic glomerulonephritis and found that 14.7% of cases were because of SLE. Lupus renal malady gives off an impression of being more common in certain ethnic gatherings 10, and this may clarify the high rate of lupus nephritis in Iraqi patients. A similar investigation of SLE in three ethnic gatherings announced that renal malady, which is characterized by American College of Rheumatology (ACR) criteria as persevering every day proteinuria of >500 mg within the sight of cell throws or biopsy proof of lupus nephritis, happened in 45% of African Americans, 42% of Chinese, and 30% of Caucasian patients, individually 10. Another multi-ethnic US companion of SLE patients revealed that renal malady happened in 51% of Africans and 43% of Hispanics however in just 14% of Caucasians 2. In a planned investigation of 216 Chinese patients with new beginning SLE, 31% patients had dynamic renal sickness as the underlying introduction. 8. The general combined frequency of renal sickness was 60% at 5 years post-SLE finding. These investigations delineated that lupus renal association is more typical in Africans, Hispanics, and Chinese than in Caucasians 9.

Conclusion

At the point when clinical introduction and the entire workup of examination were at long last joined, with exceptional accentuation on histopathology and immunofluorescent tiny examination, the accompanying classification was gotten. Cases with glomerular association optional to foundational lupus erythematosis represented 27 out of 110 (24.5%). Consequently lupus nephritis is the most incessant reason for glomerulonephritis observed in the present examination. As indicated by the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) grouping of lupus nephritis, the most incessant compose was ISN/RPS class IV (A/C) represented 14 case (12.7%), trailed by ISN/RPS class III (A/C) (8 case, 7.3%) and in conclusion by ISN/RPS class III (A) (5 cases, 4.5%). In Iraq, SLE is the fundamental driver of glomerular damage.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Hindiyah primary health care centre / Karbala province / Iraq and all experiments were carried out in accordance with approved guidelines.

References


Synthesis of Diimidazole Has Pyrazole, Isoxazole and Pyrimidine Derivatives and Evaluation as Antibacterial

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Abstract

New diimidazole contains pyrazole, isoxazole and thiopyrimidine were synthesized by using simple methods. All compounds were synthesized by using hippuric acid (1) as stating material which was obtained from benzoyl and glycine with. pyrazole derivatives (6a – b ) was obtained from reaction (5a – b) with hydrazine hydrate while reaction of (5a – b) with hydroxyl amine isoxazole derivatives (7a – b) were obtained, finally reaction of thiourea was reacted with 5a – b thiopyrimidine derivatives were get (8a –b) . FTIR and 1HNMR spectra were used to characterized derivatives several bacterial species like Pseudomonas aeruginosa, Staphylococcus aureus, and Acinetobacter baumanii were used to tested antibacterial activity

Keywords: pyrazole, isoxazole, imidazole, thiopyrimidine

Introduction

Heterocyclics were found good biological molecules like imidazole 1 molecules with five and six membered rings mostly are high activity 2. All natural products with heterocyclics are important in biologically active system 3 and medicinal to approved drugs. imidazole derivatives in heterocyclic chemistry considered most important compounds and drugs 4 such as Antioxidant activity 5 anti-microbial (6-7), antiangiogenic 8 , antitumor 9 , analgesic 10 . pyrazoles and isoxoles play important role to synthesis biologically active drugs like Antifungal 11 , antimicrobial 12 anticancer 13 , analgesics 14 , Ant helminthic 15 , Ant pathogenic 16, anti-inflammatory 17

Method

Synthesis of [(phenyl carbonyl)amino]acetic acid(1)

Glycine (0.1mol ) in 15ml of 1N NaOH was cooled to 5 and then benzoyl chloride (0.1 mole ) was added to cold drop wise . The reaction mixture was stirring for one hour. The aqueous layer was acidified with 2N HCl and the product was collected and recrystallized from ethanol and product was collected as white powder

Synthesis of (Z)- 2-phenyl -4-benzylidene oxazol-5(4H)-ones (2a - d)

To a mixture of compound (1) ( 0.01 mole ), acetic anhydride (20 ml) and acetic acid (5 ml), (0.01 mol) of aromatic aldehyde was added. And the mixture was refluxed for 4hr and the temperature was reached to 80°C. The reaction mixture was allowed to cool. , then poured into crushed ice and it was allowed stirred for 30 min. the product was recrystallized from ethanol.

Synthesis of 3-(2-amino)-5-arylidine-2-phenyl-3,5-dihydro-4H-imidazol-4-one (3a – d)

To a mixture of (2a – d) derivatives (0.01 mole) in (20ml) triethyl amine , (0.01 mole) of hydrazine was added. And then it was refluxed 2 h. after that the mixture was cooled to RT. And the desired compound was obtained after recrystallizing from ethanol.

Synthesis of 1-[(4Z)-4-(arylidene) -5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl] thio urea (4a – d)

Potassium thiocyanate was added to a mixture of compounds [3a - d] (0.01 mole) in ethanol (27 ml) and then stirred for 30 min. the mixture was  poured in to could water and then filtered, the solid (desired compound) was recrystallized from ethanol.

Synthesis of (5Z)-5-(4-chlorobenzylidene)-3-(4-oxo-2-thioxoimidazolidin-1-yl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (5a – d)
To solution of (4a – d) (0.01 mole), in 30 ml of dry benzene chloro acetyl chloride was added and the mixture was heated in water bath at (60 OC) for 3 hrs. with stirring after that the solvent was evaporated, and the product desired compound was recrystallized from appropriate solvent.

**Synthesis of 1-[3-(Aryl)-5-phenyl-3,3a-dihydroimidazo[4,5-c]pyrazol-6(2H)-yl]-2-thioxoimidazolidin-4-one (6a – b)**

To solution of (5a – b) (0.01 mole), in 20 ml of acetic acid hydrazine hydrate was added and the mixture was heated (refluxed) for 5 hrs. with stirring after that mixture poured to water (200ml), and the product desired compound was recrystallized from appropriate solvent.

**Synthesis of 1-[3-(Aryl)-5-phenyl-3,3a-dihydro-6H-imidazo [4,5-c]isoxazol-6-yl]-2-thioxoimidazolidin-4-one (7a – b)**

To solution of (5a – b) (0.01 mole), in 20 ml of acetic acid hydroxyl amine hydrochloride was added and the mixture was heated (refluxed) for 5 hrs. with stirring after that mixture poured to water (200ml), and the product desired compound was recrystallized from appropriate solvent.

**Synthesis of 1-[6-(4-chlorophenyl)-8-phenyl-2-thioxo-1,2,5,6-tetrahydro-9H-purin-9-yl]-2-thioxoimidazolidin-4-one**

To solution of (5a – b) (0.01 mole), in 20 ml of acetic acid thiourea was added and the mixture was heated (refluxed) for 5 hrs. with stirring after that mixture poured to water (200ml), and the product desired compound was recrystallized from appropriate solvent.

**Results and Discussion**

Scheme (1) shown all compounds were synthesized

Ar = p-NO$_2$, p-Cl, p-Br, p-NMe$_2$

Synthesis of target (1) was done by the reaction of amino acid with benzoyl chloride through nucleophilic mechanism. (93%). m.p (186-188), color (White). The FT-IR spectrum of compound (1), shows stretching vibration of (OH) of carboxylic acid at (2610-3390) cm$^{-1}$ and appearance of new band at (3350) cm$^{-1}$ for NH.

Compound (1) was treatment with aryl lead to formation of compounds (2a - d)

2a :yield (79%), FT-IR cm$^{-1}$ C=N(1646), C=O(1729), (C=)ar (1601,1551), (C-H)ar (3083), (NO)$_2$,(1534-13), 1H-NMR(ppm), s,(6.92) for(C=CH) , (7.25-7.76) (m, aromatic protons).

2b :yield (82%), FT-IR cm$^{-1}$ C=N(1657), C=O(1721), (C=)ar (1603,1500), (C-H)ar (3035), (C-Cl) , (1097) , 1H-NMR(ppm), s,(6.88) for(C=CH) , (7.44-7.89) (m, aromatic protons).

2c :yield (75%), FT-IR cm$^{-1}$ C=N(1704), C=O(1640,1509), (C-H)ar (3050), (C-Br) 975. 1H-NMR(ppm), s,(6.76) for(C=CH) , (7.54-7.91) (m, aromatic protons).

2d :yield (80%), FT-IR cm$^{-1}$ C=N(1632), C=O(1720), (C=)ar (1603,1508), (C-H)ar (3066), (C-H)alph (2884-2987), 1H-NMR(ppm), s, (3.11) for(NMe$_2$) , s,(6.35) for(C=CH) , (7.47-7.81) (m, aromatic protons).

Treatment of (2a – d) with hydrazine gives (3a – d)

3a :yield (78%), FT-IR cm$^{-1}$ NH$_2$ ( 3345,3213 ), C=N(1656), C=O(1701), (C=)ar (1610,1503), (C-H)ar (3079) , (NO)$_2$,(1545-1330). 1H-NMR (ppm), s, (5.66) for(NH$_2$) , s,(6.48) for(C=CH) , (7.51-7.77) (m, aromatic protons).

3b :yield (69%), FT-IR cm$^{-1}$ NH$_2$ ( 3401,3234 ), C=N(1651), C=O(1709), (C=)ar (1600,1500), (C-H)ar (3060), (C-Cl) , (1109), 1H-NMR (ppm), s, (5.23) for(NH$_2$) , s,(6.31) for(C=CH) , (7.51-7.77) (m, aromatic protons).

3c :yield (73%), FT-IR cm$^{-1}$ NH$_2$ ( 3389,3143 ), C=N(1666), C=O(1712), (C=)ar (1599,1501), (C-H)ar (3069), (C-Br) 977. 1H-NMR (ppm), s, (5.46) for(NH$_2$) , s,(6.76) for(C=CH) , (7.55-7.92) (m, aromatic protons).

3d :yield (61%), FT-IR cm$^{-1}$ NH$_2$ ( 3345,3207 ), C=N(1643), C=O(1718),(C=)ar (1607,1500),(C-H)ar (3070), (C-H)alph (2879-2980), 1H-NMR (ppm), s, (3.61) for(NMe$_2$) , s, (5.66) for(NH$_2$) , s,(6.48) for(C=CH) , (7.11-7.69) (m, aromatic protons).

Reaction of compounds (3a – d) with KSCN in ethanol, compounds (4a – d) was formed

4a :yield (71%), FT-IR cm$^{-1}$ NH$_2$ ( 3311,3225 ), NH ( 3112), C=N (1646), C=O(1731), (C=)ar (1601,1551), (C-H)ar (3083) , (NO)$_2$,(1534-1330), 1H-NMR (ppm),
Compounds (6a – b) , (7a – b) and (8a – b) were obtained by cyclizing compounds (5a – b) with hydrazine , hydroxyl amine and thiourea respectively.

6a : yield (65%), FT-IR cm⁻¹, NH (3213), NH of pyrazole, (3171), C=N (1645), C=O thio imidazole (1735), (C=)ar (1611,1500), (C-H)ar (3060), (NO₂), (1555-1535). 1H-NMR (ppm), s, (4.29) for (CH₂ , thio imidazole ) , s, (9.22) for (NH ) , s,(8.27) for(NH , pyrazole), (7.53-7.98) ( m ,aromatic protons).

6b : yield (61%), FT-IR cm⁻¹, NH (3269), NH of pyrazole, (3199) C=N(1661), C=O thio imidazole (1739) (C=)ar (1600,1501) , (C-H)ar (3071) ,(C-Cl), (1021). 1H-NMR (ppm), s, (4.19) for (CH₂ , thio imidazole ) , s, (9.82) for (NH ) , s,(8. 79) for(NH , pyrazole), (6.89-7.24) ( m ,aromatic protons).

7a : yield (71%), FT-IR cm⁻¹, NH (3245), C=N (1651), C=O thio imidazole (1743) , (C=)ar (1605,1516), (C-H)ar (3079) , (NO₂), (1532-1530) . 1H-NMR (ppm), s, (4.02) for (CH₂ , thio imidazole ) , s, (9.46) for (NH ) , (6.92-7.48) ( m ,aromatic protons).

7b : yield (77%), FT-IR cm⁻¹, NH (3211), C=N(1670), C=O thio imidazole (1728) (C=)ar (1609,1511), (C-H)ar (3079),(C-Cl), (1011), 1H-NMR (ppm), s, (4.10) for (CH₂ , thio imidazole ) , s, (9.32) for (NH ) , (6.89-7.24) ( m ,aromatic protons).

8a : yield (57%), FT-IR cm⁻¹, NH (3311), NH of pyrimidine , (3165) C=N (1649), C=O thio imidazole (1741) , (C=)ar (1601,1511), (C-H)ar (3092) ,(NO₂), (1539-1535) . 1H-NMR (ppm), s, (4.20) for (CH₂ , thio imidazole ) , s, (9.71) for (NH ) , s,(8.16) for(NH , pyrimidine), (6.72-7.30) ( m ,aromatic protons).

8b : yield (54%), FT-IR cm⁻¹, NH (3302), NH of pyrimidine, (3190) C=N(1644) , C=O thio imidazole (1733) (C=)ar (1600,1500) , (C-H)ar (3085) ,(C-Cl), (1077) . 1H-NMR (ppm), s, (4.51) for (CH₂ , thio imidazole ) , s, (9.76) for (NH ) , s,(8.36) for(NH , pyrimidine), (6.99-7.59) ( m ,aromatic protons).
Table 1. Antibacterial Activity of derivatives (4a – d, 5a – d, 6a – b, 7a – b and 8a – b).

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Staph. aureus</th>
<th>E. coli</th>
<th>Proteus mirabilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>++++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4b</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4c</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>4d</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5a</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>5b</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>5c</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5d</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>6a</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>6b</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>7a</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>7b</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>8a</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>8b</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Antibacterial activity of derivatives

It might be observed that derivatives which that tested were active but 8a, 8b, 4b and 6a have high activity toward all types of tested bacterials while compound 5a and 5d have weak activity toward all types of tested bacteria compound 8a was showed high inhibition with E. coli and Proteus mirabilis.

Conclusion

Financial disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Dentistry, University of Babylon, Hillah city, Iraq and all experiments were carried out in accordance with approved guidelines.

References

1. AJK Atia Synthesis and antibacterial activities of new metronidazole and imidazole derivatives, Molecules 2009;14 (7):2431-2446


Novel Approach and Cloud Point Extraction Method for Determination of Acetazolamide Drug

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¹Chemistry Department, College of Science, Babylon University, Iraq

Abstract

Acetazolamide was hydrolyzed to primary aromatic amine by using (0.4) M NaOH under reflux. The product was evaluated by two a ways. The first way, Diazotization coupling reaction (approach) as simple, sensitive, rapid and selective Spectrophotometric method, using 8-hydroxyquinoline as Chromogenic reagent to give Azo dye (red) in basic medium. The second way involves applying Cloud point extraction, using Triton-x₁₁₄ as surfactant. The Azo dye was diagnosed by FT-IR, ¹HNMR and UV-Visible technique. The analytical data for Approach and Cloud point extraction method, involve concentration rang (5-150), (0.5-6) µg.mL⁻¹, molar absorptivity (2.3×10³), (1.3×10⁴) L.mol⁻¹.cm⁻¹, Sandall’s sensitivity (0.096) µg.cm⁻² (0.017) µg.cm⁻² and detection limits (0.952) µg.mL⁻¹ and (0.043) µg.mL⁻¹ respectively. In addition the measurement enrichment factor (100) and preconcentration factor (6.30), The proposed methods don’t affect by the existence of excipients so the methods were applied successfully in determining Acetazolamide in pharmaceutical preparations.

Keywords: Hydrolysis Acetazolamide, spectrophotometric determination, Cloud point extraction, Diazotization coupling reaction, 8-Hydroxyquinoline.

Introduction

Acetazolamide a carbonic anhydrase inhibitor, which is used primarily to reduce intraocular pressure by decreasing aqueous humor formation, therapeutically for treatment of glaucoma, epilepsy and as a diuretic and has been used clinically since 1954 ¹⁻⁴. Acetazolamide in either medicinal forms or biological fluids were estimated in several techniques and methods have been declared in the literature, including HPLC for the quantification of acetazolamide in human and rat plasma ⁵⁻⁷, LC/MS and GC/MS ⁸⁻¹⁰, LC-UV ¹¹⁻¹³, and spectrophotometry for determination acetazolamide and other sulfonamide drugs ¹⁴⁻¹⁹. Applications of cloud point extraction techniques for estimation of some elements and drugs ²⁰,²¹. The present paper involves a novel determination of acetazolamide in pure and pharmaceutical formulations by Diazotization coupling reaction (approach) and Cloud point extraction spectrophotometric method, depending on basic hydrolysis of acetazolamide to primary aromatic amine, and coupling the product with 8-Hydroxyquinoline as a chromogenic reagent to give Azo-dye in alkaline medium.

Experimental

Instruments

The scanning of all spectrums and measurements of the absorbance at selected wavelengths achieved by T80 UV-Visible Spectrometer PG Instrumental Ltd, UK, with quartz cell matched 1 cm, Infrared spectra were registered using FT-IR, Shimadzu, Japan, ¹HNMR spectrum was registered using NMR Burker DPX 400 spectrophotometer operating at 300 MHz. the chemical shift δ is quoted in ppm relative to DMSO-d⁶, while the pH was adjusted using 340i pH-meter WTW, Germany, and Heating-Cooling Water Bath – Haak Fe, Sartorius

Chemicals and reagents

Highest purity of Acetazolamide (C₄H₉N₆O₃S₂) was gained from state company for drug Industries and Medical Appliance-(SDI) Samarra-Iraq, 8-hydroxyquinoline (C₉H₇NO),Sodium nitrite (NaNO₂),Absolute ethanol(C₂H₅OH),Sodium hydroxide (NaOH), Sodium carbonate (Na₂CO₃) were gained from the BDH Company with Purity 99.00%. Hydrochloric acid (HCl) was gained from a BDH Company with
concentration 37.00%. Triton X-114 from Arcos organics, New Jersey, USA with purity 100%. Pharmaceutical preparations that were used in this study were Cidamex and Diamox 250mg/acetazolamide from CID Egypt and France respectively.

**Hydrolysis of Acetazolamide**

0.1 g of Acetazolamide was hydrolyzed in alkaline medium by using 0.4 M NaOH under reflux for 2 hours. During the reaction, the secondary amide is converted to the primary amine. The synthetic path of the product is shown in Scheme I. The hydrolysis product was cooled and diluted to suitable volume using distilled water to obtain a stock solution (2000 µg·mL⁻¹). More dilute solution was prepared daily by neutralizing of stock solution with dilute hydrochloric acid and dilute to final suitable volume using distilled water. The tablet sample (Cidamex and Diamox 250mg/acetazolamide) were prepared in the same manner.

2 mol. from the hydrolysis product was converted into dizonium salt ion by using 0.3 mL HCl 1 M and 0.2 mL NaN₂ 1%. The dizonium salt ion was coupled with 1 mol. 8-hydroxyquinoline in alkaline medium to give azo-dye. The synthetic path of the azo-dye is shown in Scheme II. The azo-dye was precipitate, purified and diagnosed by FT-IR, ¹H-NMR and UV-Visible technique as shown in Fig.2, 3 and 4.

**General procedure**

**General procedure of Approach method**

The calibration curve of acetazolamide was constructed by using a series of (10 mL) volumetric flask. Increasing volumes (0.1-3 mL) from Acetazolamide solution after hydrolysis (500 µg .mL⁻¹), mixed well with (0.3, 0.2 mL) from Hydrochloric acid (1 M) and (1%) Sodium Nitrate respectively, and stand for (5 min) to complete formation of dizonium salt. Then added (2.5 mL) 8-Hydroxyquinoline (0.03M) and (0.7 mL) Sodium Hydroxide (0.4 M). The volume was made up to mark with distilled water. The absorbance for all solutions was measured at (502 nm) at (25°C) against solution blank.

**General procedure of Cloud point extraction method**

A series of (10 mL) volumetric flask, increasing volumes (0.05-0.6 mL) from a solution of Acetazolamide after hydrolysis (100 µg .mL⁻¹) mixed well with (0.1, 0.2 mL) from Hydrochloric acid (1M) and Sodium Nitrate (1%) respectively, and stand for (5 min) to complete formation dizonium salt. Then added (0.5 mL) 8-Hydroxyquinoline (0.005M) at pH=7.2 by using Sodium carbonate (0.2 M). The volume was made up to the mark with distilled water. The solutions were transferred into a centrifuge tube with added (0.5 mL) Triton X-114. The mixture was transferred into hot water bath about (10 min) at (55°C) to form a cloud solution. The mixture separated into two phases by centrifuge (10 min) at (3500 ramp), The aqueous phase decanted and the surfactant-rich phase diluted with (0.3 mL) absolute ethanol. The absorbance of final solutions was measured at (502 nm) against blank solution was prepared in the same away.

**Result and Discussion**

**Identification of the prepared drug and Azo Dye**

Identification of the Hydrolysis product and Azo Dye. The hydrolysis product was prepared as explained in paragraph 2.3. The qualities experimental were involved tests for amine group by using Nitrous acid test and Azo-dye test. The hydrolysis product (5-amino-1,3,4-thiadiazole-2-sulfonamide) give yellow solution result from a reaction between a primary aromatic amine with concentrated HCl and NaNO₂ to formation diazonium salt ion, while the acetazolamide before hydrolysis not reaction. The two compounds were tested also by azo-dye test. The hydrolysis product after formation diazonium salt ion was coupled with 8-Hydroxyquinoline in alkaline medium to give azo-dye, while the Acetazolamide before hydrolysis not reaction. This azo-dye was prepared by taking a stoichiometric amounts was precipitate, purified and diagnosed by FT-IR, ¹H-NMR and UV-Visible technique.

IR spectrum of azo-dye showed in Fig.1. one peak at 3417 cm⁻¹ assigned for stretching (O-H) of phenolic group, peak at 1426 cm⁻¹ assigned of stretching (N=N), and other peak 1575,1145 and 879 cm⁻¹ assigned for stretching (C=N) thiadiazole, (S-C) and (C-S-C) groups respectively. This mean the formation of the aromatic primary amine group by hydrolysis of Acetazolamide and converted the amine in to diazonium salt in the presence of HNO₂ and coupling the last with 8-Hydroxyquinoline in alkaline medium. The ¹H-NMR spectrum (DMSO-d6, 400 MHz) of azo-dye complex showed chemical shifts at δ 1.66 (1H, -SO₂NH₂), Singlet broad band at δ 3.5 refer to water in the solvent, DMSO, where its band δ 2.5, δ 5.5 (1H, aromatic -C=O-H), δ 7.1(2H,C-H₆₅)
quinoline), δ 7.4 and 7.5 (3H,2H, C-H\textsubscript{22} quinoline), δ 8.3 and 8.85 (2H,1H, C-H\textsubscript{21} quinoline)\textsuperscript{26-28} as shown in Fig.1. The qualitative and quantitative study of drug after hydrolysis, also done by UV-Visible technique. The Azo-dye complex (color product) scanned at (700-400 nm), and give a maximum absorption λ\textsubscript{max} at (502 nm) versus blank solution. While the scan of the blank solution versus water doesn’t give any absorption at λ\textsubscript{max} for colored product as shown in Fig.3. This property was adopted in the estimation of trace amounts from Acetazolamide in pure and pharmaceutical preparations. The effect of various experimental conditions as concentration, volumes from 8-Hydroxyquinoline reagent, volumes of HCl (1M), volumes of (1%) Sodium Nitrate, different types of bases, various volumes of Sodium Hydroxide (0.4 M) and temperatures of formation of (40 µg.ml\textsuperscript{-1}) azo dye were studied. The starting point involves using (0.5mL) from (1M) Hydrochloric acid with (0.5 mL) from (1%) Sodium nitrate, stand for (5 min), then (0.5mL) from (0.05M) 8-Hydroxyquinoline, and (0.5mL) from (0.4 M) Sodium hydroxide were added in (10 mL) volumetric flask. The absorbance of the solutions was measured at (λ\textsubscript{max}=502nm) against blank solutions after (5 min) since the beginning of the coupling reaction. The effect of various experimental conditions shown in Fig.2.

Selection Optimal Experimental Conditions of CPE method

The effect various concentrations, volumes of 8-Hydroxyquinoline reagent, volumes of HCl (1M), optimum pH value, volume of Triton X-114 5%, also effect of temperature and incubation time on formation of surfactant of (5 µg.ml\textsuperscript{-1}) azo dye were studied. The starting point involves (0.3 mL) of Hydrochloric acid (1M) fellow (0.2 mL) of Sodium nitrate (1%), stand for (5 min ), then (0.3 mL) from 8-Hydroxyquinoline (0.005M), at (pH = 7) Sodium carbonate with (0.3 mL) Triton X-114 5% were added in (10 mL) volumetric flask. The mixture was heated (10 min) at 55°C in water bath. The mixture was separated by centrifuge Ramp=3500 at (10 min). The surfactant rich phase was diluted with (0.3 mL) absolute ethanol and the absorbance of the solution was measured at (λ\textsubscript{max}=502nm) against the blank solution The effect of various experimental conditions shown in Fig.2.
Calibration curve

After fixing all the optimum conditions for the reaction of acetazolamide after hydrolysis with 8-Hydroxyquinoline of approach and cloud point extraction method the calibration curves were constructed as shown in Fig.3. The all analytical values were calculated with accuracy and precision result are summarized in Table.1. The results shown each method has a good accuracy and precision.

Table 1 Summary of analytical value of calibration curves with accuracy and precision resulting of approach and CPE method with

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Approach method</th>
<th>CPE method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Regression equation</td>
<td>Y = 0.0103x+0.0099</td>
<td>Y = 0.0593x+0.0024</td>
</tr>
<tr>
<td>2 Slope</td>
<td>0.0103</td>
<td>0.0593</td>
</tr>
<tr>
<td>3 Intercept (a)</td>
<td>0.0099</td>
<td>0.0024</td>
</tr>
<tr>
<td>4 Correlation coefficient</td>
<td>0.9981</td>
<td>0.9992</td>
</tr>
<tr>
<td>5 Linear Range (µg.mL⁻¹)</td>
<td>5-150</td>
<td>0.5-6</td>
</tr>
<tr>
<td>6 Molar absorptivity (Ɛ) (L.mol⁻¹.cm⁻¹)</td>
<td>2.3×10³</td>
<td>1.3 ×10⁴</td>
</tr>
<tr>
<td>7 Sandall's sensitivity (S) (µg.cm⁻²)</td>
<td>0.096</td>
<td>0.017</td>
</tr>
<tr>
<td>8 Limit of Detection (µg.mL⁻¹)</td>
<td>0.952</td>
<td>0.043</td>
</tr>
<tr>
<td>9 Limit of Quantitation LOQ (µg.mL⁻¹)</td>
<td>3.174</td>
<td>0.140</td>
</tr>
<tr>
<td>10 Preconcentration factor</td>
<td>---</td>
<td>100</td>
</tr>
<tr>
<td>11 Enrichment factor</td>
<td>---</td>
<td>6.3</td>
</tr>
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<table>
<thead>
<tr>
<th>Concentration µg mL⁻¹</th>
<th>Taken</th>
<th>Found</th>
<th>Error*%</th>
<th>Recovery*%</th>
<th>RSD*%</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n= 5</td>
<td></td>
</tr>
<tr>
<td>Acetazolamide</td>
<td></td>
<td></td>
<td></td>
<td>Approach method</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.929</td>
<td>-0.175</td>
<td>99.824</td>
<td>0.362</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.603</td>
<td>-0.793</td>
<td>99.206</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>59.450</td>
<td>-0.916</td>
<td>99.080</td>
<td>0.963</td>
</tr>
<tr>
<td>Cloud point extraction method</td>
<td>1.5</td>
<td>1.47</td>
<td>-2.00</td>
<td>98.00</td>
<td>0.212</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.98</td>
<td>-0.50</td>
<td>99.50</td>
<td>0.163</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.05</td>
<td>1.00</td>
<td>100.10</td>
<td>0.071</td>
</tr>
</tbody>
</table>

Stoichiometry of Reaction and mechanism

The methods of Mole ratio and Continuous variation, Jobs method were used to detect the stoichiometry of azo-dye formation from reaction 8-Hydroxyquinoline reagent with acetazolamide drug. The results obtained in Fig.4 shown that 2:1 acetazolamide to 8-hydroxyquinoline was formed at 502 nm. And The average conditional stability constant of the colored products in water at optimum conditions was $6 \times 10^{12}$ L² mol⁻². The azo dye has high stability because the acetazolamide have electron with drawing group (sulfonamide) makes $\text{O} \rightleftarrows \text{N}$ group more positive charge and 8-Hydroxyquinolnic have electron donating group makes ring very active so easy formation of high stability azo-coupling reaction between acetazolamide and 8-hydroxyquinoline[26]. The proposed mechanism of reaction illustrated in Scheme.II.
**Pharmaceutical applications**

The proposed methods approach and cloud point extraction were applied successfully for determination three concentrations form Acetazolamide in (Cidamex and Diamox 250mg) tablets as shown in the Table 3, after studying the effect of additives by adding separately excess amounts about (10:1) from additives to (40 µg.mL⁻¹) acetazolamide solutions under optimum reaction conditions followed in the calibration curves. As shown in a Table. 2.

**Table 2. Determination of (40 µg.mL⁻¹) from Acetazolamide in the presence of additives**

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Acetazolamide (40 µg.mL⁻¹)</th>
<th>Error%</th>
<th>Recovery%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration found µg.mL⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pvp</td>
<td>39.83</td>
<td>-0.425</td>
<td>99.75</td>
</tr>
<tr>
<td>Lactose</td>
<td>40.05</td>
<td>0.125</td>
<td>100.13</td>
</tr>
<tr>
<td>Starch</td>
<td>39.69</td>
<td>-0.775</td>
<td>99.225</td>
</tr>
<tr>
<td>Mg stearate</td>
<td>40.07</td>
<td>0.175</td>
<td>100.175</td>
</tr>
</tbody>
</table>

*Average of five determinations

**Table 3. Application of proposed methods on pharmaceutical preparation for Acetazolamide**

<table>
<thead>
<tr>
<th>(Cidamex) 250 mg</th>
<th>Concentration µg mL⁻¹</th>
<th>Error*%</th>
<th>Recovery*%</th>
<th>RSD*%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Taken</td>
<td>found</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.74</td>
<td>-0.650</td>
<td>99.35</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.41</td>
<td>-1.172</td>
<td>98.83</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>60.06</td>
<td>0.111</td>
<td>100.11</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.477</td>
<td>-1.53</td>
<td>98.46</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.996</td>
<td>-0.10</td>
<td>99.90</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.988</td>
<td>-0.24</td>
<td>99.76</td>
</tr>
<tr>
<td>(Diamox) 250 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.29</td>
<td>-1.777</td>
<td>98.22</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.83</td>
<td>-0.330</td>
<td>99.67</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>59.88</td>
<td>-0.186</td>
<td>99.81</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.495</td>
<td>-0.333</td>
<td>99.67</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.962</td>
<td>-0.950</td>
<td>99.05</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.972</td>
<td>-0.560</td>
<td>99.44</td>
</tr>
</tbody>
</table>
Evaluate the results of the proposed methods

The standard method for estimation Acetazolamide in the British pharmacopoeia was applied for determination of acetazolamide in pure drug and Pharmaceutical Preparations. The results of standard method comparison with proposed methods approach and cloud point extraction (F and t test value). The results summarized in the Table.4. Shown no significant differences between the two methods.

Table 4. Application of F, and t test for comparison between proposed and standard methods

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>Proposed method</th>
<th>Standard method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rec. *%</td>
<td>Rec. *%</td>
</tr>
<tr>
<td>Pure Acetazolamide</td>
<td>99.37</td>
<td>0.0009</td>
</tr>
<tr>
<td>Cidamex</td>
<td>99.43</td>
<td>0.0081</td>
</tr>
<tr>
<td>Diamox</td>
<td>99.23</td>
<td>0.0100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>Proposed method</th>
<th>Standard method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rec. *%</td>
<td>Rec. *%</td>
</tr>
<tr>
<td>Pure Acetazolamide</td>
<td>99.20</td>
<td>0.0120</td>
</tr>
<tr>
<td>Cidamex</td>
<td>99.37</td>
<td>0.0025</td>
</tr>
<tr>
<td>Diamox</td>
<td>99.39</td>
<td>0.0049</td>
</tr>
</tbody>
</table>

F-Value (experimental) = 0.1000, Critical F-Value (19.000)
t-Value (experimental) = -0.9944, Critical t-Value (2.776)

Conclusions

A simple, rapid, sensitive and new selective Spectrophotometric methods have been developed, not affected by excipients, successfully applied for determination of trace amounts of acetazolamide drug in pure and pharmaceutical formulations based on basic hydrolysis of acetazolamide and coupling the hydrolysis product with 8-hydroxyquinoline reagent depending on the diazonium coupling reaction.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science, Babylon University, Iraq and all experiments were carried out in accordance with approved guidelines.

References


The Effect of Pilates Exercises in the Repair of the Aberration and Balance of the Muscles of Shoulder Girdle as a Beginning for the Fitness

Hussein Ali Kadhim¹, Haidar Shmkhi Jabar¹

¹Physical Education and Sports Sciences, Thi-Qar University, Iraq

Abstract

Fitness refers to the good health of athletes, then, it is necessary for every athlete to duty the importance of management and agreement in the training of muscle groups then everything related to the substratum and muscle balance and evaluate it by using the Pilates way to identify its elements and secrets so as to raise the substratum of the athlete and rehabilitation of distortions and deviations, creating healthy habits, and spreading fitness awareness among athletes through working to improve strength, lengthening and muscular balance of the shoulders. Thus, the researcher used the experimental approach by using the experimental design with one suitable group of the research, while, the sample of the research that made up of (12) athletes, that has been chosen by the researcher by using the intentional method for those who are suffering from the aberration of the shoulder fall. The researcher made sure to applied the Pilates exercises by using repeat contraction that aims to improve muscle balance, strength and flexibility of the sample of the research, with (8) weeks and consists of (24) Training modules, (3) modules a week, the most important conclusion in the research is that the using of Pilates contribute to reduce and rehabilitation the aberration of the shoulder fall, if the essential strength of the shoulder girdle muscles that assist in developing the muscular balance, it can be concluded that these exercises agreed with the aberration of shoulder fall and muscle balance.

Keywords: Health of Athlete, Fitness, Muscle Balance.

Introduction

Allah the almighty is giving the health to mankind to be crown for them, Allah the almighty says in the Glorious ¹. We have indeed created man in the best of moulds, the human body is very accurate and complex, it is created to the fullest level to be ready to adapt to all the requirements of the environment, Fitness refers to the good health of athletes, then, it is necessary for every athlete to duty the importance of management and agreement in the training of muscle groups, which required to planning in order to achieve a high ² level of muscle balance whether it is in the body parts (right – left) or in one part for the muscles (stretching and shrinking), especially in the joints and extremities that work in the activities of flinging balls and beating on the basis of the muscles of the shoulder girdle, then ignore the muscles experiences of balanced force of the left and right shoulders through the performance ³ of movements of the main goal leads to appearance of the aberration of shoulder fall, the favorite arm in the activities of throwing and beating in the two games (volleyball - handball) ⁵. Therefore, the existence of such a kind of aberrations in the body changes its mechanics in the performance of these different skills and dispersion of power in the side paths do not work in the same skill then it be the deviation of the area that is the most at risk and cramp ⁶. The significance of research lie in the study of all concerning with the substratum and muscle balance and evaluate it by using the Pilates way to identify its elements and secrets so as to raise the substratum of the athlete and rehabilitation of distortions and deviations ⁷, creating healthy habits, and spreading fitness awareness among athletes through working to improve strength, lengthening and muscular balance of the shoulders. The balance of muscle is the main part to make a good health (fitness), also this method prepares the body type to another perfect body.
The Methods and Practical Procedures of the Research:

The Methodology of the Research

The researcher used the experimental method by using the experimental design with one group which is suitable for the research.

The Sample of the Research

The sample of the research that made up of (12) athletes of first club athletes (volleyball- handball) in Thi-Qar, that has been selected by the researcher by using the intentional method for those who are suffering from the aberration of the shoulder fall. The researcher made a similarity of the following variables:

Table 1: Shows the Coefficient of Skewness to Measure Pre-Test of the Research Sample in Some Main Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Measurement Cell</th>
<th>The significance of the statistical characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean X.</td>
</tr>
<tr>
<td>Age</td>
<td>Year</td>
<td>19</td>
</tr>
<tr>
<td>Training Age</td>
<td>Year</td>
<td>3.5</td>
</tr>
<tr>
<td>Length</td>
<td>M.</td>
<td>178</td>
</tr>
<tr>
<td>Weight</td>
<td>Kg.</td>
<td>69.70</td>
</tr>
</tbody>
</table>

The previous table shows that all the values of the Coefficient of Skewness between (-1,1), so this indicates to the similarity of samples in the research variables before make the main experience.

The Research Tests

The Aberration of the Shoulder Fall: It is detected by drawing a line between the two non-sticky papers placed on the lateral top of the shoulder joint (anatomical points) on both sides of the body. If the anatomical point forms a current angle (0 °) with the frontal axis (axial axis), that means this is not aberration, however, if the degree of angle increases, this indicates that there is a quantum deviation and the angle is measured in degree by the program (Kinovea) as in Fig. (1).

The Tests of Muscle Balance of Muscles That is Working on the Shoulder Joint

The maximum strength of the underlying muscles (capture, rumbles, dimensions, roundness, internal rotation, external rotation) of the shoulder joint was calculated by way of the number of times a movement was performed with a load of weight 5 kg, and then the maximum force value of the right shoulder and shoulder (1) and then calculate the balance of muscle balance through equation (2) the closer the ratio of 100% indicates that there is a balance between the muscles of the body (shoulders):

\[
RM-1 = \frac{\text{raised weight} \times 100}{\text{Number of repetitive repetitions} \times 2} \quad (1)
\]

Muscular balance = high shoulder force ÷ strength of low shoulder × 100 \( \ldots \) (2)

The Main Experience

The exercises were conducted after reviewing a number of sources related to sports training related to the subject of the research. After presenting it to the experts, the exercise was started in the form of repetition contract to improve the muscle balance and development of muscle strength and elasticity for the members of the research sample on 15/1/2019 until 20 (3) units per week (Sunday - Tuesday - Thursday) for all muscles and in the method of directing the endurance (3-1) between the weeks while the orientation between the days (2-1), “The number of repetitions (3-5) and the number of groups
of (3-5) groups may be included researcher intensity training by giving exercises on both sides and then per party either using different frequencies or varying intensity of training for each side of the body where focused The researchers were trained to maintain the strength and increase the lengthening of the low shoulder while the shoulder training was high by increasing the strength and maintain the level of muscle extension and since the training intensity used in the development of flexibility is (100%) the intensity of performance will be using the researcher to use endurance guidance based on the maximum exercise time intensity (100%) directed by hard training.

The Analysis and Discussion of the Results

Explain and discuss the results of differences in shoulder fall deviation for pre and post-tests of the groups

Table (2): The Values of means X., standard deviations and level of development for shoulder fall deviation for pretests and posttests of the research group

<table>
<thead>
<tr>
<th>No.</th>
<th>Variables</th>
<th>Measurement cell</th>
<th>Test</th>
<th>Development level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shoulder Fall</td>
<td></td>
<td>Pre-test</td>
<td>3.583</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post-test</td>
<td>1.416</td>
</tr>
</tbody>
</table>

The Experimental Research:

We see that the values of shoulder fall deviation of the research group have evolved by (39.534) as it appears that the value of the deviation of the shoulder fall by the dimensional test has evolved significantly from what it was in the tribal test and the researchers attribute the reason for this ratio of evolution to the physical awareness played by the researcher in educating those affected by the deviation of the fall The shoulder has led to positive results for the research, and this is confirmed by raising awareness of the dictionary is one of the methods used in preventing deformities, especially deformities that did not reach the stage of composition. By presenting the table for the deviation of the shoulder fall, we found there a clear decrease in the degree of this deviation and the researchers attribute the reason for this and generally to the practice of the sample members to the training of the rehabilitation using the method of Pilates, and that this improvement was attributed by the researchers the full commitment of the members of the sample performance all Pilates training vocabulary, which contained physical exercises that means devices and aids to treat and rehabilitate this condition, has been repeated (3) times a week for twelve weeks. Hence it can be said that the goal of the research was achieved and was recognized the effect of exercises using the method Pilates prepared to correct the deviation of shoulder fall which contributed to the body’s correct ability, as well as improving the muscular balance of the shoulder girdle and increasing its flexibility, and strengthening the muscles, it is a comprehensive exercise that works to rehabilitate the body from all aspects.

Table (3): Values of Means X., standard deviations and the level of development of the relative index of the muscular balance of the pre and post-tests of the research group

<table>
<thead>
<tr>
<th>No.</th>
<th>Variables</th>
<th>Measurement Cell</th>
<th>Test</th>
<th>Development level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Muscle balance of the holding muscles of the shoulder</td>
<td>%</td>
<td>Pre</td>
<td>87.252</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>96.322</td>
</tr>
<tr>
<td>2</td>
<td>Muscle balance of extensor shoulder muscles</td>
<td>%</td>
<td>Pre</td>
<td>89.947</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>95.744</td>
</tr>
<tr>
<td>3</td>
<td>The muscular balance of the distal muscles of the shoulder</td>
<td>%</td>
<td>Pre</td>
<td>88.582</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>95.238</td>
</tr>
</tbody>
</table>
4 Musculoskeletal balance of the proximal muscles of the shoulder % Pre 87.758 2.820 7.401 Post 94.773 1.766
5 The muscular balance of the inner rotation rotator muscles % Pre 88.444 1.712 8.664 Post 96.834 1.858
6 The muscular balance of the outer rotation muscles of the shoulder % Pre 79.399 7.908 13.132 Post 91.403 9.584 9.417

We can see that the values of muscle balance of the shoulders of the post-test has evolved significantly from the post-test and attributed the researchers cause this development to increase the growth of muscle strength in a balanced balance of the right shoulder compared to the left shoulder and thus contributed to modify the body from the previous situation to the ideal situation which should be. The two researchers attributed this to the nature of exercises applied by the research group Pilates exercises performed by the research sample on a regular basis and within different frequencies and times of constancy specified, contributed to increase the muscle balance in the muscles of the shoulder girdle as well as to get the ideal shape of the shoulders, Musculature between the underlying motor muscles (rounded to the inside) and the corresponding muscles (rounded out) not only happens in the force but also occurs in the lengthening, The essence of the exercises applied by the research sample was to focus on an ideal combination of strength and length, which achieves the goal of researching that the proposed program of balance in muscle strength has an important effect on the development of muscle balance in general that compatibility between fist muscle groups and relaxed muscle groups will helps to increase the equal strength of the muscles of the body in order to achieve an acceptable physical balance of the body, meaning the shape or position taken by the body during movement or stillness helps to maintain the center of the body’s weight within the base of the balance of the parts of the body, especially the shoulders in a hierarchical form from the top down as a result of the development of the balanced strength of the shoulder girdle muscles, which in turn help to move the weight of these parts gradually, balanced and equal between the bones, joints, muscles and ligaments of the body.” Keeping the muscles in balance with increasing strength is the first requirement to develop the muscle’s ability to produce maximum strength in the maximum range of motion at the highest possible rate of speed, as well as to increase the muscle capacity of the left arm (non-strike or aimed) during the technical performance of the events of throwing and beating Has a significant impact on the improvement of the ability of the strike arm or as a result of the principle of the transfer of the impact of training from the arm of non-working to the labor force, and also find that the rate of progress of the arm is preferred than the preferred arm due to neglect in the previous training programs and the varying levels of strength and ability of the shoulders agree with the main goal of research is to balance the muscle strength between muscle groups working and non-working and this is what achieved exercises for balance in muscle strength.

Conclusions
Through the results between the pre and post-tests, the application of the exercise Pilates therapeutic treatment on the devices and tools helped significantly improve the strength and flexibility of the shoulders of the muscles of the shoulder girdle as well as reduce the degree of deformity of all injured. That is, the curriculum was in line with the deviation of the shoulder fall. The rate of development between the results of the pre and post-tests of the variables during the study was effective and in favor of the post-test of the deviation of the shoulder fall and muscle balance.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Physical Education and Sports Sciences, Thi-Qar University, Iraq and all experiments were carried out in accordance with approved guidelines.

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Effect of Specific Tactical Exercises in Some Biochemical Indicators and Psychological Endurance and Achievement of 400 M Hurdles for Young Players

Mahmoud Nasser Radi, Ali Bukheet Hassan, Muntadher Mohammed Ali

Faculty of Physical Education and Sports Sciences / University of Kufa

Abstract

This study dealt with several topics including the introduction of the research and the importance of the use of specialized tactical training during the training units, which are manifested in the development of biochemical indicators, psychological tolerance and performance of the effectiveness of 400 m hurdles, so researchers thought to conduct this study and go in the process to identify the results that will be achieved. In the results to the lack of the use of specialized lactic exercises, which are based on scientific foundations and that would make adaptations functional, in addition to the effectiveness of this need of self-sufficiency of large runners because it is a difficult activity that requires the will and determination to win, if The research problem involves two main axes: the first is the low speed restriction and tolerance of the special speed in the 400 m hurdles, and the second axis in the search is to obtain a high level of functional efficiency of some biochemical indicators associated with special speed and the tolerance of the speed Therefore, the researchers considered going into this experiment.

Keywords: biochemical indicators, psychological endurance, achievement

Introduction

The achievement of sport requires the use of the scientific method of modern planning in various fields, where it began to take a large space to create a sophisticated world using it in all methods and scientific methods and in all areas of life 1, including sports, which is one of the important areas that reflect the culture of peoples. The emergence of modern science in the field of sports, especially in the science of training and physiology, the great impact in the development of the sports side and thus the evolution of athletes by upgrading their levels to the best compared to the previous level 2. The effectiveness of the arena and the field of sports events reflected the scientific progress achieved and reflected on most of the activities 3, including digital levels of times in track (running, jogging, walking, barriers and barriers) and distances in the field (jump, throw and jump) and points in the composite competitions (Decathlon for men – sevenfold for women) 4. Including the 400 m hurdles, which are very difficult to perform and their skills, and therefore require skill and physical training to suit the difficulty of the players during the competitions. Hence, it was necessary to use specialized lactic training because it has a positive effect on the development of functional efficiency 5 and upgrading the level of training of the player and the training and psychological state as the exercise exercises used, which is one of the most important factors of success and progress in performance provided that it is built on the basis of scientific correct to contribute In order to increase the efficiency of the athlete to achieve the best achievement, hence the importance of research in the use of tactical training specialized during the training units, which are reflected in the development of biochemical indicators and psychological tolerance and performance of the effectiveness of 400 m hurdles. So researchers thought to conduct this study and go into the process to identify the results that will be achieved. The 400 m hurdles are difficult games that require integration of physical elements and skill performance as well as
They were divided into two groups (experimental and control) equally by random method. Table (1) builds the community and the research samples.

Table (1). Shows community and sample research

<table>
<thead>
<tr>
<th>Number of members of the exploratory experiment</th>
<th>percentage</th>
<th>Number of main experiment personnel</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>22.2%</td>
<td>41</td>
<td>77.7%</td>
</tr>
</tbody>
</table>

Sampling homogeneity and equivalence of the two research groups:

**Homogeneity of the sample:**

In order to adjust the variables that affect the accuracy of the search results, the researchers sought to verify the homogeneity of the research sample, which is related to the morphological measurements (length, mass mass, temporal age). The researchers used the torsion factor before applying the main experiment to the control and experimental groups, As shown in table (2).

**Measurement method:**

1. Five minutes after the player’s 400m hurdles test, this is the best time to measure lactic.

2 - The measuring tape is placed in the location assigned to the device.

3. After placing the tape, the code number of the tape will appear. A blood sample will then be taken through the use of a complaint through which a finger is pricked and then pressed to allow us to extract the blood drop.

4. Note through the screen device and after raising the finger for a few seconds will appear on the screen the proportion of lactic acid in the blood and according to the specified percentage.

The record: A blood drop from the finger that has been pricked by a LACTATE PRO 2 device is used to obtain the drop of blood and place it in the measuring tape placed in the device . The blood is drawn by the medical staff, And in recent years used modern devices and simple can be carried by hand to measure the amount of lactic acid in the blood after the implementation of the
physical effort for about five minutes according to the consensus of expert experts after the implementation of the physical effort required to know the movement of the escalation of the amount of lactate blood and landing after the physical effort to ensure the transfer of the largest amount of The lactic acid from the muscles to the blood and put this sample of blood in the device, measuring the amount of lactic acid after several seconds and read the figure that appears in the screen of the device, which represents the measurement (Mmol / L³), and Yoshida, 1984 that lactate responses in The veins are better responsive to lactate arteries. 2

2- Measuring the level of concentration of dehydrogenase (LDH) in the blood:

The concentration of the enzyme dehydrogenase (LDH) was measured after the completion of the 400 m hurdle test. The laboratory sits on a chair immediately after 5 seconds, and the venous blood is withdrawn by (2 cc) by the chemist from The research community (14) hostility, which is sufficient amount as indicated by the instructions attached with the measuring tape, which is placed in the tubes (medical) written on the name of the player and number, and was transferred to the laboratory of Sadek Specialist directly, where the analysis of the enzyme By the specialist doctor.

Second - Psychological Endurance Scale:

In order to reach the goals that were developed, the researchers adopted the psychological stress scale prepared by 3.

The scale may be from (64) paragraphs, see Appendix (2).

Third: Test run 400 m hurdles:

- Objective of the test: measuring the real achievement of the competition.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Measuruing unit</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Calculated T value</th>
<th>Significant Test level</th>
<th>Significant type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>s ± p</td>
<td>s ± p</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and Discussion

Table (2) Shows the computational circles, standard deviations, the calculated value of (t) of the interrelated samples, the level of significance of the test and the significance of the difference to the pre and post - tests of the control group of the variables investigated.
**Table (2)** Shows the computational circles, standard deviations, the calculated value of (t) of the interrelated samples, the level of significance of the test and the significance of the difference to the pre and post - tests of the control group of the variables investigated.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Measuring unit</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Calculated T value</th>
<th>Significant Test level</th>
<th>Significant type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid mmol / L</td>
<td>mmol / L</td>
<td>14.60</td>
<td>0.62</td>
<td>13.01</td>
<td>8.75</td>
<td>0.000</td>
</tr>
<tr>
<td>L.D.H Unit / L</td>
<td></td>
<td>509.762</td>
<td>20.181</td>
<td>489.098</td>
<td>11.891</td>
<td>3.619</td>
</tr>
<tr>
<td>Heart rate after voltage Beat / min</td>
<td></td>
<td>184.85</td>
<td>2.54</td>
<td>180.57</td>
<td>0.78</td>
<td>4.66</td>
</tr>
<tr>
<td>Psychological endurance degree</td>
<td></td>
<td>62.149</td>
<td>4.56</td>
<td>68.857</td>
<td>4.70</td>
<td>6.60</td>
</tr>
<tr>
<td>Completion of 400 m hurdles m / sec</td>
<td></td>
<td>58.428</td>
<td>0.567</td>
<td>56</td>
<td>0.213</td>
<td>4.16</td>
</tr>
</tbody>
</table>

As for the difference in the experimental group of the variable (heart rate after the effort), the researchers attributed to the use of the special lactical training that was prepared and applied to the members of the experimental group, because the runners were exposed to the effort (anaerobic), seeing 11 Physical exertion is considered the most important factor affecting the heart rate, as the rise of the pulse during the effort is normal in response to the effort exerted to meet the body’s energy needs, which the heart and circulatory system to provide them by increasing the heart rate or the size of the blow “ . 12

**Table (4).** (T) for the independent samples and the level of significance of the test and the significance of the differences between the results of the test (post-dimensional) of the control and experimental groups of the variables investigated.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Measuring unit</th>
<th>Control</th>
<th>experimental</th>
<th>Calculated T value</th>
<th>Significant Test level</th>
<th>Significant type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid mmol / L</td>
<td>mmol / L</td>
<td>13.01</td>
<td>0.28</td>
<td>11.92</td>
<td>0.63</td>
<td>4.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

moral
The differences between the control and experimental groups were due to the quality of the exercises. The results showed that there were significant differences between the two groups (control and experimental) in the concentration of lactic acid in the blood and for the benefit of the experimental group. The specialized lactic prepared and designed by the researchers according to the physiological bases, which is compatible with the prevailing energy exchange system for the effectiveness of 400 m. Based on the scientific foundations of sports training and sports physiotherapy, as these exercises were prepared according to the system of lactic energy and this helped the athletes to get rid of lactic acid quickly, and researchers believe that sports training leads to increase the work of organizations to eliminate the biological increase of lactic acid, The exercises resulted in these differences after the effort between the two groups and after training for the concentration of lactic acid in the blood, which led to a state of improvement in the experimental group and thus an improvement in the work of the functional devices we note the low concentration of lactic acid. The concentration of lactic acid in the blood of well-trained athletes is less comparable to non-trained or less effective training if they do the same training or effort.

**Conclusions**

Based on the research results reached within the research community, the following conclusions were reached: specialized lactic exercises helped to develop the concentration of lactic acid in the blood. The specialized lactic exercises contributed to the high concentration of the enzyme (L.D.H) in the blood. The specialized lactic exercises were in the level of achievement in the sample research.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Faculty of Physical Education and Sports Sciences / University of Kufa and all experiments were carried out in accordance with approved guidelines.

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Comparative Molecular Study between Some Plant Extract and Tinidazole of Hydatid cysts in Basrah, Southern Iraq

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Abstract

Hydatid disease or Echinoccosis is one of the serious public health problems. This study was designed as a comparative molecular study between some plant extract and tinidazole of sheep hydatid cysts in Basrah province. In this study, 213 sheep were examined and the number of those infected with hydatid cysts was 75 (35.2 %). The results showed that hydatid cyst were detected successfully by performing PCR technique. In addition, *Quercus aegilops* has been shown the best plant extract for destroying all genes, the second plant extract which is *Capparis spinosa* showed destroying of two genes (*G6-7, COI*) and failed to destroy (*sh4-1*). Furthermore *Prosopis fracta* has shown to destroy only (*G6-7*) and tinidazole failed to destroy any of these genes used in this study.

Key words: Comparative Molecular Study. Plant Extract with Tinidazole.

Introduction

Hydatidosis is one of the very important parasitic defect of domestic animals that cause economic losses and public health disease worldwide. It is caused by adult or larval stages of cestodes (metacestodes), which is belonged to the genus *Echinococcus* and the family *Taeniidae*, also known as Hydatid Disease, is an infection caused by the larval stage of the flat worm *Echinococcus granulosus*. It has a worldly distribution and is one of the most general parasitic zoonosis. The metacestodes progress are fluid - filled hydatid cysts, in which protoscoleces are construct as the following generation of tapeworms. In liver and lung, cyst develop. Their growth cause acute defect and death in the intermediate host. Protoscoleces inside entire hydatid cysts in organs of perished or slaughtered animals stay infectious for up to 2 weeks, according to the temperature and other conditions. It has been observed that tinidazole has the ability to rupture the helix structure of DNA and prevent building of nucleic acid which leads to destroy the cells and the parasite. Many types of medical plants have been used to treat some parasitic infection. Arab people have traditionally used *Quercus sp.* to treat dysentery (which is caused by *Entamoeba histolytica*), stomach cancer and bloody vomiting, as well as many disorder like eczema, cancer healing and vomiting. This is due to the presence of active substance (Phenols and Tannins) in the plant. Acorns serves an paramount role in early human history and it were a source of food for many cultures around the world. In the past, the poorer would origin eat acorns in their food. According to Edible and Useful Plants of California, by Charlotte Clark, “people who use acorns today concur that they resemble other nuts in oiliness and taste”. In general, acorn flour contains significant quantities of calcium, magnesium, phosphorus, potassium, sulfur, fat and protein. Many species of *Capparis spinosa* extract, many of these species are reported from Iraq, northern to southern of the country. The plant is well known for the edible buds and fruits (caper berry), which are consumed pickled. *Prosopis fracta* is belonged to Memosaseae family. It is a grapple shrub with 30-100cm height, grows extravagantly in Iraq.

Materials and Method

In this study, A total 213 organs (lung and liver) of sheep infected with hydatid disease were examined to determine the effect of some plant extract comparing with drug used for treatment (tinidazole) from the period between October 2018 to March 2019. The hydatid cysts were taken from the infected organs and
put in a clean container with 70% ethyl alcohol and transported to the laboratory of Veterinary Parasitology at College of Veterinary Medicine / University of Basrah. The samples were divided to five groups, each group has 10 samples as follows: group one was a control, represented by cyst without any treatment, group two was treated by tinidazole, group was three treated by *Capparis spinosa*, group four was treated by *Prosopis fracta* and group five was treated by *Quercus aegilops*. The extract or tinidazole after prepared to the plain tube were added to each group, followed by adding part of the germinal layer of the cyst for 24 hours. A part of cyst of each group was processed for DNA extraction.

The aqueous extract was prepared by distal water and plant extract according to with some modification. Twenty five gram of plant powder were added to 100ml distal water, and left for 24 hours on magnetic stirrer (IKA-combinimag) type RCT, the extract was then filter by vacuum pump using special filter paper type Whatman No-1. The extracts were concentrated and streams by rotary vacuum evaporator in type Rota Vapor-RE, Buchi, at Biochemistry Laboratory colleges of science / University of Basrah. After drying them, they were kept in dark bottle until used.

**DNA Extraction:**

DNA was extracted from liquid (Protoscolices) and germinal layer using Wizard® Genomic DNA Purification Kit (Promega/USA) following the manufacturer’s instructions. The concentration of extracted DNA was determent by NanoDrop spectrophotometer at 260 nm and 280 nm and stored at -20°C.

**Polymerase Chain Reaction:**

DNA of hydatid cyst was amplified by performing PCR technique using (GoTaq Hot Start Green Master Mix, Promega) following the manufactures instructions. Three pairs of primers were used for the detection of *Echinococcus* were used in this study: 

- **COI:** F: 5’ TTGAATTTGCCACGTTGGAATGC 3’, R: 5’ GAACCTAACGACATAATGA 3’ with product size 792bp
- **sh1-4:** F: 5’ GTTATAAGGCGCTTCGTTGTTGG 3’, R: 5’ CGTACGATTAGTTTCACAATATATGA 3’ with product size 295bp
- **G6-7:** F: 5’ TGGGGTAGTTACAATAGTTATTC 3’, R: 5’ CATATTCAATGAGTACGATTA 3’ with product size 234bp. The annealing temperature for all PCR are 56°C.

The PCR conditions are as follows: initial denaturation for 5min at 95°C, followed by 35 cycles of 95°C for 30sec, 56°C for 30sec, 72°C for 30sec. The reaction was then held at 72°C for 10min, and then cooled down 4°C for 5min (table 2). The PCR product was then detected on agarose gel stained with ethidium bromide, used 100-1000bp (KAPA BIOSYSTEM) and 1kb DNA ladder (Promega).

**DNA Sequencing and Sequences analysis**

PCR product was sent to Macrogene (Korea) company, for sequencing. The sequences were edited and aligned using Parbi-Doua and NCBI BLAST programs. Contrasted the results with data gained from Gene Bank published ExPASY program which is available at the NCBI online. Phylogenetic analysis was performed by using the NCBI program.

**Results and Discussion**

This study showed that the total number of the examined sheep was 213 and the total number of infected sheep was 75. The total percentage of infection was (35.2%) which is agreed and disagreed with who showed that the prevalence of infection was 1.5%, 5.9% in north and middle of Iraq, respectively. The result of infected liver and lung in sheep by hydatid cyst is -shown in figure (1, A and B). This difference could be accounted on the basis of differential management practices, natural resistance, drug treatment and nutrition. The live and dead protoscolices were stained with green and red color, respectively (Figure 1, C).

The results of this study showed that the percentage of infection is high in sheep in Basrah province. Greater endeavor is wanted to control the transmission of hydatid cysts from slaughter house by the suitable disposal of infected residue, especially in sheep. This will reduce the transmission of cysts from slaughter house to potential hosts in this region. Now, veterinary authority put rules to control this disease through the creation good standardization abattoirs system to raise knowledge of farmers about hydatid cysts and removal stray dogs from Basrah province.

The results of the present study are compatible with other studies in different parts of the world which showed that the sheep strain is the most important pattern accountable for human injury and a broad range of intermediate hosts.
No effective chemotherapy is presently accessible for the medical treatment of cystic and alveolar hydatid disease in human \(^{29}\). However recently several anthelmintic drugs (which have shown encouraging results in the reduction to the larval cystic mass) \(^{30}\), there is an evidence effect of the drug tinidazole and praziquantel on lethal the protoscolices \(^{31,32}\).

**Fig. (1): Sheep infected by hydatid cyst (A) in liver (B) in lung.**

(C) Live and dead protoscolices isolated from sheep.

**Molecular study**

In this study, the effect of plant extract or drug against *Echinococcus* was evaluated through the determining of specific genes of three groups: control, extract and treated by tinidazole.

Three genes, including strain *G6-7* (234bp), strain *sh4-1* (295bp) and strain *COI* (792pb) were detect in this study.

Fig.(2) showed the results of PCR (*G6-7, sh4-1, COI*) in group one (control group) and group two (treated by tinidazole), the result showed PCR amplification for *G6-7* 237bp, *sh4-1* 295bp and *COI* 792bp. While fig. (3) showed group four (treated by *Prosopis fracta*), the results of PCR the amplification for *sh4-1* 295bp and *COI* 792bp and absent for *G6-7* because the effect of plant extract. Fig.(4) appeared the results of PCR (*G6-7, sh4-1, COI*) in group three (treated by *Capparis spinosa*), the result showed that *sh4-1* 295bp was amplified but absent in *G6-7, COI* because effect of plant extract. The same figure showed the last group (group five) appeared treated by *Quercus aegilops*, the result showed absent of any amplification because effect of plant extract. The high efficacy of *Quercus aegilops* come from phenol and tannins properties \(^{33}\), Tayler study pointed out because tannins polar properties give the inhibition effect many type of microorganism, as well as limited the inhibition effect of protease enzyme \(^{33}\). For all that, this study reported the *Quercus aegilops* is the best extract against three genes used in current study, table (4) appeared all the result of genes in all groups.
Fig. (2): Results of PCR of control and tinidazole groups. lane (1) is the ladder 1kb DNA ladder (Promega), lanes (2,8) for G6-7, lanes (3,6,9) for sh4-1, lane (10) for COI. Samples treated with tinidazole are represented in lanes (11,13,15,17,19) for G6-7, lanes (12,14,18) for sh4-1, lanes (16) for COI.

Fig. (3): Results of PCR for the fourth group (treated by Prosopis fracta), lanes (1,20) is the ladders 100-10000bp (KAPA BIOSYSTEM), lanes (9,10,12,13) for sh4-1, lanes (15,16) for COI.

Fig. (4): Results of PCR for the third and fifth groups (treated by Quercus aegilops). lanes (1,21) is the ladders, lanes (2-20) show no PCR product. Group three (treated by Capparis spinosa), lanes (22,41) is the ladders, lanes (24,25,27) for sh4-1.
The results of this study agree with Hosseinzadeh in sheep genes strain \((G6-7, \, sh4-1, \, COI)\) \(^{34}\), these authors extracted DNA and used \(G7-6\) and \(sh4-1\) genes in order to detect \(E. \, granulosus\) in sheep. \(COI\) gene aligned \((792\text{bp})\) sequence matrix of partial of gene \(cox1\) which contained 124 variable sites \((15)\). \(COI\) gene was used for the determination and identification of \(Echinococcus\) \(^{16}\).

The results showed that the effect of \(Quercus \, aegilops\) extract on hydatid cyst was big in the absence of all genes in PCR product. On the other hand, the effect of \(Capparis \, spinosa\) extract was observed on only two genes \((G6-7\, \text{and} \, COI)\), whereas the effect of \(Prosopis \, fracta\) extract was observed only on \(G6-7\) gene (Table 1). \(Prosopis \, spp.\) were also known to have medicinal effects. It was demonstrated to have cytotoxic effects on its fruits, which has a significant activity against lung carcinoma \(^{35}\). The very important point was in the tinidazole group which gave positive amplification results for all genes which means the frailer to brake any of the gene.

### Table (1) Effect of tinidazole, \(Capparis \, spinosa\), \(Prosopis \, fracta\), \(Quercus \, aegilops\) against three genes with control group.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Control</th>
<th>Tinidazole</th>
<th>(Capparis , spinosa)</th>
<th>(Prosopis , fracta)</th>
<th>(Quercus , aegilops)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G6-7)</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(sh4-1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>(COI)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Percentage</td>
<td>100 %</td>
<td>33 %</td>
<td>66 %</td>
<td>66 %</td>
<td>0 %</td>
</tr>
</tbody>
</table>

### Sequencing

The results of sequencing determined the identity of \((COI, \, G6-7, \, sh4-1)\) genes. Sequence similarity of \(G6-7\) was 97\% with Austria isolation, while the \(sh4-1\) was 99\% with Oman isolation, 79\% with Estonia isolation which was recorded in GenBank in accession number \(\text{KX039965.1}\) and the partial gene recorded by \(^{36}\) and \(COI\) was 99\% identical with Australia isolation.

### Phylogenetic Analysis

The phylogenetic trees were constructed using NCBI website according to the compartment between sequences published in a recent study and other sequences of the other \(E. \, granulosus\) which were published in GenBank. Figure 5 show the phylogenetic analysis of gene \(COI\) for \(E. \, granulosus\), rooted neighbor joining phylogenetic tree. This tree shows the distribution and phylogenetic relationships between \(E. \, granulosus\) in Iraq and other countries.

### Conclusion

The results showed that hydatid cyst were detected successfully by performing PCR technique. In addition, \(Quercus \, aegilops\) has been shown the best plant extract for destroying all genes, the second plant extract which is \(Capparis \, spinosa\) showed destroying of two genes \((G6-7, \, COI)\) and failed to destroy \((sh4-1)\). Furthermore \(Prosopis \, fracta\) has shown to destroy only \((G6-7)\) and tinidazole failed to destroy any of these genes used in this study.

### Financial Disclosure: There is no financial disclosure.

### Conflict of Interest: None to declare.

### Ethical Clearance: All experimental protocols were approved under the Veterinary Medicine colleges / Basrah university, Iraq and all experiments were carried out in accordance with approved guidelines.

### References

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Histological, Histochemical And Immune Histochemical Study of Effect Aging on the Parotid Salivary Glands in Buffalo

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Abstract
The present work were carried out on twenty parotid salivary glands were collected from head of buffalo (slaughtering of Basra) and divided into two equal groups (one year age and five year age). The results were showed that the parotid salivary gland consist of spherical serous acini connected with branched ducts, intercalated ducts which attached with striated ducts, these duct involved the intra lobular ducts. The larger duct named interlobular duct, myoepithelial cells located between the basal membrane and secretory acini and ducts. The results revealed that the effect of aging on the histological structures of parotid salivary gland by using routine stain, histochemical stain stereiological histologically, and immunohistochemistry. The high intensity of connective tissues in the parotid salivary glands with age of five years as compared to the age of one years (normal intensity), also showed fibrosis in the parotid gland of five year age as compared with parotid gland of one year no appear any fibrosis, stereological histologically of the mean diameter of acini for parotid gland of five years age increased significantly p<0.05 (13±0.33 micron) while acini of one years age (10.4±0.02 micron), the mean diameter of intercalated duct for parotid gland of five years age increased significantly p<0.05 (40.13±2.61 micron) as compared of intercalated duct for age one years (26.56±1.22 micron). The histochemical results by using PAS stain showed low intensity of glycogen for parotid gland of five years age as compared to the group of one years age. The immunohistochemistry (VEGF) showed decrease in the parotid salivary gland of five year age as compared to the group of one years age.

Keywords: salivary glands, aging, hypo salivation.

Introduction
The salivary apparatus consist of three major salivary gland and several minor salivary glands ¹. The parotid glands were the one and largest of the three major salivary glands located at the side of maxilla opposite to ear and resemble to pancreas ². The parotid salivary glands divided in to lobules by connective tissues, each lobules contain several secretory serous acini, these acini attached to striated ducts which led to inter lobular duct. The parotid salivary gland secret serous saliva which act as protection, antimicrobial, mineralization, lubrication, epithelial repair and buffering ³. The aging is term used in human, animals, plants and fungi caused by many factor, the first is free radicals which attack the cells and damage the DNA, the second is the imuno system depression which promote the cells and tissues for attack. The third cause is the glycation and oxidative stress ⁴. The glycogen consider as the main storage unit of glucose in the tissues and play an important role in the salivary glands ⁵ in rat. The aim of this study is to investigate the effect of aging on the histology, some histochemistry and immunohistochemistry of the parotid salivary glands.

Materials and Method
Twenty parotid salivary glands were collected from buffalo (slaughtering of Basra) and divided into two equal groups. Group I with one year age and Group II with five year age. The deparrafinized sections were stained with hematoxyline eosin for routin histological study and PAS reaction for glycogen content ⁶, as well as some deparrafinized section were processed for VEGF immunohistochemistry. The mounted section were photographed with light microscope evaluated statistically using t-test program ⁷.
Results

The present study showed that parotid salivary gland consist of spherical serous acini connected with branched ducts. There were intercalated ducts which attached with striated ducts, these duct involved the intra lobular ducts. There were larger duct named interlobular duct(Fig.1). There are two type of secretory cells (mucous cells and serous cells, the secretory granules were located in the upper part of cell. There were myo epithelial cells located between the basal membrane and secretory acini and ducts, there were connective tissue dividing the glands to lobes and lobules by trabeculli which contain nerves and blood vessels(Fig.2(B)). When comparing the two group ,the intensity of connective tissues were increased in the parotid salivary glands with age of five years as compared to the parotid salivary gland with age of one years (normal intensity)(Fig.2) .There were a few fibrosis in the parotid salivary gland of five year age as compared with parotid salivary gland of one year which showed no any fibrosis(Fig.3).

The steriological histologically of the mean diameter of acini for parotid gland of five years age observed increased significantly p<0.05 (13±0.33 micron) as compared to the parotid of one years age(10.4±0.02 micron). The steriological histologically of the mean diameter of intercalated duct for parotid gland of five years age observed increased significantly p<0.05 (40.13±2.61 micron) as compared to the parotid of one years age(26.56±1.22 micron) (Fig.1).

The intensity of PAS reaction(glycogen content) were decreased in the parotid salivary glands of five years age as compared to the group of one years age(Fig.4).

The VEGF immuonohistochemistry showed that VEGF distributed in the basal membrane around the acini, ducts and around the blood vessels in connective tissues in the parotid salivary glands. The results showed that the intensity of VEGF which showed as brown color were decreased in the parotid salivary gland of five year age as compared to the group of one years age (Fig.5).
Discussion
The present study showed that parotid salivary gland consist of spherica serous acini connected with branched ducts. These result coincide with\(^8\) in buffalo;\(^9\) in goat and sheep;\(^10\) in rhesus monkeys;\(^11\) in barking deer;\(^12\) in human and rodent;\(^13\) in local rabbit;\(^14\) in rat;\(^15\) in mice and\(^16\) in rabbits. These result disagreed with\(^17\) in castrated donkey;\(^18\) in dog;\(^19\) in mammals;\(^20\) in dog, cat and ferret, who observed that parotid gland was a mixed gland serous and mucous.
There were intercalated ducts which attached with striated ducts. These results agree with the study of the African palm squirrel, who reported the presence of a large number of intercalated duct in the parotid gland with only serous cells. These duct involved the intralobular ducts. There were larger ducts named interlobular ducts. These results agree with study of 21 in camel; 22 in castrated donkey and 8 in buffalo, who reported the intercalated duct and striated duct were referred to intralobular duct, disagree with 23 in buffalo who reported that the intralobular ducts in buffaloes without striated duct.

There were myoepithelial cells located between the basal membrane and secretory acini and ducts agree with study of 24 in Dungarian hamster, disagree with 25 in rat who reported that, the serous acini lack myoepithelial cells.

The intensity of connective tissues were increased in the parotid salivary glands with age of five years as compared to the parotid salivary gland with age of one year agree with 26 and Scott and co-workers e.g., 27,28 in human; 29 in rats; 30 in human; 31 in rats, who said the major salivary gland tissue represented by acinar cells with age, serous and mucous acinar cells are lost or decrease in the number and replaced by fat, connective tissue due to aging. The increase in adipose tissue by aging may be attributed to the reduction in the hormone Testosterone which associated with aging 32 in human; 33 in rats.

There were a few fibrosis in the parotid salivary gland of five year age as compared with parotid salivary gland of one year which showed no any fibrosis, the result agree with 1 in human; 34 in human; 35 in rat.

The mean diameter of acini for parotid gland of five years age observed increased significantly p<0.05 (13±0.33 micron) as compared to the parotid of one year age(10.4±0.02 micron) increase in the diameter of both the serous and mucous acini has been shown in this study agree with 36 in human; 37 in rats.

The mean diameter of intercalated duct for parotid gland of five years age observed increased significantly p<0.05 (40.13±2.61 micron) as compared to the parotid of one year age(26.56±1.22 micron) This observation agrees with 38 in rats; 39 in human, who stated that there is an increase in the ductal diameter with aging.

The intensity of PAS reaction(glycogen content) were decreased in the parotid salivary glands of five years age as compared to the group of one years age agrees with 40 in mice, who mentioned that the fibrotic regions were rich in collagens and it has been investigated that the total collagen density was lower in young mice than in older mice. In addition, acinar atrophy and dilated interlobular ducts were observed in old mice.

The results showed that the intensity of VEGF which showed as brown color were decreased in the parotid salivary gland of five year age as compared to the group of one years age agrees with (5,6) in rat, they observed that there were age-related decreases in the number of acinar cells, increase in eosinophilic zymogen granules in cells, collagen accumulation in fibrotic areas and dilatation in interlobular ducts. Also, while type I collagen and MMP-2 immunoreactivity were moderate in the salivary glands of the young mice, they were high in the salivary glands of the old mice.

**Conflict of Interest:** The author has no disclosures to report.

**Source of Funding:** Self.

**Ethical Clearance:** Not required.

**References**


Study the Protective Role of Vitamin (B6 and B12) on the Some of the Blood Physiological, Biochemical Parameters and Histological Induced by Treatment of Sodium Fluoride Male White Rat

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1Assist. Prof. Physiology/ College of Education for Girls University of Kufa

Abstract
The study was conducted in the Faculty of Pharmacy / Karbala University for the period from 12/2018 to 7/2019. The first group was orally injected with a saline solution with a concentration of 0.9 ml and was considered as a control group. The second group was injected with sodium fluoride at a concentration of 20 mg / kg body weight (Positive), while the third group was injected with sodium fluoride with the same previous concentration with vitamin B6 leaves Group G was also injected with sodium fluoride with the same previous concentration with vitamin B12 at a concentration of 25 μg / kg body weight. In the fifth group, sodium fluoride and vitamins B6 and B12 were injected with the same concentrations mentioned above with 1 mg / kg body weight. Ml of each of the solutions above and for all groups where the duration of the dosage lasted 21 days and once a day. a significant decrease in the ratio of body weight, red blood cells, hemoglobin, hematocrit, high density lipoprotein and glutathione peroxidase, whereas white blood cells, platelets, cholesterol, triglycerides, low density lipoproteins and Malondyaldehyde increased significantly in the sodium fluoride treatment group with control group.

Keywords: vitamin (B6 and B12) , blood physiological , biochemical parameters

Introduction
Vitamins are organic compounds essential for improving the functioning of the human body including biotin, folic acid, niacin, pantothenic acid, riboflavin, thiamine, vitamin A, pyridoxine, copolamine, vitamin C, vitamin D, vitamin E and vitamin K. The body needs small amounts and is obtained from a healthy diet 22. As for the importance of water-soluble vitamins, vitamin B6 had a role in the prevention of cardiovascular disease 16, immunological functions and memory diseases 43 and kidney stones. In addition, it may also be useful in the treatment of gastrointestinal diseases such as premenstrual syndrome (PMS) 55, side effects of oral contraceptives, nausea and vomiting during pregnancy (26,52), depression and carpal tunnel syndrome. Vitamin B12 also helps prevent certain diseases, especially neural tube defects 32, cardiovascular disease 37, cancer 14, depression, Alzheimer’s disease and dementia 12.

Fluorine is a rare natural element and is often found in the soil and is flammable, calcined, toxic, with fluorine compounds forming about 0.08% of the earth’s rocks 42. Fluorides in general are highly toxic to cells and tissues of the body because they cause calcium deposition. They also cause high blood pressure, respiratory failure and general paralysis. They also have a relationship to cancer. They inhibit the effectiveness of the enzyme necessary for protein synthesis and DNA synthesis. Medical research on the effect of fluoride ion on the human was found to be cancer of the treated cells. It was also found that mice injected with additional doses of fluoride ion had liver cancer 23.

Material and Methods:

Blood tests
The Automated Cell Counter (Sysmex-800, Japan) is used to calculate the blood count of different blood cells.

The sample (tube containing the blood and anticoagulant) is injected into the designated area of the
device. The blood parameters of WBC, red blood cell count (RBC), platelet count (PLT), hematocrit ratio (HCT), and hemoglobin level (Hb) are estimated by using a small amount of sample Blood and with high accuracy and short period of time.

**Measure serum cholesterol level:**

According to what he described

Measuring the concentration of high-density lipoprotein cholesterol (HDL-c):

It was measured according to what he described.

**Measuring the concentration of low-density lipoprotein cholesterol LDL-c.**

Its concentration is calculated according to the following equation:

\[
\text{Concentration of LDL} = \text{total cholesterol} - \left(\frac{\text{triglyceride}}{5} + \text{HDL concentration}\right).
\]

**Assay of Glutathione Peroxidase (GPx) Activity:**

Measuring the concentration of malondyaldehyde (MDA) in the serum.

According to what he described.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Standard Error ± Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLT</td>
</tr>
<tr>
<td>control</td>
<td></td>
</tr>
<tr>
<td>NaF</td>
<td>46.67±734.80</td>
</tr>
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<td>vitamin NaF+B6</td>
<td>49.67±453.20</td>
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<tr>
<td>vitamin NaF+B12</td>
<td>28.35±307.80</td>
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<tr>
<td>vitamin NaF+B6+B12</td>
<td>20.78±302.40</td>
</tr>
<tr>
<td>LSD</td>
<td>106.014</td>
</tr>
</tbody>
</table>

Table (1) shows different blood standards in different treatments

**Result and Dissection**

**Change blood standards**

The results of Table (1-1) showed a significant decrease (P <0.05) in the number of red blood cells (RBCs) of G2 and G3 compared with the control group G1 and the other groups G4 and G5., G4, G5 when compared with G2.

In terms of hemoglobin Hb, there was a significant decrease (P <0.05) in G2 and G3 compared to control group G1. When comparing groups G3, G4 and G5 among them except control group G1 with G2, <0.05) for hemoglobin.

In terms of hematocrit HCT, P <0.05 was also observed in G2 and G3 compared with G1. When comparing G3, G4, G5 with G1, G2 with significant G (P) <0.05) for the hematocrit ratio.

The results of the white blood cell count (WBC) differed with the previous results. A significant increase was observed (P <0.05) in groups G2, G3, G4 but did not reach the level of significance in group G5. When comparing totals except G1 with G2, Moral decline (P <0.05).

The number of platelet plaques was significantly higher (P <0.05)

A significant difference between the treated groups with control below the probability level of 0.05

b, c significant difference between the treated groups below the probability level of 0.05
The results of the present study showed a significant decrease in the number of red blood cells and the proportion of hemoglobin and hematocrit (which is the proportion of the volume of red blood cells packed to the full volume of blood) while increased the proportion of white blood cells and platelets. Reduced numbers of red blood cells, hemoglobin and packed red blood cells may be due to deficiencies in hematopoietic factors (iron, folic acid, folate B9 and vitamin B12), which are essential factors for normal blood formation and crucial for the manufacture of DNA. In the cellular divide, a deficiency of vitamin B12 and folic acid will cause dysfunction in the process of cell multiplication, especially in the red blood cell manufacturing system, leading to anemia (17, 40).

The results of the microscopic examination of the tissue sections of the liver, kidneys and rats treated with sodium fluoride have enhanced the morphological growth of white blood cells and platelets, which can explain why this was reported when exposure to high concentrations of substances and compounds increases the number of pellets The white blood of lymphocytes increases due to the immune system’s sense of fluorine ion as a defense against inflammatory processes in the liver, kidneys, spleen and bone marrow.

Our current findings agree with many previous studies that the interaction of toxic substances (sodium fluoride) with red blood cells may affect their ability to carry Hb and thus reduce its content. reported a slight increase in WBC values, while our results did not agree with what observed a decrease in white blood cell count.

B6, B12, G3, G4 and G5 were treated to modify the levels of blood parameters studied by having an important role in the formation of red blood cells and in the synthesis of hemoglobin, which is important for red blood cells to transport oxygen and therefore increase the proportion of hematopoietic as the amount of percentage volume (%) of red blood cells in the blood.

Vitamin B6 deficiency has an important role to play in the weakness of the immune system and resistance to inflammation, as it reduces the body’s ability to secrete white blood cells responsible for regulating the functions of the immune system.

**Change in the values of cholesterol, triglycerides, high-density lipoproteins and low-density lipoproteins**

Table (1-2) showed elevated cholesterol. In the G2 group was significantly higher (P <0.05) than the control group G1, while the rest of the groups were between high and low, not reaching the moral level.

When comparing the G3, G4, and G5 groups with the G2 poisoning group, they observed a significant decrease (P <0.05).

TG triglycerides in G2 were significantly higher (P <0.05) and G3, G4, and G4 were higher than G1, but did not reach the moral level.

When comparing the results in the same groups with the G2 poisoning group, a significant decrease was observed (P <0.05).

The low HDL ratio was also significantly decreased (P <0.05) in the G2 poisoning group for the control group. The other G3, G4 and G5 groups were also high but slightly higher than the G1.

When comparing the results of the same groups with the G2 poisoning group, the increase was significantly higher (P <0.05).

There was also a significant increase in LDL (P <0.05) in the G2 poisoning group from the G1 control group. It was also observed that the results of the other values were slightly higher, not reaching the moral level.

When comparing the results of these groups G3, G4, G5 with the G2 poisoning group, the results were significantly lower (P <0.05).

The results of the present study showed a significant increase in the concentration of cholesterol, triglycerides and LDL with a significant decrease in HDL level in males of adult rats treated with sodium fluoride at a concentration of 20 mg / kg compared to G1 control group. In his study of the blood and tissue effects of sodium fluoride in local rabbits.

The results of the present study differed with the results of the study of with low blood cholesterol level in pigs treated with sodium fluoride at a concentration of 150 mg / kg body weight.

While the treatment groups G3, G4, G5, sodium fluoride, vitamin B6 and B12 were separated once and again, they led to a marked improvement in the values of cholesterol and triglycerides. If LDL levels are high, they are deposited on the walls of the blood vessels, leading to narrowing and reducing their elasticity,
which leads to blockage. As a result, there is a problem in blood flow, which is called atherosclerosis. When it occurs in the arteries that feed the heart, it increases the risk of heart attack. It is worth mentioning here that HDL helps to prevent the deposition of LDL cholesterol on the walls of the blood vessels and take it out of the circulatory system as vitamin B12 contributes to the reduction of cardiovascular disease and levels Low-density lipoprotein, high blood pressure, low levels of high-density lipoprotein, obesity and diabetes.

The role of both vitamin B6 and B12 as antioxidants and in the repair of hepatic tissue cells will thus rid the body of excess cholesterol. Its role in liver cell repair leads to the liver’s return to its normal state, secretion and regulation of the levels of important lipoproteins necessary for blood plasma.

**Table (2) Shows different levels of lipid profile in different treatments**

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Standard Error ± Mean</th>
<th>LDL</th>
<th>HDL</th>
<th>TG</th>
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<tr>
<td>control</td>
<td>5.74±102.99</td>
<td>1.82±33.66</td>
<td>1.03±46.13</td>
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<td>NaF</td>
<td>6.18±182.97</td>
<td>1.63±18.92</td>
<td>3.40±69.17</td>
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<tr>
<td>vitamin NaF+B6</td>
<td>6.37±108.32</td>
<td>2.26±28.80</td>
<td>4.22±55.58</td>
<td>5.05±128.36</td>
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<tr>
<td>vitamin NaF+B12</td>
<td>6.50±104.01</td>
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<tr>
<td>vitamin NaF+B6+B12</td>
<td>6.38±107.92</td>
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<td>LSD</td>
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<td>3.488</td>
<td>1.463</td>
<td>0.746</td>
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</table>

- **b, c** significant difference between the treated groups with control below the probability level of 0.05.

- **LSD** a significant difference between the treated groups below the probability level of 0.05.

Change in the values of Glutathione Peroxidase GPX and Malondialdehyde.

The decrease in the ratio of Glutathione peroxidase decreased significantly (P <0.05) in the G2 group compared with G1 control group, as well as in the other groups G3, G4, G5 but the decrease did not reach the moral level.

When comparing the results of these groups with the G2 poisoning group, it was significantly higher (P <0.05), approaching the G1 control group.

**Figure (1) Shows different levels of Glutathione peroxidase in different treatments.**

- **b, c** significant difference between the treated groups below the probability level of 0.05.

(P <0.05) in G2 and P <0.05 in G3 and G4 were also significantly higher in the G5 group but decreased to Moral comparison with G1 control group.
When compared with G2, the G3, G4 and G5 groups were all significantly reduced (P < 0.05).

**Figure (2)** Shows different levels of Malondyaldehyde in different treatments.

- a significant difference between the treated groups with control below the probability level of 0.05.
- b, c significant difference between the treated groups below the probability level of 0.05.

**Conclusion**

The results showed a significant decrease in the ratio of body weight, red blood cells, hemoglobin, hematocrit, high density lipoprotein and glutathione peroxidase, whereas white blood cells, platelets, cholesterol, triglycerides, low density lipoproteins and Malondyaldehyde increased significantly in the sodium fluoride treatment group with control group.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Education for Girls University of Kufa, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Outcome Following Medical and or Surgical Intervention (ESPAL) for the Treatment of Epistaxis in ENT Unit in Al-Diwaniyah Teaching Hospital: Cohort Study

Ali Najm Abdullah Alibadi1, Wasam Abbas Abdulhsen Albusalih1

1ENT specialist / Al-Diwaniyah Teaching Hospital / Department of surgery / Al-Diwania / Iraq,

Abstract

Aim of the study: In the current study the aim was to evaluate a sample of Iraqi patients with epistaxis regarding medical and surgical interventions and their outcomes. The study was carried out in Ear Nose and Throat (ENT) unit in Al-Diwaniyah Teaching Hospital, Mid-Euphrates region, Iraq. The study started on January 2018 and ended on June 2019. The main variables included in the study were age, residency, level of education, occupation, clinical presentation (cold versus emergency), severity of bleeding, type of intervention (Medical versus surgical), presence or absence of associated disease and response to treatment. Medical intervention included, direct pressure, anterior nasal packing and chemical cauterization with silver nitrate; whereas, surgical intervention included transnasal endoscopic sphenopalatine artery ligation (TESPAL). Bleeding was more frequently of emergency presentation rather than cold in association with surgical group, more severe in surgical group and characterized by more frequent attacks per month in surgical group in comparison with medical group. Response to medical and surgical intervention was good and satisfactory in all patients even after follow up for 6 months at least. There were negligible adverse effects in association with surgical intervention.

Key words: transnasal endoscopic sphenopalatine artery ligation, epistaxis, Iraq

Introduction

One of the most frequent emergencies daily faced by ENT specialists is epistaxis (nose bleed) 1. Basically nose bleeds are classified into anterior and posterior ones. Anterior bleeding is the most common variety and the source of bleeding is often from Little’s areas (Kiesselbach plexus). On the other hand, posterior nose bleeding is the least common and the most likely source of bleeding is the sphenopalatine artery (2, 3). The disease may be primary in which no cause can be identified or secondary for some other conditions such as trauma and coagulopathies (1-3). Epistaxis is most often in children between 2 and 10 years and elderly between 50 and 60 years of age. It is estimated that 60 % of population experienced epistaxis at some point of their life. However, only 10 % of cases deserve medical intervention since most of cases are mild and self-limited 4. From etiological perspective, local causes include trauma, septal deviation, local steroids and cannulation; whereas, systemic causes included alcoholism, coagulopathies (hemophilia and von Willebrand disease) vascular malformations and hypertension (5-7). Epistaxis may associate the use of a number of medications such as anticoagulants (warfarin), non steroidal anti-inflammatory drugs (ibuprofen) and platelet aggregation inhibitors (clopidogrel). Neoplastic disorders should be suspected in case of headaches, facial pain, unilateral nasal blockage and facial deformity (5-7). Nosebleeds are the consequences of blood vessel rupture within the nasal mucosa. Rupture of mucosal blood vessels may be spontaneous, caused by trauma, secondary to use of a number of medication, or secondary to other neoplastic or non neoplastic disorders. When blood pressure is abnormally high, the duration of bleeding may be prolonged. Similarly the use anticoagulant medications and having coaglupathies increase the duration of bleeding 8. The most important step in management includes the identification of bleeding site whether anterior or posterior. Medical interventions such as direct pressure, anterior nasal packing, chemical cauterization with silver nitrate, anterior nasal balloon
and thrombogenic gels are usually effective in controlling anterior nasal bleeding. If these measures fail to control bleeding, a posterior source will be suspected and treated properly. Posterior nasal packing may be helpful, surgical intervention and ligation of sphenopalatine artery may be needed to control posterior nasal bleeding (9-11). In the current study we prospectively evaluated a sample of Iraqi patients with epistaxis regarding medical and surgical interventions and their outcomes.

Patients and Methods

The present cohort study included 65 patients of epistaxis who were treated medically and or surgically and followed up for at least 6 months. The study was carried out in Ear Nose and Throat (ENT) unit in Al-Diwaniyah Teaching Hospital, Mid-Euphrates region, Iraq. The study started on January 2018 and ended on June 2019. The age range of included patients was form 4 to 65 years. The main variables included in the study were age, residency, level of education, occupation, clinical presentation (cold versus emergency), severity of bleeding, type of intervention (Medical versus surgical), presence or absence of associated disease and response to treatment.

Medical intervention included, direct pressure, anterior nasal packing and chemical cauterization with silver nitrate; whereas, surgical intervention included transnasal endoscopic sphenopalatine artery ligation (TESPAL).

The study was approved by institutional ethical approval committee and a verbal consent was obtained from every participant after full illustration of the study aim and procedures.

Obtained data were transformed into SPSS (version 23) spread sheet. Numeric data were expressed as range, mean and standard deviation, whereas, categorical data were expressed as number and percentage. Independent sample t-test was used to evaluate mean difference between two groups; while chi-square test was used to study association between categorical variables. The level of significance was considered at $P \leq 0.05$.

Results

The present study included 65 patients with epistaxis. Those patients were categorized according to type of intervention into two groups. The first group included 52 patients who responded well to medical treatment and considered as medical intervention group. The second group included 13 patients who failed to respond to proper medical treatment and needed surgical intervention in the form of endoscopic sphenopalatine artery ligation. The latter group was considered the surgical intervention group. The demographic characteristics of patients enrolled in the current study are demonstrated in table 1.

The age range of all patients was from 4 to 65 years, that of medical intervention group was from 4 to 65 years and that of surgical intervention group was from 9 to 40 years. Mean age of medical intervention group was more than that of surgical intervention group, 26.15 ±8.13 years versus 18.02 ±16.55 years; however, the difference was statistically insignificant ($P = 0.095$), table 1. The study included 38 (58.5 %) males and 27 (41.5 %) females; there was no significant difference in the frequency distribution of patients according to gender between medial group and surgical group ($P = 0.378$), table 1. There was also no significant difference in the frequency distribution of patients according to residency, urban versus rural, between medial group and surgical group ($P = 0.378$), table 1. In addition, there was no significant difference in the frequency distribution of patients according to occupation between medial group and surgical group ($P = 0.318$), table 1. The distribution of patients according to level of education was also shown in table 1.

Idiopathic cases were seen in 44 (67.7 %) patients. Most of cases belonging to surgical group had idiopathic cause (93.3 %). Associated disorders were encountered mainly in patients with medical intervention and included trauma, chronic hypertrophic allergic rhinitis, dryness of nasal mucosa, common cold, diabetes mellitus, hypertension and immune thrombocytopenic purpura (ITP), as shown in table 2.

The characteristics of bleeding episodes are shown in table 3. Bleeding was more frequently of emergency presentation rather than cold in association with surgical group, more severe in surgical group and characterized by more frequent attacks per month in surgical group in comparison with medical group, as shown in table 3.

Response to medical and surgical intervention was good and satisfactory in all patients even after follow up for 6 months at least. There were negligible adverse effects in association with surgical intervention.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n = 65</th>
<th>Medical Intervention n = 52</th>
<th>Surgical Intervention n = 13</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td>0.091†</td>
</tr>
<tr>
<td>Range</td>
<td>4 - 65</td>
<td>4 - 65</td>
<td>9 - 40</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>19.65 ±15.54</td>
<td>26.15 ±8.13</td>
<td>18.02 ±16.55</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>38 (58.5)</td>
<td>29 (44.6 %)</td>
<td>9 (13.8 %)</td>
<td>0.378 ¥</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>27 (41.5)</td>
<td>23 (35.4 %)</td>
<td>4 (6.2 %)</td>
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<tr>
<td>Residency</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>Urban, n (%)</td>
<td>40 (61.5)</td>
<td>7 (10.8 %)</td>
<td>33 (50.8 %)</td>
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<td>Rural, n (%)</td>
<td>25 (38.5)</td>
<td>6 (9.2 %)</td>
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<td>Occupation</td>
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<td></td>
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<tr>
<td>Student, n (%)</td>
<td>8 (12.3)</td>
<td>8 (12.3 %)</td>
<td>0 (0.0 %)</td>
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<td>Self-employed, n (%)</td>
<td>40 (61.5)</td>
<td>31 (47.7 %)</td>
<td>9 (13.8 %)</td>
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<td>Unemployed, n (%)</td>
<td>17 (26.2)</td>
<td>13 (20.0 %)</td>
<td>4 (6.2 %)</td>
<td>NS</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate, n (%)</td>
<td>2 (3.1)</td>
<td>2 (3.1 %)</td>
<td>0 (0.0 %)</td>
<td></td>
</tr>
<tr>
<td>Primary, n (%)</td>
<td>37 (56.9)</td>
<td>35 (53.8 %)</td>
<td>2 (3.1 %)</td>
<td></td>
</tr>
<tr>
<td>Secondary, n (%)</td>
<td>15 (23.1)</td>
<td>12 (18.5 %)</td>
<td>3 (4.6 %)</td>
<td></td>
</tr>
<tr>
<td>University, n (%)</td>
<td>11 (16.9)</td>
<td>3 (4.6 %)</td>
<td>8 (12.3 %)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; †: Independent samples t-test; ¥: Chi-square test; NS: not significant at \( P \leq 0.05 \)
Table 2: Associated diseases

<table>
<thead>
<tr>
<th>Associated Disease</th>
<th>Total n = 65</th>
<th>Medical Intervention n = 52</th>
<th>Surgical Intervention n = 13</th>
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<tbody>
<tr>
<td>Idiopathic, n (%)</td>
<td>44 (67.7 %)</td>
<td>32 (61.5 %)</td>
<td>12 (92.3 %)</td>
</tr>
<tr>
<td>Trauma, n (%)</td>
<td>2 (3.1 %)</td>
<td>2 (3.8 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>Chronic hypertrophic allergic rhinitis, n (%)</td>
<td>1 (1.5 %)</td>
<td>1 (1.9 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>Dryness of nasal mucosa, n (%)</td>
<td>8 (12.3 %)</td>
<td>8 (15.4 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>Common cold, n (%)</td>
<td>1 (1.5 %)</td>
<td>1 (1.9 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3 (4.6 %)</td>
<td>3 (5.8 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4 (6.2 %)</td>
<td>3 (5.8 %)</td>
<td>1 (7.7)</td>
</tr>
<tr>
<td>Immune thrombocytopenic purpura</td>
<td>2 (3.1 %)</td>
<td>2 (3.8 %)</td>
<td>0 (0.0 %)</td>
</tr>
</tbody>
</table>

Table 3: Characteristics of bleeding episodes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n = 65</th>
<th>Medical Intervention n = 52</th>
<th>Surgical Intervention n = 13</th>
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<tr>
<td>Presentation</td>
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<td></td>
<td></td>
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<tr>
<td>Emergency, n (%)</td>
<td>12 (18.5 %)</td>
<td>7 (10.8 %)</td>
<td>5 (7.7 %)</td>
</tr>
<tr>
<td>Cold, n (%)</td>
<td>53 (81.5 %)</td>
<td>45 (69.2 %)</td>
<td>8 (12.3 %)</td>
</tr>
<tr>
<td>Bleeding severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild, n (%)</td>
<td>44 (67.7 %)</td>
<td>42 (64.6 %)</td>
<td>2 (3.1 %)</td>
</tr>
<tr>
<td>Moderate, n (%)</td>
<td>11 (16.9 %)</td>
<td>4 (6.2 %)</td>
<td>7 (10.8 %)</td>
</tr>
<tr>
<td>Severe, n (%)</td>
<td>10 (15.4 %)</td>
<td>6 (9.2 %)</td>
<td>4 (6.2 %)</td>
</tr>
<tr>
<td>Frequency of bleeding attacks/ month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, n (%)</td>
<td>12 (18.5 %)</td>
<td>12 (18.5 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>2, n (%)</td>
<td>3 (4.6 %)</td>
<td>3 (4.6 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>12, n (%)</td>
<td>18 (27.7 %)</td>
<td>18 (27.7 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>16, n (%)</td>
<td>14 (21.5 %)</td>
<td>12 (18.5 %)</td>
<td>2 (3.1 %)</td>
</tr>
<tr>
<td>20, n (%)</td>
<td>7 (10.8 %)</td>
<td>0 (0.0 %)</td>
<td>7 (10.8 %)</td>
</tr>
<tr>
<td>24, n (%)</td>
<td>2 (3.1 %)</td>
<td>1 (1.5 %)</td>
<td>1 (1.5 %)</td>
</tr>
<tr>
<td>30, n (%)</td>
<td>9 (13.8 %)</td>
<td>6 (9.2 %)</td>
<td>3 (4.6 %)</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>12 (8)</td>
<td>12 (14)</td>
<td>20 (7)</td>
</tr>
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</table>
Discussion

Nose bleeding is common in daily clinical practice and some cases are true emergency that needs prompt and rapid intervention in order to prevent further complications (1-4). Anterior nasal bleeding is usually easy to control using conventional medical approaches than posterior nasal bleeding. These medical interventions included direct pressure, anterior nasal pack and silver nitrate cauterization. However, posterior nasal bleeding frequently associated with severe and recurrent attacks of bleeding that requires surgical intervention, often in the form of endoscopic sphenopalatine artery ligation. Posterior epistaxis often arises from the posterior septum and the posterior lateral nasal wall, which constitutes 5 to 10% of patients. Due to the difficulties in spotting the location of bleeding, its treatment is more difficult than anterior epistaxis (9-11).

In the current study we included 65 cases of epistaxis that have been managed in the unit of ENT in Al-Diwaniyah teaching hospital and then we categorized them according to type of intervention into medical group and surgical group aiming at identifying possible demographic characteristics, associated disorders or characteristic of nose bleed that can predict the need for surgical intervention.

In the current study, regarding demographic characteristics, those patients who required surgical intervention were relatively young; however, the difference in mean age between both groups did not reach statistical significance. Probably those young patients has posterior nose bleeding more than those older patients; however, the lack of statistical significance makes this observation related to chance factor rather being a significant association. Indeed, several authors reported that those who need inpatient treatment are usually older than those receiving outpatients treatment (12, 13).

In the present study, associated diseases were more frequent in group of medical intervention and the majority of cases of surgical group were idiopathic. These findings seems contradictory to majority of previous reports (17-20); however, it may be merely statistical finding and increasing sample size in the future may reveal better idea regarding the impact of primary and secondary nasal bleeding on treatment approach.

In the current study following up of patients revealed significantly more attacks in association with surgical intervention in comparison with medical interferences; however, we believe that the source of this recurrent attacks is attributed to secondary infection and subsequent mucosal abrasion evidenced by that bleeding severity is much less than original one before operative interference reflecting mucosal oozing rather than true arterial bleeding.

In the current study both medical and surgical interventions were efficient in producing high rate of satisfactory good results. However, some cases did not respond to medical intervention and needed surgical intervention to control nose bleeding.

Conclusion

when medical treatment fails to control nose bleeding, transnasal endoscopic sphenopalatine artery ligation is effective and safe procedure with negligible adverse outcomes.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Diwaniyah Teaching Hospital / Department of surgery / Al-Diwania / Iraq and all experiments were carried out in accordance with approved guidelines.

References


Abstract

Aim of the study: we planned and conducted the current cohort study to evaluate the safety and efficacy of this new contraceptive method in a sample of Iraqi women.

Patients and Method: This cohort study was carried out at some governmental health institutes such as Al-Diwaniyah Maternity and Child Teaching hospital and Al-Shamiyah teaching hospital and some private clinics. The beginning of the study is dated back to the 2nd of January 2016; the study continued till end of June 2019 and included 53 women. Inclusion criteria included any women with implanon implant that was recently implanted. The variables to be evaluated included age, residency, gravidity, parity and abortions in addition to principal outcomes, complications (amenorrhea, bleeding and development of ovarian cysts) and pregnancy six months following removal of the implant. All women participating in the current study were followed up till at least six months after implant removal.

Results: The outcome of implanon use was assessed following a period of follow up that ranged from 2 to 3 years with a mean of 2.89 years, as shown in table 2. Contraceptive failure was no seen in any case with a failure rate of 0.0 %. Amenorrhea was the most frequent side effect and it has been seen 18 (34.0 %). Ovarian cyst was seen in 6 cases accounting for (11.3 %) and bleeding was seen in 2 cases only accounting for (3.8 %). Complications, amenorrhea, ovarian cysts and bleeding were not significantly correlated to any of demographic or obstetric characteristics of the study group (P > 0.05).

Key words: safety, efficacy, implanon implant, Iraqi women

Introduction

Control of the number of family members and regulation and timing of child birth become well accepted issue in our community since decades. This because of economic concern partly, as increasing family size is by itself an economic burden (1-3). In addition some cultural attitudes have been positively directed toward the quality of siblings, their education and their positive participation in the society (4). So that having little number of well educated children and of good socioeconomic status becomes the goal of Iraqi family since 80s of the previous century. Thus various methods of contraception became sought by Iraqi couples. Indeed, none of contraceptive methods is free from failure rate or adverse health issues (5,6). The methods of contraception are diverse; however, they can be grouped into two major groups, hormonal and non hormonal methods. Hormonal methods include oral birth control pills (7), skin patches (8), vaginal ring (9) and hormone releasing contraceptive coils (10). Non hormonal methods included male condom, female condom (11), copper coils (12), diaphragms (13), chemical creams, gel or suppositories (14) and natural control methods (15). The intrauterine device (IUD) is on top of the list of contraceptive methods used worldwide and it is estimated to be used by more than 168 million women globally (16). Recently in the United States, IUD gains wide acceptance by women within all ages and parities as an efficient method of avoided unintended pregnancy (17). In addition, researchers have shown that pregnancy rates in association with oral contraceptive pills and implants are about 20 times more frequent when
compared with IUD. As it was mentioned previously, there are basically two forms of contraceptive methods, hormonal based and non hormonal ways. Implanon is one of the relatively newly introduced hormonal based contraceptive methods. It is an etonorgestrol implant. It is composed of a single rod that is to be inserted in the upper arm. Etonorgestrol is a synthetic active metabolite derived from a synthetic progestin and has high affinity to bind progesterone receptors. It acts to prevent pregnancy by several ways including the inhibition of luteinizing hormone (LH) secretion, an important hormone for natural ovulation, making the cervical mucus more viscous thereby inhibiting passage of sperm, and rendering the uterine mucosa less receptive for embryo thereby interfering with implantation. Implanon was recently introduced into our country and its efficacy and safety is yet to be determined since published national research work with this regard is somewhat lacking. Therefore, we planned and conducted the current cohort study to evaluate the safety and efficacy of this new contraceptive method in a sample of Iraqi women.

**Patients and methods**

This cohort study was carried out at some governmental health institutes such as Al-Diwaniyah Maternity and Child Teaching hospital and Al-Shamiyah teaching hospital and some private clinics. The beginning of the study is dated back to the 2nd of January 2016; the study continued till end of June 2019 and included 53 women. Inclusion criteria included any women with implanon implant that was recently implanted. The variables to be evaluated included age, residency, gravidity, parity and abortions in addition to principal outcomes, complications (amenorrhea, bleeding and development of ovarian cysts) and pregnancy six months following removal of the implant. All women participating in the current study were followed up till at least six months after implant removal. The study was approved by the institutional approval committee and verbal consent was obtained from all participants following full illustration of the aim and procedures of the current study. Data were then transformed into an SPSS (version 23) spread sheet. Numeric data were expressed as mean, standard deviation, median, interquartile range (IQR) and range, whereas, categorical data were expressed as number and percentage. Correlation was calculated using Spearman correlation test and the level of significance was chosen at \( P \leq 0.05 \).

**Results**

The demographic characteristics of women enrolled in the current study were demonstrated in table 1. The study included 53 women with an age range of 17 - 40 years and a mean age of 26.70 ± 6.27 years. Women less than 20 accounted for 5 (9.4 %), women between 20 and 39 accounted for 42 (79.2 %) and women older than 40 accounted for 6 (11.3 %). According to residency there were 47 (88.7 %) women from urban areas and 6 (11.3 %) from rural areas. Regarding obstetric history, the range of gravidity was from 0 to 9 with a median of 4, the parity was ranging from 0 to 8 and the median was 3 and abortion ranged from 0 to 5 with a median of 0. Regarding mode of delivery the range of vaginal delivery was from 0 to 9 with a median of 1 per woman, whereas, the range of cesarean section was from 0 to 5 with a median of one cesarean section per woman, as shown in table 1.

The outcome of implanon use was assessed following a period of follow up that ranged from 2 to 3 years with a mean of 2.89 years, as shown in table 2. Contraceptive failure was no seen in any case with a failure rate of 0.0 %. Amenorrhea was the most frequent side effect and it has been seen 18 (34.0 %). Ovarian cyst was seen in 6 cases accounting for (11.3 %) and bleeding was seen in 2 cases only accounting for (3.8 %), as shown in table 2.

Complications, amenorrhea, ovarian cysts and bleeding were not significantly correlated to any of demographic or obstetric characteristics of the study group \((P > 0.05)\), as shown in table 3. Pregnancy after removal of the implant was seen in 17 women (32.1 %).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
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<td>Number of cases</td>
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</tr>
<tr>
<td>Age</td>
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</tr>
<tr>
<td>Range</td>
<td>17 - 40</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.70 ± 6.27</td>
</tr>
<tr>
<td>&lt; 20, n (%)</td>
<td>5 (9.4 %)</td>
</tr>
<tr>
<td>20-34, n (%)</td>
<td>42 (79.2 %)</td>
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<tr>
<td>≥ 35, n (%)</td>
<td>6 (11.3 %)</td>
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<td>Residency</td>
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Table 1: General characteristics of the study sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Urban, n (%)</th>
<th>Rural, n (%)</th>
<th>Gravidity</th>
<th>Parity</th>
<th>Vaginal delivery</th>
<th>Cesarean section</th>
<th>Abortion</th>
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<tbody>
<tr>
<td></td>
<td>47 (88.7 %)</td>
<td>6 (11.3 %)</td>
<td>0 - 9</td>
<td>0 - 8</td>
<td>0 - 7</td>
<td>0 - 5</td>
<td>0 - 5</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>4 (3)</td>
<td>3 (2)</td>
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<td>2 (3)</td>
<td>0 (2)</td>
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</table>

n: number of cases; SD: standard deviation; IQR: inter-quartile range

Table 2: Outcome of implanon use

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n</th>
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<tbody>
<tr>
<td>Contraceptive failure</td>
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<td>0.0</td>
</tr>
<tr>
<td>Complication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>18</td>
<td>34</td>
</tr>
<tr>
<td>Ovarian cyst</td>
<td>6</td>
<td>11.3</td>
</tr>
<tr>
<td>Bleeding</td>
<td>2</td>
<td>3.8</td>
</tr>
<tr>
<td>Pregnancy following 6 months implanton removal</td>
<td>17</td>
<td>32.1</td>
</tr>
<tr>
<td>Duration of implant (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>5.7</td>
</tr>
<tr>
<td>2.5</td>
<td>6</td>
<td>11.3</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>83</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>2.89</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 3: Correlations of complications to demographic and obstetric characteristics of women enrolled in the study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Amenorrhea</th>
<th>Ovarian Cyst</th>
<th>Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Age</td>
<td>0.029</td>
<td>0.838</td>
<td>0.178</td>
</tr>
<tr>
<td>Residency</td>
<td>-0.005</td>
<td>0.973</td>
<td>-0.128</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.038</td>
<td>0.786</td>
<td>0.256</td>
</tr>
<tr>
<td>Parity</td>
<td>0.029</td>
<td>0.836</td>
<td>0.259</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>0.097</td>
<td>0.491</td>
<td>0.201</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>-0.003</td>
<td>0.984</td>
<td>-0.083</td>
</tr>
<tr>
<td>Abortion</td>
<td>0.043</td>
<td>0.762</td>
<td>0.015</td>
</tr>
<tr>
<td>Duration</td>
<td>0.223</td>
<td>0.108</td>
<td>-0.015</td>
</tr>
</tbody>
</table>
Discussion

The avoidance of unintended pregnancy by Iraqi couples became well known trend in our community and the search for a suitable method for contraception with minimal adverse effects and almost zero percent failure rate became an urgent need in daily obstetric clinical practice. For that reason, now and then new modalities of contraception enters into daily obstetric practice, but basically they are always of either hormonal or non-hormonal principle of action.

Implanon is well recognized as an effective mode of contraception in developed countries. It is a single road etonorgestrol implant that is can be inserted in the upper arm and remains for a period of 3 years. The active ingredient is Etonorgestrol which is a synthetic active metabolite derived from a synthetic progestin and has high affinity to bind progesterone receptors. It produces its effects by several mechanisms such as the inhibition of luteinizing hormone (LH) secretion, an important hormone for natural ovulation, making the cervical mucus more viscous thereby inhibiting passage of sperm, and rendering the uterine mucosa less receptive for embryo thereby interfering with implantation.

In the current study, Impalnon use was evaluated in a cohort study and women were followed up for a period that lasted for an average of 2.89 years. The use of Implanon was associated with zero percent failure rate and minimal anticipated side effects such as amenorrhea and bleeding in addition to 6 cases of ovarian cyst that have resolved spontaneously. In addition, pregnancy was successful after Implanon removal in 32.1% of cases with in a period of six months after implant removal.

In addition, the cost of Implanon was also satisfactory for all participants. The safety and efficacy of Implanon has been reported by several previous reports. Some side effects have been previously reported. Damage to antebrachial cutaneous nerve at time of insertion or removal has been observed by Wechselberger et al. Damage to ulnar nerve at time of insertion has been seen by Osman et al. There was spontaneous extrusion of Implanon in one case 3 days after insertion. Extrusion has also been seen by Harrison-Woolrych and Hill. Splitting of Implanon in 2 halves in situ more than one year after insertion has been observed by Agrawal and Robinson; however, none of these adverse outcomes has been seen in our study.

Therefore, in view of available data of the current research and previously published data about Implanon single rod contraceptive implant, a conclusion can be inferred as following: Implanon is effective and safe mode of contraception and recommended to be used in daily obstetric practice in our community.

Conclusion

The use of Implanon is highly effective as a contraceptive mode since it was associated with 0% risk of contraceptive failure and it was safe because of minimal tolerable side effects in almost all included women.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Diwaniyah child and maternity hospital / Department of Obstetrics and gynecology / Al-Diwania / Iraq and all experiments were carried out in accordance with approved guidelines.

References


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Sperm Activation of Asthenospermic Infertile Patient by Using Swim-up and Swim-down Techniques

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Directorate of Education Babylon, Iraq

Abstract
The study included ten semen samples collected from infertile patients with asthenospermia. Each sample was divided into two equal parts where the sperm were activated by swim up techniques up on a part of the sample while the second part was used to activate the sperm by swim down. The results showed a significant decrease \((p < 0.05)\) in both the recovered sperm concentration and the concentration of leukocytes after activation with both techniques compared with their values before activation. A significant increase was observed in the percentage of sperm motility style A+B and a significant decrease in sperm motility percent style C after activation compared with their values before activation. The significant difference was not observed \((p >0.05)\) when comparing between the two techniques. There was also no significant difference \((p > 0.05)\) in the percentage value of the abnormal sperm percent before and after activation. The study concludes that the techniques of swim up and swim down are effective in activating the sperm of infertile patients who are infected with asthenospermia.

Key words: swim up, swim down, sperm activation

Introduction
Assistive Reproductive technologies have developed rapidly in recent years. These technologies have grown and became with therapeutic value for the infertile couples in both developed and developing countries. According to statistics on Intra cytoplasmic sperm injection (ICSI), the number of children are that borne by using ICSI was only 237809 in 2004. Intra Uterine Insemination (IUI) technique using which is the sperm of the husband is another technique of assisted reproduction which is the first choice for its ease, efficiency and low costs. IUI is used for infertility due to cervical factors, male infertility factors, mild endometriosis, and unexplained infertility. The high pregnancy rates resulting from the use of the IUI technique with the husband’s sperms and the low rates of failure and risk made it the most common; pregnancy rates reached 8 – 20% per cycle. In IUI technique, is injected recovered motile sperms after activation. IUI is acceptable when the activated sperm concentration is 0.8 -5 million / ml and pregnancy rates may be increased if the number of motile sperm are higher than the amount mentioned. A recent study confirmed that the pregnancy ratios index is based on the total number of motile sperm which is deposed in the uterus, the age of the wife, the appropriate timing of insemination, the duration of sperm preparation and the choice of appropriate and technique for infertility factor.

Swim up technique is the most common technique of sperm activation to suit most male infertility factor, while activation of the sperm by using the swim down technique is effective as it is the natural movement of the sperm. Asthenozoospermia is the most important factor of male infertility. Therefore, the present study aimed to activate the sperm of infertile patients who suffer from asthenospermia by using swim up and swim down techniques to compare the efficiency of two mentioned techniques for activation the sperms of the asthenospermia.

Material and Method
Ten samples of seminal fluid were collected from infertile patients who suffered from asthenospermia by masturbation after a period of interruption of intercourse for three days. Seminal Fluid Analysis was performed for all semen samples according to the WHO method. Each semen sample was divided into two equal parts to perform sperm activation with Swim up and Swim down...
techniques.

1- Swim up technique

Placed 1 ml of semen with 1 ml of activation medium (Ham`F-12) in a tube and mix well to be tilted at a 45° angle in an incubator at 37 c° for half an hour. The top of the medium layer was transferred to a new tube to be centrifuged rapidly at rpm 2000 for 10 minutes. The upper layer was discharged and covered the sperm pellet in the bottom of centrifuged tube with 0.5 ml of Ham`F-12 medium. They were left for half an hour in the incubator at 37 c° and then a drop was taken from the top centre and examined under a microscope to evaluate the studied sperm parameters.

Swim down technique

It was placed 1 ml of semen with 1ml of the activation medium of the Ham`s F-12 in a tube and mix well to be placed at 37°c in a incubator vertically at 90° angle for half an hour. The upper layer was discharged, the lower layer was centrifuged with 2000 rpm for 10 minutes. The upper layer was then ignored and sperm pellet deposited in the bottom of the centrifuge tube was covered with 0.5 ml of Ham`s F-12 where it was left for half an hour in the incubator at 37°c , then a drop was taken from the upper medium center and examined under a microscope to evaluate the studied sperm parameters.

Results

The results revealed in table-1- a significant decrease (p< 0.05) in the concentration sperm after sperm activation by the swim up and swim down techniques compared to the sperm concentration before activation , while significant difference was not observed in the concentration sperm between the two techniques, but the sperm concentration recovered by using swim down technique was insignificant (p>0.05) higher than those recovered by swim up technique . In terms of sperm motility at grade activity A+ B, a significant increase (p < 0.05) was observed in the sperm motility percent after activation by using swim up and swim down techniques which was compared to the values of sperm motility before activation. While the percentage for the sperm motility percent of grade C , the results were showed a significant decrease (p<0.05) in percentage of the sperm motility grade with swim down technique compared to its values before activation, while there was insignificant differences (p>0.05) was observed in swim up technique compared to sperm motility percent grade C compared to before activation . No significant difference (p>0.05) was observed in the percentage for abnormal sperm after activation compared with before activation . A significant decrease (p<0.05) was observed in the leukocytes concentration after activation by using swim up technique and swim down technique compared with value before activation , while no significant difference (p>0.05) was observed between the concentration of leukocytes between the two techniques which is used to activate sperm infertility patients who are suffering from asthenospermia.

Table (1) : The sperm parameters before and after the activation by using swimming up and swim up and swim down techniques

<table>
<thead>
<tr>
<th>Sperm parameters</th>
<th>Before activation</th>
<th>After activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration (million/ ml)</td>
<td>44.9±14.3 a</td>
<td>11.7±5.5 b</td>
</tr>
<tr>
<td>Sperm Motility Percent grade A +B</td>
<td>12.5±8.5 a</td>
<td>23.0±10.3 b</td>
</tr>
<tr>
<td>Sperm Motility Percent grade C</td>
<td>58.0±10.8 a</td>
<td>51.0±8.09 ab</td>
</tr>
<tr>
<td>Abnormal Sperm Morphology</td>
<td>30.0±15.09 a</td>
<td>21.0±11.0 a</td>
</tr>
<tr>
<td>Leukocytes concentration ( million / ml)</td>
<td>2.7±0.9 a</td>
<td>0.2±0.1 b</td>
</tr>
</tbody>
</table>
Different letters mean significant differences (p < 0.05)

**Discussion**

With the progress of reproductive technique in human, the need is required for improved sperm activation and development the increasing of the highly active motile sperm for contribute in improved external fertilization processes. For example, IUI requires activation of the sperm and isolating of the active sperm from the rest of the sperm\(^7\).

There are several effective ways to separate active sperm such as swim up technique, glass wool column technique, and density gradient\(^8\). All of these technique effectively separate active sperm from seminal plasma, but their efficiency varies with the variety of sperm parameters. The ratio of active sperms normal sperm morphology and the degree of DNA of the recovered sperm were differed significantly with difference of the techniques\(^9\). The swim up technique is represented to isolate the sperm from the sperm pellet after washing and centrifugation, it is the considered a basic and standard technique for normospermia and infertility patients, as well as for the treatment of female infertility factors\(^10\).

The washing process is necessary to remove prostoglandins, inflammatory agents and pus cells\(^11\). The technique of swimming up and other techniques are designed for obtaining motile and normal sperms. It is not necessary for these sperms to be of the highest quality, but it is important that they be able to produce acceptable pregnancy rates\(^12\).

Swim up technique does not require much experience or many rare and complex materials, so it is most practical technique for its ease and low cost\(^13\). The increase in the number of motile sperms after the activation of sperm naturally leads to an increase in the number of normal sperm morphology\(^14\). Another positive aspect of swim up technology is the removal of immotile sperms, pus cells and immature cells, which enhances the benefits of activation and preparation technique of sperm for artificial insemination\(^15\). The results of the current study showed an increase in the total number of motile sperm, the percentage of forward motile sperms (grade A+B) in the swim up technique was 23% while in the swim down it was 30%. One study has indicated that the effect of the concentration of total recovered motile sperm after perform of swimming techniques and IUI processes when less than 10 million / ml is low, and when the concentration of recovered motile sperm is more than 30 million / ml, pregnancy rates are very high and mild ratio when the concentration of recovered motile sperm is between 10 and 30 million / ml\(^16\).

The swim down technique is considered the best in term of removal of the abnormal sperm, dead sperm and other cell residues. The swim down technique shares with the other technique to got a small number of motile sperm, so this technique is preferred for people with normal sperms parameters and couples whose wives suffer from female infertility factors\(^17\).

The results of the present study showed that there was an insignificant increase in the percentage of motile sperm with progressive motility (A+B pattern) and a significant decrease in the percentage of motile sperm with no progressive motility (C pattern) when using swim down compared to swim up technique and this resulte consistently agree with the results of other study which is indicated that there was a significant increase in the number of sperm of the forward motility when using the technique of swim down compared to those sperm obtained by using swim up technique\(^18\). The swim up and swim down techniques considered as easy and highly efficient in terms of sperm activation and high-quality sperm separation for Artificial Insemination\(^19\). It was concluded from this study that the recovered sperm which is obtained after activation of the sperm by using swim up and swim down techniques with a high degree of activity qualify them for use in Artificial Insemination and the possibility of obtaining good pregnancy ratios.

**Conclusion**

A significant increase was observed in the percentage of sperm motility style A+B and a significant decrease in sperm motility percent style C after activation compared with their values before activation. The significant difference was not observed (p >0.05) when comparing between the two techniques. There was also no significant difference (p > 0.05) in the percentage value of the abnormal sperm percent before and after activation. The study concludes that the techniques of swim up and swim down are effective in activating the sperm of infertile patients who are infected with asthenospermia.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.
Ethical Clearance: All experimental protocols were approved under the Directorate of Education Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

References

The Impact of Both Types of Leukemia on the Concentrations of Alpha-TNF and serum C and Serum Immunoglobulin in Both Sexes

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1University of Kirkuk, Iraq, 2University of Tikrit \ Faculty of Education for Pure Sciences, Iraq

Abstract

Vascular leukemia is a malignant disease that affects the tissues that form the blood cells. These cells are carcinogenic and occur due to the deficiency of cells to differentiate and develop to be normal blood cells. The acute leukemia includes two types of acute and chronic, the seriousness of the disease on human life and its ability to infect all age groups, Gender The research is demonstrated to evaluate the level of tumor necrosis factor, active C protein and total white blood cells and their types in the blood of the study groups (leukemia patients and control) The research is conducted in the period from September 2018 until the end of April 2019 in the center of the province of Kirkuk specialized for tumors and blood diseases. Blood samples are collected from 141 patients with leukemia and 30 normal subjects (control group) in different age groups.

Keywords: leukemia, alpha-necrotic factor, active C protein, white blood cells

Introduction

Lungemia is a group of malignant diseases that begin in the bone marrow, infect cells that generate blood cells and multiply in the bone marrow and circulatory system. The number of red blood cells and platelets in the blood decreases. Excessive egg blood but these abnormal cells are abnormal and do not perform normal functioning. There are two types of leukemia, the first type acute leukemia, which is characterized by spleen swelling and bleeding or inflammation of the gums, and then symptoms of fatigue, exhaustion, lupus and general weakness due to anemia and appear to stained purple color on the parts of the body due to lack of platelets as well as enlargement lymph nodes (Tamimi, 2007). The condition of the patient worsens quickly and affects children (under 15 years of age) more than the elderly. The second type is chronic renal leukemia, which is the result of anorexia nervosa. It is characterized as one of the disorders of the immune system. It has about 15% of the total number of different leukemia diseases and affects all age groups, but its incidence in the elderly is more common. The causes of both types of infection are not yet known to be definitive, but scientists have attributed the causes of the disease to several factors, including viral, genetic or environmental factors such as smoking, exposure to chemicals or nuclear explosions. Of cancers, including leukemia, and that the chance of leukemia increases with aging because of the high level of free radicals in the body as age increases and the ability of the immune system to remove toxins from these free radicals. For example, laboratory tests, for example, a smear test or a full blood picture, a decrease in the proportion of hemoglobin in the blood, and a rise in total blood cells Eggs, high blood cells, lymphocytes, and only the search for aromas in the blood smear (salaf), and then the bone marrow test and the search for the aromas, and the spasms in the white blood cells are lower than in the normal state (Hoffbrand, 2006). French, British and American scientists have categorized each of the above species into several branches. Acute acute leukemia with nine branches or subtypes, acute and undifferentiated acute leukemia and leukemias, primary growth aromas, leukemias, complete growth aromatics, leukemias, leukemias, leukemias, purulent leukemia, leukemia, and chronic leukemia was categorized into leukemia with the presence of the Philadelphia chromosome and leukemia, the absence of the Philadelphia phytochemical leukemia (Hofbrand et al, 2006). The treatment of the disease lies in early
detection of the disease. Treatment immediately after the diagnosis begins by blocking the abnormal cells and then eliminating them and the stage. The first step is to strengthen and stabilize the first stage. For example, anthraxycin and cytosine compounds are used for 3 and 7 days respectively, and then Cytarabine is treated with one of the drugs. All these drugs attack normal blood cells. Bone, In addition, there are other methods of treatment, including targeted therapy, radiotherapy and biologic therapy. Materials and Methods of Work: The study was conducted at the Kirkuk Specialized Center for Oncology and Hematology. The samples are collected from patients from the beginning of September 2018 until the end of April 2019. Blood was collected from 141 patients diagnosed with leukemia by a blood diseases specialist and 30 healthy people (control) after conducting routine laboratory properties to ensure their safety from any diseases were different ages and both sexes. And withdraw 5 ml of each person’s blood and separated into two parts Part 1 2 ml Put in a test tube containing anti-clotting for tests on the total white blood cells and types and add the second part 3 ml in a glass tube and leave it for 20 minutes at room temperature and then Place the centrifuge at 3000 RPM for 15 minutes to receive the serum For the testing of the tumor necrosis factor and the effective C protein, put the first part of the blood in the Ruby autoanalyzer and give the device instructions to withdraw blood and read the full blood image The result appears on the screen G and tender instruct the device to pull the result of a tethered printer and knowledge of it after we write down the patient’s information on (name, age, sex, date) The second part of the blood after we get the serum of it we begin to conduct test checks tumor necrosis factor alpha and by the steps:

1. Prepare the solutions according to the instructions of the processed company and according to the information received from DRG International, Inc., USA,

2. Reading absorption at a wavelength of 450 nanometers by an ELISA reader

Write the results in the questionnaire for each person (patients and control).

We also carried out the effective protein c test as instructed by Spanish company Linear.

Statistical analysis: Perform all statistical analyzes in this study and calculate the mean variables and frequency distribution of the variables and compare them between the study groups through the T-Test.

Results

The results of the statistical analysis showed a high morale (p<0.01) for both the level of the tumor necrosis factor and the active c protein in the serum of patients (mean 8.79 ± 4.20, 1.90 ± 0.381) respectively, (P<0.05) in the total number of white blood cells (WBC) in the blood group of patients compared with the control group at an average of (21.6 ± 6), 15 and 6.18 ± 1.46 respectively, and showed a high morbidity of both lymphocytes and only blood cells in patients with an average of 42.7 ± 5.95 and 7.4 ± 11.9 respectively In comparison with With mean control group (mean 34.7 ± 5.9 and 1.8 ± 1.3) respectively, while a significant decrease in blood white blood cells was observed in patients’ blood with an average of 48.4 ± 19.6 compared to The control group recorded an average of 61.1 ± 6,7

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean and standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα tumor necrosis factor</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>4.76 ± 1.68</td>
</tr>
<tr>
<td>Group of patients</td>
<td>8.79 ± 4.20</td>
</tr>
<tr>
<td>Active C protein</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>&lt;From 0.5</td>
</tr>
<tr>
<td>Group of patients</td>
<td>1.9 ± 0.38</td>
</tr>
<tr>
<td>Total white blood cells</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>6.18 ± 1.46</td>
</tr>
<tr>
<td>Group of patients</td>
<td>21.6 ± 6.15</td>
</tr>
<tr>
<td>White blood cells of neutrophil</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>61.17 ± 6.77</td>
</tr>
<tr>
<td>Group of patients</td>
<td>48.4 ± 19.6</td>
</tr>
<tr>
<td>Blood cells of lymphocytes</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>34.70 ± 5.95</td>
</tr>
<tr>
<td>Group of patients</td>
<td>42.30 ± 18.3</td>
</tr>
</tbody>
</table>
While no significant difference was observed in the proportion of white blood cells of the menstrual and acid between the average group of patients compared with the control group.

**Discussion**

In this study the results of the white blood cell count were significantly higher in the group of patients compared to the control group and this result can be interpreted according to 14. When leukemia occurs, a female proliferation of leukemia cells occurs at any stage of its maturation in the bone marrow. This works on the elevation of abnormal white blood cells in the bone marrow and peripheral blood. This result can be explained according to 14 and works to reduce the performance of the work of these cells and thus reduce the immunity of the patient and therefore infected the patient at this stage microbial infections and this explains the high rate of factor necrosis alpha is a cytokine protein involved in the process against inflammation in the body in this case needs to increase by 15 C-reactive protein (C) is an anti-inflammatory protein that collects on the surface of dead, diseased or carcinogenic cells and around different types of microbes. It is present in the blood at elevated levels depending on the inflammatory state and is installed in the liver in response to factors released from the thrombocytopenia 16.

**Conclusion**

In the statistical analysis of the tumor necrosis factor alpha and b-C, the significant increase is significantly increased (p00.01) in the serum of patients with leukemia (8.79 ± 4.20, 1.90 ± 0.381 respectively) compared with the control group, (0.76 ± 1.76 ± 0.1) respectively. There is also a significant increase in the total number of white blood cells (WBCs) and lymphocytes (P00.01) Control group while white blood cells recorded a significantly lower morale compared to the control group while no significant difference may be observed in white blood cells as well as the acidity between the mean group of patients and the mean control group, the results under study show a positive relationship between the alpha-prophylactic factor C and the total number of white blood cells.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of Tikrit \ Faculty of Education for Pure Sciences, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

Psychological Factors Affecting on Performance in Speaking Skill

Raziqa Aliwy Naser Al-Khafaji¹, Jalal Azizi Farman Al-Barqawy¹, Muneer Ubeid Najem Al-Jiboory¹
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Abstract

The aim of this research paper is to evaluate performance during styles of simple perspective sensible learning due to VARK model which identifies four primary types of learners: visual, auditory, reading/writing, and kinesthetic. The study sample consists of (140 teachers of both sexes) who are distributed according to the school kind (for boys or for girls) and location, for elementary schools. The research paper is descriptive, the paper tool is a form of 25 teaching skills of learning styles. The statistic means are used in the centigrade scale, weighted average and Pearson Correlation Coefficient (PCC). The study has concluded results like visual and auditory control because of getting the highest degrees in the statistic means.

Key words: Psychological, Performance, Skill

Introduction

This language is evaluated because it is the language of Quran and it deserves a lot of interest. It is the language of Quran and Sunnah (Prophetic Tradition) as well as a language of learning and education as assured by the study of (Al-Musawi, Fatima). Al-Musawi study refers that Arabic language is a gift from God to human beings to be a means for the human to know himself. one of the factors that effects in the human and social development of the society and unites the cultural and intellectual frames of education. Most of other carriers are obliged to confess that their basic preparation is insufficient. What makes a teacher successful and distinguished is the work based on educational theories and concepts and the full understanding of educational operation sides and indicators. The successful teacher has to apply procedures that assist learners to learn and acquire targeted skills and experiences. One of the most important issue for the teachers is his knowledge about learning styles which enable him to build and design the learning units and introduce the appropriate means for his pupils rather than what is appropriate to him as a teacher. The teacher mustn’t depend on a specific method in teaching but he must be creative of his style and flexible in taking the appropriate style to achieve the educational and learning goals. Choosing the learning style is connected with the text book and teacher’s ability to use the appropriate style of the educational situation that makes the learners accept the information. This is assured by the study of which shows the sets effect in listening of pupils of English language. Some believe that factors and psychological, physical, mental and social readiness are connected with each other and considered as important factor in the achievement process. So if a pupil desire to any kind of studies increased, his achievement or degree will be increased and vice versa. It is necessary to make use of any teacher according to his competence because each teacher has something to introduce, as a part of his professional responsibility, in textbook development on the basis of continued professional preparation. The teachers’ preparation will remain as a difficult task because of the limited textbooks and educational means unless we have the efficient teacher. So it is necessary to identify the learning styles to improve the learning performance including the class interaction which leads to positive effects in the educational process. The study problem is identified by the following questions:

1- How could we know the style of each pupil of the elementary school?
2- What is the relation between the learning styles and teachers’ of Arabic language performance?
3- What is the benefit for the teacher and the pupil of elementary school to know the learning styles?
Expressions Identification:

Evaluation, as an expression, is identified by (Al-liqani and Al Jamel) as a judgment issue towards a subject (Al-liqani, 82:1996).

Al-Qala identified the evaluation as the procedures through which the data of the scientific material or a project or an occurrence or an individual is collected and studied depending on a scientific style that assures the achievement of the limited goals to take the appropriate decisions.

The performance:

It is identified by Al-Dulaimi as “a personal activity made by an individual when facing a specific task to achieve successful results”.

Learning styles:

VARK identified them as “the way in which the learner can receive knowledge”

Atiyah identified them as “motivating the learner’s mental forces and self-activity to change his behavior resulted from internal and external effects”.

Methodology

The researcher chose the descriptive method to adapt with the current study nature. That method was considered as one of the most used one in the educational and psychological researches.

Research community:

It is divided into two parts:

a- The community of governmental elementary schools in the center of Babylon province.

b- The community of teachers (of both sexes) of Arabic language in the elementary schools.

The research tool:

The research aims to evaluate the performance of teachers of Arabic language during the learning styles. So it needs monitoring form as a tool of performance evaluation. Direct monitoring is considered as one of the most appropriate means to measure the learning styles for students. It is also important to monitor the teachers educational methods due to the students kinds and interests. Direct monitoring provides a clear image about the educational behavior of teachers.

Tool validity:

Validity is considered as one of the basic conditions that should be existed in the research paper. The tool will be valid if it achieves the purpose for which it exists and can measure what is required.

Tool stability:

The research tool should be stable and it won’t unless it gives the same results when applied on individuals under the same circumstances.

Tool application:

The tool is applied as the following:

1- Making interviews with all teachers of Arabic of the fourth and fifth class and inform them about the visit purpose which is serving the scientific research.

2- The researcher makes (109) visits to the teachers of Arabic, each teacher has one or more visits.

3- Not up to the standard note is included in the notice form prepared previously.

Research results:

The researcher concluded many results, prominent among those was:

1- Students Individuals differences are not taking into consideration due to the learning styles preferred by students.

2- Visual and auditory style is controlled on most of teachers’ performance and this will lead to the lack of performing the other two styles (reading/writing, and kinesthetic).

Conclusion

The study sample consists of (140 teachers of both sexes) who are distributed according to the school kind (for boys or for girls) and location, for elementary schools. The research paper is descriptive, the paper tool is a form of 25 teaching skills of learning styles. The statistic means are used in the centigrade scale, weighted average and Pearson Correlation Coefficient (PCC). The study has concluded results like visual and auditory control because of getting the highest degrees in the statistic means.
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of basic education/University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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Effectiveness of an Instructional Programs on Patient’s Knowledge Regarding Self-Care Management after Ischemic Heart Disease

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Abstract
Cardiovascular disease remains the leading cause of morbidity and mortality worldwide, and there is a rising global burden. The effects of diet on cardiometabolic risk factors have been studied extensively. Healthy habit as a cost-effective approach to risk reduction in post-ischemic heart disease patients is proven to be beneficial. A quasi experimental study design is carried out at An Nasiriyah Heart Center in AL-Nasiriyah City, from 15th of September, 2018 to the 2nd July, 2019. A non-probability (Purposive sample) of (100) patients diagnosed with myocardial infarction divided into two group (50) patients as control group and (50) patients as study group. The data were collected through the use of questionnaire designed by researcher, which comprised of (2) parts: Part I: related to the Socio-demographic characteristics and Part II: Clinical characteristics. Part III: Assessment self-care management of IHD patients’ knowledge. Reliability of the questionnaire is determined through a pilot study and the validity through a panel of experts. The descriptive and inferential statistical procedures were used for analysis of data. Findings of the present study indicated that the instructional program recorded positive and meaningful results in improving patients’ knowledge with self-care management.

Keywords: Effectiveness, Instructional Program, Patients, Knowledge, Self-Care Management.

Introduction
Cardiovascular diseases is considered as one of the most common chronic diseases and mortality reason in the world. It is estimated that due to cardiovascular diseases, 20% of healthy life years of people will be lost by 2020. In 2000, CVD is accountable for more than 1.9 million of death in the European Union, 4.35 million deaths in Europe, responsible for 43% of all deaths in male and 55% of all deaths in female. Cardiovascular disease is developing and has become the leading reason of death in developing countries. Furthermore numerous risk factors have been linked to the development of CVD including: poor dietary habits, overweight and obesity, hypertension, insulin resistance or diabetes mellitus, alcohol consumption, smoking, poor physical activity levels, and dyslipidemia. Many of these risk factors are related and reversible through a healthy diet and increased physical activity. Adopting a healthy lifestyle can lower cholesterol, reduce recurrent myocardial infarction, need for surgery or angioplasty. One of the ways to do this is to reduce the amount of saturated fats, salts, and meats consumed. Studies have established the benefits of therapies, such as self-care management, at reduction risk factor in secondary prevention of IHD. In his study, Yancy et al. has shown 73.3% of patients felt limitation and isolation, 62% gave up hope and accepted that they did not look after themselves, 66.6% of them had frequently been hospitalized and they believed it happen due to lack of knowledge about looking after themselves. Nowadays, developing the technology has decreased the duration of diagnosis, cure and hospitalization days and patients don’t have enough time to obtain information about their disease during hospitalization. According to a study, 20% of hospitalized patients said that they have received enough information and consultation about their health conditions, while 20% of them were not satisfied about their received information and 60% have said information must be presented better. Learning self-care can direct person toward maintaining the health and cause to increase of adaptation and ability of self-
Material and Method

A quantitative research approach has been used for this study. The quasi-experimental design (two-dimensional demonstration of two-group pre-test-post-test design) conducted on patients with ischemic heart disease towards education program with application of pre-post-test approach for the study group and control group in assessing their knowledge and the application of education program for the study group. It carried out in order to achieve the initial stated objectives. The study was started from 15th of September, 2018 to the 2nd July, 2019, mission to carry out the study. A non-probability (purposive) sample of (100) patients was selected. All the patients diagnosed with ischemic heart disease and they had a medical records and they review cardiac outpatient clinics An Nasiriya Heart Center. The sample was divided into two groups each one contained (50) patients as control group and case group. The study group was exposed to an education program about knowledge of self-care management, while the control group was not exposed to such instructional program. To accomplish the study, the researcher constructed the questionnaire based on the review of previous related literature and related studies. Data were analyzed through the use of SPSS application version 0.22. Descriptive data analysis including Frequency, Percentage, Mean of score (M.S) with their Standard Deviation (S.D). Percentile Grand Mean of Score (PGMS), Percentile Global Mean of Score (PGLMS), and Relative Sufficiency (RS %). Inferential data analysis includes Chi Square statistic of the contingency, Test (ANOVA) Data, T-test (independent sample t-test), T-test (paired t-test).

For the abbreviations of the comparison significant (C.S.), we used the followings

- □ NS : Non-significant at P>0.05
- □ S: Significant at P<0.05
- □ HS : Highly significant at P<0.01

The study limited by different factors including: difficulty to reach to the national studies and previous studies regarding educational programs about nutritional regimen for myocardial infarction patients, Limited research population that could affect the generalization of research findings in all Iraqi governorates. As well as difficulty to attend some education sessions by some patients.

Table (1) Statistical distribution of both groups (case & control) by their demographic data N=100

<table>
<thead>
<tr>
<th>Items</th>
<th>Sub-groups</th>
<th>Case group Total = 50</th>
<th>Control group Total = 50</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
<td>Frequency</td>
<td>Percentage</td>
</tr>
<tr>
<td>Age</td>
<td>40-49</td>
<td>6</td>
<td>12.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>14</td>
<td>28.0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>60 and above</td>
<td>30</td>
<td>60.0</td>
<td>41</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>34</td>
<td>68.0</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>16</td>
<td>32.0</td>
<td>15</td>
</tr>
<tr>
<td>Educational status</td>
<td>Illiterate</td>
<td>12</td>
<td>24.0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Read and write</td>
<td>11</td>
<td>22.0</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>5</td>
<td>10.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>8</td>
<td>16.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Preparatory</td>
<td>6</td>
<td>12.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Institute</td>
<td>4</td>
<td>8.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bachelor and above</td>
<td>4</td>
<td>8.0</td>
<td>2</td>
</tr>
<tr>
<td>Marital status</td>
<td>Married</td>
<td>43</td>
<td>86.0</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Widowed</td>
<td>7</td>
<td>14.0</td>
<td>9</td>
</tr>
</tbody>
</table>
Table (1) shows that studied groups recorded no significant differences at P>0.05, and this reflects the validity of the selected subjects due to their similar status in the light of those variables, as well as preceding results indicating that two studied groups are thrown from the same population in the light of, and this is more reliable for this study, since any meaningful deviation between studied groups should be interpreted according to the effectiveness of applying the suggested program. Statistical distribution of case group and control group by their socio-demographic data, it explains that the highest percentage (case group 60% and control group 82%) of patients are with ages above 60 years old, and (68% of case group and 70%of control group) of them are male patients, the majority of case group (86%)and control group (82%) are married, most of the sample live in urban residents (case group74% and control group 68%), while (24% of case group and 50% of control group) are illiterate, most of (76% case group and 84% of control group) have insufficient monthly income, and are (38% of case group and 60% of control group) retired.

Table (2) Statistical distribution of both groups (case & control) by their clinical data N= 100

<table>
<thead>
<tr>
<th>Items</th>
<th>Result</th>
<th>Case group Total = 50</th>
<th>Control group Total = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
</tr>
<tr>
<td>Period of diagnosis</td>
<td>less than or equal 2 years</td>
<td>42</td>
<td>84.0</td>
</tr>
<tr>
<td></td>
<td>more than 2 years</td>
<td>8</td>
<td>16.0</td>
</tr>
<tr>
<td>Body mass index</td>
<td>under weight</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>normal weight</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>over weight</td>
<td>32</td>
<td>64.0</td>
</tr>
<tr>
<td></td>
<td>obesity</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>32</td>
<td>64.0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>18</td>
<td>36.0</td>
</tr>
<tr>
<td>if yes smoking cigar rate</td>
<td>No smoking</td>
<td>32</td>
<td>64.0</td>
</tr>
<tr>
<td></td>
<td>Cigarette</td>
<td>18</td>
<td>36.0</td>
</tr>
<tr>
<td>Period of smoking</td>
<td>10-19</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>20-29</td>
<td>9</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>13</td>
<td>26.0</td>
</tr>
<tr>
<td></td>
<td>40 and above</td>
<td>6</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>No smoking</td>
<td>18</td>
<td>36.0</td>
</tr>
<tr>
<td>No. of cigarettes</td>
<td>20</td>
<td>15</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>6</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>No smoking</td>
<td>18</td>
<td>36.0</td>
</tr>
</tbody>
</table>

Table (2) show statistical distribution of case group and control group by their clinical data, the highest percentage (84% of case group and 74% of control group) of patient have been diagnosed since two years or less, (64%of case group and 70% of control group) of patients are overweight and (64% of case group and 74% of control group ) of patients are smokers, more over (60% of case group and 72% of control group ) of the patients not educated about self-care management, more than half (64% of case group and 52% of control group) only attend hospital only for one time.

Table (3): Distribution of the studied groups according to (Studied Sub & Main Domains) with
Knowledge Regarding self-care management

<table>
<thead>
<tr>
<th>Control Groups</th>
<th>Pre</th>
<th>Post</th>
<th>Assess</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Knowledge</td>
<td>Subgroup</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Low</td>
<td>43</td>
<td>86.0</td>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
<td>7</td>
<td>14.0</td>
<td>Moderate</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Knowledge Regarding self-care management</th>
<th>Case Groups</th>
<th>Pre</th>
<th>Post</th>
<th>Assess</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Knowledge</td>
<td>Subgroup</td>
<td>No.</td>
<td>%</td>
<td>Subgroup</td>
</tr>
<tr>
<td>Low</td>
<td>47</td>
<td>86.0</td>
<td>High</td>
<td>45</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
<td>14.0</td>
<td>Moderate</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
<td>Total</td>
<td>50</td>
</tr>
</tbody>
</table>

(* ) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05. Testing based on: Wilcoxon Signed Ranks Test, and Contingency coefficient (C.C.) test.

On the subjects of studied groups concerning self-care management, results shows that there is no significant differences accounted in controlled group. While the study group subjects has assigned highly significant differences at P<0.01. Regarding compared along pre - post periods for all patients who has participated in this suggested program, there is assigned highly significant differences at P<0.01 toward study group patients.

Table (4) shows that a high significant relationship between (Pre & Post-test case groups), at (p-value = 0.000).

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Case group</th>
<th>Pre-test</th>
<th>Sig.</th>
<th>Post-test</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.044</td>
<td>.360</td>
<td>103.40</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>1.486</td>
<td>.229</td>
<td>6.400</td>
<td>.015</td>
<td></td>
</tr>
<tr>
<td>Educational status</td>
<td>.680</td>
<td>.667</td>
<td>3.293</td>
<td>.009</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>30.960</td>
<td>.000</td>
<td>884</td>
<td>.352</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>1.308</td>
<td>.282</td>
<td>3.477</td>
<td>.015</td>
<td></td>
</tr>
<tr>
<td>Monthly income</td>
<td>.483</td>
<td>.620</td>
<td>.169</td>
<td>.845</td>
<td></td>
</tr>
<tr>
<td>Residency</td>
<td>1.101</td>
<td>.299</td>
<td>550</td>
<td>.462</td>
<td></td>
</tr>
<tr>
<td>Period of diagnosis</td>
<td>.695</td>
<td>.409</td>
<td>1.038</td>
<td>.313</td>
<td></td>
</tr>
<tr>
<td>Suffering from the disease</td>
<td>.196</td>
<td>.660</td>
<td>1.928</td>
<td>.171</td>
<td></td>
</tr>
<tr>
<td>Health education toward self-caring</td>
<td>2.133</td>
<td>.151</td>
<td>3.840</td>
<td>.056</td>
<td></td>
</tr>
<tr>
<td>If yes whom getting the education</td>
<td>.681</td>
<td>.568</td>
<td>2.435</td>
<td>.077</td>
<td></td>
</tr>
<tr>
<td>No. of admissions</td>
<td>17.022</td>
<td>.000</td>
<td>353</td>
<td>.704</td>
<td></td>
</tr>
</tbody>
</table>

Sig. = significant
Discussion:

The case results show that the most frequent age group for patients’ with IHD are (60 years old and more). This result agrees with (Jernberg, et al.,) 8., they concluded in their study that (60 years old) are the dominant age group of the case sample Many studies claimed that the elderly patients’ over 60 years of age have more chances of having IHD, This fact may be related to many factors such as decrease metabolic rate for these patients’ and increase catabolic rate. Concerning the gender of the sample , the study results reveal that the majority of subjects are males. This result agrees with Tomaszewski, et al., 9 in their study they found that the dominant gender are male. In addition, the differences in sex in the broad scope of wellness and illness have been the matter of general investigation, Men consider themselves as responsible for their families and they react to ward solving any problem they face or stress without expressing of their problem to others this may become as one of the risk factor for IHD and other chronic disease, on the other hand women can be relieved from their problems by many ways for example crying, talking about the problem to friends or relative. Regarding educational level, The results of the study indicate that most of the patients’ were illiterates. This result agrees with Singh, et al., 9 their study results indicate that most participants are illiterate this may be due to the socioeconomic reasons. This may be related to culture and knowledge deficit resulting from economic and social causes related to poor living conditions. The study results show that majority of the study subjects are married, these results agree with many other studies Malik, et al., 10; Manfredini, et al., 11 their results indicated that the majority of case subjects are married, The Iraqi people are well socialized and have habits and customs they follow and one of these customs is having married in early age. Young men can face many psychological stress after many years throughout marriage. Regarding occupational status, the results show high percentage of case group are retired people. Regarding patients’ clinical data, the case results reveals that most of patients are diagnosed with angina, as it is the first signs that may occur for patient before myocardial infarction due to vasoconstriction of the coronary vessels not death part of heart muscle tissue. This finding is in agreement with finding of a previous case of Wang, et al., 18he found that there are poor knowledge level of the patients before application the educational program. A study concluded that communities need to be aware of educational programs in relation to heart diseases to reduce complications and increase knowledge of the disease. Concerning the result related to associations between post-test and demographical data. The present case reveals that there is non-significant association between post-test and demographic data of case group in relation to (Marital status, Monthly income, residency, Period of diagnosis, Suffering from the disease, training sessions and No. of admissions). The results of the present case are supported by other studies that indicated no significant difference between demographic data and post-test (Aziz & Lafi, 2013; Wigmans, 2018).

Conclusions

The majority of IHD patients are males with age 60 and above, also they are illiterate, with not enough monthly income, and most of them live in urban areas. Instructional program an appropriate and effective way to improve the patient’ knowledge about IHD self-care The study group have significant modification in relation with self-care management toward IHD at pretest and posttest The control group does not present any significant modification related to knowledge toward IHD at pretest and post test There is strong effect between the study group knowledge in pre-test and their (marital status, body mass index, Smoking Period, number of cigarettes, Number of hospital admissions).

Financial Disclosure: mThere is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing / University of Thiqar / Iraq and all experiments were carried out in accordance with approved guidelines.

References

16. Mayor S. Moderate LDL cholesterol reduction with statins shows similar benefit to aggressive lipid lowering in ischaemic heart disease. Bmj, 2016;I3437.
Abstract

In this paper, a cellulose paper was impregnated with silver nanoparticles (AgNPs) for the purpose of removing Enterococcus faecalis from drinking water. AgNPs papers were prepared by chemical reduction of silver nitrate (AgNO3) with various concentrations (0.005 M, 0.01 M, 0.015 M, and 0.025 M) using sodium borohydride (NaBH4) as a reducing agent. Two ratios of NaBH4/AgNO3 of 2:1 and 10:1 were used to show the effect of reduction on the formation and removal efficiencies of AgNPs. AgNPs papers were characterized using SEM and TEM. TEM images showed that the silver nanoparticles size in the papers varies from 1.3 to 75 nm.

Keywords: Enterococcus Faecalis, Drinking Water, Silver Nanoparticles.

Introduction

Disinfection of potable water is the specialized treatment for destruction or removal of organisms capable of causing disease; it should not be confused with sterilization, which is the destruction or removal of all life. Although disinfection methods currently used in drinking water treatment can effectively control microbial pathogens, researches in the past few decades have revealed a dilemma between effective disinfection and formation of harmful disinfection byproducts (DBPs). Three categories of human enteric pathogens are of concern in drinking water: bacteria, viruses, and ameobic cysts. Disinfection must be capable of destroying all three. Destruction or removal of these organisms is essential in providing a safe potable water supply. Some bacteria, viruses, protozoa, and larger organisms ingested from contaminated water cause diseases varying from mild illnesses to life-threatening. The Enterococcus genus is placed in the Enterococcaceae family and consists of species that occur in human and animal gastro-intestinal (GI) tracts, as well as in the guts of insects traditional fermented food and dairy products, and in various environments including plants, soil and water. Enterococcus faecalis is a non-spore-forming, fermentative, facultatively anaerobic, Gram-positive coccus. Enterococcus faecalis cells are ovoid and 0.5 to 1 μm in diameter. They occur singly, in pairs, or in short chains, and are frequently elongated in the direction of the chain. They typically have an optimum growth temperature of 35°C and a growth range from 10 to 45°C. They currently rank among the most prevalent multidrug resistant hospital pathogens worldwide as the third most commonly isolated healthcare pathogen, and are capable of causing a variety of infections including endocarditis, sepsis, surgical wound infections, and urinary tract infections. The organism has the natural ability to acquire, accumulate and share extrachromosomal elements encoding virulence traits, which help to colonize, compete with other bacteria, resist host defense mechanisms and produce pathological changes directly through production of toxins or indirectly through induction of inflammation. Nanotechnology and its application is one of the rapidly developing sciences. Silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms.

Experimental Procedure

Sampling

The samples were taken from Shatt al-Hilla, at Al-Hilla city/Iraq and during the period (November 2018 – March 2019). 500 ml of water as grabbed and kept
in precleared plastic bottle. The samples were analyzed immediately to prevent any change in their quality that may occur.

**Preparation of AgNPs papers**

A (10 cm * 10 cm * 0.8 mm) off-white paper, 100% alpha cellulose was used to be embedded with silver nanoparticles. AgNPs papers were prepared by in situ reduction of AgNO3 with various concentrations (0.005 M, 0.01 M, 0.025 M and 0.05 M) with two reduction ratio of 2:1 and 10:1. Each paper was soaked in 40 ml of AgNO3 solution for 30 minutes, then it was washed with ethanol for 1 minute to remove the excess Ag ions which not absorbed by the paper. To form AgNPs, the paper was placed in 40 ml of NaBH4 solution for 1 hr. After that, the paper was soaked in de-ionized water for 30 minutes. Then the paper was dried in the oven at 60 °C for 2.5 hrs.

**Characterization**

The synthesized AgNPs papers were characterized by Scanning Electron Microscopy (SEM), type Quanta 450 available at the University of Babylon/ College of Pharmacy and Transmission Electron Microscopy (TEM) available at Al-Nahrain University/ College of Medicine.

**Acid Digestion**

To determine the silver content in the AgNPs paper, an acid digestion of the paper was performed and then analyzes the amount of dissolved silver with an Atomic Absorption Spectrometer (AAS) (AA320N) available at the University of Babylon/ College of Material Engineering. Approximately a 100 mg of the dried AgNPs paper was reacted with 5 ml of nitric acid (HNO3) and 5 ml of water. The mixture was boiled until the paper was disintegrated. 5 ml of 30% hydrogen peroxide (H2O2) was added to the mixture to assist in the complete oxidation of the organic matter to release additional metals into the solution. The mixture was boiled again and left to be cooled, then filtered through a filter paper and then diluted by adding a 100 ml of water. The diluted mixture was tested for silver content using an AAS.

**Microbiological Test**

The raw water samples were cultured using serial dilution method, 1 ml of the sample was diluted in 9 ml of distilled water (1:10 dilution). 1 ml of 1:10 dilution mixed with 9 m; of distilled water (1:100 dilution), etc.
the filtered water samples were cultured without dilution. 0.1 ml of each sample was spread over a media plate and then the plates were incubated in 37 °C for 48 hrs in an incubator (LIB-030M).

**Results and Discussion**

**Paper Characterization**

The AgNPs papers were characterized by SEM and TEM. Fig.2 represents the images obtained by SEM to show the presence of AgNPs in paper fibers. Fig. 3 represents the images obtained by TEM to determine the particles sizes of AgNPs. Table 1 represents the particles sizes of AgNPs obtained by TEM test.

![Fig. 2: Images obtained from SEM. (a) 2:1 NaBH₄/AgNO₃ ratio. (b) 10:1 NaBH₄/AgNO₃ ratio.](image)

![Fig. 3: TEM images. (a) 2:1 NaBH₄/AgNO₃ ratio. (b) 10:1 NaBH₄/AgNO₃ ratio.](image)
Table 1: The particles sizes of AgNPs obtained by TEM test

<table>
<thead>
<tr>
<th>AgNO₃ concentration, M</th>
<th>Nanoparticle Size Range (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2:1 NaBH₄/AgNO₃ ratio</td>
</tr>
<tr>
<td>0.005</td>
<td>6.86 – 75</td>
</tr>
<tr>
<td>0.01</td>
<td>3 – 69.26</td>
</tr>
<tr>
<td>0.025</td>
<td>1.414 – 32.802</td>
</tr>
<tr>
<td>0.05</td>
<td>2 – 21.84</td>
</tr>
</tbody>
</table>

TEM images showed that an excess of sodium borohydride reductant (10:1 ratio of sodium borohydride to silver nitrate) gave more uniform and smaller nanoparticles.

Acid Digestion

Acid digestion was performed to determine the silver content of the paper. The results were obtained by using AAS (AA320N). Table 2 shows the results of the AAS test.

Table 2: Silver content of each paper

<table>
<thead>
<tr>
<th>AgNO₃ concentration, M</th>
<th>Silver content (mg Ag/g of dried paper)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2:1 NaBH₄/AgNO₃ ratio</td>
</tr>
<tr>
<td>0.005</td>
<td>3.9584</td>
</tr>
<tr>
<td>0.01</td>
<td>4.4765</td>
</tr>
<tr>
<td>0.025</td>
<td>6.3268</td>
</tr>
<tr>
<td>0.05</td>
<td>7.8669</td>
</tr>
</tbody>
</table>

The acid digestion of AgNPs papers showed silver content ranging from 3.9 to 8.7 mg Ag per dry gram of paper. The increase in silver content of the paper correlates with the increase in precursor silver ion concentration of the solution in which the papers were soaked, prior to reduction. For the same concentration of AgNO₃, the NaBH₄/AgNO₃ ratio of 10:1 resulted in more silver content than 2:1 ratio.

Bactericidal Effectiveness of AgNPs papers

Fig. 4 shows the effect of the silver content in the AgNPs paper on the removal efficiency of Enterococcus faecalis of filtered water samples and raw water samples with a NaBH₄/AgNO₃ ratio of 2:1 and 10:1 respectively.

Fig. 4: Effect of silver content on the removal efficiency of Enterococcus faecalis of raw water samples with a: (a) NaBH₄/AgNO₃ ratio of 2:1. (b) NaBH₄/AgNO₃ ratio of 10:1.
Fig. 4 shows that removal efficiency Enterococcus Faecalis of the raw water samples for both ratios ranges from 99.9% to 100% for all silver contents.

**Analysis of silver content in the effluent**

Due to possible human health effects from silver exposure, the silver content in the effluent water was analyzed by AAS. Table 4 represents the relationship between the silver content in the paper and silver release in the effluent.

**Table 4: The relationship between the silver content in the papers and silver in the effluent water.**

<table>
<thead>
<tr>
<th>AgNO3 concentration, M</th>
<th>NaBH4/AgNO3 ratio</th>
<th>Silver Content in the Effluent, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.005</td>
<td>2:1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10:1</td>
</tr>
<tr>
<td>0.01</td>
<td>2:1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10:1</td>
</tr>
<tr>
<td>0.025</td>
<td>2:1</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10:1</td>
</tr>
<tr>
<td>0.05</td>
<td>2:1</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10:1</td>
</tr>
</tbody>
</table>

As shown in Table 4, the average silver content in the effluent water for the three replicates range from 0 to 0.082 which meets the United States Environmental Protection Agency (US-EPA) guideline for drinking water of less than 0.1 mg/L [EPA, 2018]. This was due to the stability of silver nanoparticle in the cellulose paper. Sodium borohydride acts not only as a reducing agent but also as an ion stabilizer, which prevents silver ions from aggregation. Moreover, hydroxyl and ether groups in the cellulose fiber play an important role in the stabilization of metal nanoparticles.

**Conclusions**

Silver nanoparticles used in this study were well dispersed and stabilized on the paper fibers. Chemical reduction of AgNO3 by using NaBH4 as a reducing agent resulted in spherical silver nanoparticles. The NaBH4/AgNO3 ratio of 10:1 resulted in smaller sizes of silver nanoparticle and more silver content than the ratio of 2:1 for the same AgNO3 concentration. (99.9-100)% inhibition of Enterococcus Faecalis was obtained with all the concentrations of AgNO3 and NaBH4 and for both NaBH4/AgNO3 ratios.

**Financial Disclosure:** There is no financial disclosure.

**Ethical Clearance:** All experimental protocols were approved under the College of Water Resources Engineering/ Al-Qassim Green University and all experiments were carried out in accordance with approved guidelines.

**References**

Assessment of the Correlation between the Salivary Flow Rate and Dental Caries Experience among Children with β-Thalassemia Major

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Abstract

Thalassemia constitutes a group of congenital blood disorders which characterized by a defect in synthesis of one or more globin chains of human hemoglobin molecule and the resultant microcytosis and hypochromia of the RBCs. It is of two main divisions, α- and β-thalassemia and several other subdivisions. The study group composed of 40 patient years previously diagnosed with β-thalassemia major attending the thalassemia center in Thi-Qar province/Iraq. The control group, matching the age and sex of the study group, and consisted from 40 child selected from a number of primary schools. Unstimulated salivary samples was taken from each subjects under standardized conditions. The mean value of flow rate of saliva was lower among β-thalassemias (0.466±0.024) than for controls (0.829±0.048). The (mean±SE) for the primary teeth (dmfs) in β-thalassemias (1.450±0.324) was higher than that for controls (1.250±0.808), this difference was not significant (P> 0.05). The (mean±SE) of the caries experience (DMFs) in β-thalassemias (6.850±0.782) was higher than for the control group (3.600±0.489).

Keywords: β-Thalassemia major, Salivary flow rate, Dental caries experience (dmfs/DMFs).

Introduction

Thalassemia is a very worldwide common autosomal and recessive genetic disorder with a large geographical incidence difference, it is a so severe and incurable disease because prevention is the only way to evade the disease [1]. Thalassaemias are caused by markdown or complete absent in the synthesis of one or more of the globin chains that constitute the hemoglobin (Hb) unit [2,3]. About 60,000 to 70,000 newly born children are born yearly with a severe form of thalassaemias around the world [4], and unfortunately most affected children are those who born in areas of low sources of income [5], β-Thalassemia major is the most severe type of thalassemia and occurs due to a defect in the synthesis of β-globin chain [6]. Beta- homotetramers in α-thalassemia are more stable than alpha- homotetramers in β-thalassemia; therefore, in beta thalassemia α-homotetramers tend to precipitate priorly in the RBCs life span, producing marked RBCs haemolysis [7]. Few of the pro-erythroblasts beginning their maturation and can survive [8,9], the resultant few RBCs will bear an inclusion bodies that identified in the spleen, shortening the RBC lifespan and producing severe haemolytic anaemia [10]. The produced anaemia stimulates the production of erythropoietin stimulating hormone from the kidney and liver with a compensatory erythroid hyperplasia, but the marrow response is disrupted by ineffective erythropoiesis. [11]. Later on, massive bone marrow expansion will happened and the end result is skeletal deformities [12]. In thalassemias, high caries index, pallor of oral mucosa, atrophic glossitis, sialadenitis, retained deciduous teeth, shortened and spiked roots of teeth and thinning of the lamina dura could be seen [13]. Oral health, on the other hand, can also affect child’s personality, knowledge, and social relationships [14]. Dental caries is a chronic disease resulted from a complex intercommunication of a mass of cariogenic oral microorganisms which grow on the teeth surfaces within the dental [15]. Dental caries is widely spread and can affecting all ages. The experience of dental caries could

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Saliva is a heterogeneous mixture of fluids mainly produced via the major and minor salivary glands and contains oral bacteria and food debris from the gingival crevicular fluid. Saliva have an important role in the maintenance of a healthy oral environment through a variety of physicochemical and biological properties. Unstimulated (resting) saliva includes secretions that enter the mouth without any exogenous stimuli. Flow rate is the most important salivary parameter affecting oral health status and it represents the rate of salivary secretion and it is expressed in milliliters per minute. Flow rate is very important protective factor against dental caries throughout its washing and buffering effect, any minimization in the normal salivary flow rate can results in establishment of dental caries. Al-Jobouri and Al-Casey and Kataria et al.

Materials and Method

The total sample included in this study was (80) subjects. The study group was consisted of a (40) child suffering from β-thalassemia major and aged (11-12 years old). The control group, the non-thalassemic children, matching the age and gender of the study group, and consisted of a (40) child selected from the primary schools. An ethical approval was firstly obtained from the Ministry of Health and the Ministry of Education to perform the clinical examination and laboratory biochemical analysis. Also, a written consent form as well as patient information sheet were provided to each participant for gaining the acceptance of the child’s parents or his/her caregiver. Children having other diseases whose known to affect the experience of dental caries or the characteristics of saliva such as diabetes mellitus, were excluded. The oral examination was performed under standardized conditions of oral health surveys (WHO 1997). During examination, a suitable chair is used with noticing that it supports the head of the child. Clinical examination was performed using plane mouth mirror and dental probe. The reported caries experience was based on the criteria suggested by Manjie et al. Unstimulated salivary samples was taken from each subjects under standardized conditions suggested by Navazesh and Kumar. Statistical analyses were done using SPSS computer programme, version 21. Descriptive statistics including (the mean and SE) of each clinical variable were determined for all the subjects. Student t-test was used to compare the caries experience between the study and control groups. Pearson’s correlation coefficient (r) was used to assess and compare the correlations among the variables. (P> 0.05) was considered statistically not significant. (P≤ 0.05) was considered statistically significant and (P≤ 0.01) was considered to be statistically highly significant.

Results

Table (1) illustrates the results showed that the (mean±SE) in β-thalassemias (1.450±0.324) was higher than that for the control group (1.250±0.808). This difference was not significant (P> 0.05).

Table (1): Show Dental caries experience by surfaces in primary dentition among β-thalassemias and their controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>dmfs</td>
<td>1.45</td>
<td>0.324</td>
<td>1.250</td>
<td>0.808</td>
</tr>
</tbody>
</table>

* Not significant difference (P> 0.05).

Table (2) illustrates the results showed that the (mean±SE) value in β-thalassemias (6.850±0.782) was higher than that for the control group (3.600±0.489). This difference was highly significant (P≤ 0.01).
Table (2): Dental caries experience by surfaces in permanent dentition among β-thalassemias and their controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>DMFs</td>
<td>6.85</td>
<td>0.782</td>
<td>3.600</td>
<td>0.489</td>
</tr>
</tbody>
</table>

** Highly significant difference (P ≤ 0.01)

Table (3) Results showed that the salivary flow rate among β-thalassemias was lower among β-thalassemia patients than for their control subjects. This difference was highly significant (P ≤ 0.01).

Table (3): Salivary flow rate among thalassemia patients and their controls.

<table>
<thead>
<tr>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.466</td>
<td>0.024</td>
<td>0.829</td>
</tr>
</tbody>
</table>

** Highly significant difference (P ≤ 0.01)

Table (4) In primary dentition, results showed a weak negative, not significant correlation between the salivary flow rate and (dmfs) index in the study and control groups. In permanent dentition, results showed a weak negative, not significant correlation between the salivary flow rate and (DMFs) index in the study and control groups.

Table (4): Correlation coefficients of the salivary flow rate and the dental caries experience among thalassemia patients and their controls.

*NS = Not significant

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>Flow rate</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>dmfs</td>
<td>-0.097</td>
<td>0.550</td>
</tr>
<tr>
<td></td>
<td>Dmfs</td>
<td>-0.181</td>
<td>0.263</td>
</tr>
<tr>
<td>Control</td>
<td>dmfs</td>
<td>-0.147</td>
<td>0.367</td>
</tr>
<tr>
<td></td>
<td>Dmfs</td>
<td>-0.168</td>
<td>0.301</td>
</tr>
</tbody>
</table>

Discussion

In the present study, the mean value of salivary flow rate was founded to be lower among β-thalassemias (0.466±0.024) than in their control subjects (0.829±0.048). This result was also documented previously by Norri [28] and by Al-Jobouri and Al-Casey [24]. This minimization in the salivary flow rate among the study group might be attributed to the fact that the salivary glands function in thalassemic patients can be affected directly by the excessive iron deposits, and the resultant painful inflammation of the salivary glands, with either regular or diminished salivary flow [23]. However, Siamopoulou et al [29] concluded in their study that the difference in salivary flow rate between the two groups is not significant. These results were in disagreement with Luglie et al. [30] and Greenberg et al. [31] whose showed in their studies that salivary flow rate was similar in both study and control groups.

The present study also concluded that the mean value of caries experience by surfaces in primary dentition (dmfs) among β-thalassemias was higher than that recorded in their controls. This result was corresponding with Gomber et al. [32], Kaur et al. [33], Dhote et al. [34]. On the contrary, this result was disagree with Scutellori
et al. [35], Qureshi et al. [36] and Arora et al. [37] who were concluded that there is no difference between the two groups.

Furthermore, the results of the present study revealed that the mean value of caries experience by surfaces in permanent dentition (DMFs) among β-thalassemias was higher than that recorded in their controls. This results was in agreement with Hattab et al. [38], Al-Raheem et al. [39] and Al-Hadithi [40], while it was in disagreement with the results of Scutellori et al. [35] and Arora et al. [37] who were founded a similar mean value between the two groups.

In present study, there was a negative correlation between salivary flow rate and caries experience. Salivary flow rate may play an important role in relation to dental caries in which the flow rate of saliva exert cleansing activity which is critical in the clearance of food remnants and bacteria [41]. It was documented that the caries experience is obviously increased when salivary flow rate is stunted and this fact sign that the chronic decrease in flow rate is a risk factor for dental caries initiation and progression [42]. In thalassemic patients, reduced salivary flow rate can help in colonization of cariogenic S. mutans, which may have a role in the higher caries experience. Al-Zaidi [43] has documented an indubitable correlation between oral mutans streptococci and dental caries in β-thalassemia patients.

However, the higher caries experience in β-thalassemias than the normal controls can be related to factors other than a reduced salivary flow rate. This could be explained on the idea that these patients are less concern with their oral health, their parents are more worried about the serious physical condition, they paying less care to the oral health care, and seeking professional oral care just when the child experience severe pain [23, 44].

Al-Wahadni [45] and Gomber and Dewan [32] concluded in their studies that skeletal changes as enlargement in maxillary arch that occur in thalassemias in could result in protrusion of anterior segment, increased space between upper and lower teeth, overbite or open-bite which all could predispose to dental caries.

**Conclusion**

Dental caries experience was higher among beta thalassemias compared to the control group. The salivary flow rate among β-thalassemias was lower among β-thalassemia patients than for their control subjects. The reduced salivary flow rate detected in β-thalassemia major patients could be considered as a modifying factor for the increased experience of dental caries in these patients.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Dentistry/Univ. of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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Determine Effect of Carbothera Therapy on Foot and Leg Ulceration for Diabetic Patients in Endocrine and Diabetic Center at Al-Nasiriya City

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Abstract

Background: Diabetic foot is a serious complication of diabetes that aggravates the patient’s condition whilst also having significant socioeconomic impact. Increasing physicians’ awareness and hence their ability to identify the «foot at risk» along with proper foot care, may prevent diabetic foot ulceration and thus reduce the risk of amputation.

Objectives: 1. Determine the socio demographic data on diabetic foot and leg ulceration
2. Determine the relationship of socio demographic data on diabetic foot and leg ulceration
3. Determine the Effecteness of Carbothera therapy upon diabetic foot and leg ulceration improvement

Methodology: A descriptive study was conducted on a sample consisting of (50) patients is having diabetic foot and leg ulceration who were selected randomly from Endocrine and Diabetic Center in Nasiriya City. Diabetic patient who have diabetic foot and leg ulceration, carried out in Nasiriya city / endocrine and diabetic Center starting from January/ 2019 to July /2019

Results: 40% the age between 60-69 years, males was 68%, the patients residing in the city (urban) by 66%,78% of them consider that their monthly income is not enough, infected with diabetes For diabetes for a period of 10 years and more is 76%, the average age of patients was 59 years, patients discovered diabetes disease was 66% , improvement in their health through the carbothera therapy 66% indicated a positive effect on the patients, a significant mean relationship at level of 8% between the urban and rural environment variable with improved carbothera therapy indicates that therapy is more effective when the patient has a more urban than rural. In addition, a very weak and statistically insignificant relationship between the patients is other demographic data and the improvement of carbothera therapy

Keywords: Diabetic, Carbothera therapy, foot and leg ulceration

Introduction

Diabetic foot and leg ulceration is one of the most significant and devastating complications of diabetes, and is defined as a foot affected by ulceration that is associated with neuropathy and/or peripheral arterial disease of the lower limb in a patient with diabetes. The prevalence of diabetic foot ulceration in the diabetic population is 4–10%; the condition is more frequent in older patients 1-3. It is estimated that about 5% of all patients with diabetes present with a history of foot ulceration, while the lifetime risk of diabetic patients developing this complication is 15% 1. The majority (60–80%) of foot ulcers will heal, while 10–15% of them will remain active, and 5–24% of them will finally lead to limb amputation within a period of 6–18 months
after the first evaluation. Neuropathic wounds are more likely to heal over a period of 20 weeks, while neuro ischemic ulcers take longer and will more often lead to limb amputation. It has been found that 40–70% of all no traumatic amputations of the lower limbs occur in patients with diabetes. Furthermore, many studies have reported that foot ulcers precede approximately 85% of all amputations performed in diabetic patients. The risk of foot ulceration and limb amputation increases with age and the duration of diabetes. The prevention of diabetic foot is crucial, considering the negative impact on a patient’s quality of life and the associated economic burden on the healthcare system. Diabetic foot ulceration is a major health problem and its management involves multidisciplinary approach. This review aims to provide a synopsis of the current management strategies of diabetic foot ulcers, from prevention to the options for therapy. The authors believe that it may be useful to primary care physicians, nurses, podiatrists, dialectologists, and vascular surgeons, as well as all healthcare providers involved in the prevention or management of diabetic foot ulcers of skin integrity, providing a site vulnerable to microbial infection. Peripheral arterial disease is 2–8 times more common in patients with diabetes, starting at an earlier age, progressing more rapidly, and usually being more severe than in the general population. It commonly affects the segments between the knee and the ankle. It has been proven an independent risk factor for cardiovascular disease as well as a predictor of the outcome of foot ulceration. Even minor injuries, especially when complicated by infection, increase the demand for blood in the foot, and an inadequate blood supply may result in foot ulceration, potentially leading to limb amputation. The majority of foot ulcers are of mixed etiology (neuro ischemic), particularly in older patients. In patients with peripheral diabetic neuropathy, loss of sensation in the feet leads to repetitive minor injuries from internal (calluses, nails, foot deformities) or external causes (shoes, burns, foreign bodies) that are undetected at the time and may consequently lead to foot ulceration. This may be followed by infection of the ulcer, which may ultimately lead to foot amputation, especially in patients with peripheral arterial disease. Structural foot deformities and abnormalities, such as flatfoot, hallux valgus, claw toes, Charcot neuroarthropathy, and hammer foot, play an important role in the pathway of diabetic foot ulcers since they contribute to abnormal plantar pressures and therefore predispose to ulceration. Other risk factors for foot ulceration include a previous history of foot ulceration or amputation, visual impairment, diabetic nephropathy, poor glycemic control, and cigarette smoking. Some studies have shown that foot ulceration is more common in men with diabetes than in women. Social factors, such as low socioeconomic status, poor access to healthcare services, and poor education are also proven to be related to more frequent foot ulceration.

**Methodology**

Non-probability (purposive) sample application was conducted on a sample consisting of (50) patients having foot and leg ulceration who were selected from endocrine and diabetic center in Nasiriya city. An assessment format was constructed and designed depending on open format information and after reviewing the related literature, the objective of this assessment was to evaluate the need for the educational program. The assessment was conducted from January 2019 to July 2019 and the format was comp each respondent a period between 30-40 minutes. Researcher were fill out the format by himself. For those who were unable to ask to read and write, the researcher filled out the format herself with their original responses.

The data of present study were analyzed through the application of two statistical approaches, which may assist for the determination of the study results.

**Statistical analysis**

A statistical questionnaire on diabetic foot disease was prepared and a sample of 50 patients was selected. The form consisted of two axes: The first on the socio-demographic aspect of the patient and the second on the clinical medical aspect.

The data from the form were classified and the variables were coded according to the requirements of the statistical program SPSS.

Since the variables used are qualitative and not quantitative, the correlation coefficient has been used to measure whether there are links between some variables and their analysis. The Spearman correlation coefficient is based approximately the vocabulary of each of the descriptive variables in question, with each given numeric value showing its order. Using these ranks, the correlation coefficient, called “grade”, can be calculated. The correlation coefficient can be calculated through the following formula:
\[ R_s = 1 - \frac{\sum D^2}{n(n^2 - 1)} \]

Where \( n \) = number of observations

\( D \) = differences between two ranks of two observations

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
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<td></td>
</tr>
<tr>
<td>40-49 Year</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>50-59 Year</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>60-69 Year</td>
<td><strong>20</strong></td>
<td><strong>40</strong></td>
</tr>
<tr>
<td>70-80 year</td>
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<td>10</td>
</tr>
<tr>
<td>Total</td>
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</tr>
<tr>
<td><strong>Gender</strong></td>
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</tr>
<tr>
<td>Male</td>
<td>34</td>
<td>68.0</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>32.0</td>
</tr>
<tr>
<td>Total</td>
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</tr>
<tr>
<td><strong>level of education</strong></td>
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<td>Secondary</td>
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<td>Prepared</td>
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<td>postgraduate</td>
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<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Monthly income</strong></td>
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<td></td>
</tr>
<tr>
<td>Enough</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td>Enough to some extent</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td>Not Enough</td>
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<td>78.0</td>
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<tr>
<td>Total</td>
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<tr>
<td><strong>Residential area</strong></td>
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<tr>
<td>Urban</td>
<td>33</td>
<td>66.0</td>
</tr>
<tr>
<td>rural</td>
<td>17</td>
<td>34.0</td>
</tr>
<tr>
<td>Total</td>
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<td>100.0</td>
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<tr>
<td><strong>Transport</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owns</td>
<td>3</td>
<td>17.6</td>
</tr>
<tr>
<td>Non owns</td>
<td>14</td>
<td>82.4</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
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<tr>
<td><strong>housing</strong></td>
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<tr>
<td>possession</td>
<td>7</td>
<td>41.2</td>
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<td>common property</td>
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<tr>
<td>leasehold</td>
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<td>other</td>
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<td>5.9</td>
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<tr>
<td>Total</td>
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<td>100.0</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
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<td></td>
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<tr>
<td>Married</td>
<td>12</td>
<td>70.6</td>
</tr>
<tr>
<td>unmarried</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>Other (Divorce, widow)</td>
<td>3</td>
<td>17.6</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Result:

Table (1) Distribution of the Study Sample by their General Information

Table (1) shows that the percentage of age was 40% for age between 60-69 years, males was 68%, which is higher among the infected than the female rate of 32%, the patients residing in the city (urban) by 66%, the adequacy of the monthly income of the patient or not, 78% of them consider that their monthly income is not enough.

Table 1 Average age of the statistical sample of a diabetic foot and leg ulceration patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Valid</th>
<th>N</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>59.16</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td></td>
<td>8.421</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

Table 2 showed that the average age of patients participating in the study was 59 years and that the ages of patients ranged between 40 and 80 years with 50% of patients between the ages of fifty-sixty years.

Table (3) Distribution of the Study Sample by their some variable

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-Infected diagnosed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>17</td>
<td>34.0</td>
</tr>
<tr>
<td>yes</td>
<td>33</td>
<td>66.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
<tr>
<td>Improvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>17</td>
<td>34.0</td>
</tr>
<tr>
<td>yes</td>
<td>33</td>
<td>66.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
<tr>
<td>Walking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>9</td>
<td>18.0</td>
</tr>
<tr>
<td>yes</td>
<td>41</td>
<td>82.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (3) showed that the majority of patients discovered diabetes through their reviews, not the same thing. The proportion of patients diagnosed with the disease was 66% of the total sample. The percentage of patients who reported an improvement in their health through the therapy of carbothera therapy 66% indicated that the therapy had a positive effect on the patients, the form of the number of patients who responded to exercise daily, which is very useful to them, the number of patients who responded that they are walking Which were very useful to them, constituted a high rate of 82% of the patients in the sample.
Table (4) showed the majority of patients (23 patients and 46% of total patients) who responded to therapy were among the 1 to 15 session sessions supporting the patient’s response to therapy after attending only one and a half sessions (two sessions a week). The relationship between Spearman and (0.248) indicates that a significant mean relationship at an error level of 8% between the urban and rural environment variable with improved carbothera therapy indicates that therapy is more effective when the patient has a more urban than rural environment. The patient to the consequences of disease in the city be committed to therapy and get better results. While the relationship between the mean and moral level of error does not exceed 9 per cent between the variable and the presence of monthly salary of the patient due to lack of improvement in the therapy of carbothera, indicating that the therapy is more effective when the patient a monthly salary. This result is for the patient’s mental state and the state of safety provided by the continuous monthly salary.

The correlation is very weak and there is no daily walk to the patient with improved therapy of carbothera through the value of the link (Spearman) and the amount (0.007), may appear this way because the patient has difficulty walking because of the disease and declare that he walked but did not exceed the steps were not simple you remember.

Discussion of the Results

The study comprised 50 participants with confirmed diagnosis of diabetes mellitus. Were more males than females, age between 60-69 years that is (due to the customs and traditions that require men to accompany women during their visit to the hospital for therapy). The patients residing in the city (urban), Which indicates that the incidence of the disease may relate to the nature of nutrition in the city and the natural nutrition in the countryside, the adequacy of the monthly income of the patient or not, of them consider that their monthly income is not enough, which indicates that insufficient income leads to “malnutrition and the lack of a suitable environment for living”. Improvement in their health through the therapy of carbothera therapy indicated that the therapy had a positive effect on the patients, the form of the number of patients who responded to exercise daily, the number of patients who responded that they are walking Which were very useful to them, constituted a high rate of the patients in the sample, but the statistical results did not show a correlation with the improvement of their health condition due to their inability to walk already and the decline of a few daily plans, which makes it useless healthily. This agree with the study by Bakker et al. 2016 (International Working Group on Diabetic Foot Editorial Board. The development of global consensus guidelines on the management and prevention of the diabetic foot.

Conclusion

The management of diabetic foot and leg ulcers remains a major therapeutic challenge, which implies an urgent need to review strategies and therapy in order to achieve the goals and reduce the burden of care in an efficient and cost-effective way. Questions remain as to which types of intervention, technology, and dressing
are suitable to promote improvement, and whether all therapies are necessary and cost-effective as adjunctive therapies. Diabetic foot ulcers should be carefully evaluated and the gold-standard therapy should be strictly applied in order to prevent amputation. Further clinical studies are needed to support the existing evidence regarding the clinical benefit of new approaches for the therapy of diabetic ulcers, and these approaches should be used only as add-on therapies to the gold-standard wound care.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, Al-Muthanna University, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Outcome of Endoscopic Endonasal Repair of CSF Rhinorrhea using Fascia Lata Graft

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Abstract

Cerebrospinal fluid rhinorrhea occur when there is communication between skull base and the nasal cavity. Nowadays endoscopic endonasal repair of CSF rhinorrhea become the preferred surgical intervention.

Setting: ENT department at Ghazi AL Hariri Hospital for specialised surgeries, Medical City, Baghdad, Iraq.

Aim: To assess the effectiveness of fascia lata as sealing graft in the endoscopic endonasal repair of CSF rhinorrhea.

Patients and Method: Fifteen patients present to our department with possible clinical diagnosis of CSF rhinorrhea. The patients submitted to full history, examination including nasal endoscopy, radiological investigation (CT and MRI) of the nose and paranasal sinuses and skull base and CSF analysis. Operative technique was endoscopic endonasal repair using fascia lata graft. With monthly follow-up.

Results: Successful rate noted in 93% of the patients. No recurrence of meningitis attacks among those who reported meningitis on their presenting illness.

Conclusion: Endoscopic endonasal repair of CSF rhinorrhea has less operative and postoperative complication. Fascia lata graft offers a good choice for the repair.

Key words: CSF rhinorrhea, fascia lata graft and endoscopic endonasal repair.

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E-mail: drlaith2006@yahoo.com.

Abstract

A number of important etiologies may result in CSF rhinorrhea. The most common include accidental trauma, surgical trauma, congenital, tumors, and spontaneous (or idiopathic). Careful understanding of these etiologies is a prerequisite prior to devising a comprehensive diagnostic and management strategy for effective surgical repair of CSF rhinorrhea. Approximately 80% of all cases of CSF rhinorrhea occur in the setting of accidental trauma, mostly in the form of closed-head injury. Conversely, CSF rhinorrhea is noted in only 2% to 3% of cases of serious head trauma. It is imperative to establish the diagnosis of CSF rhinorrhea beyond doubt before considering surgical repair. If this is not done, patients may be subjected to unnecessary surgical intervention and its attending morbidity.

Contraindications to the endoscopic treatment of CSF fistulas include the presence of an intracranial lesion; a fracture of the posterior wall of the frontal sinus; lateral extensions of the frontal and sphenoidal sinus, and CSF rhinorrhea from a temporal bone defect. Aim of the study is to assess the effectiveness of fascia lata graft in the endoscopic endonasal repair of CSF rhinorrhea.

Patients and Method

Across sectional study was conducted between March 2014 and November 2015 at ENT department,
Ghazi AL-Hariri for specialised surgeries Hospital, Medical City Complex, Baghdad, Iraq. Fifteen patients (eight males and seven females) were with a possible clinical diagnosis of CSF rhinorrhea. The duration of symptoms ranged from two months to twelve years. All patients had failed conservative treatment.

All patients underwent a thorough clinical history, endoscopic examination of nasal cavity, and the glucose concentration of the nasal discharge (CSF) was analyzed. All our patients underwent computed tomography (CT) and magnetic resonance imaging (MRI); CT scan was helpful in showing fracture site in post-traumatic CSF rhinorrhea while MRI was beneficial in detecting meningocele. Inclusion criteria was both the traumatic and non-traumatic CSF leak. Patients with skull base tumor were excluded from the study. Endoscopic examination where done using local decongestant and topical anesthesia using 0 degree rigid scope and the findings were difficult to localize the exact site of CSF leak, but the leak was noted coming from sphenoethmoidal recess or frontoethmoidal area other patients with an obvious meningocele was noted specially in pediatric age group. Our surgical option was endoscopic endonasal repair of CSF rhinorrhea using tensor fascia lata as sealing graft. All surgeries done under general anesthesia with oral endotracheal intubation. Patients lie in slightly reverse trendelenberg position. In cases were the defect in the cribriform area or from sphenoethmoidal area ,we lateralized the middle turbinate to get access to those areas sometimes we did complete ethmoidectomy to get access to the fovea ethmoidalis, In cases were a meningocele is present we follow it till reaching its neck and visualize the defect which coming from. The most critical step in the repair was to cauterize all the mucosa surrounding the defect and denuded the bone by using a bipolar suction diathermy to help get a good ground for the graft. A fatty tissue which obtained from the patient thigh plugged into the defect (underlay) ,after that an appropriately sized tensor fascia lata graft ( 30% larger than the defect due to later shrinkage of the graft ) is insinuated into the defect a few millimeters.then a gelfoam sponge was inserted to support the graft and a Merocil (Kennedy) pack was inserted and it was removed at third or fifth day; once the packs were removed; an endoscopic examination is done to remove any crustation and to get the first look after the surgery .The patients were instructed to minimize their daily activity like weight lifting, upstairs, sex, and avoid constipation by eating rich fiber diet . Patients were advised for monthly visit for endoscopic examination and assessment.

**Results**

Fifteen patients were with diagnosed of CSF rhinorrhea their age range from two years to fifty five years. Duration of symptoms range from two months to twelve years, eight patients were male and seven patients were female (figure 1); six patients experienced CSF leak from the left side while the other nine patients had the leak from the right side (figure 2).

![Figure 1](image1.png) **Figure (1) Gender ratio**

![Figure 2](image2.png) **Figure (2) Eight patients had history of head trauma (of those, three patients had previous nasal surgery) while the remaining seven patients had non traumatic CSF rhinorrhea (figure3).**

![Figure 3](image3.png) **Figure (3) Traumatic versus spontaneous CSF leak**

Of those fifteen patients, five patients reported history of meningitis (ranging from one to several
attacks during their illness); the other patients had no such history, figure (4).

Figure (4) meningitis rate

Eight patients were founded have a meningocele during the surgery; five meningocele were originated from cribriform plate, two meningocele were originated from fovea ethmoidalis, while one patient had a sphenoidal meningocele, figures (5),(6)

Figure (5) rate of meningoceles

Regarding CSF rhinorrhea without meningocele; the sites of the leaks were; three patients had leaks from cribriform plate; three patients had leaks from fovea ethmoidalis and one patient had leak from frontal sinus.

Figure (6) origin of meningoceles

Discussion

In our country; CSF rhinorrhea had been treated surgically by neurosurgeons using open approach (craniotomy) for many years with its highly morbidities and mortalities. In the last few years a great move done in our otolaryngology department Ghazi AL Hariri hospital to treat such cases with CSF rhinorrhea by endonasal endoscopic approach. In our study male with CSF rhinorrhea more common than female. Yong-Gang Kong et al (2013), found seventeen male and five female among 22 cases with CSF rhinorrhea. 

Thibaut Van Zele et al (2013), found five female and one male among six patients. Majority of our cases (53.3%) were traumatic, which is similar to most other study. S. Schmerber et al (2006), found six pateintns had spontaneous leak and sixteen patientns had traumatic CSF leak. Muhammad Umar Farooq and Murtaza Ahsan Ansari (2011), also found the same rate. In our study all patients submitted MRI, Bernardo Cunha Araujo Filho et al (2005), Order MRI only for suspected cases of meningoencephaloceles. José Alberto Landeiro et al (2004), ordered MRI for all patients. We had five cases of meningitis, Bernardo...
Cunha Araujo Filho et al (2005), report one case among 44 cases. 9. Yong-Gang Kong et al (2013) found four cases among twenty two patients (5). Mazhar Husain et al (2006), found three patients had meningitis among nine patients. 11. Antibiotics cover used for all our patients, Friedman JA et al (2001), adopted antibiotics for all patients. 12. We have eight cases with meningocele among our patients, Yong-Gang Kong et al (2013), had two cases among 22 patients. 5. In our study, majority of our cases had leak from lateral lamella of cribiform plate 53.33%. Bernardo Cunha Araujo Filho et al (2005), found the same. (9) Silva et al (2006), found sphenoid bone involved more commonly than ethmoid bone. (13) The success rate was of CSF closure in our study was 93.33%. Kirtane et al (2005), had success rate of 96.63%. (14) Bernardo Cunha Araujo Filho et al (2005), 80% success rate. (9) Schick et al (2001), report success rate of 80%. (15)

**Conclusion**

Nowadays endoscopic endonasal repair of CSF rhinorrhea become the preferred option for the repair of CSF rhinorrhea with fascia lata graft offer a good choice for the repair in both traumatic and spontaneous CSF leaks with high successful rate.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Gazi AL-Hariri Hospital for specialised surgeries, Medical City, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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7. S Schmerber MD, Ch. Righini MD, JP Lavielle, MD. Endonasal Endoscopic Closure of Cerebrospinal Fluid Rhinorrhea, Departments of Ear, Nose, and Throat Surgery and 2Neurosurgery, Grenoble Hospital, Grenoble, France. Copyright C 2001 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.


Genotype and haplotype of HLA- class II in Type I and Type II Diabetes Mellitus in Iraqi Patients

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Abstract

Background: Diabetes is one of the common complicated disease associated with multiple influenced factors, immunological serological and molecular factors play a great role in susceptibility and diagnosis of this disease. HLA genotyping were reported to have an influencing in both types of diabetes.

Aim: The current study was conducted to investigate the association of human leukocytes genotypes in Diabetes patients.

Method: Case-control study enrolled 225 blood samples collected from patient attended to the Marjan Teaching Hospital- Hilla and 25 apparently healthy from October 2018 to May 2019. Class II HLA genotyping was performing for 60 patients with diabetes mellitus and 25 healthy unrelated controls by means of the PCR-SSP method. The diagnosis of T1D and T1ID was set up according to American Diabetes Association criteria.

Results: both types of diabetes were significantly associated with HLA-DR3.  Associations were also observed with HLA –DQA105:01, the appearance of these two alleles differs in both TID and TIID . Conclusion. Certain HLA class II alleles, haplotypes, and genotypes have related to diabetes mellitus so it can be dependent as a genetic marker for susceptibility of this disease in Iraq.

Keywords: Type II diabetic, Type I diabetes, HLA classII, genotype, haplotype.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from the defects in insulin secretion, insulin action or both.1 Type 1 and type 2 diabetes consider the two main types, with type 2 diabetes constituting for the majority (>85%) of the total diabetes mellitus prevalence, According to the latest estimates, there are 425 million people with diabetes in 2017 and this number is expected to rise in 2045 to 629 million.2 The genetic loci involved in the rejection of foreign organs knew as the major histocompatibility complex (MHC), and the MHC encodes highly polymorphic cell surface molecules. The human MHC is representing as the HLA (Human Leukocyte Antigen) system because these antigens first identified and characterized using alloantibodies against leukocytes.3 The encoding of HLA-DQ proteins are belongs to HLA-DQ genes and expressed on α and β chain at cell surface.4 The DQ region of HLA include two gene clusters, DQA1 and DQB1.5 The polymorphism of HLA had serologically significant impact and the Polymorphism at the HLA-DQB1 locus used to be determined serologically and recognized the specificities DQ1, DQ2, DQ3 and DQ4.6 The use of DNA typing techniques has increased the number of alleles. The allelic sequence diversity is also predominantly present in exon 2 and, except for DQB1*0201 and DQB1*0202, all alleles can be discriminated by PCR-SSOP in this exon. A large number of studies have demonstrated that specific alleles at the DRB1, DQA1, and DQB1 loci are strongly associated with diabetes.7,8 However, allelic variation at these loci cannot account fully for the pattern of HLA haplotype sharing among affected sib pairs.9
Material and Method

Patients and controls

Two hundred twenty-five blood samples were collected from clinically diagnosed diabetes patients who regularly admitted by medical committee specialized diabetic center of marjan hospital (Babylon) from October 2018 to May 2019, the age of patients between (2-80) years including both sex male (112) and female (113), in addition to (25) samples were taken from apparently healthy human were taken from Babylon province as control. The study was approved by the Research Ethics Review Boards of the University of Babylon. This work was done by self funding and it is a part of M.Sc project for the first author with the agreement of university.

All participants provided written informed consent. Case Ascertainment. Patients with diabetes were diagnosed by a physician on the basis of the following criteria: a fasting glycemia ≥ 1.26 g/dL, an unexplained weight loss, signs of hyperglycemia (polyuria, polydipsia, polyphagia, and asthenia). These criteria were defined according to the recommendations of the American Diabetes Association.10.

Blood sampling

For each individual enrolled in the study, 3 ml of venous blood was collected in EDTA-treated tubes for DNA extraction, which performed according to the protocols recommended by the manufacturer (Favorgen/Taiwan).

HLA genotype analysis

HLA –DQA105:01 and HLA-DR3 genotyping was performed with PCR-sequence-specific primers (PCR-SSP).11 In a 20 µl mixture of 2.5 µl DNA, 1.5 µl from each forward and reverse of the primer, 5 µl master mix and 9.5 µl nuclease free water. The DNA amplification for HLA-DQA1 is includes an initial denaturation of 2 min in 94°C, 32 cycles of amplification (every cycle consists of a denaturation of 30 s in 94°C, a hybridization of primers during 30 s in 63°C, and an extension of 30 s in 72°C), and a final extension of 10 min in 72°C.11 While the process of DNA amplification for HLA-DR3 is same as DQA1, (exception the hybridization temperature was 52°C), then the PCR products were separated in 1.5 % agarose electrophoresis system using ethidium bromide then visualized with the gel documentation, with 100 bp-ladder (Bioneer, Korea) and photographed. The sequences of primers used for the amplification of the genes are presented in table 1.

Table 1: Sequences of the couples of primers used for the amplification of the genes.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences 5-3</th>
<th>Size(bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA -DR3</td>
<td>5’CACGTTTCTTGGAGTAC3’ 5’CGTAGTTGTGTCTGAGTAGT3’</td>
<td>237 bp</td>
<td>(Fagbemi et al., 2017)</td>
</tr>
<tr>
<td>HLA- DQA1*05:01</td>
<td>5’ACGGTCCCTCTGGCCAGTA3’ 5’AGTTGGAGCGTTTAATCATGAC3’</td>
<td>186 bp</td>
<td></td>
</tr>
</tbody>
</table>

Statistical Analysis

All data were statistically analyzed according to software program version 20 SPSS statistical software (version 17; SPSS, Inc., Chicago, IL, USA). The association between TID, TIID and each identified HLA-DR/DQ alleles, haplotypes, and genotypes was assessed using the odds ratio with its 95% confidence interval (OR, CI 95 percentage).

Results

The present study reveals a noticeable variety with HLA haplotype among type I and type II diabetes. HLA alleles discriminated by PCR assay. The distribution of HLA-DQA1 show high appearance in both types, out of 30 samples 26 show positive result 86.66% in type I and out of 33 samples 26 show positive result 78.78% in type II finally, statistical analysis showed no significant differences comparing diabetes patient and control subjects table 2.
Table 2: Human leukocytes antigen (HLA) DQA1 alleles’ distribution in type I and II diabetes.

<table>
<thead>
<tr>
<th>Model</th>
<th>Sample No.</th>
<th>Patients +ve results</th>
<th>patients-ve results</th>
<th>Healthy +ve results</th>
<th>Healthy -ve results</th>
<th>OR ( CI)</th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
</tr>
<tr>
<td>Type I</td>
<td>30</td>
<td>26</td>
<td>86.66%</td>
<td>4</td>
<td>13.33%</td>
<td>21</td>
<td>84%</td>
</tr>
<tr>
<td>Type II</td>
<td>33</td>
<td>26</td>
<td>78.78%</td>
<td>7</td>
<td>21.21%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>52</td>
<td>82.53%</td>
<td>11</td>
<td>17.46%</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Agarose gel electrophoresis image for HLA –DQA1 haplotype at 100 v for 40 min. and 70 v for 30 min. M, marker 100bp, L1,2,3,4,5,6,7,8,9,10,11 give positive results and L12 give negative result.

The distribution of HLA-DR3 in type I and II showed 14 positive result with percentage 46.66% for TID and 11 positive result (36.66%) for TIID whereas the control revealed 8 positive result (32%), finally, statistical analysis showed significant differences comparing diabetes patient and control subjects, table no 3 reveals high appearance of this haplotype HLA DR3 among TID than TIID.
### Table 3: Human leukocytes antigen (HLA) DR3 alleles’ distribution in type I and II diabetes.

<table>
<thead>
<tr>
<th>Model</th>
<th>Sample No.</th>
<th>Patients +ve results</th>
<th>Healthy +ve results</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Type I</td>
<td>30</td>
<td>14</td>
<td>16</td>
<td>53.33%</td>
<td>8</td>
</tr>
<tr>
<td>Type II</td>
<td>30</td>
<td>11</td>
<td>19</td>
<td>63.33%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>25</td>
<td>35</td>
<td>58.33%</td>
<td>25</td>
</tr>
</tbody>
</table>

![Figure 2: Agarose gel electrophoresis image for HLA –DR3 haplotype at 100 v, 50 min. M, marker 100 bp, L1, 3, 4, 5,8,9,10,11 give positive results. L2, 6,7,12 give negative results.](image)

**Discussion**

Diabetes mellitus is a combined metabolic disorder that includes several complications and the incidence of diabetes has been increasing worldwide therefore various are relentlessly working out the possible role of a vast number of genes associated with this disease. Human leukocytes antigen represented many proteins that encoded by HLA genes, therefore HLA-DQ genes expressed as heterodimers of alpha and beta chains at the cell surface.

The distribution of HLA-DQA1 in the present study show high appearance in both types, out of 30 samples 26 show positive result 86.66% in type I and out of 33 samples 26 show positive result 78.78% in type II finally, statistical analysis showed no significant differences comparing diabetes patient and control subjects.

Although the high appearance of this HLA-DQA1 type in both types of diabetes TID and TIID, statistical analysis show no significant differences between patients and control, this led to conclude that this haplotype had no risky effect on this population sample of diabetes patients.
HLA allele and haplotype frequencies vary considerably across ethnic groups. Researchers pointed that HLA-DQA1∗05:01, HLA-DQA1∗03:01 play a role in diabetes susceptibility particularly TID.

The analyses of HLA disease associations in different ethnic populations, due to differences in allele frequency distributions and patterns of linkage disequilibrium, can allow important general inferences of disease risk associated with specific alleles and their combinations.

The distribution of HLA-DR3 in type I and II showed that type I diabetes revealed percentage 46.66% and type II revealed a percentage 36.66% whereas the control revealed percentage 32%. Statistical analysis showed significant differences comparing two types of diabetes patient.

On contrary of HLA DQA, HLA DR3 appear in less percentage in diabetes patients particularly in TIID. This may be due to the protective role of this haplotype in the population samples of diabetes. The increased risk of DR3/4-DQB1*0302 heterozygotes relative to DR3/3 and DR4/4 genotypes has led to the hypothesis that the trans-complementing DQ heterodimers are more effective in presenting diabetogenic epitopes to T-cells.

Type I diabetes has the strongest association with HLA-DQA1 and DR3 similar result were also reported by.

Studies on DRB1 and DQB1 allele distributions and the importance of genotype context support the genetic associations observed in previous studies.

Variable results on DQA1 and DR3 genotypes in this study observed the risky and protective role of these genotypes in TID, TIID and healthy individuals. This is compatible with the of DQ heterodimer encoding by DQA1∗0501 and DQB1∗0302 explanation.

**Conclusion**

This study was designed to assess the associations of HLA class II alleles, haplotypes, and genotypes with the risk of developing T1D and T2D in Iraq. Certain HLA class II alleles, haplotypes, and genotypes were related to diabetes and may be used as genetic susceptibility markers to diabetes. Further studies of HLA and diabetes mellitus in Iraq are needed to confirm the present results and to provide data for the development of screening assays and for better management of patients with diabetes at the onset of disease.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the college of Science for women, University of Babylon and all experiments were carried out in accordance with approved guidelines.

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Cloud Point Extraction, Preconcentration and Spectrophotometric Determination of Co (II) and Cu (II) using 15-Crown-5

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Abstract

In the current study, a cloud point extraction (CPE) with UV-vis spectrometry for detection of Cu (II) and Co (II) ions with 15-crown-5 as a chelating agent was developed to be more selective and sensitive. The CPE method is improving the analytical signal and permits the preconcentration of metals ions. After phase separation induced by change temperature to become higher more than the temperature of the cloud, the point is performed with centrifugation. The Co (II) and Cu (II) ions are determined in the enriched phase by UV-vis spectrometry. Several factors affecting the extraction efficiency were optimized, e.x. pH, the concentration of the chelating agent, non-ionic surfactant concentrations, and cloud point temperature. Under optimum conditions, a linear calibration graph in the range of 0.5-5 μg mL⁻¹ of Co (II) in the initial solution with r² = 0.9994 (n=8) and 0.2–7μg of Cu (II) in the initial solution (mL⁻1) with r² = 0.9991 (n=8) were obtained. Detection limits of 0.4and 0.1μg are for Co(II) and Cu(II) along with preconcentration factors (66 and 50) for these ions, respectively. The method was used for detection of Co(II) and Cu(II) in some food samples.

Keywords: Cloud point extraction, preconcentration, spectrophotometric

Introduction

CPE Cloud Point Extraction method which is interest with the separation method metal ions field by forming small volume of the surfactant-rich phase (colloidal system) as a result of heating non-ionic aqueous solution of surfactant to a certain temperature at which the solution become turbid ,then the colloidal solution is separated into a small volume of surfactant-rich phase and an aqueous phase(1-6). A list of advantages of this method than other analytical methods because of i-it green method to avoid hazardous solvents ii- its selectivity for obtaining high coefficient of preconcentration iii- its economic for capital cost iv- its rapidity for reduce the time(7-9) iiv-simplicity application for separation and purification of a variety samples of pharmaceutical products, vital and solid,and enviromental(10-16).

A previous study used CPE methodology coupled with flame atomic absorption,(17-22) high performance liquid chromatography,(23) Ultrasonically modified Amented,(24-25) Ultrasound,(26-27) atomic absorption spectrometry,(28)spectrophotometric technique(29) and total reflaction X-ray fluorescence(30).

Supramolecular chemistry are strong linked to molecular recognition chemistry ,which investigates now host molecules that do the recognizing quests molecules that are recognized ,although molecular recognition chemistry is sometimes called host-guest chemistry ,the main concept associated with molecular recognition is the “Lock and Key “concept proposed by Email Fisher at the end of the nineteenth century 31. According to the structures and sizes of various crown ethers are forming stable and selective complexes with metal ions including alkali and alkali earth metal catoin ,although ability of these macro cyclic ligands as electron pair donors towards neutral molecular iodine and with δ- or π- acceptors(35-37).In this present work we report the formation of complex 15Crown5 with copper and cobalt in ethanol solution. This work focused on the suitability of CPE combination with UV-Visible spectrophotometr

The experimental
The apparatus

Spectrophotometer (A Shimadzu UV–vis 2101) was used for making experiment studies. Determinations of pH were measured using a (Wellhem, Model 7110) (Germany) digital pH meter. The water bath is used for studying temperature (OPTIMA co, made in Japan). A centrifuge (Hettich co., made in Germany) was used to the separation.

Reagents and Solutions:

Doubly Distill water was used in the study. The nitrate salts of cobalt and copper (Merck) solution of 1000 μg. mL⁻¹ were prepared by dissolving the appropriate amount of nitrate salt. The solutions were produced by dilution by doubly distill water. The nonionic surfactant Triton X-114 was provided (Acros Organics company, New Jersey, made in the USA).

Suggested methods for Cloud point extraction

For making cloud point extraction, it requires 10 ml aliquot solution containing 0.5–5μg mL⁻¹ of Co(II), 0.2–7μg mL⁻¹ of Cu(II). Then, a solution containing 15C5 dissolved in Triton X-114 was added to the aliquoted solution at a suitable pH. The phase separation occurs when heated the solution in the water bath at 60°C for 15 min, So accelerating phase separation by using centrifuging the solution at 5000 rpm for 10 min. Then cooling, the surfactant-rich phase changed to viscous, and the aqueous phase was easily removed using a syringe pipette. The surfactant-rich phase was made up to 1.0 mL by adding ethanol and Trans to a quartz cell to the determination of the absorbance at 290,252 Nanometer.

Application of foods samples:

Heating spinach sample (40) gram for three hours in silica crucible on heater and the charred substances was moved to oven for heating 24 hours at 650°C. then cooled, after that treated by nitric acid (10) mL and H₂O₂ (3) mL at concentration (30) and return to the oven for two hours at 650°C, and shouldn’t any organic compound. Treatment of final residue by nitric acid (3) mL and convert by evaporated to the fumes. Final solid was kept in distal water, then keep at pH (4) made up to 25mL. Black tea one gram, green tea one gram and tomato sauce one and a half gram were digested with the mixture of 8 mL concentrated nitric acid, H₂O₂ (2) ml at a concentration (30%) in microwave and dilution it by deionized water (50) mL. Also, the blank digest too submitted for the same way. The CPE methods were used to the final solutions. The analyte ions levels were determined by the procedure provided in section 2.3.

The Results and the Discussion

Absorption spectra and Characteristics of the method:

The absorption spectra of Co (II)-15-C-5and Cu (II)-15-C-5 complexes in the surfactant-rich phase after CPE procedure against reagent blank are shown in Fig. 1 the detection wavelengths were chosen to be 290 and 252 nm. The stoichiometry of 15-Crown-5 complexes with Co (II) and Cu (II) was examined by mole ratio method, and the continuous variation was found to be 1:1. The preconcentration factor results in by dividing aqueous phase volume to the preconcentrated phase volume. Table 1 summarizes the analytical characteristics of the method under the optimum experimental conditions.
Table (1): showed parameter methods that used for detection values of cobalt and copper by using CPE.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Co (II)</th>
<th>Cu (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>290</td>
<td>252</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = 0.1981x + 0.0006$</td>
<td>$y = 0.2886x + 0.0102$</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.9993</td>
<td>0.9993</td>
</tr>
<tr>
<td>C.L. for the slope (b±t sb) at 95%</td>
<td>$0.1981 ± 0.0176$</td>
<td>$0.2886 ± 0.0011$</td>
</tr>
<tr>
<td>C.L. for the intercept (a±t sb) at 95%</td>
<td>$0.0006 ± 0.0491$</td>
<td>$0.0102 ± 0.0303$</td>
</tr>
<tr>
<td>Concentration range (µg mL$^{-1}$)</td>
<td>0.5-5</td>
<td>0.2-7</td>
</tr>
<tr>
<td>Limit of Detection (µg mL$^{-1}$)</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Limit of Quantitation (µg mL$^{-1}$)</td>
<td>1.56</td>
<td>0.65</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg cm$^{-2}$)</td>
<td>$1.0 \times 10^{-3}$</td>
<td>$6.257 \times 10^{-3}$</td>
</tr>
<tr>
<td>Molar absorptivity (L mol$^{-1}$ cm$^{-1}$)</td>
<td>$5.7726 \times 10^{4}$</td>
<td>$1.0161 \times 10^{4}$</td>
</tr>
<tr>
<td>Composition of complex (M: L)</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>RSD% (n=7) at 3 µg mL$^{-1}$</td>
<td>3.3%</td>
<td>2.8%</td>
</tr>
<tr>
<td>Preconcentration factor</td>
<td>66.66</td>
<td>50</td>
</tr>
</tbody>
</table>

Optimizing of CPE parameters:

**Effect of pH:**

The separation of ions includes primary production sufficient hydrophobic with metal-chelate to be extraction of a little amount of surfactant-rich phase\(^{(38)}\). PH test is the main parameters\(^{(39)}\). PH effect to the CPE method was applied in (1–5) range. Adjusting of PH values is with HNO$_3$. Figure (2) showed a pH effect on the percentage of extraction; it included the extraction percentage increasing with nitric acid decreasing. The final results, pH (4) were chosen.

**Effect of 15-Crown-5 concentration:**

The effect of 15-Crown-5 on the extraction and determination of Co (II) and Cu (II) complexes were investigated in the range $2.0 \times 10^{-3}$-$2.0 \times 10^{-4}$ mol L$^{-1}$, the results present in Fig. 3. The extraction will result in high concentrated of 15-Crown-5 and remained almost constant. That explains that micelles are filled by the chelating agent. At higher concentration, extraction efficiency was decreased. That due to the free 15-Crown-5 competes with the complexes in extraction to surfactant-rich phase results in low extraction\(^{(40,41)}\). Therefore, a concentration of $1.0 \times 10^{-2}$ and $9.0 \times 10^{-3}$ mol L$^{-1}$ of 15-Crown-5 was selected as the optimum concentration Co (II) and Cu (II).
Triton X-114 Effect:

One of a critical factor that in the CPE is the concentration of surfactant, Triton X-114 is non-ionic surfactant wide using in CPE due to its achieves a good cloud point extraction that means increase the extraction efficiency by reducing the phase volume ratio, results in improvement of concentration factor. Where Triton X-114 present in markets at high purity, with low point temperature, low cost and toxicity and the surfactant-rich phase (high density)\(^{(42,43)}\). The preconcentration efficiency depends on the Triton X-114 level (0.001-0.015) as range M, as shown in Fig. 3. At lower concentrations of the surfactant, the highest extraction efficiency for cobalt and copper ions was obtained with 0.007 and 0.009 M Triton X-114. The extraction decreases with increasing of the surfactant amounts. That may be belonging to increasing in viscosity and volume of the micellar phase leading to poor sensitivity\(^{(44)}\). An amount of 0.4 and Triton X-114 (0.5 MI) were selected for subsequent experiments.

Effect of time and temperature of Equilibration Incubation:

Determination of incubation is done by temperature and time. As well as, the extraction process depends on temperature. Figure (5) showed the influence time and temp of the incubation of Cobalt and copper. The cloud point temperature of Triton X-114 is (23-25)°C in (0.1–5) weight percentage, heating more than ten °C is important for getting good separation\(^{(45)}\). When temperature or incubation time is an increase, it will increase Co (II) and Cu (II) extracted amount in the surfactant-rich phase with quantitative separation after fifteen minutes in 60 °C (water bath). The prolonged times is necessary to apply it at high temperatures for satisfactory mange extraction\(^{(46)}\).

Wide ranges are used in our study (30–70) °C as temperature degree and (5–30) minutes as a time. Time (15) minutes and temperature (45) °C were optimum conditions for extraction.

The effect of centrifuge conditions:

Centrifugation was accomplished for separating the aqueous and surfactant-rich phase in the suggested procedure. (5000) recycled per minute and ten minutes are best phase separation\(^{(47,48)}\).

Effect of foreign ions:

The interferences are studied in the preconcentration stage, 15-crown-5 react cations or analytes may react with species and the extraction efficiency become low. The solution (10) ml consist of (3) \(\mu\)gmL\(^{-1}\) Co (II) and Cu(II) as Table (2).

Table (2): Effects of the matrix ions on the recoveries of the examined metal ions (\(N=3\))

<table>
<thead>
<tr>
<th>Ion</th>
<th>Added as</th>
<th>Tolerance Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>K+ ,Na+</td>
<td>KCl, NaCl</td>
<td>1000</td>
</tr>
<tr>
<td>Mg2+</td>
<td>MgCl</td>
<td>1000</td>
</tr>
<tr>
<td>Pr3+</td>
<td>Pr(NO3)3</td>
<td>500</td>
</tr>
<tr>
<td>La+</td>
<td>La2O3</td>
<td>1000</td>
</tr>
<tr>
<td>I-3</td>
<td>Resublumed</td>
<td>1000</td>
</tr>
<tr>
<td>Li+</td>
<td>LiCl</td>
<td>1000</td>
</tr>
<tr>
<td>Ag+</td>
<td>AgNO3</td>
<td>750</td>
</tr>
</tbody>
</table>

Applications and Accuracy:

The suggested procedure was used to determination of the Co(II) and Cu(II) according to Section 2.4 in Spinach, Tomato Sauce, Green tea and Black tea provided from local markets. The results are shown in Tables 1. The values measured by at ranged (96-101) %, the results indicate that confirm the accuracy of the procedure.
Table (3): Contents of analyte ions in the food sample. (N = 3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Co</th>
<th>Cu</th>
<th>Co</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td>0</td>
<td>8.61</td>
<td>2.3</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>11.08</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>14.52</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>16.92</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>0</td>
<td>0.82</td>
<td>3.4</td>
<td>100.4</td>
</tr>
<tr>
<td>Sauce</td>
<td>2.5</td>
<td>3.33</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.50</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>3.94</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Black tea</td>
<td>0</td>
<td>1.93</td>
<td>3.3</td>
<td>101.2</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>4.46</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.41</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>4.89</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Green tea</td>
<td>0</td>
<td>1.30</td>
<td>2.1</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>3.73</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>7.99</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>10.40</td>
<td>0.89</td>
<td></td>
</tr>
</tbody>
</table>

A comparison between previously studies and the suggested method of pre-concentration and Cu(II) and Co(II) determination as (Table 4) which indicates the method R.S.D. and lower detection limit and the methods are the procedure for detection small Co(II) and Cu(II) in food by use spectrophotometer.

Table 4: Comparison between merit methods with previous studies

<table>
<thead>
<tr>
<th>Sample</th>
<th>reagent</th>
<th>surfactant</th>
<th>LOD(ng mL⁻¹)</th>
<th>PF</th>
<th>detection</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>water and food samples</td>
<td>PTSC-1</td>
<td>Triton X-114</td>
<td>0.6 7</td>
<td>25</td>
<td>FAAS</td>
<td>49</td>
</tr>
<tr>
<td>Environmen tal samples</td>
<td>MPKO</td>
<td>Triton X-114</td>
<td>0.1 9</td>
<td>67</td>
<td>FAAS</td>
<td>50</td>
</tr>
<tr>
<td>tap water, snow water,</td>
<td>PAN</td>
<td>Triton X-114</td>
<td>0.1 2 g/l</td>
<td>25</td>
<td>Capillary electrophoresis</td>
<td>51</td>
</tr>
</tbody>
</table>
**Conclusion**

Extraction of ions of cobalt and copper is done with 15-crown-5 in non-ionic surfactant Triton X-114 phase. Extraction of complete elements was done by 15-crown-5 because of the hydrophobic production. The molar ratio is determined. Simplicity, Ecological safety, metrological characteristics, the sensitivity and convenience of are procedures used for extraction of the organic compounds. The results found these methods could use it for preconcentration and separation of cobalt and copper ions.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Chemistry, College of Education, University of AL-Qadisiyah, Diwaniyah, Iraq and all experiments were carried out in accordance with approved guidelines.

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Hormonal and Immunological Study in Serum of Pregnant albino rats Treated with Nickel Oxide Nanoparticles

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Abstract

The present study was aimed to elucidate the effect of Nickel oxide nanoparticle (100mg /kg b.wt) on some parameters in pregnant Albino rats which including serum reproductive hormonal level, Leutinizing Hormone (LH), Follicle stimulating Hormone (FSH) and Testosterone (Test), and some Immunoglobulin levels (IgA, IgG, IgM) for different period of pregnancy (12 and 14 days). The hormonal results showed significant (P≤0.05) increased of treatment groups as compared with control groups. Immunological results showed significant decreases in treated groups as compared with control groups, It could be concluded that increasing concentrations of NIO- NPs and durations of exposure leads to negative effects on the pregnant albino rat.

Key words: NIO, nanoparticles, albino rats, hormones, immunoglobulin.

Introduction

Nickel is a silver-white metallic chemical element that is naturally present in the Earth’s crust. Because of its unique physical and chemical properties, being tough, harder than iron, ferromagnetic, having good plasticity and highly resistant to rusting and corrosion, nickel and its compounds are widely used in industry. Nickel is an essential element for at least several animal species. These animal studies associate nickel deprivation with depressed growth, reduced reproductive rates, and alterations of serum lipids and glucose. Nickel is known as a potentially harmful element for humans. Its concentration in the environment can rise due to industrial activities. Human exposure to nickel or its compounds has the potential to produce a variety of pathological effects, which may include cutaneous inflammations such as swelling, reddening, eczema and itching on skins, and may also include allergy reactions and teratogenicity in the human body. Nickel is capable of evoking dual responses in the human immune system. Experiments conducted in humans and in rodents have shown that nickel exhibits both immune modulatory and immune toxic effects, Number of immunological and lymphoreticular effects have been reported in humans and animals exposed to nickel. In 38 production workers exposed to nickel (compound not specified), significant increases in levels of immunoglobulin G (IgG), IgA, and IgM and a significant decrease in IgE levels were observed. Significant increases in other serum proteins, which may be involved in cell-mediated immunity (including α1-antitrypsin,α2-macroglobulin, ceruloplasmin) were also noticed. Nickel and chromium significantly depressed the circulating antibody response of rats immunized with a viral antigen, with the greatest decrease in antibody titers noted in animals receiving the metal two weeks before the initial antigen dose. Several studies have examined the relationship between nickel exposures and acquired immune function. Exogenous chemicals can interfere with the normal functioning of the HPG axis, resulting in reduced fertility or even infertility in both females and males. Ni NPs effect the serum sex hormone levels (FSH, LH, E2 or T) in female and male rats. Some study demonstrate that Ni NPs increased significantly the level of serum FSH and LH, and decreased E2 this effect associated with dose-dependent in females. The results indicate the effects of Ni NPs on the female rat ovarian reserve. It is probably an indication of the decreased level of serum E2 and ovarian hormone secretion following ovarian damage with Ni NPs, which increased the level of serum FSH and LH by negative feedback. Meanwhile, the male

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rat serum FSH, LH and T content analysis showed the levels of FSH and T were decreased significantly by Ni NPs treatment.

**Materials and Method**

**Animals**

Animals with weight of 195-280 gm and aged of 2.5-3 months were obtained from the animal house of the Biology Department/College of Science at University of Babylon. Animals were put inside special cages for breeding with length of 25 cm, 18 cm width and 19.5 cm height and stayed about 30 days. The cages were covered with sawdust, which replaced three times weekly with the care of hygiene and sterilization. The animals were provided with food and water ad libitum. The animals were housed in special rooms with controlled conditions of temperature (24±10°C) and natural light periods (12 hours light/dark).

Each two females were put with one male (for mating) in special plastic cages and strung metal caps with dimensions of 40 cm length, 25 cm width and 19.5 cm height. After ensuring the pregnancy by observing vaginal plug and vaginal smear, this day regarded as 0th day of gestations (GD=0). Pregnant rats divided in five groups (n=4). Each two females were put with one male (for mating) in special plastic cages and strung metal caps with dimensions of 40 cm length, 25 cm width and 19.5 cm height.

**Nanoparticles Dose Preparation**

Determination of Nickel oxide doses were depended on the animals body weight. 25-PPM doses of nanoparticle suspension was prepared and mixed with distilled water.

**Animals Anesthesia**

The pregnant rats were anesthetized with chloroform in 12 and 14 days of pregnancy. Pregnant rats were put after anesthesia on dissection bowl, the fore and hind limbs were fixed by fine pins, the pregnant’s abdomen was opened by a sharp scissors and take blood sample by heart puncture.

**Blood collection:** Blood samples were collected via the left ventricular cardiac puncture into sterilized EDTA tubes and gel tube to separate the serum quickly and then centrifuged at 3000 rps for 5 minutes. The serum samples were stored frozen until used.

**Hormonal Assay:** Serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Estradiol were measured by using ELISA (Monobind Company, USA) for both control and treatment animals.

**Results**

Effect of Nickel Oxide nanoparticles on Luteinizing Hormone in Pregnant Albino Rats for 12, 14 Days of Pregnancy.

The result of the present study showed that the LH hormone means increases significantly (P≤0.05) in treated group (12, 14 days) with NIO (100 mg / kg b.wt) (1.1 ±0.010, 1.28 ± 0.043) (pg/ ml) respectively, as compared with control group (0.8263 ± 0.02512, 0.836 ± 0.0251) for 12, 14 days respectively (Figure-1).

![Figure 1](image1.png)

**Figure (1):** Effect of Nickel Oxide Nanoparticles on Luteinizing Hormone in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

Effect of Nickel Oxide Nanoparticles on Follicle Stimulating Hormone in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

FSH means in present study increases significantly (P≤0.05) in treated group with NIO (100 mg / kg b.wt) (1.023 ±0.032, 1.16 ± 0.051) (pg/ml) as compared with control group (0.8 ± 0.004, 0.8 ± 0.003 pg/ml) 12 and 14 days respectively (Fig.2).

![Figure 2](image2.png)

**Figure (2):** Effect of Nickel Oxide Nanoparticles on Follicle Stimulating Hormones in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.
Effect of Nickel Oxide nanoparticles on Testosterone Hormone in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The present study showed significant increases (P≤0.05) of Testosterone in treated group with NIO (100 mg / kg b.wt) (0.61 ±0.001, 0.63 ±0.001 pg/ml), as compared with control group (0.541 ±0.013, 0.52 ±0.08 pg/ml) for 12 and 14 days respectively (Figure-3).

Effect of Nickel Oxide nanoparticles on Immunoglobulin A in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The result in (Figure-4) revealed that the IgA, showed significant (P≤0.05) decreases in the treated group with NIO (100 mg / kg b.wt) (16.29 ± 0.292, 14.3 ± 0.22 pg/ml) as compared with control group (19 ± 0.386, 18.63 ± 0.52 pg/ml) for 12 and 14 days respectively.

Effect of Nickel Oxide Nanoparticles on Immunoglobulin G in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The result in (Figure-5) revealed that the IgG, showed significant (P≤0.05) decreases in the treated group with NIO (100 mg / kg b.wt) (95 ± 1, 82.66 ± 1.52 pg/ml) respectively, as compared with control group (119 ± 1, 118.3 ± 1.52 pg/ml) for 12 and 14 days respectively.

Effect of Nickel Oxide Nanoparticles on Immunoglobulin M in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The result in (Figure-6) revealed that the IgM, showed significant (P≤0.05) decreases in the treated group with NIO (100 mg / kg b.wt) (16.35 ± 0.19, 14.46 ± 0.21 pg/ml) respectively, as compared with control group (19.62 ± 0.06, 18.24 ± 0.02 pg/ml) for 12 and 14 days respectively.

Discussion

Effect of Nickel Oxide Nanoparticles on Luteinizing, Follicle stimulating and Testosterone Hormones in Pregnant Albino Rats for 12 and 14 days of pregnancy.

The present study showed significant (P≤0.05) increases in treated group with NIO (100mg / kg b.wt) as compared with control group for 12 and 14 days of pregnancy this change of hormones could be resulted from the hypothalamic–pituitary–gonadal (HPG) axis is the hormone system whereby the hypothalamus secretes so-called releasing hormones, which are transported via the blood to the pituitary gland. There, the releasing hormones induce the production and secretion of gonadotropins (i.e., LH and FSH), which in turn are transported by the blood to the gonads (i.e., the ovaries and testes).

Generally speaking, in females, LH and FSH stimulate the ovarian follicle that contains the maturing egg to produce estradiol. After ovulation has occurred, LH also promotes production of progesterone. Both hormones participate in a negative feedback mechanism through most of the menstrual cycle, suppressing GnRH release from the hypothalamus and LH release from the pituitary (11).

Exogenous chemicals can interfere with the normal functioning of the HPG axis, resulting in reduced fertility or even infertility in both females and males, the effects of Ni NPs on aspects of serum sex hormone levels (i.e., FSH, LH, E2 or T) in female and male rats. Increases of Ni NPs level in serum FSH and LH, with significant and
dose-dependent in females. It is probably an indication of the decreased level of serum E2 and ovarian hormone secretion following ovarian damage with Ni NPs, which increased the level of serum FSH and LH by negative feedback 12.

The change of hormone reproductive levels indicates the abnormal reproductive axis function, which correlated with male and female infertility 13. However, the mechanisms by which NPs alter the functions of HPOA ultimately resulting in female infertility have not been investigated thoroughly 15. Meanwhile, the amount of studies about NPs having negative effects on HPOA is relatively small. It is certain that NPs with size of 36 nm were significantly accumulated in cerebrum and cerebellum translocation via the olfactory nerve and increased with the exposure time 16. The sizes of NPs less than 90 nm could disturb the balance of GnRH, FSH and LH, such as Ni, PEG-b-PLA (17) 18.

Other study showed that gold nanoparticles reduced the level of testosterone. Another assumption is that the nanoparticles could affect the gene expression of the protein that is involved in the transport of cholesterol into the inner membrane of mitochondria and increased the synthesis of steroids. It is also possible that nanoparticles by reducing the gene expression of the mitochondrial membrane protein Star, inhibit the cholesterol transportation into the inner membrane of mitochondria, and eventually inhibit the conversion of cholesterol to pregnenolone and reduced the level of testosterone. Karpenko (2013) studied toxic effects of cerium oxide nanoparticles on sex hormones and concluded that nanoparticle reduced glandular and testosterone secretion, the gold nanoparticles reduced the level of testosterone 19.

Effect of Nickel Oxide Nanoparticles on Immunoglobulin A, G and M in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The results of the current study as in the table (1) revealed that the IgA, IgG and IgM, showed significant (P≤0.05) decreases in the treated group of (12, 14) days with NIO (100mg/kg b.wt), as compared with control group. The decreases of Immunoglobulins levels in pregnant females due to that the nanoparticles can also suppress the immune system which can weaken immune response against infections and cancerous cells. These immunosuppressive properties, on the other hand, can make nanoparticles useful in preventing transplant rejection, in treating inflammatory and autoimmune diseases, and in delivering immunosuppressive drugs 20.

Nanoparticle properties are responsible for immunosuppressive effects. While some nanoparticles are used to deliver immunosuppressive drugs, others have their own immunosuppressive properties. Shen et al. 22 have shown that Fe3O4 nanoparticles weaken the antigen-specific humoral response and T cell cytokine expression in ovalbumin-challenged mice. Mitchell et al. 23,24 reported that multi-walled carbon nanotubes (MWCNTs) suppressed systemic humoral immunity in mice. Some nanoparticles have been shown to possess anti-inflammatory properties. CeO2 nanoparticles were reported to reduce ROS and the level of inflammatory cytokines IL-6 and TNF-α in murine macrophages 25. After the immunization of mice with a C60 fullerene derivative conjugated to bovine thyroglobulin, they produced IgG antibodies specific to fullerenes. Other researchers were not able to detect fullerene-specific antibodies, even when they used a carrier molecule 25.

Conclusion

The hormonal results showed significant (P≤0.05) increased of treatment groups as compared with control groups. Immunological results showed significant decreases in treated groups as compared with control groups. It could be concluded that increasing concentrations of NIO- NPs and durations of exposure leads to negative effects on the pregnant albino rat.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Babylon University/College of Science, Iraq and all experiments were carried out in accordance with approved guidelines.

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18. Åkerlund E. Cellular Effects of Nickel and Nickel Oxide Nanoparticles: Focus on Mechanisms Related To Carcinogenicity [Internet]. 2018.


Assessment of Mental Health of Healthcare Professionals Working in the Infectious Diseases Units: Comparative Study

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Abstract

Background: Health care professionals working in infectious diseases units or hospitals are at higher risk for being affected by certain contagious pathogens than other professional groups through direct contact, equipment, and contaminated supplies; therefore, they are working under ongoing stress that negatively impacting their psychological well-being.

Aims: This study aims to assess the mental well-being of healthcare professionals working in infectious diseases units and compare these results to the score of other healthcare professionals working in other units.

Methodology: A comparative study design using a convenience sample of (N=300) healthcare professionals (150 working in the infectious diseases units and 150 working in different units). The General Health Questionnaire-28 was used to assess the mental health of study subjects.

Results: Recent findings indicate a statistically difference between study group and comparative group (p<=0.05), which means that healthcare providers working in the infectious disease units are at higher risk for psychological disturbance than the comparative group. Moreover, other work factors are found to have significant relationship with professionals’ mental health, such as infection prevention trainings, years of experience in infectious diseases units, availability of personal protection equipment and hand hygiene, and number of patients in the unit.

Conclusion: Working in high risk work environment increases the concern about personal safety and impose ongoing stress, which negatively influence professional’s mental well-being. More training about infection prevention and provide personal protection materials help reducing the risk of disease transmission among patients and to health care providers.

Keywords: Emotional Intelligence, Work-related Stress, Psychiatric Nurses

Introduction

Most healthcare professionals are vulnerable to the risks of workplace; especially those who expose to blood, body fluids, and injuries that could be contaminated with infectious microorganisms (15,20). Death rates are more likely to be higher among healthcare professionals (especially nurses and doctors) who work in infectious diseases units, as a result of their role as frontline professionals in caring for clients with different infectious diseases (14). Health care professionals working in infectious diseases units or hospitals are at higher risk for being affected by certain contagious pathogens through direct contact, equipment, or contaminated supplies; consequently, they are working under an ongoing stress that affects their psychological health. Working in such units increases the demand on health professionals to increase the quality of hospital care, controlling infection, and protecting themselves at the same time, which impose additional stress and impact the professionals’ general health outcomes (4). The purpose of study is to assess the mental health outcomes of healthcare professionals working in the infectious diseases units and measure the difference in the mental health outcomes among healthcare professional working in the infectious diseases units and other units. Mild stress is consider essential to promote professionals’ productivity and responses; however, when the stress level is high, it negatively impact the
health of individuals. Workplace challenges do not often reported to psychological complaints; however, psychological health of professionals is impacted when they are unable to adapt effectively with these challenges. Stress is described an imbalance among the perceived needs, understand resources, and ability to cope with those needs, which can cause physical and psychological distress. Stigma is not only affecting clients with infectious diseases, it is another issue that faces healthcare professional who care for those clients as a result of the possibility of infection transmission to healthcare care employees. Consequently, it is also considered one of the main causes of perceived stress, depression, and anxiety. Professional mental health is directly and indirectly influenced by the stigma associated with infectious diseases through stress. The Importance of risk awareness and patients safety have been indicated in several research. According to, “nurses are expected to maintain a high level of awareness towards an infectious disease outbreak and ensure the safety of the public.” This critical role highlights the reason behind the high level of stress among nurses who work in communicable diseases units. Identifying the risk factors that cause professional stress among health professionals is a crucial approach to create healthy workplace environment and maintain professionals’ mental well-being.

### Methodology

#### Study Design and Sampling Plan:

Comparative study design using a convenience sample of 300 healthcare professionals for both groups (150 in the study group and 150 in the comparative groups). Health care providers who participated in this research study were selected regardless of their gender group, specialty, or work time. This strategy was used to clarify the general characteristics of healthcare providers of either group. About 400 healthcare providers received the questionnaire and agreed to be a part of the study; however, only 310 subjects responded to the questionnaire (150) subjects from the first group and (160) subjects from the comparative group. To make the sample size equal in the both groups, 10 subjects were randomly excluded from the comparative group.

#### Study instrument:

The General Health Questionnaire (GHQ28) is used to assess the mental health of healthcare providers working in the infectious diseases units and the mental health of the comparative group. This scale was a developed as a screening tool to detect those likely to have or to be at risk of developing psychiatric disorders. This screening tool was developed by Goldberg in 1978. It is a 28-items that is measure a four levels scale ranged from (0 to 4) respectively; 0 indicates no, 1 indicates less than usual, 2 indicates more than usual, 3 indicates more often than usual. The levels of psychological distress among healthcare providers are determined based on the sum of items scores (minimum score 0 and maximum score 84). The cut score is 23/24 and individuals with higher scores are considered having higher level of psychological distress. This scale is includes four subscales in which each scale consist 7 items, divided as follow: 1) Items (1-7) measure somatic symptoms; 2) Items (8-14) measure anxiety/insomnia; 3) Items (15-21) measure social dysfunction; 4) Items (22-28) measure severe depression. The Arabic version of the scales had good reliability level, Cronbach’s alpha level (0.89).

### Ethical Considerations

Healthcare professionals were asked for voluntary participation. Research objectives, risks, and benefits were explained to participants to help them decide whether to participate or not. After they agreed to be a part of the study, participants were given anonymous questionnaire to maintain participants’ confidentiality.

### Results and Discussion

The recent study indicates that there is a statistically significant difference between study group (healthcare providers working in infectious diseases units) and comparative group (healthcare providers working indifferent units) in respect to their mental health. More than half of the healthcare professionals working in the infectious diseases units were symptomatic for psychological distress; whereas, 30% of the comparative group (working in non-infectious units) were symptomatic for psychological distress. indicate that nurses working in high demand or high risk environment anticipated a greater likelihood of developing depression and stress symptoms as a consequence of the continuous concern about their safety. mentioned that healthcare professional who work in hospitals settings are more likely to be vulnerable to depression, and the risk is greater than the risk compared to the general population. However, highlighted that healthcare workers who are in contact with communicable diseases are at higher risk.
Health practitioners need to emphasize the importance of using personal protection equipment for infection prevention and control, as well as studies have highlighted the importance of providing control, availability of the personal protective working conditions, including training about infection control, risk perception. Significant relationships were indicated that those employees who had higher baseline depression and anxiety than those who care for clients with non-communicable diseases. These psychological effects related to the nature of environment as a high risk, loss of social support (isolation), stress attached to their role, and risk perception. Health care professionals who had direct contact with the affected patients were at great risk for physical and psychological risks. Even though, providers uses personal protection equipment, they still experience barriers of communication and difficulties in diagnosis and caring process, which extent the time needed for each patients and increase the risk for stress. Working in such conditions indicated considerably higher social isolation and felt considerably more vulnerable to somatic symptom, sever anxiety, depression, and fatigue. Analysis of variance (ANOVA) was used to find the differences among healthcare providers in response to psychological disturbances as a result of working in high risk environment. The findings indicate that technicians had the highest mean score than other groups of professionals, which represents their vulnerability to psychological disturbance, followed by physicians and nurses. The study of compared the phenomenon of burnout among healthcare professionals working in the same situation. Researchers indicate a significant difference across professional groups in terms of being subjected to burnout and emotional disturbance. The difference was explained by several factors, including the responsibilities and roles assigned for each group, as well as the type of patients they care for. Being at the frontline in contact with patient can also increase the vulnerability of work stress. In addition, training programs can have positive impacts on reducing work-related stress. Significant relationships were found between psychological health and professionals’ working conditions, including training about infection control, availability of the personal protective equipment, and availability of hand hygiene. Several studies have highlighted the importance of providing training for infection prevention and control, as well as the importance of using personal protection equipment for all healthcare professionals working in the infectious diseases units to prevent the potential risk and reduce work stress. The study that carried out by showed that healthcare professionals who had not received infection prevention training were less likely to have knowledge of infection prevention than those who had received infection prevention training. Therefore, lack of knowledge increases their vulnerability to stress. Moreover, training has positive impact on the attitude of the respondents in the study group compared to the control group. Health practitioners need to safeguard themselves with barriers such as gloves, face masks, gowns, protective eyewear and face shields to reduce work-related microorganism transmission. Despite that, the threat cannot be secured completely, such as the threat results from needlestick injury, using personal protective equipment (PPE) regularly can protect both the professional and the patient from potentially infectious body fluids. The research of linked the increased risk for professionals’ infection to their noncompliance in using hand hygiene before and after caring process. Most providers of healthcare underestimate the significance of hand hygiene as it is not correctly stressed. As a result, many nurses lack the expertise and resources necessary to promote their compliance. Re-education; therefore, enables them by emphasizing the importance of compliance with hand hygiene, which increases the quality and safety of provided healthcare services. In addition, studies have also shown that personal protection equipment are not all available for healthcare professionals; however, even with the availability of required equipment, some professionals are careless to use the protective materials. Therefore, hand hygiene is critical to reduce the risk of infection transmission among patients and prevent the risk for healthcare providers.

**Conclusion**

This study aims to assess the mental well-being of healthcare professionals working in infectious diseases units and compare these results to the score of other healthcare professionals working in other units. Workplace stress has been found in all healthcare providers as a result of various types of stressor factors, which impact their mental health. However, healthcare providers who are in contact with infectious diseases are at higher risk for depression, stress, anxiety, and sleep disturbance than others who work in different healthcare settings. Consequently, feeling stressed and concerned about personal safety impose a negative effect on professionals’ mental health. More training

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The reference text in the document: [1]. A literature review study was conducted on the workplace factors that influence the psychological health of healthcare professionals involved in caring for clients with various infectious diseases. This research discovered that those employees had higher baseline depression and anxiety than those who care for clients with non-communicable diseases. These psychological effects related to the nature of environment as a high risk, loss of social support (isolation), stress attached to their role, and risk perception. Health care professionals who had direct contact with the affected patients were at great risk for physical and psychological risks. Even though, providers uses personal protection equipment, they still experience barriers of communication and difficulties in diagnosis and caring process, which extent the time needed for each patients and increase the risk for stress. Working in such conditions indicated considerably higher social isolation and felt considerably more vulnerable to somatic symptom, sever anxiety, depression, and fatigue. Analysis of variance (ANOVA) was used to find the differences among healthcare providers in response to psychological disturbances as a result of working in high risk environment. The findings indicate that technicians had the highest mean score than other groups of professionals, which represents their vulnerability to psychological disturbance, followed by physicians and nurses. The study of compared the phenomenon of burnout among healthcare professionals working in the same situation. Researchers indicate a significant difference across professional groups in terms of being subjected to burnout and emotional disturbance. The difference was explained by several factors, including the responsibilities and roles assigned for each group, as well as the type of patients they care for. Being at the frontline in contact with patient can also increase the vulnerability of work stress. In addition, training programs can have positive impacts on reducing work-related stress. Significant relationships were found between psychological health and professionals’ working conditions, including training about infection control, availability of the personal protective equipment, and availability of hand hygiene. Several studies have highlighted the importance of providing training for infection prevention and control, as well as the importance of using personal protection equipment for all healthcare professionals working in the infectious diseases units to prevent the potential risk and reduce work stress. The study that carried out by showed that healthcare professionals who had not received infection prevention training were less likely to have knowledge of infection prevention than those who had received infection prevention training. Therefore, lack of knowledge increases their vulnerability to stress. Moreover, training has positive impact on the attitude of the respondents in the study group compared to the control group. Health practitioners need to safeguard themselves with barriers such as gloves, face masks, gowns, protective eyewear and face shields to reduce work-related microorganism transmission. Despite that, the threat cannot be secured completely, such as the threat results from needlestick injury, using personal protective equipment (PPE) regularly can protect both the professional and the patient from potentially infectious body fluids. The research of linked the increased risk for professionals’ infection to their noncompliance in using hand hygiene before and after caring process. Most providers of healthcare underestimate the significance of hand hygiene as it is not correctly stressed. As a result, many nurses lack the expertise and resources necessary to promote their compliance. Re-education; therefore, enables them by emphasizing the importance of compliance with hand hygiene, which increases the quality and safety of provided healthcare services. In addition, studies have also shown that personal protection equipment are not all available for healthcare professionals; however, even with the availability of required equipment, some professionals are careless to use the protective materials. Therefore, hand hygiene is critical to reduce the risk of infection transmission among patients and prevent the risk for healthcare providers.
about infection prevention help raising professionals’ knowledge and practice of preventing infection from being transmitted to other patients or to the healthcare providers. Likewise, using personal protective equipment and hand hygiene play an important role in reducing the risk of infection among healthcare professionals. Therefore, it is important for healthcare professionals to be aware of the effective strategies that reduce their vulnerability to the risk of infection. More training about infection prevention and provide personal protection materials help reducing the risk of disease transmission among patients and to health care providers, which indeed helps reducing the psychological stress among healthcare professionals.

Table 1: psychological disturbance among healthcare professionals: The study group

<table>
<thead>
<tr>
<th>Psychological Status</th>
<th>Overall Psychological disturbance</th>
<th>Somatic symptoms</th>
<th>Anxiety\insomnia</th>
<th>Social dysfunction</th>
<th>Severe depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F.</td>
<td>%</td>
<td>F.</td>
<td>%</td>
<td>F.</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>73</td>
<td>48.7</td>
<td>66</td>
<td>44.0</td>
<td>66</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>77</td>
<td>51.3</td>
<td>84</td>
<td>56.0</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100.0</td>
<td>150</td>
<td>100.0</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 2: Psychological disturbance among healthcare providers: The comparative group

<table>
<thead>
<tr>
<th>Overall Psychological Disturbance</th>
<th>F.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>106</td>
<td>70.7</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>44</td>
<td>29.3</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3: Difference between study group and comparative group in response to psychological disturbance.

<table>
<thead>
<tr>
<th>Independent Samples Test</th>
<th>Levene’s Test for Equality of Variances</th>
<th>‘t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>31.977</td>
<td>.000</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>4.833</td>
<td>252.522</td>
</tr>
</tbody>
</table>

Table 4: Mean scores of healthcare providers in response to psychological distress:

<table>
<thead>
<tr>
<th>Healthcare professionals</th>
<th>Mean</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurse</td>
<td>.8473</td>
<td>84</td>
</tr>
<tr>
<td>Physician</td>
<td>.8979</td>
<td>21</td>
</tr>
<tr>
<td>Pharmacist and Pharmacist Assistant</td>
<td>.4404</td>
<td>6</td>
</tr>
<tr>
<td>Technicians</td>
<td>1.130</td>
<td>39</td>
</tr>
</tbody>
</table>

*The higher the mean score, the higher the risk for mental disturbance
**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Emotional Intelligence and Work-related Stress among Nurses Working in Psychiatric Hospitals

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Abstract

Background: Emotional Intelligence is a broad concept that connects various personality and cognitive traits of human beings. The concept of emotional intelligence is described as the individual’s capability of recognizing their own emotions and the emotions of others, and practice this understanding to navigate important interactions successfully.

Aims: The purpose of this study is to assess emotional intelligence among nurses working in psychiatric hospitals, as well as the relationship between emotional intelligence and workplace stress.

Methodology: A descriptive correlational study design using a convenience sample of (N=109) nurses working in two main psychiatric hospitals. Two separated scales (The Emotional Intelligence Scale-34 items and The Work-related Stress scale-24 items) was used to meet the study objectives.

Results: The study findings indicate that emotional intelligence levels were ranged between moderate to high among study participants. However, the majority had moderate work-related stress about (61%). Nurses’ work stress was found to have significant relationship with their emotional intelligent level.

Conclusion and Recommendations: Despite the positive levels of emotional intelligence nurse have, working in psychiatric hospitals is still cause of stress for nurses. The statistical relationship found in this study highlights the importance of enhancing emotional intelligence among nurses working in psychiatric hospitals settings by engaging them in effective training programs to maximize their abilities of adjustment and reduce their levels of work-stress.

Keywords: Emotional Intelligence, Work-related Stress, Psychiatric Nurses

Introduction

Emotional Intelligence (EI) is a broad concept that connects different personality and cognitive traits of humans, including emotional processing, emotional understanding, integrating affects and management of feeling states, ¹⁶. The concept first introduced by Salovey and Mayer in 1990, and it was defined as cognitive skills that introspectively can detect and regulate affective states and assess the emotional state of others. In general, emotional intelligence is described as the ability of an individual to recognize own emotions and others emotions, and to practice this understanding to navigate important interactions successfully. A clear understanding of EI and its difficulties is helpful in the practice of nursing; due to the understanding of how emotions merge with cognition. Generally, EI helps nurses to improve relationships, manage their patients more effectively, make better decisions, and positively affects the quality of care provided to patients and families. The study aims to assess emotional intelligence among nurses working in psychiatric hospitals, as well as the relationship between emotional intelligence and workplace stress. Emotional intelligence is mainly focus on people with the ability to express, evaluate, and adjust enthusiasms and emotions of themselves and others, as well as those who can properly guide and control the thought and performance. Therefore, emotional intelligence is considered as an effective power to deal with job stressors. Also, nurses with high emotional intelligence and emotional managements are able to direct positive feelings exchanged by healthcare individuals to decrease the negative effects of job stress.
experience and suffer from stress and health problems have less traits related of their work characteristics and less contact with patients (1, 8). Emotions can have an impact on job outcomes and emotional intelligence, which could explain the differences among individuals in dealing with work stress. 8 Emotional intelligence can have a direct influence on the quality of the provided healthcare services or indirect influence, through other mediator, variables such as individual's job satisfaction and professional conflict. Studies have shown that nurses communication skills and job satisfaction play significant role in the emotional intelligence of nurses and the quality of the provided services 3. Moreover, work-related outcomes are more likely to be impacted when nurses feel stressed and insecure in their work environment. 15.

Methodology

Study Design and Sampling Plan:

A descriptive correlational study design using a convenience sample of (N=109) psychiatric nurses working in the main psychiatric hospitals in Baghdad Province. Both male and female nurses were included regardless of their working shift. Their age ranged between 20 – 60 years old, with at least one years of experience in the psychiatric hospital.

Study instrument:

Two separated scales were used to meet the study objectives. The first scale is the Revised Emotional Intelligence Scale-(34) items. The original emotional intelligence scale was developed by 4, which contains (65 items); then, it was reduced to (34 items). The revised scale is widely used to measure the emotional intelligence cross-cultures. This scale is a self-reported instrument that is measured and rated on three levels rating scale; 1= not applicable to me, 2= applicable to some extent, 3= highly applicable. The levels of emotional intelligence are determined based on mean scores of the total items as follow: 1) nurses with means scores of (1 - 1.66) are considered having low emotional intelligence; 2) nurses with means scores of (1.67 – 2.33) are considered having moderate emotional intelligence; 3) nurses with means scores of (2.34 - 3) are considered having high emotional intelligence. The second scale is the Karasek Professional-Related Stress Scale was adopted and modified to be used in this study to assess workplace stress among psychiatric nurses. The original scale was developed by Karasek in 1980 2. This scale is also a self-reported instrument; however, it is measured and rated on four levels rating scale; “1=strongly disagree, 2= disagree, 3= agree, 4= strongly agree”. Coding for negative statements was reversed for statistical purpose. The levels of work stress are determined based on total scores of the scale items as follow: 1) nurses with total scores of (58 – 69) are considered having high work related stress. 2) nurses with total scores of (70 – 81) are considered having moderate work-related stress; 3) nurses with total scores of (82 - higher) are considered having low level of work-related stress.

Scale Validity and Reliability

The Revised Emotional Intelligence Scale and The Karasek Professional-Related Stress Scale were translated to Arabic by two bilingual content experts. The Arabic versions of both scale were presented to panel of content experts related to the fields of mental health, psychology, and community health to maintain the validity of the Arabic versions. Both scales had good reliability levels with a Cronbach’s alpha level (0.89) for the emotional intelligence scale and a Cronbach’s alpha level (0.78) for the work stress scale.

Ethical Considerations

Nurses were asked for voluntary participation. Research objectives, risks, and benefits were explained to participants to help them decide whether to participate or not. After they agreed to be a part of the study, participants were given anonymous questionnaire to maintain participants’ confidentiality.

Results and Discussion

This study indicates that majority of the study sample (60.6%) had high level of emotional intelligence, and (39.4%) had moderate level. The results of this study show that female nurses have higher level of emotional intelligence, than male nurses. Recently, there are some studies have been conducted to study nurses’ emotional intelligence in several fields. Empathy, emotional awareness, and problem solving were the most common attributes of emotional intelligence that have been reported by nurses. Researchers discovered that nurses’ emotional intelligence was higher than other professionals. This difference is related to the fact that nurses get more training about caring process and spiritual care (12,14). Another research was conducted in the Netherlands showed higher emotional intelligence scores among mental health nurses compared to the
general population. Engaging in more ethical behavior have been indicated by nurses who reported high levels of emotional intelligence. Moreover, it has been found that the high level of emotional intelligence among clinical nurses play a role in organizational justice, specifically regarding interpersonal and informational aspects of relationships. The outcomes of this study show moderate level of work-related stress was identified in (60.6%) of psychiatric nurses; while (14.7 %) had high level of stress related to their workplace. Nursing personnel are considered as professionals with increased level of stress, anxiety, and depression due to their daily routine, which is particularly demanding since they are forced to cope with pain, sadness, and death. Stress also comes from the fatigue resulted from an increased workload, as well as the inability of nurses to cope with the emotional needs of patients and their families. Nurses who work in psychiatric settings are more likely to experience work violence than other nurses who work in different healthcare settings. Violence is negatively impact the provided quality psychiatric care and increase the level of stress among nurses. Shu-Fen Niu et al. (2019) have highlighted alarming percentages about the experienced violence by nurses that cause higher level of work-related stress among nurses population. Researchers indicates that more than 50% of the psychiatric nurses involved in their study experienced physical violence and more than 80% experienced psychological violence by psychiatric patients. Resilience of nurses is another concept could explain the work-related stress among psychiatric nurses participated in this study. Most recent systematic review study was conducted on 38 articles that targeted nurses resilience and work-related factors. Researchers pointed out that “Job demands (stress, burnout, posttraumatic stress disorder, and workplace bullying) were negatively associated with resilience, while job resources (coping skills, self-efficacy, social support, job satisfaction, job retention, and general wellbeing) were positively related to resilience”. A significant inverse relationship between emotional intelligence and work-related stress was identified among psychiatric nurses, in which that stress decreased when emotional intelligent increased. Several studies have highlighted the link between emotional intelligence and work-related stress among nurses. Nurses who have a high level of emotional Intelligence build relations within an organization and with the people they serve; whereas, those with low level of EI may tend to create problems for the organization through their individual behaviors. Therefore, developing emotional intelligence should be emphasized in order to overcome stress at workplace and accomplish goals and cope with stress. The study of represent that emotions play an important role in a profession that requires not only technical expertise, but mainly relies on the psychological and physical care of human beings. The positive effects of EI with respect to the quality of nursing work and the ability to mitigate the impact of a difficult working environment via the contribution of emotional self-management and the personal stress management capacity in environments that is full of stress. EI enables nurses to effectively manage and deal with stress at work. Furthermore, the ability of individuals to regulate emotion can have positive effect on group cohesiveness and that the emotional intelligence level of team leaders can improve team empowerment and minimize stress among workers. It has been found that longer careers, job retention, and participation in clinical ladder programs are related to higher emotional intelligence scores of clinical staff nurses. Moreover, there is relationship between emotional intelligence and nurse reports of self-compassion that affect positively on their performance, and higher nurse emotional intelligence is associated with lower levels stress and less burnout. In addition, less confrontational anxiety was associated with the ability to regulate emotion.

Conclusion

The recent study aims to measure the phenomenon of emotional intelligence among nurses working in psychiatric hospitals and the relationship with their stress at work. The findings show that more than half of nurses working in psychiatric hospitals had high emotional intelligence level. However, despite the positive results, working in psychiatric hospitals is still a source of stress for nurses. The statistical relationship appears in this study between emotional intelligence and workplace stress highlights the importance of enhancing EI among nurses, as it improves the capacity to exercise skills in a manner that benefits patients, families, peers, and themselves as practitioners. Furthermore, increased emotional intelligence can lead to more successful management, job satisfaction, organizational commitment, better patient health outcomes, and less workplace stress.
Table 1: Emotional intelligence levels among psychiatric nurses

<table>
<thead>
<tr>
<th>Emotional Intelligence Levels</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate emotional intelligence</td>
<td>43</td>
<td>39.4</td>
</tr>
<tr>
<td>High emotional intelligence</td>
<td>66</td>
<td>60.6</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Work-related stress among psychiatric nurses

<table>
<thead>
<tr>
<th>Work-related Stress levels</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild stress</td>
<td>27</td>
<td>24.8</td>
</tr>
<tr>
<td>Moderate stress</td>
<td>66</td>
<td>60.6</td>
</tr>
<tr>
<td>High stress</td>
<td>16</td>
<td>14.7</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3: Relationship between emotional intelligence and work-related stress

<table>
<thead>
<tr>
<th>Dependent and independent variables</th>
<th>Emotional Intelligence</th>
<th>Work-related Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotional Intelligence</td>
<td>Pearson Correlation: 1</td>
<td>.345**</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>.000</td>
</tr>
<tr>
<td>N</td>
<td>109</td>
<td>109</td>
</tr>
<tr>
<td>Work-related Stress</td>
<td>Pearson Correlation: .345**</td>
<td>1</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>109</td>
<td>109</td>
</tr>
</tbody>
</table>

Table 4: Regression analysis to predict work-related stress from nurse’s emotional intelligence.

<table>
<thead>
<tr>
<th>Dependent &amp; Independent Variables</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>2.364</td>
<td>.223</td>
<td>10.612</td>
<td>.000</td>
</tr>
<tr>
<td>Emotional intelligence</td>
<td>.354</td>
<td>.093</td>
<td>.345</td>
<td>3.807</td>
</tr>
</tbody>
</table>

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing/University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

References


The Relationship between Smoking and Urokinase Gene 3’-UTR T/C Expression on Occurrence of Bladder Cancer

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2College of Medicine, University of Kerbala, Kerbala, Iraq

Abstract

Bladder cancer (CA Bladder) is the malignancy that affects the lining of the bladder (which is the most common types of urinary epithelial cancers that associated with the most invasive types of cancers and has the highest incidence of recurrence and infections). This study was designed to investigate the role of cigarette smoking on expression of urokinase gene that has a prominent role in the incidence of CA bladder. The samples were collected from 90 patients after being clinically diagnosed by the specialist surgeon of Imam Hussein Center for Cancer Diseases, Holy Kerbala, Iraq who were heavily smokers (more than one pocket/day). These patients were compared to 90 non-smoker patients with CA bladder and 90 persons who apparently healthy individuals as a control groups, DNA was extracted from all blood samples. The level of gene expression was correlated with cycle threshold value calculated by using real time PCR.

The results showed significant association between smoking and occurrence of CA bladder (by increased expression of urokinase 3’-UTR T/C gene) in comparison to both control and non-smoker patients groups.

Keywords: CA bladder DNA, cigarette smoking, Urokinase gene 3’-UTR T/C, real time PCR.

Introduction

The genetic investigations for polymorphisms help to distinguish between the different inherited forms of the gene and their impact on the occurrence of many human diseases include cancer 6. The extent of the risk factors like smoking and genetic predisposition of many affect the occurrence of many diseases such as cancer, heart disease and diabetes 1. The urokinase-type plasminogen activator uPA system is a responsible for transforming plasminogen into plasmin, which has different physiological functions 10. It additionally assumes a key signaling protein in disease intrusion and metastasis both locally and spread away for far destinations 4.

The urokinase gene is located at chromosome 10q24 9. Polymorphisms of a C/T transversion at the 3’ UTR (+4065 nucleotide) were demonstrated by C/T substitution in exon 6 and a T/C substitution in intron 7 8. Among the risk factors that participate in the occurrence of CA bladder, smoking was detected as one of major one 3. High levels of urokinase have been depicted in bladder tumors especially in those who were heavily smokers 5.

Methodology

Blood samples were collected from males of age group ranging from 50-60 years old by the Imam Hussein Center for Cancer and Hematology at the Imam Hussain Teaching Hospital in Holy Karbala province, Iraq. The samples were include a 90 heavily smoker patients after the clinically determination of bladder malignancy (transitional cell carcinoma) by authorized specialist, 90 patients with CA bladder with no history of smoking for at least the past 25 years, and 90 apparently healthy non-smoker individuals as a control group. Both patients and control groups were matched regarding age, body weight, past medical history and any associated confounding factors. This study was extended for period from January to August 2018. DNA was extracted from all blood samples by DNA extraction kit (Bioneer,Korea). Polymerase chain reaction (PCR) was adopted for amplification of gene, The Premieres
were Designed by STS Accession No. G27040: forward primer 5'-CCGCAGTCACA-C-CAAGGAAGAG-3' and backward primer 5'GCCTGAGGGTAAAGCT-ATTGTCGTGCAC-3'.

The RT-PCR protocol involve the following steps: (a) initial one cycle of denaturation for 5 minutes at 95°C, (b) followed by annealing at 58°C for 40 seconds, (c) then extension at 72°C for 40 seconds, (d) with final step of holding the specimen at 8°C. Real time PCR was used for quantitative assessment of urokinase gene 3'-UTR T/C expression by measuring cycle threshold (cT) value (which is inversely proportional to the level of gene expression).

Statistical Analysis

Data were statistically represented as mean±SD and analyzed by detection of variance of significance using ANOVA test by sigma plot software version 12.5.

Results and Discussion

Smoking was thoroughly studied as a predisposing factor for many human disease include cardiovascular disorders, respiratory diseases malignancies, neurological deficits and many other organic dysfunction. In current study, the aim was to demonstrate the Table (1): the cT value of urokinase gene 3'-UTR T/C (expressed as Mean±SD) estimated by real time PCR.

<table>
<thead>
<tr>
<th>Group Name</th>
<th>No.</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA smokers</td>
<td>90</td>
<td>12.584±2.692</td>
</tr>
<tr>
<td>CA non smokers</td>
<td>90</td>
<td>23.213±3.569</td>
</tr>
<tr>
<td>Healthy non smokers</td>
<td>90</td>
<td>24.865±2.861</td>
</tr>
</tbody>
</table>

The difference in cT values were quite significant between smoker patients as compared to both non-smoker patients and non-smoker healthy persons (12.584±2.692 versus 23.213±3.569 at P<0.001 and 12.584±2.692 versus 24.865±2.861 at P<0.001 respectively). On the other hand, no significant difference was detected between non-smoker patients in comparison to non-smoker healthy individuals (23.213±3.569 versus 24.865±2.861). These findings suggested that smoking has potential inducing effect for expression of urokinase gene 3'-UTR T/C which has crucial role in occurrence of CA bladder in heavily smokers. Other finding was noticed that the occurrence of CA bladder in non-smoker patients may be attributed to other sort of predisposing factor or gene.

This association was mentioned in certain study, which suggested that chronic exposure to cigarette smoking had strong association with development of urothelial carcinoma.

In another study done to evaluate the effect of smoking on soluble 3'-UTR isoforms (regulatory pathway of bronchial epithelial cell function), similar results were founded supporting the potential role of smoking as predisposing factor for CA bladder in our study.

This effect of smoking was explained in certain study as a result of the effect of smoking on p53 pathway that mediate apoptotic signaling that responsible for eradication of cancer cells.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Education for Pure Sciences, University of Kerbala, Kerbala, Iraq and all experiments were carried out in accordance with approved guidelines.

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Effect of Carcinogenic Substance (7,12 Dimethylbenz[a]Anthracene (DMBA)) on Tissue, Hematology Character and Enzyme Activity in Rat

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Abstract

This study aimed to investigate the effects of 7,12 dimethylbenz[a]anthracene (DMBA) on some blood parameters and hepatic histopathology in rats and antioxidant enzyme. twenty female Wistar albino rats, weighing 180–200 g, were randomly divided into two group. DMBA group (positive group) who received 20 mg DMBA/kg body weight/ (single Douse). Control group (negative group) don’t received any thing. The animals in the groups were sacrificed at the end of the 90 days: The histological structure of the liver tissues in the control group was normal. the liver exhibited hydropic degeneration and coagulation necrosis in hepatocytes, severe dilation in the sinusoids, congestion in the central and portal regions, DMBA groups, was degenerative and necrotic changes were detected. MDA levels increased in the DMBA group , compared to the control group, Antioxidant activity CAT, SOD and GPX, the results showed increased enzyme activity in positive control compared with negative groups, ,the hematological parameter was increase in WBC, PLT, GRA, and LYM and decrease in RBC value .

Keywords: 7,12-dimethylbenz[a]anthracene (DMBA); Blood parameters; Antioxidant enzyme; MDA; Rat.

Introduction

Cancer, also termed as malignant neoplasm, is a type of diseases where in a group of cells show abnormal proliferation, invasion and sometimes metastasis 1. Cancer begins when cells in a part of the body start to grow out of control 2. There are more than 100 types of cancer have been identified 3. The substances that promote cancers are called carcinogens, and agents that have the ability to change DNA in ways that are inherited by daughter cells are called mutagens. Most cancers are related to environmental, lifestyle, or behavioral exposures 4. The term “environmental”, as used by cancer researchers, refers to everything outside the body that interacts with humans 5. Other chemicals, like benzene, ketones, vinyl chloride, ethylene bromide, and dichloro-diphenyl-trichloroethane (DDT), are known carcinogens 6. The accumulation of multiple factors, carcinogens and altered genes, transform a normal cell into a cancerous one. Carcinogens that have the ability to promote cancer have various sources 7. The factors responsible for cancer development are classified as exogenous and endogenous 8. The first group includes nutritional habits (food preservation and preparation), socio-economic status, lifestyle, physical agents (ionising and non-ionising radiation), chemical compounds (natural and synthetic) and biological agents (Helicobacter pylori, Epstein Barr virus, human T lymphotropic viruses I and II, human papilloma virus and the hepatitis B virus 9.

Material and Method

Experiential animals and Tumor induction in rats:

Twenty female of Wistar albino rats and aged between (7-8) weeks were weighing between 150 – 250 g used in this study. All they were kept in ventilated cages, with temperature of 25±2°C. A 12:12 h light:dark cycle
is also regulated for these animals. Balanced rodent food pellet and water is provided. The rats were randomly assigned to 2 groups of 10 rats each.

The groups were as follow:

Group 1: Received a single dose of DMBA (positive control) 20 mg DMBA/kg body weight/ (single dose)

Group 2: Did not received any treatment (negative control).

**DMBA administration:** Mammary tumors were induced by 7, 12-

Dimethylbenz (a) anthracene (DMBA) \(^{10}\). A single dose of DMBA dissolved in corn oil was given by oral gavage to two groups using the syringe and needles. DMBA was purchased from Sigma Aldrich and dissolved in corn oil. The concentration of the solution was 20 mg DMBA per 1 ml corn oil for each rat \(^{10}\).

**Preparation of tissue sample**

The liver was exteriorized and excised. All specimens were immediately fixed in 10% formaldehyde solution. After fixation they were processed in usual manner, and embedded in paraffin for subsequent histopathological examination for liver. A scoring system (of no abnormalities, mild, moderate, severe) was used to classify the liver changes according to the severity of the damage and extent of histological changes. The histological sections were evaluated by a pathologist without prior knowledge of the treatment given to the animals \(^{11}\).

Blood parameters were determined in whole blood by the using rat mode of veterinary practice with a blood cell counter (Abocus Junior Vet-5, Austria). Measurements of biochemical parameters were made with a Modular PP autoanalyzer (Mindray BS800, China).

**Results**

Histopathological findings: The control group showed normal histological structure in the liver tissue (Figure 1A). In the DMBA group, the livers exhibited dilatation of the sinusoids, cholangiohepatitis in the portal region, and congestion in the sinusoidal and portal regions (Figure 1B). Hematological parameters also change in negative group compared of positive group Table 1, in positive group : white blood cell (WBC) , LYM, MON, GRA and PLT while other parameter such as RBC was decrease. The state of free radicals and antioxidants is given in Table 2. As seen in Table 2, the levels of MDA increased in liver groups compared to the control group, also SOD, GSH-Px, CAT, and GSH values decreased.

**Figure1; A:** DMBA group showing cholangiohepatitis in portal region, mild dilation in sinusoids, hyperemia, and hydropic degeneration in hepatocytes;  
**B:** Control group showing liver tissue with a normal histological structure

**Table 1. Some hematological parameters in the**
### Table 2. Some biochemical parameters in the groups (values are mean n=4)

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Negative control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WBC (10^3 /mm^3)</td>
<td>8.2</td>
<td>15.1</td>
</tr>
<tr>
<td>2</td>
<td>RBC(10^3 /mm^3)</td>
<td>8.8</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>LYM %</td>
<td>67%</td>
<td>78.5 %</td>
</tr>
<tr>
<td>4</td>
<td>MON%</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>GRA %</td>
<td>18.8 %</td>
<td>94 %</td>
</tr>
<tr>
<td>6</td>
<td>MCV(fl)</td>
<td>56.6</td>
<td>58.7</td>
</tr>
<tr>
<td>7</td>
<td>MCH(Pg)</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>MCHC(g/dl)</td>
<td>32.3</td>
<td>32.8</td>
</tr>
<tr>
<td>9</td>
<td>PLT(10^5 /mm^3)</td>
<td>338</td>
<td>737</td>
</tr>
</tbody>
</table>

**Discussion**

7, 12-dimethylbenz (α) anthracene (DMBA) is a well known carcinogen and immunosuppressor used in rodent models to study cancer. DMBA is reported to induce mutations by making DNA adducts. Although, it is a well known skin carcinogen, yet many researchers have reported the deleterious effect of DMBA in liver. Liver is the primary site of metabolism and is often prone to damage by xenobiotics. Evidently, liver cancer is the second most common cause of cancer deaths worldwide. Several haematological and haematochemical parameters were changed when treated with DMBA and found that DMBA induced hepatocellular carcinoma. Experimental studies showed that DMBA-induced skin, oral, mammary and ovarian tumors. The carciogenic and mutagenic effect of DMBA requires its metabolic activation by mixed function oxidases. The hydroxylation of DMBA at 7-methyl group is a crucial step towards its carcinogenesis. Further metabolism of DMBA leads to formation of a wide range of metabolites with varying toxicity. Among these, trans-3,4-dihydrodiol-1,2-epoxide is the carcinogenic product of DMBA. The metabolic products of DMBA, when present inside body, hampers ROS-antioxidant balance by overproduction of free radicals and the body in turn reacts by modulating activities of antioxidant enzymes to curb the damaging effects of an increased ROS. Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, region, season, environmental heat, maintenance, and nutrition. In the present study, the effects on blood parameters and hepatic, histopathology of fluoride. Oxidative products derived from mutagen metabolism, such as DMBA, might impair vital cellular function by damaging proteins and lipid membranes. Consequently, these changes induced by the chemical carcinogen 7,12-dimethylbenz[a]anthracene, have been reported to be leukemia, and the development of anemia. Reactive oxygen species (ROS) are important pathological agents for many diseases. Increased oxygen radical production and lipid peroxidation are associated with the pathogenesis of many diseases and the toxic effects of a wide range of compounds increase in total leukocyte count (WBC), eosinophil, neutrophil and monocyte values for rats exposed to DMBA was also reported by. On other hand find MDA content increase significantly with rat treated with DMBA only.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, University of Warith Al-anbiya’a, Iraq and all experiments were carried out in accordance with approved guidelines.

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Psychological Factors Affecting on Developing Reading Skills and Evaluation of Reading Strategy Knowledge

Wathiq Al – Husseini¹, Asst. Prof. Dr. Diaa Oued Al Arnousi¹, Qassim Raheem¹

¹University of Babylon / College of Basic Education, Iraq

Abstract

The questions are one of the most common and used methods in the educational process and in schools all over the world and are the most important educational strategies. Because questions are a great place in teaching, which is the mainstay of the dialogue method and is like the driving force in the lesson. The educators say (If you want to become a highly competent educator, it is important that you begin to apply and develop educational questions). These questions also occupy great importance in the educational process after which the standard means of knowing the readiness of the students and the level of information they received, in a good question, we achieve the desired goal and achieve a desirable educational product. Besides, the exams are a means to help educators to make different educational decisions, so that, there have been wide concerns calling for the reform of the test questions and the evidence of that conferences that discussed this problem, studies and research aimed at diagnosing them in order to address, improve and develop their defects to achieve best results in education.

Key words: evaluation, Arabic Language Questions, six Grade Students, Standard Specification Table.

Introduction

Education is the basis for the reform of humanity and its success. It is a force that can develop individuals, sharpen their talents, sharpen their minds and ideas. The education of the individual means comprehensive education that is integrated in all aspects, spiritual, mental, physical, psychological, social and aesthetic.¹ The method of education is the language that benefits the individual in understanding cultural aspects is the means of understanding and the means of education and the collection of cultures, a tool for the transfer of ideas, but is a tool of thinking and sense and feeling.² In summary: Language is one of the tools of public life, and it does not perform its duty unless fulfills its purpose for, and does not reach its real status with its people unless they are concerned about what they are and about.² Through this way, the Arabic language is one of the most important elements of our Arab nation. It is the language of the Holy Quran, which says: “We have sent down Arab villages” (Yousif / 2). The Holy Quran absorbed the characteristics of the language and it’s all stylistic features and its characteristic expression Until become the basis for the validity of the language and the state return to know the secrets and facts.³ Thus, Arabic is one of the most important elements of our Arab nation and by which the nation held fast and boycotted the sermons. As well as it is the language addressed by God Almighty Prophet Muhammad (peace be upon him and peace) as it is the language that came down the Koran by saying: (We sent down Arab villages that you may know) (Yousif / 2). The Holy Quran absorbed the characteristics of the language and it’s all stylistic features and its characteristic expression Until become the basis for the validity of the language and the state return to know the secrets and facts.⁵ The Arabic language is a branch of a large group called the Semitic languages, and the first to be known as the German orientalist Schlutzer in 1781.³ The evaluation of exam questions is a component of the curriculum, because it is the process of ensuring the achievement of goals, the relationship between the evaluation process and the educational goals, the evaluation process aims at knowing the extent to which these goals are achieved, or the extent of their progress towards the educational goals to be achieved. The importance of the evaluation is clear because of its great impact in the process of change and development in the educational process, because on the basis of a sound objective evaluation can be achieved a...
lot of educational principles, in addition to showing and treating weaknesses and showing positive aspects and strengthening them. There are some important terms that should be clarified as follows:

1. Evaluation: “It is the last stage that depends on measurement and decision-making, and then the feedback that makes teaching and teaching circular rather than linear.”

2. Questions: The field in which the student acquires knowledge and mental skills and intellectual capacities and works on their development and development.

3. Exam: A systematic objective method for measuring a sample of a person’s skills or abilities or a group of individuals in a particular time and place.

4. Bloom classification: A classification that contains a range of public areas, which include the possible educational data, and the expected educational process.

The theories and models of evaluation are very numerous and difficult to define each model that has many positives that make it suitable under conditions. And certain educational programs, and no matter how many calendar models, for the good qualities that should be characterized by any model of the calendar and any measure or any test are:

1. The Objectivity: - Objectively means that the procedures of evaluation and its components or measurements are accurate without falsehood away from self-impact and personal impressions of the transient to be true and consistent.

2. It is appropriate that the evaluation be appropriate in its tools, procedures and results for the subject of evaluation.

3. The Inclusion: - that the evaluation process is comprehensive so that the study programs or the subject to be evaluated in all its aspects and not by a certain aspect of it.

4. The Stability: that the evaluation process results in the same results if the results are re-analyzed or the application of measurement and evaluation tools is repeated.

5. The Honesty, ie, the evaluation means what is intended to evaluate it cannot study something and measure something else.

6. The Easiness: - That the evaluation and its tools process can be easily applied within the available material and human resources available and under the local conditions designed for them. The researcher will discuss the classification of Bloom and the criteria adopted in the analysis of the questions of Arabic grammar for sixth grade students. The scientific and literary branches in detail in the third chapter.

The results of the study are as follows:

1. The questions of reading subjects in the primary stage are markedly concerned with the level of understanding and remembering. The percentage of questions of comprehension (47.3%) and the level of remembering (40.8%). The levels of application, analysis, installation and evaluation were very small.

2. The questions of reading topics are concerned with the skills of direct understanding of the text, and the skills of understanding the conclusion of a greater interest in higher thinking skills such as the skill of criticism and taste, the proportions were respectively: (57.6%), the deductive understanding (34.5%), the critical comprehension (6.9%), and the final taste (1.7%). The test questions focused on the questions of understanding the direct meaning by 68% and the deductive questions by 32%.

3. Reading comprehension questions (50.3%), comprehension level (31.1%) and application (9.1%), followed by the remaining levels at low rates.

4. The predominant type of questions in subjects of reading is the most important questions (34.8%), while the balance of questions is (51.4%) compared with (48.6%) for the objective questions.

The research methodology and procedures:

It includes a presentation of the procedures that have been carried out to achieve the research objectives, starting with the research methodology and descriptive design, defining the research community and its design, preparing the research materials and tools, and presenting the statistical means used.

The Descriptive Design: The researcher followed descriptive research methodology to analyze the content.
of test questions because it is the appropriate method for analyzing questions, which is one of the research methods used in education and psychology. The aim of this approach is to provide data and facts about the problem of the subject to be interpreted and to identify its implications.

**The research community and design:**

The research society includes the general (ministerial) examination questions for the Arabic grammar for the third grade in the Republic of Iraq for the academic years of the academic year (2014/2015) to the academic year (2018/2019), and the first two and the third and the number of (11) exam paper, (5) papers for the first round and (5) exam papers for the second round examination paper for the third round and as Annex 1, as the research community represents the questions of the ministerial examinations of the sixth scientific grade therefore, the whole research community will be a basic sample that will be adopted by the researcher in his procedures.

**Table (1) shows the research community and its sample:**

<table>
<thead>
<tr>
<th>No.</th>
<th>Academic year</th>
<th>No. of main questions</th>
<th>No. of sub-questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2014-2015 attempt /1</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>2014-2015 attempt /2</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>2014/2015 2014-2015 attempt3</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>2015/2016 2014-2015 attempt 1</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>2015/20162014-2015 attempt 2</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>2016/2017 2014-2015 attempt2</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>2016/2017 attempt2</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>2017/2018 attempt 1</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>9</td>
<td>2017/2018 attempt 2</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>2018/2019 attempts 1</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>11</td>
<td>2018/2019 attempts 2</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>77</strong></td>
<td><strong>223</strong></td>
</tr>
</tbody>
</table>

**The research Tool:**

A - Sources of building the tool:

The structure of the criteria for the evaluation of the ministerial examinations of Arabic grammar through:

- Building an open survey questionnaire addressed to teachers and specialists in the methods of teaching Arabic language and measurement and evaluation.

- See a collection of previous studies and literature relevant to the current research topic that examines the standards of good questions. By means of exploratory resolution and the sources of tool construction, the orthodontic criteria that analyzed the test were built. The study consisted of six criteria and these criteria were analyzed by the questions of the ministerial examinations of the grammar of the Arabic language for the sixth grade in the scientific branches on the grounds that these criteria are the most important criteria that
should be available in the examination questions are:

- Independence.
- Direct and indirect questions.
- Objectivity.
- Inclusion.
- Bloom’s levels of knowledge.
- Technical aspects.

B - Tool credibility:

These criteria were presented to a sample of experts and arbitrators with expertise and experience for the purpose of identifying the validity and importance of each paragraph of the questionnaire, in order to achieve the apparent honesty. A standard was established to measure each paragraph (valid, not valid, observations). In this respect, Ebel points out that the test is true if its verbs measure what has been measured 13.

The researcher relied on the arbitrators to indicate the validity of the paragraphs of the questionnaire in their precise reference to the paragraphs, which is an indicator of the apparent authenticity of the questionnaire, and after collecting the questionnaires from the arbitrators, the researcher determined the proportion of agreement (90%) and more than the opinions of experts on the validity of the paragraph, because it represents the view of the majority, Agreement as follows:

- Independence 100%
- Objectivity 100%
- Bloom levels of the field of knowledge 100%
- Inclusive 100%
- Direct and indirect questions 95%
- Technical aspects 97%

In addition, some experts and arbitrators suggested that another criterion be increased, namely the proportion of correspondence to the textbook questions. The criteria were as follows:

- Independence
- Objectivity

- Bloom’s levels of knowledge
- Inclusion
- Correspondence to textbook questions
- Direct and indirect questions
- Technical aspects.

Steps to analyze the test questions:

A - Reading the contents of my book Arabic grammar rules for the sixth grade scientific reading good to know all content and topics.

B - Reading the questions a good reading and answer them as appropriate.

C) The question which contains several branches, each of which is treated as an independent question.

D - matching the questions to the criteria adopted by the researcher.

E) Unloading the results of the analysis into the criteria for analyzing the questions.

Evaluation criteria for ministerial examination questions:

There are scientific educational foundations that must be available in any exam to trust its results and benefit from it. The good exam is the one that is fit to perform the purpose for which it was set up in the fullest manner. Such a test will not be complete unless there is information on its validity for measurement.

Bloom’s Levels of Knowledge:

Many of the studies in Bloom’s classification (1956) indicate that this classification is one of the most important works that can help to analyze questions in general and in a procedural manner to contain all levels that reflect the desired objectives of the education process 14.

The Bloom classification is one of the most important attempts to address behavioral goals and the most widely used. It has an advantage in the identification and identification of educational goals 15.

Bloom’s classification helps teachers gain a clear idea of the patterns of behavior emphasized by a particular set of educational schemes. As well as it represents
an educational system and benefit for all teachers, administrators and those interested in education and researchers who are in the Department of specialization in the issues of curriculum and evaluation 16

Therefore, the researcher chose this classification because it is one of the most common classifications because it contains a very wide range of behavioral patterns of goals that most educational programs and systems are expected to achieve. It also benefits educators in general, and teachers who seek to define their educational goals in special behavioral terms. 17.

Those who are interested in measurement and evaluation, led by Bloom, set behavioral goals in three areas. A meeting of the University of Chicago in 1956 resulted in an agreement to classify goals into three areas:

(Cognitive / mental, emotional / emotional, and motor).

Statistical means:

The researcher used the following statistical means:

- percentage
- Equation (scott) to find the stability of analysis.

Conclusion

The ministerial examinations are of great importance, because it represents the boundary between the stage and the other, especially since each stage characteristics and advantages differ from the stage that precedes it, especially the transition from secondary level to university; The current research problem revolves around the validity of ministerial examination questions for the Arabic language for the sixth scientific grade and the extent to which the characteristics of good questions. In the light of the criteria standard and the analysis of questions according to this standard. The importance of the current research stems from the importance of the Arabic language and its importance to all the subjects, the importance of the ministerial examinations also stems from the fact that they are the most important evaluation tools currently used in our schools.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon / college of Basic Education, Iraq and all experiments were carried out in accordance with approved guidelines.

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Comparison Between Photoselective Vaporization of Prostate by Diode Laser and Monopolar Transurethral Resection of Prostate in Single Center Experience

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Abstract

Background: Transurethral resection of prostate has been regarded as the gold standard surgical management of benign prostatic hyperplasia (BPH). With the evolution of new technologies, laser prostatectomy emerged as an alternative.

Aim of the study: To compare the rate of outcome of diode laser vaporization of prostate and Transurethral Resection of Prostate in Al Diwaniya governorate.

Patients and Method: From October 2017 to October 2018, forty patients with surgical indications of prostatectomy were enrolled in this study. Their ages ranged from 60-75 years with a mean age 68.6 years and prostate size ranged from 40-80 ml with a mean size 62.175 ml. Twenty patients underwent diode laser vaporization of prostate and another 20 patients underwent Transurethral Resection of Prostate. The choice based on surgeon preference and patient ability. Preoperative evaluation was done for all patients by history, physical examination and investigations. International Prostate Symptoms Score, maximum flow rate, digital rectal examination, prostate specific antigen, abdominal ultrasound, urinalysis, blood urea, s.creatinine, complete blood count were all done preoperatively. One pint of compatible fresh blood prepared.

Results: The rate of significant bleeding and transfusion in diode PVP and TURP was (zero vs. 35% respectively), in PVP and TURP the mean irrigation amount perioperatively was (10.10 vs. 33.15 pints respectively), mean operative time was (70.80 vs. 50.00 min respectively), mean hospital stay was (16.03 vs. 30.09 hours respectively), mean time to urethral catheter removal was (24 vs. 81.71 hours respectively), postoperative complication rate (mainly dysuria) was (75% vs. 65% respectively), mean postoperative IPSS was (14.40 vs. 14.20 respectively), mean postoperative Qmax was (15.45 vs. 16.30 ml/sec respectively). There was no statistical difference between both groups in regard to lowering IPSS or increment in Qmax. The mean cost was (1500 vs. 500.42 dollars) in diode PVP and TURP respectively.

Conclusion: Diode laser vaporization of prostate is a good alternative to TURP with comparable results in treatment of BPH especially in patients taking anticoagulant drugs who cannot stop their medication. However, the high cost of this procedure and dysuria as a complication may prevent the common use of this technique.

Keyword: Diode photoselective vaporization of prostate, Transurethral resection of prostate, Benign prostatic hyperplasia.

Introduction

Benign Prostatic Hyperplasia (BPH) is a histologic diagnosis that refers to the proliferation of smooth muscle and epithelial cells within the prostatic transition zone and periurethral zone.¹ BPH is commonly characterized by lower urinary tract symptoms (LUTS).² The
prevalence of histologically diagnosed BPH increases from 8 percent in men aged 31 to 40, to 40 to 50 percent in men aged 51 to 60, to over 80 percent in men older than age 80. The prevalence of moderate or severe LUTS for men in the fifth, sixth, seventh, and eighth decades of life to be 26, 33, 41, and 46 percent, respectively. Patients often presents with LUTS as storage irritative symptoms (frequency, urgency and nocturia) due to bladder response to obstruction (increased pressure) and voiding obstructive symptoms (straining, weak stream, intermittency and feeling of incomplete bladder emptying). BPH is a progressive condition that can lead to serious long-term complications such as acute urinary retention (AUR), renal insufficiency, development of gross hematuria, bladder calculi, urinary incontinence, and recurrent urinary tract infections (UTIs). Medical history can establish the severity of LUTS by IPSS. PSA should only be performed if life expectancy is greater than 10 years and if a diagnosis of prostate cancer would modify the management approach. Medical treatment includes Alpha 1 antagonist, 5-alpha reductase inhibitors, Anti-muscarinic agents and Phosphodiesterase-5 inhibitors, when medical treatment fails to control patients complaints, surgical intervention is mandatory; however, surgery is associated with a number of complications. Surgical approach includes TURP. Laser prostatectomy is either coagulation or vaporization (or both), resection or enucleation depending on the wavelength applied. The usage of TURP is gradually decreasing (nearly 5%/year). Types of laser procedures includes HoLEP which can be used in large glands (100-150) and has comparable efficacy and good outcome compared to open prostatectomy and TURP but longer operating time. In this study, we want to compare the rate of intraoperative bleeding and the need for blood transfusion, the amount of irrigant, operative time, hospital stay, time of urethral catheterization, postoperative complications and the cost of diode laser vaporization of prostate and TURP in Al Diwaniya governorate.

Patients and methods

From October 2017 to October 2018, forty patients with surgical indications of prostatectomy were enrolled in this study. Their ages ranged from 60-75 years with a mean age 68.6 years and prostate size ranged from 40-80 ml with a mean size 62.175 ml. Twenty patients underwent diode laser vaporization of prostate and another 20 patients underwent Transurethral Resection of Prostate. The choice based on surgeon preference and patient ability. Preoperative evaluation was done for all patients by history, physical examination and investigations. International Prostate Symptoms Score, maximum flow rate, digital rectal examination, prostate specific antigen, abdominal ultrasound, urinalysis, blood urea, s.creatinine, complete blood count were all done preoperatively. One pint of compatible fresh blood prepared.

Results

Our patients data were comparable between the two groups with a mean age of 68.6±4.14 years (range 60-75 years) and mean prostate size of 62.175 ± 10.885 ml (range 40-80 ml). Their mean PSA was 3.615 ng/ml (range 1.5-8ng/ml). Regarding vesical stones, one patient (5%) of laser group was with 18 mm stone and 3 patients (15%) of TURP group with 18, 13 and 15 mm vesical stones which were removed before beginning of the procedure. Four patients (20%) were on anticoagulants who could not stop their medications (all of them on warfarin 5 mg, 2 patients with cardiac stents, 1 patient with prosthetic mitral valve and 1 patient with deep lower limb venous thrombosis). All of them were enrolled in laser group as shown in table 1.

Table 1: General characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Laser n = 20</th>
<th>TURP n = 20</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year) mean ±SD</td>
<td>68.50 ±4.14</td>
<td>68.70 ±4.03</td>
<td>0.878*</td>
</tr>
<tr>
<td>Size of prostate (ml) mean ±SD</td>
<td>59.35 ±10.885</td>
<td>65.00 ±9.03</td>
<td>0.082*</td>
</tr>
<tr>
<td>PSA(ng/ml) mean ±SD</td>
<td>3.62 ±2.07</td>
<td>3.62 ±1.72</td>
<td>0.993*</td>
</tr>
<tr>
<td>Presence of stones &lt; 20 mm, n (%)</td>
<td>1 (5%)</td>
<td>3 (15 %)</td>
<td>0.598 †</td>
</tr>
<tr>
<td>Patients on anti-coagulant, n (%)</td>
<td>4 (20 %)</td>
<td>0 (0 %)</td>
<td>0.106 ¥</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; *: independent samples t-test; †: Yates correction for continuity; ¥: Fischer exact test; NS: not significant

The indications for intervention in both groups were the same and included thirteen patients (32.5%) with moderate to severe IPSS (who were already on
medical treatment), recurrent urinary retention in 10 patients (25%), gross hematuria in 4 patients (10%), recurrent urinary tract infection in 10 patients (25%) and deterioration in renal function in 3 patients (7.5%). Table 2

**Table 2: indications of surgery**

<table>
<thead>
<tr>
<th>Laser group No</th>
<th>TURP No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate – sever IPSS</td>
<td>7 35%</td>
</tr>
<tr>
<td>Recurrent urinary retention</td>
<td>5 25%</td>
</tr>
<tr>
<td>Gross hematuria</td>
<td>2 10%</td>
</tr>
<tr>
<td>Recurrent UTI</td>
<td>4 20%</td>
</tr>
<tr>
<td>Deterioration in renal function</td>
<td>2 10%</td>
</tr>
</tbody>
</table>

The mean operative time in laser group was 70.8 min. (range 60-80 min.) , while in TURP group was 50 min. (range 40 – 60 min.) (figure 11). Regarding intraoperative complications, no patient developed significant intraoperative bleeding in laser group. In our study, we determined significant intraoperative bleeding as reduction in blood pressure that required transfusion, while seven patients (35 %) in TURP group developed significant intraoperative bleeding and required intraoperative blood transfusion. No patient in laser group developed obturator reflex in comparison with TURP group in which 3 patients (15 %) developed obturator reflex, as shown in table 3.

**Table 3: operative time and intraoperative complications**

<table>
<thead>
<tr>
<th>Laser group</th>
<th>TURP group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean operative time(min.) ± SD</td>
<td>70.80 ± 5.07</td>
<td>50.00±6.19</td>
</tr>
<tr>
<td>Intraoperative bleeding</td>
<td>0 (0 %)</td>
<td>7(35%)</td>
</tr>
<tr>
<td>Obturator reflex</td>
<td>0(0%)</td>
<td>3(15%)</td>
</tr>
</tbody>
</table>

**:** independent samples t-test. *: Fischer exact test . SD :standard deviation. HS :highly significant. NS :not significant.

**Table 4: postoperative care**

<table>
<thead>
<tr>
<th>Laser group</th>
<th>TURP group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean amount of irrigation (pints) ±SD</td>
<td>10.10±2.59</td>
<td>33.15±5.24</td>
</tr>
<tr>
<td>Mean hospital stay (hours ) ±SD</td>
<td>16.03 ±3.89</td>
<td>30.09 ±10.32</td>
</tr>
<tr>
<td>Mean time of urethral catheterization (hours ) ±SD</td>
<td>24</td>
<td>81.71±10.34</td>
</tr>
<tr>
<td>Mean cost (dollars ) ±SD</td>
<td>1500</td>
<td>500.42±195.32</td>
</tr>
</tbody>
</table>

*Independent samples t-test. **One sample t-test; SD: standard deviation; HS: highly significant at at P ≤ 0.01

The mean follow up period was two months. All of the patients were assessed clinically by calculating IPSS and Q max .The mean pre-operative IPSS was 17.65 (range 13 – 27 ) and significantly decreased post operatively in both groups (mean IPSS 14.3 with range of 9-18 ), with the mean IPSS of laser group decreased from 20.1 to 14.4 and that of TURP decreased from 18.75 to 14.2.
Table 5: IPSS and Q-max before and after operation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Laser n = 20</th>
<th>TURP n = 20</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>IPSS (pre-operative)</td>
<td>20.10</td>
<td>4.30</td>
<td>18.75</td>
</tr>
<tr>
<td>IPSS (post-operative)</td>
<td>14.40</td>
<td>3.05</td>
<td>14.20</td>
</tr>
<tr>
<td>Q-max (ml/s) (pre-operative)</td>
<td>10.30</td>
<td>1.66</td>
<td>10.20</td>
</tr>
<tr>
<td>Q-max (ml/s) (post-operative)</td>
<td>15.45</td>
<td>1.15</td>
<td>16.30</td>
</tr>
<tr>
<td>P †</td>
<td>&lt; 0.001</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td></td>
<td>HS</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; S: significant *: independent samples t-test; †: Paired samples t-test; NS: not significant at P \( \leq 0.05 \); HS: highly significant at P \( \leq 0.01 \)

Regarding post-operative complications: no patient in laser group had frank hematuria in the immediate post-operative period compared to 2 patients (10 %) in TURP group. TUR syndrome did not occur in neither group. Four patients (20 %) in laser group had UTI in comparison to 5 patients (25 %) in TURP group. Acute retention occurred in 3 patients (15 %) in each group. Eight patients (40%) in laser groups complained from dysuria compared to 3 patients (15%) in TURP group. The overall complications rate was 75 % vs. 65 % for laser and TURP groups respectively. As shown in table 6.

Table 6: Post-operative complications

<table>
<thead>
<tr>
<th>Complication</th>
<th>Laser n = 20</th>
<th>TURP n = 20</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-operative bleeding</td>
<td>0 (0%)</td>
<td>2 (10 %)</td>
<td>0.487 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>TUR syndrome</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>---</td>
</tr>
<tr>
<td>UTI</td>
<td>4 (20 %)</td>
<td>5 (25 %)</td>
<td>1.000 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Acute retention</td>
<td>3 (15 %)</td>
<td>3(15 %)</td>
<td>1.000 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Dysuria</td>
<td>8 (40 %)</td>
<td>3 (15 %)</td>
<td>0.077**</td>
</tr>
</tbody>
</table>

n: number of cases; *: Fischer exact test; †: ¥: Yates correction for continuity; **: Chi-square test; NS: not significant at P \( \leq 0.05 \)
Discussion

In this study, we compared the rate of intraoperative bleeding, the amount of irrigation fluid needed, operative time, hospital stay, time to urethral catheter removal, postoperative complications, follow up results and the cost. In our study, no patient in laser group developed significant bleeding, while in TURP 35% of patients had significant bleeding and needed transfusion. Kuntz RM reported that bleeding and need for transfusion is reduced significantly in diode laser PVP compared to TURP. Erol A. et al reported 1 patient with diode PVP developed bleeding that needed catheterization and irrigation. Seitz M. et al stated no patient had significant bleeding in diode PVP. Our study is comparable to these results. Reich O. et al reported rate of 7.1% of blood transfusion in TURP in 10,654 patients, while in our study it is 35 %. This high rate may be due to small sample study. The amount of irrigation needed for laser group is much less (mean 10.10 pints) than TURP group (33.15 pints) and this is because less encountered bleeding in laser group. Mithani MH. et al had mean of 16.44 pints for laser group. In our study, the operative time of laser group is longer than that of TURP by about 20 minutes. Razzaghi MR also reported higher operative time of laser because the time it takes for the light energy absorption by water and Hb. In our study, the mean hospital stay for laser and TURP groups was 16.03 and 30.09 hours respectively. This less stay for laser can be attributed to less bleeding and uncomplicated procedure. Tugcu V. et al concluded also less hospital stay. In our study, the urethral catheter was removed after 24 hours for laser group, while in TURP the mean time to remove the catheter was 81.71 hours. This is an advantage. Cetinkaya et al reported 1.45 day for laser and 2.63 day for TURP which is comparable to our results. The overall complication rate of laser and TURP groups in our study was 75% and 65% respectively.

Conclusion

Diode laser vaporization of prostate is a good alternative to TURP with comparable results in treatment of BPH especially in patients taking anticoagulants who cannot stop their medication and this advantage make it better than TURP. However, the high cost of this procedure and dysuria as a complication may reduce the common use of this technique.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to Declare.

Ethical Clearance: All experimental protocols were approved under the Al-Diwaniyah teaching hospital/department of urology/Al-Diwaniyah province, Iraq and all experiments were carried out in accordance with approved guidelines.

References

10. Andersson KE, Yoshida M. Antimuscarinics and the overactive detrusor—which is the main


Tympanometric Findings among Adults with Chronic Nasal Obstruction Due to Sinonasal Pathology

Salim Hussain Ibrahim

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Abstract

Background: The nasal obstruction is one of the causes that affect the middle ear function, but the physiological mechanism of this effect is not well understood. This study aims at 1st; evaluation of the middle ear function among adults with chronic nasal obstruction due to sinonasal pathology using tympanometry, and 2nd, at comparison between the anatomical and mucosal inflammatory causes of nasal obstruction and its impact on the middle ear function.

Patients and methods: A prospective case study of (310) adult patients with chronic nasal obstruction were subjected to tympanometry for study of middle ear pressure in the private ENT clinic for a period of two years in the Salah Al-Deen governorate.

Results: this study found that (13%) of adults with chronic nasal obstruction had abnormal tympanogram (type B or C), (17.2%) was due to mucosal inflammation and (7%) due to anatomical and structural cause. Commonly one ear affected (unilateral) was (67.5%). Myringotomy and aspiration of middle ear found that (64%) had OME and VT was inserted.

Conclusion: The study statistically proved that adults with chronic nasal obstruction due to mucosal inflammation differs from others due to anatomical and structural causes in the followings; 1. The incidence of abnormal tympanogram (type B and C) is more. 2. The type B (OME) was more than type C (Eustachian tube dysfunction). 3. In the treatment, it was found that the need to VT insertion was more.

Keywords: Tympanometry, Chronic nasal obstruction, Otitis media with effusion, Nasal polyposis, Chronic rhinosinusitis.

Introduction

One of the major ENT complaints is chronic nasal obstruction. A definite relation between nasal obstruction and middle ear diseases, where Eustachian tube dysfunction (ETD) caused by nasal obstruction, that may cause middle ear hypoventilation resulting in otitis media with effusion (OME), that may be definitely diagnosed through use of otoscopic findings associated with a B tympanogram (1,2,3). Some previous researches have been carried out on how the nasal obstruction that influence middle ear function, but the physiologic impact of this effect is not well defined (4,5,6). The evaluation of the middle ear function with a high rate of success in the management and follow up of middle ear diseases can be carried out by Tympanometry, because of its compliance, flexibility of the ear drum to changing air pressure 7. This study aims at 1st; evaluation of the middle ear function among adults with chronic nasal obstruction due to sinonasal pathology using tympanometry, and 2nd, at comparison between the anatomical and mucosal inflammatory causes of nasal obstruction and its impact on the middle ear function.

Patients and Method

A prospective case study of 310 adult patients with chronic nasal obstruction (>3 months) were subjected to tympanometry (620 ears) for study of middle ear pressure in a private ENT clinic during a period from 1st January 2017– 1st January 2019, in the salah El-den
governorate. Iraq. Male were 184 (59%) and female were 126 (41%), age ranging from 18–63 years (mean age 31 years). The diagnosis of nasal pathology was based on detailed history taken, proper ENT examination includes nasal endoscopy and investigation includes computed tomography of the nose and paranasal sinuses. Ear examination includes otoscopic examination, Tuning fork tests. Tympanometry, PTA when abnormal tympanometry. Exclusion criteria includes nasoapharyngeal mass or tumor, recent history of acute rhinitis or middle ear infection, chronic suppurative otitis media, previous nasal or ear surgery, tympanosclerosis, no case with nasal malignancy. Patients were classified according to the causes of nasal obstruction in to;

1. Patients with nasal obstruction due to anatomical and structural causes includes Septal deviation, hypertrophied turbinates, concha bullosa, nasal valve collapse, synchia, choanal atresia.

2. Patients with nasal obstruction due to Mucosal inflammation, such as allergic rhinitis with or without nasal polyposis (NP), non allergic rhinitis, and chronic rhinosinusitis (CRS).

Tympanometry was carried out using viola middle ear analyzers from inventis audiology equipment / Italy to conduct automatic tympanometry tests at low frequency (226Hz)

The tympanometer probe was inserted into the clean ear canal. The types of tympanogram are classified according to Jerger/Fiellau-Nikolajsen in to the following;

1. Type A is a normal peaked curve with pressure between +50 and -99 daPa, Compliance from 0.3-1.5 ml. Suggests normal middle ear functioning

2. Type B is a flat curve without peak, Compliance less than 0.3 ml suggests middle ear effusion.

3. Type C is a peak curve with negative pressure (-100 or more negative), compliance from (0.3-1.5 ml). Suggest Eustachian tube dysfunction.

Treatment involves surgical correction of nasal obstruction according to the causes ,and myringotomy procedure was carried out for the ear with type B and type C tympanogram , when there is fluid aspirated and the ventilation tube (VT) is inserted.

Results

From total 310 patients with chronic nasal obstruction 40 patients (13%) had abnormal tympanogram (type B or C). (Table 1). An abnormal tympanogram was found in patients with chronic nasal obstruction due to the mucosal inflammation was 31/180 patients (17.2%), and in patients due to anatomical causes was 9/130 patients (7%),Which is statistically significant association p-Value 0.018. (Table 1). Patients complaining of nasal obstruction due to mucosal inflammation the type B/C tympanogram were as follows, in allergic and non-allergic rhinitis was 7/57 patients (10.5%), nasal polyposis (NP) was 17 /94 patients (18%), and in chronic rhinosinusitis (CRS) was 7/29 patients (24%), anatomical and structural causes 9/130 (7%) as shown in (Table 2). Patients who had an abnormal tympanogram in one ear (unilateral) were 27/40 patients (67.5%) and in both ears (Bilateral) were 13/40 patients (32.5%) (Table 2). Regarding the type of tympanogram, the normal type A was in 567/620 ear (91.5%), type B was 25/620 ear (4%),and type C was 28/620 (4.5%), this statistically very strong association p-value =0.005 (Table 3).

Patients who underwent surgical treatment were 31/40 patients (77.5%) (9 patients refused to carry out the operation or were missing). Total number of ears who have abnormal tympanogram was 39 ear ( 8 patients were in both ears and 23/patients in one ear). Those patients have undergone myringotomy and aspiration of middle ear and found that 25/39 ear (64 %) fluid was aspirated (OME) so that a VT was inserted. Shah VT was inserted in 23/39 patients (59%) and Good T-tube in 2/39 patients (5%) who had adhesive otitis media, this results was statistically significant association P-value =0.05 (Table 5) The study found that the Shah VT was inserted in 23/33 ear (70%) of patients with chronic nasal obstruction due to mucosal inflammation ,and in 2/6 ear (33%) of patients with anatomical causes. (Table 4) The research found that 24/40 (60%) of Patients who had abnormal tympanogram were complaining from hearing loss or fullness of ears and tinnitus and (40%) of patients deny the presence of such ear symptoms.
Table 1: Relation between causes of nasal obstruction and abnormal tympanogram.

<table>
<thead>
<tr>
<th>Pathological causes of nasal obstruction</th>
<th>Patients No</th>
<th>Abnormal tympanogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical and structural causes</td>
<td>130 (42%)</td>
<td>9 (7%)</td>
</tr>
<tr>
<td>Mucosal inflammation</td>
<td>180 (58%)</td>
<td>31 (17.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>310 (100%)</td>
<td>40 (13%)</td>
</tr>
</tbody>
</table>

$X^2=5.58$, P.value=0.018, d.f.=1

Table 2: The relation of sinonasal pathology and abnormal tympanogram.

<table>
<thead>
<tr>
<th>Sino-nasal pathology</th>
<th>Patients No (%)</th>
<th>Abnormal tympanogram</th>
<th>Total</th>
<th>Unilateral</th>
<th>bilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical and structural causes*</td>
<td>130 (42%)</td>
<td>9 (7%)</td>
<td>7 (78%)</td>
<td>2 (22%)</td>
<td></td>
</tr>
<tr>
<td>Nasal polyposis**</td>
<td>94 (30.3%)</td>
<td>17 (18%)</td>
<td>11 (62%)</td>
<td>6 (38%)</td>
<td></td>
</tr>
<tr>
<td>Allergic and non allergic rhinitis</td>
<td>57 (18.4%)</td>
<td>7 (10.5%)</td>
<td>5 (71%)</td>
<td>2 (29%)</td>
<td></td>
</tr>
<tr>
<td>Chronic rhinosinusitis</td>
<td>29 (9 %)</td>
<td>7 (24%)</td>
<td>4 (62.5%)</td>
<td>3 (37.5%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>310(100%)</td>
<td>40 (13%)</td>
<td>27 (67.5%)</td>
<td>13 (32.5%)</td>
<td></td>
</tr>
</tbody>
</table>

* Includes ;Septal deviation ,hypertrophied turbinates, concha bullosa,nasal valve collapse, Congenital choanal atresia.

**Includes ;Antrochoanal polyp.

Table 3: The relation between causes of nasal obstruction and type of tympanogram.

<table>
<thead>
<tr>
<th>Pathological causes of nasal obstruction</th>
<th>Ear No</th>
<th>Type of tympanogram</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type A</td>
</tr>
<tr>
<td>Anatomical and structural causes</td>
<td>260</td>
<td>249 (95.8%)</td>
</tr>
<tr>
<td>Mucosal inflammation.</td>
<td>360</td>
<td>318 (88%)</td>
</tr>
<tr>
<td>Total</td>
<td>620</td>
<td>567 (91.5%)</td>
</tr>
</tbody>
</table>

$X^2=10.77$, P-value=0.005, d.f.=2
Table 4: Relation between the causes of nasal obstruction and surgical procedure on the ear.

<table>
<thead>
<tr>
<th>Causes of nasal obstruction</th>
<th>Surgical procedure</th>
<th>Grommet insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>patients</td>
<td>Myringotomy only</td>
</tr>
<tr>
<td>Anatomical &amp; structural causes</td>
<td>5</td>
<td>4 (67%)</td>
</tr>
<tr>
<td>Mucosal inflammation</td>
<td>26</td>
<td>10 (30%)</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>14 (36%)</td>
</tr>
</tbody>
</table>

X²=2.9, P-value=0.05, d.f.=1

Discussion

The incidence and severity of middle ear diseases has been found to be related to the cause of nasal obstruction, whether anatomical and structural causes or mucosal pathologies. There are several suggested theories about the effect of nasal obstruction on the Eustachian tube, one theory suggest that nasal obstruction leads to turbulence of air flow in the nasopharynx. The turbulent air flow may leads to Eustachian tube dysfunction (ETD) by the following supposed mechanisms, 1. Dryness of mucosal film leads to increased viscosity of the mucous and the surface tension at the tubal orifice interfering the opening of the Eustachian tube. 2. The air pollutant and microorganisms deposited in the (ET) orifice causing tubal or pre tubal inflammation and obstruction. 3. Stimulation of the mechanoreceptors, leading to reflex alteration of (ET) function. Many studies have been conducted to assess the correction of nasal obstruction due anatomical causes and the study of its impact on the middle ear dysfunction. Nanda et al, & Osama G. et al have found that septoplasty improve hearing and middle ear function by improving ET function, but Şahin, et al found that no effect of septoplasty on middle ear pressure and ET function. The whole mucosa of the upper respiratory tract, including the nasal cavity, sinuses, nasopharynx, eustachian tube, and middle ear, which belong to a system called the rhino-pharyngotubal unit or the “unified airspace”. Therefore, the presence of inflammatory disease that affects the nasal and middle ear mucosa is considered the more likely physiologic mechanism that leads to middle ear diseases.

The mucosal inflammation in Allergic rhinitis, NP and CRS contribute to congestion, including increased venous engorgement, nasal secretions from nasal glands and tissue swelling (edema), the accumulated secretion and edema block the eustachian tube. This can reduced middle ear ventilation leading to negative middle ear pressure. There is a significant association between OME and persistent symptoms of allergic rhinitis, these results suggest a direct involvement of the middle ear mucosa. Parietti-Winkler et al reported an association between OME and nasal polyposis. They concluded that the mechanism causes of OME in NP and CRS is more likely the presence of inflammatory disease that affects the nasal and middle ear mucosa, and less likely the obstruction of the ET by polyps or by congestion of the nasopharyngeal mucosa. There is a study concluded that the OME seem to be an inflammatory disease that extends to the middle ear rather than a consequence of the nasal disease itself. Because even when the symptoms of nasal obstruction are well controlled, the OME can develop or persist.

The current study found that patients with CRS and NP are at greater risk of developing abnormal tympanogram (24%) and (18%) respectively (Table 2). as found by other authors Rennie et al, study on Eustachian tube dysfunction found that 69% were due to CRS with NP, 28% CRS without NP, and 14% due to allergic rhinitis. In CRS the mucopurulent or purulent Secretions pass over the pharyngeal end of Eustachian tube and it can lead to inflammation of ET and hypertrophy of tubal tonsil results in ET obstruction. Patel et al (2016) found that symptoms of ET dysfunction are frequent in CRS, and respond well to
endoscopic sinus surgery. The current study found that the VT was indicated in 70% of patients with sinonasal mucosal inflammation because of glue like aspirate of the middle ear (OME). While in the anatomical causes VT was indicated in (33%). This results goes with Mary, et al who found that by 4, where they found that patients with severe CRS with NP are at greater risk of developing OME, and often highly viscous middle ear effusion.

Limitation in this study that combined pathology may be found commonly septal deviation and/or hypertrophied turbinates with allergic rhinitis, nasal polyposis or chronic rhinosinusitis. In this study they were classified with mucosal inflammation causes. This study statistically proved that adults with chronic nasal obstruction due to mucosal inflammation differs from patients with nasal obstruction due to anatomical and structural causes in the followings; 1.The incidence of abnormal tympanogram (type B and C) is more. 2.The type B (OME) was more than type C (Eustachian tube dysfunction). 3. In the treatment, it was found that the need to VT insertion was more. This study has the following recommendations recommendation: It is preferable to do tympanometry for patients with chronic nasal obstruction ,especially when it is due to NP and CRS, even if they not complain of hearing loss, tinnitus or fullness in the ear.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Tikrit Medical College, Iraq and all experiments were carried out in accordance with approved guidelines.

References

The impact of Mindfulness Education on Elementary School Students

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¹Asst. prof. Dr. Ibtisam Al-Zaywaini, ²University of Babylon / College of Basic Education, Iraq

Abstract

The current research aims at evaluating the reading for the fourth grade of primary school in the light of the habits of mind and followed the researcher descriptive approach to suitability the current research procedures to achieve the research objective, the researcher prepared a list that included the criteria of habits of mind based on this researcher prepared a questionnaire (6) standards and (141) paragraphs after verifying the authenticity of the tool from its presentation to a group of arbitrators the stability of the analysis using Holistic equation (0.83) The tool consists of (128) paragraphs distributed in six areas The researcher has developed three alternatives (valid), (invalid), (need to be modified), and the researcher used the appropriate statistical means for their research. The data obtained from the analysis process are: Holistic equation, centimeter weight, square cay, and weighted mean.

Keywords: Evaluation, Arabic Reading Book, Habits of Mind

Introduction

The students are the real wealth of the nation and society must be prepared for life, including the development of writing and reading skills, the maximum extension of the hand of reform is the book must be a systematic evaluation of the curriculum to learn the extent to which the goals set for them ¹ Reading is very important in the elementary stage as a natural entry point for learning and primary school fails miserably if it fails to teach its students to read because the student’s success and progress in all subjects depend on his literacy ability is not material with content that can be taught separately from other subjects But is a fundamental part of every other material. It has become clear that there is a need for something new if schools are to start from a traditional mindset that is focused on capacity only so that students can have a kind of mind-set to create a productive self-fulfilling life and therefore the need for effective and effective mental habits such as the need to develop educational goals that reflect The belief that ability is a repertoire of skills that the individual stores and continues to be constantly expanding as one can increase the skill of thinking through man’s efforts. Education in its general form is a familiar process for all reflect an activity produced by human societies across many times and places, It is not only a reflection of social, economic and political life, but it is an important tool in the development and formation of this life, including from different social systems the prosperity of nations and the attainment of their goals are linked to their concerns. These nations raise and nurture a strong generation to take responsibility for the leadership of the nations and hand them over from fathers to sons, therefore, attention to education means providing the right direction and congratulating the environment for the emergence of righteous individuals. Language has a great role in the twenty-first century because it is a new world in which information prevails in all spheres of life as the individual is able to get the information he needs as quickly as possible in the least effort and language is the basic goal through which he knows what he wants ¹. One of these languages is the Arabic language that every speaker has the right to be proud of and is magnified by our Arabic language, which is characterized by the beauty of its methods and the strength of its expression and its vast wealth of words and meanings. reading is the means of the numbers of the individual scientifically and morally is a means of personal harmony and social assistance to the individual to autonomy and adaptation with others and reading helps to quickly understand and gain desired attitudes and behaviors. The textbook offers opportunities for the teacher to use many of the teaching methods, especially those that need to have a textbook in the hands of the student when the teacher assigned
a student or several students to read a paragraph of the book to develop information or a comparison with external information is the basic premise in front of students to the world of research, knowledge, thinking and knowledge.

**Methodology**

The researcher followed descriptive research methodology to analyze the book of Arabic reading for the fourth grade of primary school because it means describing the facts to identify the data related to a particular educational phenomenon and follow closely and analyze and interpret and investigate the relationships and connotations and then access to the explanatory and complementary instructions can be used. The researcher relied on the analytical descriptive approach because it is a method that is compatible with the nature of the research and its requirements. The descriptive approach is a method for studying scientific phenomena or problems through scientific description and then reaching logical explanations with signs and bar Yen and that research is descriptive of a wider use of research in educational and psychological field it is usually looking at the present and diagnose existing phenomena and the relationships with the discovery by classifying them and their works on the interpretation of those phenomena. 

**Identify the research community and its sample:**

The research community is identified and identified by the researcher to define their research community as well as to define their own identity so that the current research community is determined the fourth grade reading book in the Republic of Iraq, the tenth edition 1437/2018. Karim Obaid Al Waeli and Dr. Abdul Abas Abdul-Jassim and Turki Abdul Ghafoor Al-Rawi, the book consisted of (147) page excelled by the researcher’s title book title, the exclusion of these parts of the content is familiar to previous studies and does not affect the analysis procedures directed towards the content related to the objectives of the book and its subjects, which is the subject of physical and moral construction of the student and has the ability to achieve educational experiences, information and facts Concepts, values and trends. Here is a description of this book:

A table showing the content of the reading book, the number of pages, their sequence and percentages

<table>
<thead>
<tr>
<th>No.</th>
<th>Contents</th>
<th>Pages no.</th>
<th>Its no.</th>
<th>No. of excluded pages</th>
<th>The remain no.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Title and author page</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>Scientific supervisor page</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0.680</td>
</tr>
<tr>
<td>3</td>
<td>Introduction</td>
<td>3-4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1.360</td>
</tr>
<tr>
<td>4</td>
<td>Books' subjects</td>
<td>5-145</td>
<td>140</td>
<td>0</td>
<td>140</td>
<td>97.27</td>
</tr>
<tr>
<td>5</td>
<td>Book contents</td>
<td>146-147</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1.136</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>1-147</strong></td>
<td><strong>147</strong></td>
<td><strong>7</strong></td>
<td><strong>140</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

**The Research Tool:**

As regards to lack of a ready and appropriate tool to gather information commensurate with the purposes and objectives of the research, the researcher adopted the questionnaire as a tool for research and to prepare this tool, the researcher followed the following steps:

- **List habits of reason:**

In order to reach the list of habits of mind and use that list in the analysis of the book of reading for the fourth grade of primary The researcher adopted the following steps:
1 - Review previous studies on the habits of reason

2 - Access to literature and books that dealt with the habits of mind

3 - The preparation of the preliminary list of the habits of reason based on the researcher’s knowledge of past efforts

4 - Directing an open survey questionnaire for supervisors and teachers of the Arabic language in primary schools, including the following questions:
   - What habits should be read in the fourth grade reading books? Please specify in terms of relevance to the primary stage
   - Do you think that the book of Arabic reading meets the habits of mind appropriate for the primary stage?

5. Distribution of the preliminary list of the habits of the mind to a group of specialists in the Arabic language to analyze them and determine whether the principles and their sub-indicators belong to the domains received and have been compiled and studied and used to reach the final list.

The tool’s credibility:

Honesty is one of the important characteristics of educational tests and standards psychological and different and is one of the old concepts so it is subjected to a lot of modification and change as a result of the spread and the expansion of the movement of tests and standards in the various humanities and the truth of several types of the researcher has used what fits the nature of her research which is the apparent truthfulness of the initial examination of the content of the instrument and is reached by the consensus of the arbitrators’ assessment of the measure of the instrument to which it was prepared and to achieve the credibility of the tool based on the credibility of arbitrators and specialists in Arabic language and literature and curricula and methods of teaching as well as those who teach Arabic reading for the fourth grade of primary school. The researcher presented the tool in its initial form in order to ascertain the apparent honesty. The arbitrators expressed their observations about the tool and took the researcher. The researcher has adopted (80%) of the opinion of the arbitrators on the validity of the paragraphs, this is indicated by Bloom, as 80% and more of the arbitrators are evidence of an investigation virtual honesty.

The Analysis:

A – The analysis unit: The researcher used the unit of thought in its analysis of the book of Arabic reading, if it is one of the most important units of analysis and the largest and may be the unity of the idea or phrase contains the idea that revolves around the subject of analysis.

B- The analysis steps:

1- Reading each topic of the Arabic reading book for the fourth grade of the primary to identify the ideas contained in the subject in general

2 - Second reading of each line in the subject for the purpose of reaching the phrase or sentence that contains a particular idea

3 - The identification of the phrase containing a specific idea added by the researcher to the principles prepared in advance and work to determine the suitability of this idea with each paragraph of this principle in a special analysis form

4 - Give a repeat of each paragraph correspond to the idea extracted

5 - Calculate the frequencies obtained by each paragraph in one principle and then calculate the frequencies of each principle individually.

The stability analysis:

While the method of content analysis is a scientific tool, there is a need to satisfy the conditions of objectivity, and achieving objectivity requires knowledge of the stability of the analysis. In terms of consistency, the results obtained from applying the analysis tool to the same sample are not changed and one of the most prominent methods of verification of the stability of the most common analysis of the presentation of a sample of the analyzed article on a group of experts and specialists and the people of know-how and experience in the analysis of content.

So the researcher used two experts who have knowledge in the analysis of the content given each of them a random sample and 10% of the analyzed material from the topics of the book of reading and the researcher calculated the correlation between them and the two experts was the correlation coefficient between them and
the first analyst (92.0) And between them and the other analyst (85.0%)

The statistical means:
The researcher used the program (spss) statistical means to analyze the results as follows:
1 - The weighted average to measure the extent to which each concept of the questionnaire
2 - Square Kai to calculate the honesty of building concepts
3 - Holistic coefficient to calculate the stability of concepts
4 - Percent weight to be used in interpreting the results.

Results
The researcher reached the following results: Taking into account the book of Arabic reading of some habits of the mind, which means taking into account individual differences between students. The habits of the mind give a wide range in the educational process to help the teacher to understand the minds of students and enhance their role in providing opportunities for students to learn and think in different ways. The planning and preparation of lessons through the habits of the mind left a great impact on the students, which led to an understanding of the students to read and assimilate well.

Conclusion
The researcher has developed three alternatives (valid), (invalid), (need to be modified), and the researcher used the appropriate statistical means for their research. The data obtained from the analysis process are: Holistic equation, centimeter weight, square cay, and weighted mean.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon / college of Basic Education, Iraq and all experiments were carried out in accordance with approved guidelines.

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Prevalence of Bronchiolitis among Hospitalized Children less than Two Years in Babylon Province

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² Assistant Professor, Ph.D. in child and adolescent health nursing, Babylon University, College of Nursing, Hilla City, Iraq

Abstract

Background: Bronchiolitis is one of viral lower respiratory tract infections that constitute a heavy burden to public health in the Pediatric population worldwide, which occurs most frequently before age 2 years in the winter and early spring months and most frequently induced by a viral infection (respiratory syncytial virus).

Settings and Design: A survey descriptive study conducted in Babylon Province through-out the period of “1st January 2019 to 30 March 2019” (three) special hospitals of pediatrics.

Method and Materials: Non-probability “purposive” sample consists of 3374 subjects, which collected through a review of patient records for (2016, 2017, 2018) in the statistical units in Babylon hospitals. The information of data was collected retrospectively through-out the special questionnaire obtained from the patient’s sheets in the statistical unit.

Statistical analysis used: The data are coded and tested by the application (SPSS) through the descriptive and inferential analysis.

Results: The analysis of the study indicated that the majority of the children (62.7%) were aged (1-6) months old who lived in rural areas, the male percentage was higher than the female. In addition, the study indicated that the prevalence of bronchiolitis progressed during the last three years.

Conclusions: The study concludes that the higher morbidity of bronchiolitis was in 2018, most of them admitted to the hospital in January months. Also, the duration of hospital stay was > 4 days.

Key-words: Epidemiology, Prevalence, Bronchiolitis, children.

Introduction

Respiratory disorder is considered the most frequent reason for hospitalization and illness in children¹. Bronchiolitis is the most relevant infection of the LRTI during infancy through the 1 year of life. “Respiratory syncytial virus” (RSV) is the main cause of this condition as it is considered a major leading cause of increasing the rate of morbidity and hospitalized patients number and cost³. As long as, the epidemiology of RSV in developing countries has identified that the most common viral cause of LRI globally⁴, bronchiolitis constitutes a heavy burden to public health in the Pediatric population worldwide, particularly in the United States, which causes significant morbidity and mortality in infants and young children⁵. Bronchiolitis mortality rate is roughly 2 per 100,000 infants and is higher in developing than in developed countries, and statistical data showed that globally there are 150 million new cases of bronchiolitis annually⁶. Also, it has been reported that hospitalization of children during seasonal Respiratory syncytial virus epidemics about 2-3% of all infants less than 1 year⁷. In the United States, there is significant season-to-season variation in RSV with the period of the peak prevalence varying by as much as 7 weeks (ranging from early January to late February) between seasons⁸. In Egypt, during the period between “October 2016 and March
found that most of the children 53.3% were diagnosed with bronchiolitis and 31.7% were diagnosed with pneumonia. The median duration of the hospital stay four days. Prophylaxis is considered the most effective and safe method that is used in the prevention of respiratory syncytial virus (RSV) disease in infants.

Methodology

The study aims to determining the prevalence of bronchiolitis among hospitalized children less than two years

Study design: A cross-sectional descriptive study that conducted through the period of 1 January 2019 to 30 March 2019.

Setting study: The study conducted in in Babylon province at “Pediatric Teaching hospitals”.

Sample of study: A total of 3,374 is selected by a convenience non-probability sample through a review of statistical records which were selected out from the main hospitals in Babylon province.

Study instrument: The information of data was collected retrospectively throughout the special questionnaire obtained through a review of patient records for (2016, 2017, 2018) in the statistical units in Babylon hospitals.

Data Analysis: The data are coded and tested by the application “statistical package of social science (SPSS)(Version 24)”. A descriptive analysis includes frequencies and percentage; inferential data analysis included the correlation coefficient, ANOVA test.

Results

Figure (1) the prevalence of bronchiolitis disease in Babylon province for the last three years (2016-2017-2018).

Table (1) Distribution of Bronchiolitis prevalence data from statistical records in the hospital.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-6</td>
<td>2,116</td>
<td>62.7</td>
</tr>
<tr>
<td>7-12</td>
<td>770</td>
<td>22.8</td>
</tr>
<tr>
<td>13-18</td>
<td>313</td>
<td>9.3</td>
</tr>
<tr>
<td>19-24</td>
<td>175</td>
<td>5.2</td>
</tr>
<tr>
<td>Total</td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2,051</td>
<td>60.8</td>
</tr>
<tr>
<td>Female</td>
<td>1,323</td>
<td>39.2</td>
</tr>
<tr>
<td>Total</td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Residency</strong></td>
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<td></td>
</tr>
<tr>
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<td>1,420</td>
<td>42.1</td>
</tr>
<tr>
<td>Rural</td>
<td>1,954</td>
<td>57.9</td>
</tr>
<tr>
<td>Total</td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Duration of hospitalization stay</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>less than 4 days</td>
<td>2,581</td>
<td>76.5</td>
</tr>
<tr>
<td>≥ 4 days</td>
<td>793</td>
<td>23.5</td>
</tr>
<tr>
<td>Total</td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Month of admission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>2,170</td>
<td>64.3</td>
</tr>
<tr>
<td>February</td>
<td>783</td>
<td>23.2</td>
</tr>
<tr>
<td>March</td>
<td>421</td>
<td>12.5</td>
</tr>
<tr>
<td>Total</td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Outcome of discharge</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>2,182</td>
<td>64.7%</td>
</tr>
<tr>
<td>Un recover or chronic</td>
<td>542</td>
<td>13.4%</td>
</tr>
<tr>
<td>Dead, un known</td>
<td>740</td>
<td>21.9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Years of admission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>1,442</td>
<td>42.7</td>
</tr>
<tr>
<td>2017</td>
<td>1,176</td>
<td>34.9</td>
</tr>
<tr>
<td>2016</td>
<td>756</td>
<td>22.4</td>
</tr>
<tr>
<td>Total</td>
<td>3,374</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (1) reveals that the majority of children were male in the past three years which signified as (60.8%) with (62.7 %) of babies aged less than 6 months; most of
them admitted in January and recovered within a short period of hospitalization stay (less than 4 days). The highest percent 57.9% were lived in the rural area.

The findings in the table above also reported that the prevalence of bronchiolitis was raised in the last three years, particularly in 2018.

**Discussion**

**Prevalence of bronchiolitis disease for the last three years (2016-2017-2018)**

The findings in the figure (1) reveals that the higher morbidity of bronchiolitis was in 2018. The researcher indicated in the present study that bronchiolitis was increased gradually in the last three years (2016, 2017, 2018) consequently. This study go along with a retrospective study carried out in Italian about “Comparing of two different epidemic seasons of bronchiolitis”, which done to evaluate the differences of bronchiolitis frequency and characteristics of the bronchiolitis-related hospitalization between two seasons; S1(2015-16), S2(2016-17). The author indicated that the bronchiolitis morbidity rising from 17% in 2015-16 to 26% in 2016-17. Also, it showed an increased rate of hospitalization. Another study conducted at AL-Zarqa Government Hospital, Jordan. It’s goal to analyze the epidemiology of RSV infection on 271 children less than 24 months with bronchiolitis, from January 1997 and May 1999. The results indicated gradual rising with peak incidence were in 1999. Rationally speaking, Iraqi children’s displacement and migration, particularly during the winter season, live in a crowded situation with bad earnings and health facilities that increased the incidence of communicable diseases with missing or dropping out in the immunization follow-up timetable.

**Distribution of Bronchiolitis prevalence data from statistical records**

In related to the prevalence of bronchiolitis as shown in the table (4.6), the study underhand proved that the majority sample was males aged less than six months who lived in a rural area, admitted in January, 76.5% stayed less than four days in the hospital which increased within two years progressed. a prospective study at Al-Bashir Hospital, Jordan, on children’s admission < 24 months. The author mentioned that the majority sample 60% were male, (53%) were < 6 months, with the annual peak during January and February. In reason, low immunity and high incidence of RSV in children less than 6 months during the winter season. Furthermore, the research showed that the duration of hospital stay was 4 days, the length of stay as indices for the severity of bronchiolitis and linked with absence of breastfeeding, reduced age, greater viral load and sepsis. Certain study indicated that the length of hospital stay was 3 days. The period varied in this study from 4 days in 2010 to 3 days in 2015, and the mean length of stay similar to that reported in Spanish studies. In the term of discharge outcome in the present study, more than half 64.7% were well status. The outcome discharge and LOS are dependent on effective management. Evidence study conducted at Meherpur General Hospital, about the RSV outbreak to determine the etiology and explore possible risk factors. Which indicated that no death among analysing cases, (36%) Still sick after 5 days of illness and (39%) recovery within 5 days.

**Conclusion**

The study concludes that predominant age is (1-6) months with a majority to male than female, the higher prevalence of bronchiolitis was in 2018, LOS was less than four days in majority of children, with improved status.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, University of Babylon, Hilla City, Iraq and all experiments were carried out in accordance with approved guidelines.

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Nanotechnology issues included in the subjects of the Department of Biology in the Colleges of Education

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Abstract

This research aimed to: Recognize how much including in content of subjects in the Department of Biology College of Education in accordance with the Nanotechnology Issues. To achieve this aim, the researchers built a standard for the Nanotechnology Issues, were verified validity by presentation to a group of arbitrators specialists in the methods of teaching the Biology, and that standard is finalized be from (8) Nanotechnology Issues and emerged of them (90) sub- Issues, then the researchers analyzed the content of subjects of the Department of Biology in Colleges of Education for the academic year (2018-2019) in the light of this criterion as the number of analyst was (12201) page, and it adopted the explicit idea and implicit idea as units for registration and repetition unit of the census, The results were: weakness of subjects in the Department of Biology in Colleges of Education to contain Nanotechnology Issues compared with the ratio based on the opinions of experts (20%), (16) only of 90 terms were achieved, that was equal of (17.77%)

Keywords: Nanotechnology - Nanotechnology Issues - Understanding Students

Introduction

In the midst of rapid and successive developments in most fields of science and knowledge, university institutions and research centers are undertaking these developments as an important tributary to them and a rich resource for their productions in the invention, discovery or development, and therefore university education adopts those developments learning and education at the very least, and perhaps the most important science that split its path to global scientific interest is nanotechnology because of the science it has enormous applied fields in various sciences, including Biology, and the first to be the outcome of this science is abundant and based on university students, including students of Biology departments in the colleges of to be educators in the future and need to develop their understanding and awareness of scientific knowledge achievements and provide the latest science and cognitive transformations. Technological Despite its many applications, importance, challenges and gravity, it did not receive the required attention from the thought and educational research in Iraq, as it notes the weakness of educational institutions in keeping with the amazing development in this field through its programs and plans

4. Through the information obtained by the researchers from the exploratory questionnaire distributed to a group of teaching specialists in the departments of Biology in the colleges of education and the number (9) include a question on the importance of nanotechnology and the most important issues related to the content of subjects in the departments Biology in the colleges of education, the researchers found that there is a certain desire and urgency of the teachers to address the topics and issues related to nanotechnology because of its global interest in scientific institutions and research centers, and its impact on the intention of the cognitive process for students and their lives, and the above is determined by the following research problem by asking:

Second: The Importance of Research:

Human societies are striving to pay attention to future science in order to face the challenges and developments of the times. Today, our world is witnessing rapid and successive changes as a result of major technological and scientific developments in all fields of life and at all levels. Humanity, and education, and educational practices allow the learner to take advantage of technological innovations in the development of achievement and skills. Among
the technological innovations are nanotechnology which takes care of the study and characterization of nanotechnology materials and their properties at a size less than (100 nm), and precise control of the interaction of molecules and rearrange the atoms and place them in order to produce new materials unprecedented, so that it is possible to design smaller robots from the head of a pin can enter and movement in the blood vessels and be able to perform accurate operations within them and treat blood clots tumors and incurable diseases.  

Given the importance of nanotechnology in education, many conferences have been held, most notably one, Nanotechnology organized by the University of Jordan in Amman from 10-13 / 10/2008, in cooperation with the University of Illinois at Urbana-Shampeen, under the name of “developed nanomaterials” (14) In Iraq initiatives have emerged related to nanoscience and technology in light of the national education strategy and education that started work formulated and organized since (2009) and launched in (2013) for (10) years, and in this regard published the University of Technology (128) research in the field of nano, and the University of Baghdad with (120) research {15} and given all the above interest in technology Nano and its issues in all fields have emerged many studies and research, including the study Ahmed and others (2015) that sought to investigate the impact of an educational program – learning according to the concepts of renewable energy and know its impact on technological enlightenment in the Department of Chemistry, and showed results the program assists students in acquiring knowledge and concepts related to nanoscience and technology and in technological development.  

Nano materials:

Hijazi defines nanomaterials as “very small materials that are prepared in the laboratory or already present in nature and whose measurements of lengths or diameters of granules range from 0.1 to 100 nanometers.”  

Forms of nanomaterials: nanomaterials are prepared in various forms including:

The Fullerene: nanoparticles composed of triple bonded carbon atoms, which give the shape of pellets that have a structure similar to graphite, but instead of containing pure hexagonal form, they contain pentagonal forms and potentially sevenfold carbon atoms.  

Thin Films: a thin layer of a specific material, less than 100 nanometers thick, and their length and width may be in micrometers. These thin layers are used in the field of semiconductors such as silicon and gold bars.  

Nanofibres

Perhaps the most famous nanofibers are those made of polymer atoms because the ratio of surface area to volume is large in the case of nanofibers, and this gains those fibers characteristic mechanical properties such as rigidity and tensile strength and others, which can be used in biomedicine, and in organ transplantation such as joints, and transport of medicines.  

Nanoballs:

One of the most important carbon nanoparticles, which ends in the class of Foloreinat, of the material C60, but they differ slightly from the composition as they are multiple crust.  

Second: Field Applications in Pharmacy:

Nanotechnology is one of the most important scientific fields that scientists rely on in the development of drug mutations that change the concepts of treatment and medication for many diseases, and perhaps the most important applications of nanotechnology in pharmacy:

1- Nano-biotics: compounds that have the ability to identify bacteria and viruses, and then eliminate them without exposure to any other cell

2 - delivery of the drug to tissues: It depends on the manufacture of nanomaterials that work to improve the bioavailability of the drug (Bioavailability) This means the presence of drug molecules in the target place of the body  

Nanotechnology can offer drug delivery solutions in the following areas:

a- Drug Coating: Using pharmaceutical packaging materials such as liposomes and polymers (such as polylactide –PLA, and lactide combined with PLGA glycolide)

b- Drug carriers: Another type of drug delivery system, nanotechnology offers important, manageable solutions to link with the drug, the target and the imaging material.

Research Approach
The researchers adopted the descriptive analytical approach for the analysis of subjects in the Department of Biology in the Colleges of Education.

**Search procedures**

First: Research Community (Community of Subjects)

The community of subjects covered represents all subjects for the four stages of the Biology department in the Colleges of education.

Second: Research sample (sample content of subjects): The analysis process included the content of subjects in the Department of Biology in the College of Education, as the number of analyzed subjects (15) of the 35 articles, or 43% of the total community. The total analyzed pages are (12201) pages, (1689) pages of the first stage materials, (2291) pages of the second stage materials, (4367) pages of the third stage materials and (3854) pages of the fourth stage materials. The scientific, drawings, tables and charts after the indexes and glossary of terms for the analyzed materials were excluded.

**Analysis of the content of the subjects of the Department of Biology**

The researchers relied on the explicit idea and the implicit idea as the two recording units because the subjects in the Department of Biology are scientific materials and expressions are often clear and explicit, but their use of the idea implicit, because some topics can suggest ideas that reflect the issues or sub-issues of the standard nanotechnology.

**Validity of the analysis**

To ensure the validity of the analysis conducted by the researchers were hired two experts * in the methods of teaching Biology and presented to them three subjects (cytology, biology, algae) with the standard of nanotechnology issues that were built and ideas extracted from the content of analyzes to make sure The analysis was valid, and they unanimously agreed on the validity of the analysis, which was prepared by the researchers in truth to the analysis he conducted.

**Stability of analysis**

In order to be objective analysis and to obtain acceptable stability, the researchers used two types of stability:

1- Agreement over time

To calculate the stability coefficient in this way, the researchers re-analysis after thirty days as the value of the coefficient of stability calculated (0.98) using the Holste equation, a very high value and reflect a high degree of stability.

2- Agreement between analysts

The researchers used external analysts to analyze the content *. This was done by selecting a random sample of the total analyzed content of (12201) pages. The proportion of the stability sample represented about (20%) (2790 pages), which included books (cell biology, biology, algae) and using the equation (Holsti) was extracted the stability coefficients calculated in this way and was equal to (0.82) For the researcher with the first analyst and 0.80) For the researcher with the second analyst (0.83) between the first and second analyst, and thus the coefficient of stability is good, and some studies have indicated that the acceptable stability ranges between (0.50 or 0, 60) as in Table 1

**Table (1) values of stability factors**

<table>
<thead>
<tr>
<th>Agreement through time</th>
<th>Researchers after 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>Agreement between analysts</td>
<td></td>
</tr>
<tr>
<td>Between the researchers and the first analyst</td>
<td>0.82</td>
</tr>
<tr>
<td>Between the researchers and the second analyst</td>
<td>0.80</td>
</tr>
<tr>
<td>Between the first and second analyst</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Determine the spoken ratio to compare the results of the analysis

Depend that the percentage (20%) to be a hypothetical ratio to compare the results of the analysis based on the agreement of the arbitrators and experts on this ratio, the researchers presented a questionnaire to find out the ratio (2). The agreement was (80%) of the experts and arbitrators on this ratio.

The results will be presented according to the research objective and discussed as follows:
Identify the extent to which the content of subjects is included in the Department of Biology, College of Education for Nanotechnology Issues.

After the subjects were subjected to the four stages, the pages subjected to analysis included (12201) pages and the results were as shown in Table (2).

**Table (2) Frequencies and Percentages of Nanotechnology Issues in the Subjects of Biology Departments**

<table>
<thead>
<tr>
<th>No</th>
<th>Subjects</th>
<th>Total iterations of major and minor issues</th>
<th>Percentage of Origin (16) element of a case Sub- extracted</th>
<th>Order</th>
<th>percentage For the subject Realized% of Origin (90) element Issue by standard</th>
<th>percentage Realized from all the subjects Tuition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Materials of the first stage</td>
<td>4</td>
<td>25%</td>
<td>second</td>
<td>4.44%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Materials of the second stage</td>
<td>1</td>
<td>6.25%</td>
<td>Third</td>
<td>1.11%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Materials of the third stage</td>
<td>11</td>
<td>68.75%</td>
<td>First</td>
<td>12.22%</td>
<td>17.77%</td>
</tr>
<tr>
<td>4</td>
<td>Materials of the fourth stage</td>
<td>0</td>
<td>0%</td>
<td>Fourth</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16</td>
<td>100%</td>
<td></td>
<td>17.77%</td>
<td></td>
</tr>
</tbody>
</table>

From the data recorded in Table (5) it is clear that the subjects in the third stage were the most interested in the contents and issues of nanotechnology, where it obtained 68.75% of the percentage out of (16) elements of the sub-issue extracted, followed by the first stage by 25% and the second by 6.25% and then the fourth by (0%) and the researchers attribute this to the algae materials and practical environment and educational laboratory. In the third stage, it received the most attention, followed by the first stage, which was the theoretical and practical biology and theoretical cell which focused on the issues of nanotechnology and microscopes. Then the second stage materials represented theoretical histology material while the fourth stage materials were weaker and poorest as it did not get any repetition despite the importance of the fourth stage materials in the formation of specialization for students of the Department of Biology in the colleges of education and this is a take on the materials of that stage, which was expected to the strongest of the four stages because the first stage represents the beginning of specialization and the second and third stages represent a transitional stage to the end of the specialization of the fourth stage.

As for the issues, the diversity of interest in the main and sub-issues and their components, as in Table (3).

**Table 3: Frequencies and percentages of paragraphs achieved from the Nanotechnology Standard**

<table>
<thead>
<tr>
<th>No</th>
<th>The main issues</th>
<th>Repeat paragraphs</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introductory issues of nanotechnology</td>
<td>11</td>
<td>68.75%</td>
</tr>
<tr>
<td>2</td>
<td>Agricultural issues</td>
<td>1</td>
<td>6.25%</td>
</tr>
<tr>
<td>3</td>
<td>Environmental issues</td>
<td>2</td>
<td>12.5%</td>
</tr>
<tr>
<td>4</td>
<td>Issues related to the dangers of nanotechnology</td>
<td>1</td>
<td>6.25%</td>
</tr>
<tr>
<td>5</td>
<td>Future issues</td>
<td>1</td>
<td>6.25%</td>
</tr>
<tr>
<td>6</td>
<td>Medical and health issues</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Industrial and economic issues</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Food issues</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16</td>
<td>100%</td>
</tr>
</tbody>
</table>
Through Table (3) we note that the issues in general did not get sufficient attention in the subjects of the four stages, which indicates the weakness of keeping up with these materials to the developments of science and its applications, including nanotechnology, but when looking at the same table we note that the identification issues of nanotechnology have been obtained more interest of the subjects has obtained (11) repetitions out of (16) recorded for all subjects and the rate of (68.75%) and the researchers attribute this to the fact that most of the study material deals with these tariff issues as an introduction to nanotechnology as well as the standards and microscopes that adopt this technique.

**Conclusions**

Through the results of the research, the researchers reached the following conclusions: The subjects of the Department of Biology in the colleges of education were weak in their inclusion of nanotechnology issues. The subjects of the third stage in the Department of Biology got the highest frequency, followed by the first stage and then the second stage, while the fourth stage did not receive any repetition. The nanotechnology knowledge issues with the greatest interest in the content of the subjects were the four stages.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of Al-Qadissiyah, College of Education, Iraq and all experiments were carried out in accordance with approved guidelines.

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Color Stability of Different Aesthetic Resin Composite Materials: A Digital Image Analysis

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Abstract

This study aimed to evaluate and compare the color stability of three commercially available resins composites after exposure to different staining solutions using digital image analysis. A total of ninety-disc shape specimens of a microhybrid composite (Amelogen plus), ultrafine hybrid composite (Essentia), and nanohybrid composite (Beautifil II) were produced in Teflon mould (n=30/resin composite type). Specimens of each resin composite were then divided into 5 subgroups (n=6/subgroup) and immersed in 200 ml of Pepsi, orange juice, tea, coffee and artificial saliva (control group), respectively. Digital images of the specimens were taken before (baseline) and after 28 days immersion against a black and white background. Adobe Photoshop CS6, Ver.13.0.1 graphic program (AdobeSystems Inc., San Jose, CA, USA) was used to analyze the digital images and calculate the change in color (ΔE). Data were submitted to statistical analysis using one-way ANOVA and Tukey Posthoc test at a significance level of p<0.05. This study concluded that all resin composites exhibited color changes after immersion in all staining solutions. However, Amelogene Plus showed better resistance to staining compared to Beautifil II and Essentia.

Keywords: aesthetic resin, composite materials, image analysis

Introduction

Composite restorative materials are the most popular esthetic materials used in dental practice. The color instability of composite materials may be the main reason for the replacement of restorations. Discoloration of composite resin caused by extrinsic or intrinsic factor. Intrinsic factors include physical-chemical discoloration reactions in the composite matrix, in surface and deeper layers of the material, triggered by heat, humidity, or UV irradiation. Chemical discoloration has been attributed to a change or oxidation in the amine accelerator, oxidation in the structure of the polymer matrix, and oxidation of the unreacted pendant methacrylate groups1. Extrinsic factors are related to the surface adsorption of staining agents from exogenous sources or due to the accumulation of plaque2. Consumption of certain drinks such as Tea and coffee may affect the physical properties and aesthetic of composite restoration. The effect of drinks on the properties of restorative resins may be directly related to the amount and frequency of its intake2. The aim of this study was to evaluate and compare the color stability of three commercially available resins composite: Amelogen Plus (Microhybrid), Essentia (ultrafine hybrid), Beautiful II (Nanohybrid) after exposure to different staining solutions for 28 days using digital image analysis. The null hypothesis tested was that there is no difference in color changes (ΔE) between the tested composite materials after 4 weeks of degradation of the restorative materials and their adsorption of staining agents3.

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immersion in different staining solutions.

**Materials and Method**

One microhybrid composite (Amelogen plus, Ultradent), one ultrafine hybrid composite (Essentia, GC), and one nanohybrid composite (Beautifil II, Shufo) were evaluated in this study. For each brand, the enamel shade was selected. A total of ninety-disc shape specimens of composite materials were produced in Teflon mould (10 mm diameter x 1 mm thick). After filling the mould with the composite, the discs were covered with glass slides, to exclude atmospheric oxygen, and then cured by visible light for 40 s, using a dental curing unit (Optilux, Demetron Res Crop, Danbury, USA) with an irradiance of 850 mW.cm⁻², which monitored with a radiometer (Kerr/Demetron, Danbury, USA). Light-curing was repeated on the opposite surface for another 40 s. After which, specimens were stored in an incubator at 37°C for 24 hours. Specimens of each resin composite brand were then divided into 5 subgroups (n=6/subgroup) according to the immersion solutions represented by Pepsi, orange juice, tea, and coffee or artificial saliva which constituted the control group. The digital images acquisition of all specimen groups was taken before immersion (baseline) by digital imaging technique using an SLR camera (Nikon D5200, Nikon Corporation, Japan) with a 105mm camera macro lens (Sigma 105 EX Macro, Sigma, Japan). The camera was fixed perpendicularly on stand clamp holder (10 cm distance from the specimen) and set on manual mode, which allowed total control of shutter speed 1/6, ISO 1250, f-stop 3.5. These measurements were remained unchanged during taking shots and three images were obtained for each sample against a white as well as a black background. For the digital imaging method, four fluorescent tubes were mounted on a costume made photostand with tubes perpendicular to the front plane that hold the discs, being 20 cm away from the specimen and illuminating at an angle of 45°. Two 6,500-K fluorescent tubes (Philips PL-C 18W/865, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands) were placed in the lower sockets and were combined with two 2,700 K (Philips PL-C 18W/827) fluorescent tubes placed in the upper sockets. After baseline images acquisition were made, specimens were then immersed in 200 ml of each staining solution and kept in an incubator at 37°C for 28 days. Staining solutions were changed every week to avoid bacteria or yeast contamination. After the staining period, the specimens were gently rinsed with distilled water and air-dried. Imaging measurements were repeated for each sample to determine the color variation.

The digital images were transferred to a personal computer (PC), saved as TIFF images and were analyzed using the Adobe Photoshop CS6, Ver.13.0.1 graphic program (AdobeSystems Inc., San Jose, CA, USA). For standardized calculations, a measurement template was created in the middle third of the samples that consisted of a square area of 50 pixels. The CIE L*a*b* values of these particular areas were calculated using the eyedropper tool. The color data obtained directly in color picker palette tab for (L, a and b) parameters (Figure1). Color changes (ΔE) were calculated as follows:

\[ \Delta E = \sqrt{(L_1^*-L_2^*)^2+(a_1^*-a_2^*)^2+(b_1^*-b_2^*)^2} \]

where \( L^* \) is lightness (0 = black; 100 = white), \( a^* \) is green-red component (\( -a^* = \) green; \( +a^* = \) red), \( b^* \) is blue-yellow component (\( -b^* = \) blue; \( +b^* = \) yellow), subscript 1 is the baseline measurement before the immersion and subscript 2 is the measurement after 28 days immersion in staining solution.

![Figure 1: Adobe photoshop program shows how to pick the color data from the sample (L=47, a=-8, b=10) red dotted area.](image)
Data of color change acquired from black, white backgrounds were submitted to statistical analysis using one-way ANOVA and Tukey Posthoc test for multiple comparisons between groups at a significance level of $p<0.05$. Statistical analysis performed using the Statistical Package for the Social Sciences (Version 24; SPSS Inc., IBM, Chicago, Illinois, USA).

**Results**

Tables 1 and 2 show $\Delta E \pm (SD)$ of all tested composite resins after 28 days immersion in different staining solutions on a black and white background, respectively. The lowest $\Delta E$ value observed for Amelogen Plus composite resin in artificial saliva on a black background (1.42±(0.45)), on the other hand, the highest $\Delta E$ value observed for Essentia composite resin in coffee on white background (28.0±(1.12)). Regarding staining solutions, coffee caused the highest $\Delta E$ in all composite resins, followed by tea, orange, Pepsi and artificial saliva, as shown in tables 1 and 2.

In the black background data set (Table 1), there were significant differences in $\Delta E$ in each brand of resin composite between the different staining solutions ($p<0.05$), except between the artificial saliva and Pepsi, there was no significant difference in $\Delta E$ in all brands of resin composites ($p>0.05$). Also, there were significant differences ($p<0.05$) in $\Delta E$ between the three composite resins in each staining solution except between Amelogen Plus and Beautiful II in artificial saliva, Pepsi, orange and tea ($p>0.05$), there was no significant difference in $\Delta E$. Similarly, there was no significant difference in $\Delta E$ between Essentia and Beautiful II composite resins in orange staining solution ($p>0.05$), as shown in table 1.

### Table 1: Change in color $\Delta E \pm (SD)$ measured on the black background of all tested composite after 28 days immersion in different staining solutions.

<table>
<thead>
<tr>
<th>Black background</th>
<th>Artificial Saliva</th>
<th>Pepsi</th>
<th>Orange</th>
<th>Tea</th>
<th>Coffee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelogen Plus</td>
<td>1.42±(0.45)</td>
<td>2.53±(0.58)</td>
<td>4.41±(0.95)</td>
<td>8.66±(0.81)</td>
<td>11.72±(0.56)</td>
</tr>
<tr>
<td>Essentia</td>
<td>2.87±(0.29)</td>
<td>3.69±(0.46)</td>
<td>5.52±(0.73)</td>
<td>12.09±(1.08)</td>
<td>22.23±(1.06)</td>
</tr>
<tr>
<td>Beautiful II</td>
<td>1.76±(0.3)</td>
<td>2.18±(0.83)</td>
<td>5.14±(0.16)</td>
<td>9.82±(0.87)</td>
<td>13.39±(0.93)</td>
</tr>
</tbody>
</table>

Superscript small letters represent non-significant difference between relevant groups.

In the white background data set (Table 2), there were significant differences in $\Delta E$ in each brand of resin composite between the different staining solutions ($p<0.05$) except in Amelogen Plus and Beautiful II there was no significant difference in $\Delta E$ between the artificial saliva and Pepsi ($p>0.05$). Also, there were significant differences ($p<0.05$) in $\Delta E$ between the three composite resins in each staining solution. However, there was no significant difference in $\Delta E$ between Amelogen Plus and Beautiful II in coffee solution ($p>0.05$), as shown in table 2.

### Table 2: Change in color $\Delta E \pm (SD)$ measured on the white background of all tested composite after 28 days immersion in different staining solutions.

<table>
<thead>
<tr>
<th>White background</th>
<th>Artificial Saliva</th>
<th>Pepsi</th>
<th>Orange</th>
<th>Tea</th>
<th>Coffee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelogen Plus</td>
<td>1.8±(0.58)</td>
<td>2.8±(0.68)</td>
<td>5.9±(0.6)</td>
<td>11.85±(0.51)</td>
<td>18.0±(0.93)</td>
</tr>
<tr>
<td>Essentia</td>
<td>5.18±(0.52)</td>
<td>7.85±(0.47)</td>
<td>12.03±(0.32)</td>
<td>18.22±(0.79)</td>
<td>28.0±(1.12)</td>
</tr>
<tr>
<td>Beautiful II</td>
<td>3.01±(0.73)</td>
<td>4.07±(0.82)</td>
<td>7.92±(0.57)</td>
<td>14.75±(1.42)</td>
<td>19.64±(1.99)</td>
</tr>
</tbody>
</table>

Superscript small letters represent non-significant difference between relevant groups.
Discussion

As a composite restorative material are continuously exposed to saliva, food stains and beverage pigments, it is important to determine its susceptibility to color change. In the present study, we investigated the effects of beverages tea, coffee, Pepsi and orange juice on the color stability of different composite materials. The artificial saliva constituted the control group. The results of the present study showed that there is a difference in color changes $\Delta E$ between the tested resin composites after immersion in different staining agents.

For standardization of staining conditions, we perform an “in vitro” study to control all variables such as food or drinking habits, tooth brushing. All the tested composites were of enamel translucency A2 shade, enamel translucency was selected because it is used as the most external layer and being in contact with staining agents. The immersion time for each composite group was 28 days in accordance with previous studies. Ardu et al. in 2017 reported that the immersion time for 28 days simulated about 2 years of drink consumption. Ertas et al. considered 28 days is equivalent to about 2.5 years of clinical aging (24 h in vitro staining corresponds to 1 month in vivo).

Digital imaging system is a reliable method for determining the color of teeth and gingiva when used with the appropriate calibration protocols. In this study, the digital imaging system was selected to evaluate the color changes against white and black background which were used to allow a double evaluation to simulate two different clinical situations; black background can mimic the situation, where no tooth structure exists in the back. i.e., class IV composite filling. White background can mimic class I, II, III, V and veneers, where one of the walls is still present.

Results of this study revealed that all tested composite showed significant color changes after immersion in the tested solution. Amelogen Plus was more stain-resistant followed by Beautiful II, while Essentia seems to be the most prone to staining. It seems that Essentia extensively colored in these staining solutions. The slightly different ranking obtained with the two different backgrounds may be related to different opacity of the composites which might modify the color perception on different backgrounds. The general behavior trend of the tested composites is confirming that Essentia has less color stability than Amelogene Plus and Beautiful II.

Findings of this study may depend on the hydrophilicity of the resin matrixes of these three resin composites and their filler particles. The differences in the staining resistance between these resin composites may be attributed to the different filler size and load which may affect the overall resin content in the cured composite. Essentia contains prepolymerized filler of resin, which together with the resin matrix, may increase the resin content in the composite, hence the water sorption and staining susceptibility consequently. It has been reported that increased resin content in the composite may increase the water sorption significantly especially in the first week of immersion in the staining solution.

The structure of resin matrix and filler type may have a direct impact on the susceptibility to extrinsic staining. The presence of UDMA, Bis-MEPP, Bis-EMA in the Essentia resin composite in this study did not enhance the staining resistance as has been proposed by previous studies. This may be due to the concurrent presence of Bis-GMA and TEGDMA in resin composition, which were present in the other two tested resin composites in this study.

Regarding the staining solution tested, coffee showed the most staining capacity, followed by tea, orange, Pepsi and artificial saliva (Control group). Changes in $\Delta E$ value were greater for the tested staining solutions than for the control group, so the staining effect of a staining solution varied according to its ingredients. These results are in agreement with many previous studies which shown various staining potential of these drinks and this may be related to their composition and properties.

The highest values of $\Delta E$ reached was in coffee and this could be related to its acidity (pH 4.5), both adsorption and sub-surface absorption of coffee colorant. The degree of polarity of the staining agent determines their degree of penetration into the resin materials. Less degree of polarity, such as coffee, can easily penetrate into the polymer matrix. Both coffee and tea contain a large amount of staining agents like Gallic acid, which could be another reason for the staining capacity of these materials. This finding is in agreement with the study conducted by Ardu et al. Some studies reported that a solution with a lower pH can attack the composite material...
surface, causing some changes and increasing pigment absorption. Although Pepsi had a low pH, which can damage the surface integrity of the materials, it did not cause as much staining as coffee and tea. This may due to the absence of yellow colorant, which is abundant in coffee and tea, these findings were in agreement with other studies which found that coffee and tea produced more discoloration than cola.

Conclusion
This study concluded that staining solutions affected the color stability of the tested resin composites significantly in a clinically unacceptable level, except for Amelogene Plus and Beautiful II in artificial saliva and Pepsi, which were acceptable clinically. Also, Essentia resin composite performed inferior to Amelogene Plus and Beautiful II in terms of color stability.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry, University of Baghdad, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References
Bergamot Essential Oil Effect against Candida Albicans Activity on Heat Cure Acrylic Denture Base

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Abstract

The aim of the current study is to evaluate the antifungal efficacy of several different percentages of Bergamot Essential Oil (BEO) incorporated into heat cure PMMA denture base material against Candida albicans.

Materials and Method: Eighty samples were divided into eight groups including six various percentages of BEO (2%, 3%, 4%, 5%, 6% and 7% by volume) and 0% BEO as a control group, in addition to 1.4% by weight of nystatin which represent the positive control group. All these additives were incorporated into heat cure PMMA denture material. After 48h incubation in distilled water, all samples were assessed by Candida albicans colonies viable count test.

Results: For Candida albicans activity test; the experimental groups (2%, 3%, 4%, 5%, 6% and 7% of BEO) showed highly significant decrease in the mean values of the viable count of Candida albicans when compared to the control group (0% BEO) (p<0.01). In contrast, a non-significant difference among experimental groups and 1.4% nystatin group.

Conclusion: Bergamot essential oil was successfully incorporated into heat cure PMMA denture base material and could act as potential antifungal agent with a drug delivery system against Candida albicans. It seemed that adding of 5% and 6% BEO was the most beneficial effects against the growth of fungi.

Key words: Heat cure PMMA, Denture base, Bergamot essential oil, Nystatin, Candida albicans

Introduction

Walter Wright discovered poly(methyl methacrylate) (PMMA) after publishing the results of his clinical evaluation of PMMA in 1937, since it is introduced has become most superior, popular and satisfactory of all polymeric denture base materials. Acrylic denture base is used in removable dentures fabrication, and its popularity and universal use has attributed to low cost, light weight, ease of processing, ease of reparation, low water sorption and solubility, biocompatibility, satisfactory aesthetic properties.

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and accurate fit, in spite of that this material until now remain not ideal denture base material due to the presence of several drawbacks like susceptibility to microbial colonization and formation of biofilm on its surface and this make the denture a source of different infections because it harbor the microorganisms in addition to lack of manual dexterity among the old age patients which make the effective biofilm removal not possible. Denture stomatitis is a disease related to denture use, and considered chronic atrophic oral candidiasis, which affect up to 65% of denture wearer, its etiology is multifactorial, but Candida albicans considered the main pathological microorganism which is the mostly isolated type from the oral cavity in patient with denture stomatitis. The most common line that is used in treatment of denture stomatitis is prescribing of topical antifungal medicines, but maintaining optimal oral drug dose and lacking of motor dexterity of geriatric patient who had impaired cognition, limit their use
and make it challenging to get a maximum benefit of these topical drugs. To overcome these obstacles it is better to incorporate the antifungal drugs into denture base materials. Unfortunately fungal resistance and side effects of these drugs make it necessary to obtain naturally derived medicaments as a substitution to these synthetic drugs. Herbal medicines are a powerful alternative treatment for microbial infections in the oral cavity which have less or no side effects; and this made a worldwide trend to make a lot of researches about them in order to find biologically safe herbal-based medicines with effective antifungal properties. Plants oils are herbal medicines and various researches, recently, have been done to test their antifungal efficiency against Candida albicans and they reported that these oils are considered a promising line of therapy which have effective antifungal properties and can be used for treatment of denture stomatitis. One of the recent research was done through the incorporation of virgin coconut oil into heat cured acrylic-based denture soft lining and proved the antifungal efficiency of this oil against Candida albicans. Bergamot is the popular name for Citrus bergamia Risso et Poiteau, Citrusbergamia is defined as “a hybrid between a sour orange (C. aurantium L.) and lemon (C. limon L. Burm.F.) or a mutation of the latter. Other authors considered it a hybrid between a sour orange and lime.”

Materials and Method

Regular- conventional heat cure acrylic resin denture base material (Vertex, Netherlands) was utilized. Bergamot essential oil (bergaptene-free) from (Aura Cacia pure essential oils, USA) was added to the liquid part of acrylic resin in six different percentages (2%, 3%, 4%, 5%, 6% and 7%), these percentages were selected to discover which one is as effective as 1.4% nystatin group (positive control group). This proportion of nystatin was calculated by the conversion of International Units (U) to milligrams. Every 6079U corresponded to 1 mg of nystatin. Therefore, for this group (500,000U) the amount of nystatin used was 90 mg for every 6.6 g of acrylic powder. In general, 500,000 units was the most common concentration used by many authors and result in decreased yeast count compared to control group. Also this percentage of nystatin start to change the color of acrylic resin into orange so it is objectionable to use it above this percentage especially in aesthetic area.

Total of eighty samples were prepared, 10 samples for control group, 10 samples for each percentage of BEO groups (2%, 3%, 4%, 5%, 6% and 7% BEO) and 10 samples for positive control group. All samples were tested by viable count of Candida albicans colonies test at the same time and circumstances and they stored in distilled water for 48h prior to test procedure to reach the state of standardization.

Candida albicans colonies viable count test

Samples preparation

Plastic models were prepared with dimensions of 10×10×2.3 mm in length, width and thickness, respectively using laser cutting machine. Then, the models were invested in freshly mixed type IV extra hard dental die stone to create stone molds where the acrylic resin samples will be packed. For control group the acrylic resin denture base materials were proportionate and mixed as directed by the manufacturer instructions. Regarding BEO incorporated samples, the required amount of oil were measured by micropipette and subtracted from the volume of monomer, then mixed manually in dry clean glass beaker with monomer. Following which, the mixture was added to acrylic powder and mixed thoroughly.

Isolation and identification of Candida albicans

Candida albicans were obtained from oral cavity of patient with an indication of a denture induced stomatitis by using cotton swab which then cultured on the surface of a sabouraud dextrose agar SDA (Oxoid, United kingdom) plates and incubated at 37°C for 48 h. The identification of candida albicans was done according to the following order: Firstly, the colony morphology (macroscopic examination) in which the Candida albicans appear as creamy, pasty, smooth and convex colonies on SDA. Secondly, microscopic examination using Grams stain procedure, candida seen as small gram-positive oval or budding yeast cells. Thirdly, Germ tube formation. Fourthly, Biochemical identification using analytical profile index API Candida system (bioMérieux, France) and API 20 C AUX system.

Assessment of Candida albicans viable count

Candida albicans suspension of about 10^7CFU/mL which equal to 0.5 McFarland standards was prepared by diluting a small quantity of inoculums in a test tube.
containing normal saline and measuring this solution with McFarland densitometer device as shown in Figure 1(A). Then, 100µL of Candida suspension was added to each tube contain 9.9 mL of sabouraud dextrose broth (Oxoid, England) to obtain 10 mL broth mixture this done by using micropipette. The samples after that immersed in tubes (Figure 1 (B)) and incubated at 37 °C for 24 h. After incubation, about 100µL was taken from all tubes of broth mixture and added to other tube contain 9.9 mL normal saline so tenfold dilution obtained. A second dilution made from this dilution by the same procedure as in Figure 1(C). About 100 µL taken from the second dilution and spreaded on SDA plates by using disposable inoculation loops (Figure 1(D)). The SDA plates incubated aerobically at 37 °C for 24 h. This dilution was chosen because it demonstrated a countable range of 30-300 CFU.

Following incubation, the Candida albicans colonies were visible on SDA plates, all these colonies counted and this viable count analyzed statistically so the material antifungal efficacy (AFE) was calculated using following formula (Equation 1):

\[
AFE [%] = \frac{V_c - V_t}{V_c} \times 100 \% \quad (1)
\]

In this formula the number of viable colonies of control samples was represented by Vc and number of viable colonies of experimental samples was represented by Vt

Statistical Analysis

The data of this research were collected and analyzed using SPSS (statistical package for social science – version 21) computer software. Descriptive statistics were made which include Means, Standard deviation, Minimum, Maximum. Inferential statistics include ANOVA (one-way ANALYSIS OF VARIANCE) test which was used to evaluate the significance of difference among the mean values of all groups, then multiple comparison using Dunnett t-tests which treat one group as a control, and compare all other groups against it was used.
Results

The viable count of Candida albicans colonies test was performed on six experimental groups (2%, 3%, 4%, 5%, 6% and 7% BEO) and two control groups (negative control 0% BEO group and positive control 1.4% nystatin group for comparison). After 48 h of samples incubation in distilled water, the results of viable counts of Candida albicans showed a reduction in the mean values for experimental groups in comparison to negative control group. So 5% and 6% groups showed the lowest mean value among all groups, while other experimental groups (2%, 3%, 4% and 7%) revealed higher mean values than positive control group. Antifungal efficiency (AFE) for the experimental groups are as follows: 55.53% for 2% BEO group, 67.21% for 3% BEO group, 68.64 % for 4% BEO group, 76.43% for 5% BEO group, 74.18 % for 6% BEO group, 66.80% for 7% BEO group and 69.87% for 1.4% nystatin group.

Descriptive statistics (mean, standard deviation, maximum and minimum) and statistical test of viable count of C. albicans results using One- Way ANOVA for comparison of means of all studied groups listed in Table (1) the difference between groups was highly significant. For multiple comparison purpose the Dunnett t (2-sided) a test was used. In this test the 1.4% nystatin group taken as control group and all other groups compared against it. The 0% BEO group showed a highly significant difference while all other experimental groups showed a non-significant difference in comparison to 1.4% nystatin group as listed in Table (2).

Table (1): Descriptive statistics of viable count of Candida albicans, using One-Way ANOVA

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (CFU/mL)</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>162.667</td>
<td>18.877</td>
<td>142.000</td>
<td>179.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>72.333</td>
<td>10.116</td>
<td>66.000</td>
<td>84.000</td>
<td>27.245</td>
<td>0.000 HS</td>
</tr>
<tr>
<td>3%</td>
<td>53.333</td>
<td>15.631</td>
<td>39.000</td>
<td>70.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4%</td>
<td>51.000</td>
<td>11.000</td>
<td>40.000</td>
<td>62.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>38.333</td>
<td>13.051</td>
<td>28.000</td>
<td>53.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6%</td>
<td>42.000</td>
<td>17.692</td>
<td>26.000</td>
<td>61.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7%</td>
<td>54.000</td>
<td>11.136</td>
<td>42.000</td>
<td>64.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4% Nystatin</td>
<td>49.000</td>
<td>4.583</td>
<td>44.000</td>
<td>53.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Multiple comparisons test of Candida albicans (CFU/mL) among the groups using Dunnett t (2-sided) a test

<table>
<thead>
<tr>
<th>Multiple Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable: Canalb</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dunnett t (2-sided)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) groups</td>
</tr>
<tr>
<td>control</td>
</tr>
<tr>
<td>2%</td>
</tr>
<tr>
<td>3%</td>
</tr>
<tr>
<td>4%</td>
</tr>
<tr>
<td>5%</td>
</tr>
<tr>
<td>6%</td>
</tr>
<tr>
<td>7%</td>
</tr>
</tbody>
</table>

*The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.
Medicinal Plants extracts are very good replacement to antimicrobial drugs with less or without side effects, as a result this encouraged the worldwide tendency towards herbal-based medicines and a lot of researches were done to obtain herbal medicine that is biologically safe and have excellent antifungal properties. Essential oils are examples of naturally derived herbal medicaments which are concentrated, hydrophobic liquids extracted from plant and have wide spectrum of pharmacological activities, these essential oils considered as a promising therapeutic line for oral infections and in the last few decades a lot of kinds of oils have been tested for efficacy against Candida albicans, each one of them has special active component that affect the fungi in particular mechanism. Bergamot essential oil has been used for its antiseptic, anti-inflammatory, diaphoretic, appetizing, and analgesic effect.

**Figure 2**: Viable counts of Candida albicans after 48 hours incubation of: a) Control samples; b) some experimental samples with BEO; c) positive control sample (1.4% nystatin). Medicinal Plants extracts are very good replacement to antimicrobial drugs with less or without side effects, as a result this encouraged the worldwide tendency towards herbal-based medicines and a lot of researches were done to obtain herbal medicine that is biologically safe and have excellent antifungal properties. Essential oils are examples of naturally derived herbal medicaments which are concentrated, hydrophobic liquids extracted from plant and have wide spectrum of pharmacological activities, these essential oils considered as a promising therapeutic line for oral infections and in the last few decades a lot of kinds of oils have been tested for efficacy against Candida albicans, each one of them has special active component that affect the fungi in particular mechanism. Bergamot essential oil has been used for its antiseptic, anti-inflammatory, diaphoretic, appetizing, and analgesic effect.

**Conclusion**

Within the limitations of the present study; the following conclusions can be obtained that Bergamot essential oil was incorporated successfully into heat cure acrylic resin denture base material and worked as a powerful antifungal herbal medicament against Candida albicans that is comparable with the effect of nystatin. Moreover, the samples with 5% and 6% BEO revealed a better antifungal efficiency compared to all other control and experimental groups.

**Financial Disclosure**: There is no financial disclosure.

**Conflict of Interest**: None to Declare.

**Ethical Clearance**: All experimental protocols were approved under the College of Dentistry/University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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Antimicrobial Activity And Characterization of Some Oxazole, Thiazol And Quinoline


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Abstract

New Heterocyclic compounds derivatives comprising 1,3-oxazole, chalcone ,thiazole, pyrimidine, quinolone moieties are reported. New derivatives of Quinazolin-4 (3H)-one ring comprising Schiff’s bases,(1,3,4-Thiadiazole),(1,3,4-Oxadiazole) and (1,2,4-Triazole), Thiaurease moieties are reported. Compounds (1), (2) and (5) were synthesized by reaction of benzyol chloride with urea, thiourea and anthranilic acid respectively , then compounds (1)and (2) were converted into(3a-c) and (4a-c) derivatives. While compound (5) reaction with urea to convert to compound (6) which was converted to (7a-c) . chalcone derivatives (9a,b ) were readily obtained by reaction of compound (8) with different aldehydes, Compounds (9a , b) were converted into (10a,b) and (11a,b) The structure of these compounds has been established on the basis of their spectral data FTIR and 1H NMR. These compounds were tested for invitro antibacterial activity against Escherichia coli, Sepidermidis, S.aureusand Klebseillastandard methods. These synthesized compounds have been shown moderate to good antibacterial activity.

Keywords: 1,3 oxazole, chalcone, thiazole, pyrimidine, quinolone, antimicrobial activity.

Introduction

Heterocyclic compounds had been receiving considerable attention due to their pharmacological and pesticidal importance. The heterocyclic nitrogen compounds like quinazolinone derivatives has a vital role in synthetic drugs and biological processes. A Quinazolin-4-one derivative possessing broad spectrum of biological and pharmacological activities such as antifungal, antimicrobial, bronchodilator, antihistaminic, anti-inflammatory, 1,3-Oxazole and thiazole derivatives possess a broad spectrum ofpharmacological activities such as antibacterial, antiviral, anti-inflammatory, antitumor. The biological significance of the pyrimidine derivatives has led to the synthesis of substituted pyrimidineand their derivatives, pyrimidinederivatives have been important role in medical applications. One possible reason for their activity is the presence of a pyrimidine base in cytosine, thymine and uracil, which are essential building of nucleic acids ,RNA and DNA. Pyrimidine derivatives possess several interesting biological activities such as antimicrobial, antitumor, antifungal, antimalarial, anticancer, antiallergic, antitubercular activities and anti-inflammatory activities. Many Pyrimidinederivatives are used for thyroid drugs and leukemia.

Materials and Method

Apparatus

Melting point were determined in open capillary tubes and were uncorrected and the purity of the compounds were checked by TLC . The IR Spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer, using KBr discs. 1H NMR Spectra of prepared derivatives were recorded in DMSO with TMS as internal standard on a Varian-Mercury 300 MHz Spectrometer the reaction were followed.

Procedures

Synthesis of N-(aminocarbonyl)-benzamide (1)

A mixture of benzyol chloride(1. 1 mole), and urea (1. 1 mole) in dry benzene(30ml) were mixed in round bottom flask and refluxed for 15 hrs, resulting mixture was poured in to crush ice and the solid filtered and
Synthesis of N-(aminocarbonothioyl)-benzamide (2)

A mixture of thiourea (1 mole) and benzoyl chloride were dissolved in benzene (30 ml) then refluxed for (16 hrs.), the mixture was cold and filtered to obtained the solid, and purified by recrystallization from appropriate solvent table (1).

Synthesis of N-substituted acetamide (General method)

In a dry and clean conical flask (0.01 mol) of substituted aromatic amine was dissolved in 15 ml dry benzene with continuous shaking and (0.015 mol) of chloro acetyl chloride was added drop wise by dropping funnel, the mixture was heated in water bath for (1 hr) after the reaction complete, the product which separated were filtered and washed with sodium bicarbonate (5%), water, dried and recrystallized from alcohol.

Procedure for Synthesis of derivatives(3a-c)

A mixture of compound (1) (0.01 mole) and (0.01 mole) of 2-chloro-N-(substituted phenyl)acetamide in ethanol were refluxed for 24 hrs and allowed to stand undisturbed over night, the product separated on cooling was filtered and purified by recrystallization.

General Procedure for Synthesis of derivatives (4a-c)

A mixture of compound (2) (0.01 mole) and (0.01 mole) of 2-chloro-N-(substituted phenyl)acetamide in ethanol were refluxed for 24 hrs and allowed to stand undisturbed over night, the product separated on cooling was filtered and recrystallized from appropriate solvent table (1).

Synthesis of (2-phenyl)-3-carboxamidequinazoline-4-(3H)-ones (5)

A mixture of benzoyl chloride (1.4 gm, 0.01 mole) and anthranilic acid (1.38 gm, 0.01 mole) in triethyl amine (30 ml) were stirred at 0-5°C for 1 hr, then further stirred for additional 1 hr at room temperature. A paste mass obtained which was washed thoroughly with sodium carbonate (10%) to remove unreacted acid, the solid separated by cooling and was dried and purified by recrystallization.

Synthesis of (2-phenyl)-1,3-benzoxazin-4(H)-ones (5)

A mixture of benzoyl chloride (1.4 gm, 0.01 mole) and anthranilic acid (1.38 gm, 0.01 mole) in triethyl amine (30 ml) were stirred at 0-5°C for 1 hr, then further stirred for additional 1 hr at room temperature. A paste mass obtained which was washed thoroughly with sodium carbonate (10%) to remove unreacted acid, the solid separated by cooling and was dried and purified by recrystallization.

Synthesis of (2-phenyl)-3-carboxamidequinazoline-4-(3H)-ones (6)

Compound (5) (2.23, 0.02 mole) and urea (0.6 gm, 0.02 mole), in pyridine 20ml, the reaction mixture was refluxed for (12 hrs) in oil bath at (180-200°C), then the reaction mixture was poured into to ice cold water containing conc. HCl. The separated solid was filtered, washed and recrystallized from appropriate solvent table (1).

General Procedure for synthesis of derivatives (7a-c)

2-chloro-N-(substituted phenyl)acetamide (0.01 mole) and (2-phenyl)-3-carboxamide quinazoline-4-(3H)-ones (6) (0.01 mole) of in ethanol were refluxed for 24 hrs and kept the product over night, the solid separated on cooling was filtered and purified by recrystallization.

Synthesis of N-(4-acetylphenyl)benzamide (8)

To a mixture of 4-amino acetophenone (0.01 mole, 1.39g) in 25 ml of benzene and benzoyl chloride (0.01 mole, 1.4g) was refluxed for 3 hrs according to literature procedure [12]. After that, mixture was kept overnight. The product thus obtained was recrystallized from appropriate solvent table (1).

Synthesis of N-(4-(3-4-substituted aryl) acryloyl) phenyl)benzamide (9a9b)

A mixture of (substituted benzaldehyde) (0.01 mole) and compound (8) (0.01 mole) and (20%) 10 ml NaOH in ethanol (30 mL) was stirring for 24 hrs at room temperature. After that, mixture was poured onto crushed ice to get precipitate. The product thus obtained was purified by recrystallization table (1).

Synthesis of N-(4-(2 mercapto-6-(substituted aryl)-1,6-dihydropyrimidin-4-yl)phenyl) benzamide (10a,b)

A mixture of required chalcone (9a,b) (0.01 mole) and thiourea (0.01 mole) in 1,4 dioxan (10 ml) and acatalytic amount of glacial acetic acid are taken in around bottom flask and heated under reflux for about 24 hrs. The reaction mixture was poured into cold water with stirring, the product was filtered and purified by recrystallization.
Synthesis of N-(4-(2-hydroxyl-6-(substituted aryl)-1,6-dihydropyrimidin-4-yl)phenyl)benzamid (11a,b)

A mixture of N-(4-(3-4-substituted aryl)acryloyl)phenyl)benzamide (9a,b) (0.01 mole) and urea (0.01 mole) in the presence of KOH (1gm) in 20ml ethanol was heated under reflux for 12 hrs, then cooled and poured in ice cold water, the solid mass was obtained by filtration, recrystallization table (1).

Results And Discussion

All compounds were prepared according to the following scheme:

The structures of prepared compounds were identified by FTIR (table 2) and $^1$H NMR spectra (table 3). All results spectral data were in correspondence to expected values. The purity of prepared compounds were checked by using TLC chromatography. The physical properties of compounds are listed in (table 1). Compound (1) was synthesized from benzoyl chloride and urea with dry benzene. The IR spectra of this compound, show the appearance of bands at 3360, 3342, 3225, 3068 and 1674 cm$^{-1}$ which could be due to v (NH$_2$, NH), v C-H aromatic and v C=O amide, respectively. The $^1$H NMR spectra measured in DMSO-d$_6$ at 25°C revealed a multiplet from 8.41 to 7.64 ppm for aromatic protons, a singlet at 11.58 ppm (N-H, 2H) and 3.7 ppm (NH$_2$). Also the reaction between benzoyl chloride and thiourea with dry benzene lead to the formation of compound (2). The spectroscopic observation of compound (2) is given show the appearance of bands at 3360, 3342, 3225, 3068 and 1674 cm$^{-1}$ which could be due to v (NH$_2$, NH), v C-H aromatic and v C=O amide, respectively. The $^1$H NMR spectra showed a singlet at 11.6 ppm (N-H, 2H), a singlet at 7.1 ppm (C-H oxazole, H), a multiplet from 8.4-7.84 ppm (ph-H, 9H). Compounds (4a-c) were synthesized by the reaction of compound (2) with 2-chloro-N- (substituted phenyl) acetamide in ethanol to give 5-membered heterocyclic system (substituted 1,3 thiazol). In compound (4a), the transformation is confirmed by observed the appearance 3257, 3088, 1672 cm$^{-1}$ which could be due to N-H, C-H aromatic and C=O as well as the appearance of C=N stretching band at 1626 cm$^{-1}$. The $^1$H NMR spectra, the signal of (N-H, 2H) and (C-H thiazole ring, H) protons were appeared at 11.7 and 7.1 ppm, as well as the signal of (ph-H, 8H) protons were appeared between 8.6-7.8 ppm. The reaction of benzoyl chloride with anthranilic acid in basic media lead to the formation of compound.

Antimicrobial activity

The activity of antibacterial and antifungal were studied by using cup-plate agar diffusion method. The inhibition zones were measured in mm. Amoxicillin and mefenamic acid (500mg/ml) were used as standard drugs for antimicrobial activity. The compounds were screened for antibacterial activity against klebsilliha, Escherichia coli, Pseudomonasaeruginosa and Staphylococcus aureus in Mullar Hinton agar, and the results are shown in the tables 4a, 4b, 4c and 4d. The results of antibacterial screening, indicate that compounds (4b, 7a, 11b) show activity against S. aureus and Sepidermidis than E. coli and Klebseilla and compared these results with standard drugs (mefenamic acid and amoxicillin). While only the derivative 10b show good activity against Klebseillamore the activity for standard drugs. The results obtained by antifungal activity, it is found that the compounds (3a, 4a, 7a and 7b) show good activity against conidid fungi and these results compared with standard drugs (mefenamic acid and Amoxicillin).
Table (1): FTIR spectra of the synthesized compounds

<table>
<thead>
<tr>
<th>No.</th>
<th>(v) (N-H)</th>
<th>(v) (C-H)</th>
<th>(v) (C=O)</th>
<th>(v) (C=C)</th>
<th>(v) (C=N)</th>
<th>Others</th>
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<tr>
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<td>3225</td>
<td>3068</td>
<td>1674</td>
<td>1618-1540</td>
<td>-</td>
<td>3360,3342(NH2)</td>
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<tr>
<td>2</td>
<td>3257</td>
<td>3053</td>
<td>1665</td>
<td>1612-1523</td>
<td>-</td>
<td>3381-3320(NH2)</td>
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<tr>
<td>3a</td>
<td>3232</td>
<td>3076</td>
<td>1664</td>
<td>1610-1515</td>
<td>1637</td>
<td>-</td>
</tr>
<tr>
<td>3b</td>
<td>3283</td>
<td>3092</td>
<td>1647</td>
<td>1600-1520</td>
<td>1626</td>
<td>-</td>
</tr>
<tr>
<td>3c</td>
<td>3225</td>
<td>3081</td>
<td>1651</td>
<td>1609-1519</td>
<td>1633</td>
<td>-</td>
</tr>
<tr>
<td>4a</td>
<td>3257</td>
<td>3088</td>
<td>1672</td>
<td>1610-1500</td>
<td>1626</td>
<td>-</td>
</tr>
<tr>
<td>4b</td>
<td>3274</td>
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<td>1600-1518</td>
<td>1622</td>
<td>-</td>
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<tr>
<td>4c</td>
<td>3282</td>
<td>3094</td>
<td>1678</td>
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<td>1630</td>
<td>-</td>
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<tr>
<td>5</td>
<td>-</td>
<td>-</td>
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<td>1608</td>
<td>1309(C-N)</td>
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<td>6</td>
<td>3100</td>
<td>3088</td>
<td>1632</td>
<td>1604-1527</td>
<td>1633</td>
<td>1109(COC)</td>
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<td>1673</td>
<td>1584-1529</td>
<td>1641</td>
<td>-</td>
</tr>
<tr>
<td>7b</td>
<td>3213</td>
<td>3038</td>
<td>1682</td>
<td>1597-1517</td>
<td>1623</td>
<td>-</td>
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<td>3230</td>
<td>3064</td>
<td>1662</td>
<td>1600-1532</td>
<td>1635</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>3159</td>
<td>-</td>
<td>1602</td>
<td>1597-1539</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1597</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1598</td>
<td>-</td>
<td>1674(CH=CH)</td>
</tr>
<tr>
<td>10a</td>
<td>3270</td>
<td>-</td>
<td>-</td>
<td>1572-1536</td>
<td>1610</td>
<td>1514,1336(NO2)</td>
</tr>
<tr>
<td>10b</td>
<td>3257</td>
<td>-</td>
<td>-</td>
<td>1575</td>
<td>1612</td>
<td>1649(CH=CH)</td>
</tr>
<tr>
<td>11a</td>
<td>3265</td>
<td>-</td>
<td>-</td>
<td>1576</td>
<td>1609</td>
<td>1518,1328(NO2)</td>
</tr>
<tr>
<td>11b</td>
<td>3259</td>
<td>-</td>
<td>-</td>
<td>1557</td>
<td>1606</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (2): Proton NMR signals of the synthesized compounds in DMSO-d<sub>6</sub>

<table>
<thead>
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<th>(\delta) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.58 (s,2H,NH), 3.7 (NH2), 7.64-8.41 (m, 5H, phH)</td>
</tr>
<tr>
<td>2</td>
<td>12. 33(s, H,NH), 3.89 (s,NH2), 7.7-8.2(m, 5H, phH)</td>
</tr>
<tr>
<td>3a</td>
<td>11.6(s,2H,NH), 7.84-8.4 (m, 10H, oxa- Hand ph- H)</td>
</tr>
<tr>
<td>3b</td>
<td>12.1(s, 2H,NH), 7.92-8.31 (m, 10H,oxa-Handph-H), 2.1 (s,3H,CH&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
<tr>
<td>3c</td>
<td>11.4(s, 2H,NH), 7.7-8.2(m, 9H,oxa-H, ph-H and pyrmi-H )</td>
</tr>
<tr>
<td>4a</td>
<td>11.6(s,2H,NH), 7.6-8.6(m, 10H ,Thaiz-Handph-H)</td>
</tr>
<tr>
<td>4b</td>
<td>12.1(s,2H,NH), 7.5-8.4 (m, 10H, Thaiz-Hand ph-H) 2.23 (s,3H,CH3)</td>
</tr>
<tr>
<td>4c</td>
<td>11. 7(s,2H,NH), 7.8 -8.6 (m, 9H, Thaiz H, ph-H and pyrmi-H )</td>
</tr>
<tr>
<td>5</td>
<td>7.55 -8.61 (m, 9H, Aromatic protons)</td>
</tr>
<tr>
<td>6</td>
<td>6. 1 (s,2H,NH2), 7.4-8.56 (m, 9H, Aromatic protons)</td>
</tr>
<tr>
<td>7a</td>
<td>9.33 (s, H,NH), 7.7-8.21 (m, 14H oxa-Handph-H )</td>
</tr>
<tr>
<td>7b</td>
<td>7.6 (s, H,NH), 7.5-8.1 (m, 14H, oxa-Handph-H ), 2.1(s,3H,CH&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
<tr>
<td>7c</td>
<td>9.4 (s, H,NH), 7.7-8.50 (m, 13H, oxa-H, ph-H and pyrmi-H )</td>
</tr>
<tr>
<td>8</td>
<td>11.98 (NH),2.3(s,3H ,CH3) 7.7-8.50 (m, 9H, Aromatic protons)</td>
</tr>
<tr>
<td>9a</td>
<td>10.8 (NH),7.8-8.6(m,13H, Aromatic protons) 6.3-6.7(d,2H,CH=CH)</td>
</tr>
<tr>
<td>9b</td>
<td>11.04 (NH), 7.4-8.8(m,13H, Aromatic protons) 6.5-6.8(d,2H,CH=CH)</td>
</tr>
<tr>
<td>10a</td>
<td>11.06 (NH),5.3(s,1H,OH) ,7.6-8.4(m,14H,pyrmi-H and ph-H)</td>
</tr>
<tr>
<td>10b</td>
<td>11.08 (NH), 5.1(s,1H,OH) ,7.7-8.9(m,14H, pyrmi-H and ph-H)</td>
</tr>
<tr>
<td>11a</td>
<td>11.1 (s,H,NH), 12.4(s,1H,SH) ,7.4-8.8(m,14H) pyrmi-H and ph-H)</td>
</tr>
<tr>
<td>11b</td>
<td>11.98 (s,H,NH), 12.6(s,1H,SH) ,7.55-8.90(m,14H, pyrmi-Hand ph-H)</td>
</tr>
</tbody>
</table>
Table 3. Antimicrobial activity of oxazole derivatives represented by% inhabitation against different bacterial and fungal species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition Zone against (in mm)</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram negative</td>
<td>Gram positive</td>
</tr>
<tr>
<td></td>
<td>E. Coil</td>
<td>klebsiella</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3b</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>3c</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Mefanamic</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Amoixillin</td>
<td>36</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 4. Antimicrobial activity of thiazol derivatives represented by% inhabitation against different bacterial and fungal species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition Zone against (in mm)</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram negative</td>
<td>Gram positive</td>
</tr>
<tr>
<td></td>
<td>E. Coil</td>
<td>klebsiella</td>
</tr>
<tr>
<td>4a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4b</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>4c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mefanamic</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Amoixillin</td>
<td>36</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 5. Antimicrobial activity of quinazoline 4-one derivatives represented by% inhabitation against different bacterial and fungal species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition Zone against (in mm)</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram negative</td>
<td>Gram positive</td>
</tr>
<tr>
<td></td>
<td>E. Coil</td>
<td>klebsiella</td>
</tr>
<tr>
<td>7a</td>
<td>20</td>
<td>11</td>
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<td>7b</td>
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<td>11</td>
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<tr>
<td>7c</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Mefanamic</td>
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<td>14</td>
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<tr>
<td>Amoixillin</td>
<td>36</td>
<td>9</td>
</tr>
</tbody>
</table>
Conclusion

Different heterocyclic derivatives containing thiazole, quinazolin-4-one, oxazol and pyrimidine in structure were prepared and characterized using spectroscopy techniques. Four routes in the synthesis, the first synthesis of oxazol derivatives 3a-3c by reaction of benzoyl chloride with urea, after that cyclization product with (substituted phenyl) acetamids. In the same method prepared thiazole derivatives 4a-4c but with thiourea. The third route includes preparation of quinazolin-4-one derivatives 7a-7b by reaction of benzoyl chloride with anthranilic acid and reaction product with urea n de cyclization by (substituted phenyl) acetamids. In the final scheme synthesis was prepared pyrimidine derivatives 10a-11b. These derivatives have been evaluated in vitro for their antimicrobial activities against several microbes like klebsilliha, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus, addition of antifungal activity.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Chemistry, College of Science-University of AL-Mustansiriyah-Baghdad-Iraq and all experiments were carried out in accordance with approved guidelines.

References


Nurses Staff Knowledge Regarding Standard Hand Hygiene Precautions in Rania City/Kurdistan Regional Government of Iraq

Blend B. Ameen
College of Nursing, University of Raparin,

Abstract
Objectives: -To assess nurses staff level of knowledge regarding standard hand hygiene precaution, and to find out the association between their level of knowledge and some of socio-demographic characteristics such as (gender, and level of education).

Method: Quantitative design, a descriptive study was carried out at Rania City. To achieve the objectives of the present study, non-probability purposive sample was used. The study sample was (66) nurses who were providing nursing services at two hospitals (Rania teaching hospital and Rania maternity and pediatric hospital). 10 of samples were excluded for a pilot study.

A questionnaire was constructed by the researcher for the purpose of the study technique and it was used as a tool for data collection. The data were collected through using an interview technique (face to face) approach.. Data were analyzed through the application of descriptive statistical analysis, such as: (frequency, percentage, mean of scores), and inferential statistical analysis (Pearson’s chi-square and correlation coefficient), by using the statistical package of social sciences (SPSS) version (20).

Results: The study indicated that the most of samples aged between 32-36 years old and represents 28.6% of the study samples. In addition, the highest percentages of the nursing staffs were female 73%. Concerning of the educational level, more than half of nurses graduated from the medical institute 66 %. And the largest study sample have 6-10 years of experiences 28.5%The findings of the study reveal that the nurses have a good level of knowledge about hand hygiene (HH) and appropriate time to do HH. The majority of the stay sample routinely used HH and they disinfectant their hands either by washing hands with water and soap or use alcohol-based jell for, while some of the nurses don’t wash their hands either because they are busy, they forget to do it or even the products are not available or not easy to reach. The study also approves that there was a statistically no significant association between the knowledge of nurses and gender and years of experiences.

Keywords: Hand hygiene, Hand washing, Alcohol-based hand rub, Knowledge, Nurses

Introduction
Nurses do hand hygiene even by washing hands with water and soap or using alcohol-based hand rub to prevent spread of infection to them or even to patients. With that there are many moments that the nurses do not do it either because they are busy, they forget to do it or the products are not available or not easy to reach. In addition, they do not wash their hands correctly because of lack of knowledge about standard HH. Hand hygiene is a simple, low cost and an effective way to prevent separation of infections. Cleaning your hands can prevent the spread of germs and safe the nurse and the patient lives. Hand hygiene refers to either hand washing with antimicrobial soap or hand disinfecting with an alcohol-based hand-rub. The aim of hand hygiene is to remove dirt and limit the microbial counts on the skin, to prevent cross transmission of pathogens between patients 1. The New England Journal of Medicine reports that a lack of hand-washing remains at unacceptable levels in most medical environments, with large numbers of doctors and nurses
routinely forgetting to wash their hands before touching patients, thus transmitting microorganisms\(^2\). One study done in ICUs in Michigan (2003), showed that proper hand-washing and other simple procedures can decrease the rate of catheter-related bloodstream infections by 66 percent\(^3\). The World Health Organization (WHO) has issued guidelines for procedural hand washing in order to reduce the prevalence of hospital associated infections, but lack of knowledge amongst health care workers is associated with poor compliance\(^4\). To reduce the spread of germs, it is better to wash the hands or use a hand antiseptic before and after tending to a sick person. All staff in hospital should deal with hand hygiene actively because this is one of the easiest ways to help keep patients as safe and healthy as possible. So there is a need to check up nurses’ knowledge and awareness toward hand hygiene periodically in order to ensure prevent spared of infection in health care setting and to stop patients and health care workers from getting infections.

**Method**

A quantitative design, descriptive study was carried out in in the Rania city from August 2, 2018 to the end of March 2019. In order to assess nursing staff level of knowledge regarding standard precaution (Hand hygiene) in Rania city. Rania is a city located in the Slimani Governorate Kurdistan Region/Iraq. A non-probability purposive sample of (66) nurses at the Rania teaching hospital and maternity and pediatric hospital. Who were providing nursing services at hospitals, 10 of samples excluded for a pilot study. Through an extensive review of relevant literature, and previous studies a questionnaire was constructed by the researcher for the purpose of the study and it is used for data collection. The questionnaire consisted of three parts: 1\(^{st}\) part includes socio-demographic characteristics of nurses, staff, 2\(^{nd}\) part consists of (7) question about HCWs compliance regarding hand hygiene, and 3\(^{rd}\) part includes (18) items, which include (2) sections. Section (1) consists of (11) items, about knowledge of study samples regarding the hand hygiene, section (2) consists of (7) items, about the most appropriate timing of hand hygiene actions that prevent transmission of germs to the patient. The data were collected through the use of interview technique (face to face). Items were measured by using two levels of (Likert) scale and rating as, the Yes answer (2), No answer (1)\(^5\). 1999). To content validity of the tool was established through penal of (5) experts. Reliability of the questionnaire was determined through the use of (test-retest) technique by using Pearson’s coefficient of correlation formula. (\(r = 78\)). This means that the questionnaire format was adequately reliable as a tool for data collection. Data were analyzed through the application of descriptive statistical analysis, such as: (frequency, percentage, mean of scores, Pearson’s chi-square and correlation coefficient and independent sample test), in addition use the statistical package of social sciences (SPSS) version (20). The mean of the score (1 - 1.33) was considered a low level of knowledge, (1.34 - 1.67) considered a moderate level of knowledge, and (1.68 – 2) considered a good level of knowledge.

**Results**

Figure (1). When nurses worked with his/her colleague and he/she forgot to disinfect their hands before touching the patient.

The bar indicate when the nurses worked with his/her colleague and he/she forgot to disinfect their hands before touching the patient, in what percent do you remind your colleague. The bar chart shows that (28.57%) percentage of nurses about (75-100%) remind his colleagues, (21.43%) says they will remind them in a percentage between (25-50%). (33.93%) give a percentage between (50-75%). In addition to (8.93%) says I never be reminded him to do hand hygiene and (7.14%) give between (0-25%).
Table (1): Level of knowledge of the study sample regarding the hand hygiene action with (2) levels of scale and frequency, percentage, mean of scores, and comparative significant.

<table>
<thead>
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<th>No</th>
<th>Items</th>
<th>Yes</th>
<th>No</th>
<th>MS</th>
<th>Comparative significant</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>%</td>
<td>F</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Hand hygiene (HH) reduces chances of spreading infections</td>
<td>56</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>Perform HH by means of hand rubbing or hand washing</td>
<td>49</td>
<td>87.5%</td>
<td>7</td>
<td>12.5%</td>
</tr>
<tr>
<td>3</td>
<td>Handwashing: washing hands with plain or antimicrobial soap and water</td>
<td>53</td>
<td>94.5%</td>
<td>3</td>
<td>5.5%</td>
</tr>
<tr>
<td>4</td>
<td>Alcohol-based handrub formulation: an alcohol-containing preparation (liquid, gel or foam) designed for application to t for reducing the number of viable microorganisms on the hands</td>
<td>48</td>
<td>85.25%</td>
<td>8</td>
<td>14.25%</td>
</tr>
<tr>
<td>5</td>
<td>Wearing gloves did not replace the need for HH</td>
<td>36</td>
<td>64.25%</td>
<td>20</td>
<td>35.75%</td>
</tr>
<tr>
<td>6</td>
<td>Washing hands when visibly soiled, otherwise use hand rub</td>
<td>28</td>
<td>50%</td>
<td>28</td>
<td>50%</td>
</tr>
<tr>
<td>7</td>
<td>Duration of the washing hands procedure when visibly soiled 40-60 seconds</td>
<td>44</td>
<td>78.5%</td>
<td>12</td>
<td>21.5%</td>
</tr>
<tr>
<td>8</td>
<td>Duration of the hand rub procedure: 20-30 seconds</td>
<td>42</td>
<td>75%</td>
<td>14</td>
<td>25%</td>
</tr>
<tr>
<td>9</td>
<td>HH contributes significantly to keeping patients safe</td>
<td>55</td>
<td>98.25%</td>
<td>1</td>
<td>1.75%</td>
</tr>
<tr>
<td>10</td>
<td>Hand need to be dried after hand hygiene</td>
<td>42</td>
<td>75%</td>
<td>14</td>
<td>25%</td>
</tr>
<tr>
<td>11</td>
<td>Jewelry, ring, and watch should be removed during washing</td>
<td>47</td>
<td>84%</td>
<td>9</td>
<td>16%</td>
</tr>
<tr>
<td>12</td>
<td>HH must be done immediately before aseptic procedure</td>
<td>44</td>
<td>78.5%</td>
<td>12</td>
<td>21.5%</td>
</tr>
<tr>
<td>13</td>
<td>HH must be done after handling objects and device such as soiled linen, trash and equipment.</td>
<td>51</td>
<td>91%</td>
<td>5</td>
<td>9%</td>
</tr>
<tr>
<td>14</td>
<td>HH must be done after contact with blood, body fluids secretions or excretions, mucous membrane.</td>
<td>56</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

G.I= Good Knowledge M.I= Moderate Knowledge L.I= Low Knowledge M.S= Mean of score S= Severity

This table indicated that the mean scores were good for knowledge of study samples regarding the hand hygiene actions and most appropriate timing that prevent transmission of germs to the patient.
Table 2. The association between nurses level of knowledge regarding hand hygiene and gender

<table>
<thead>
<tr>
<th>Knowledge of nurses</th>
<th>Levene's Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>0.049</td>
<td>0.825</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table shows there is no significant association between knowledge of nurses regarding hand hygiene and gender at level of P value ≤ 0.05.

Table 3. The association between nurses level of knowledge regarding hand hygiene and years of experiences Pearson correlation

<table>
<thead>
<tr>
<th>Years of Employment</th>
<th>Knowledge of nurses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Correlation</td>
<td>1</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.631</td>
</tr>
<tr>
<td>N</td>
<td>56</td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>0.066</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>1</td>
</tr>
<tr>
<td>N</td>
<td>56</td>
</tr>
</tbody>
</table>

This table shows there is no significant association between knowledge of nurses regarding hand hygiene and years of employment at level of P value ≤ 0.05.

Discussion

Hand hygiene is one of the essential ways to prevent or reduce the spread of infection in the health care setting. Periodically assessing knowledge of nursing staff regarding hand hygiene is an important ways to provide them with update information. In relation to the sociodemographic characteristics of the nurses’ the results show more than a quarter percentages of the nurses their ages between (32-36) years old. More than two-third of the study sample were female, due to Rania city has two hospitals; (Rania teaching hospital and Rania maternity and pediatric hospital). Most of the nurses in maternity and pediatric hospital were female. Concerning the level of education, more than half percentage of the samples are graduated from the medical institute, at the same time only a small percentage of the nurses were graduated from nursing college 12%. Regarding the years of experiences, above the quarter percentage of nurses’ has (6 - 10) years, while only five percentages
of nursing staff have (16 – 20) years’ experience. The result is an agreement with the Alireza Sharif study, the result of the study shows most of the nurses (38.5%) had working experience 5-10 years. But another study done in Mozambique which titled (Cold chain management: Knowledge and Practice in PHC facilities) that one-third of workers had between (2 to 5) years which constituted 32%. Concerning source of information, more than 70% of nurses had a self-reading either from reading books or from internet to increase or update their knowledge about HH. In addition, more than half of nurses took the training course about HH. The hospital administration, always tried to help their nursing staff to participate in course training in order to increase the staff’s knowledge and experiences about hand hygiene. While the diagram 1 shows that the more than seventy percentage of the study sample routinely used hand hygiene either by washing hands with water and soap or use alcohol-based handrub, but nearly 30% did not do hand hygiene routinely. This is not acceptable in hospitals because maybe they become source of transmission of infection. Healthcare workers’ hands are the source for the carrying of pathogens from patient to another and within the healthcare environment. The health care worker commitment to the highest degree of practices remains low. The study also shows the reason that makes the nurses did not do hand hygiene due to they are too busy, out of product or the product are way or located in an inconvenient location to get in time. In addition, they forget to do HH sometimes. Even though these excuses are not reasonable for nurses or other health care worker, they need to do hand hygiene in order to prevent or reduce the transmission of infection in healthcare facilities. Health experts say poor hand hygiene is one of element in hospital-borne infections that kill tens of thousands of Americans each year. One every 25 patients in U.S. hospitals get a hospital-acquired infection as part of his or her care despite modest progress in controlling those pathogens inside medical facilities, the Centers for Disease Control and Prevention (CDC) reported in its most comprehensive look at a stubborn and dangerous health care problem. The result of this study also shows depicted that no significant association between knowledge of nurses regarding hand hygiene with gender and years of experiences. The mean knowledge score was not associated with gender (p=0.82), and years of employment (p=0.63). This may back to some reasons like the small percentage of study samples, and this is out of hand of the researcher due to Rania city has only two hospitals, and only nurses includes in the study. Another reason is the most of the study samples have the same level of education (most of them graduated from the institute). There is consistent with a study done about the knowledge of hand hygiene among the healthcare workers of two teaching hospitals in Mashhad. The result shows there was no significant difference in the knowledge level of the participants who had received formal training in hand hygiene and those who had not. Also, the mean knowledge score was not associated with age, gender, department, and the profession.

Conclusion

According to the results of the study, nurse have good knowledge regarding HH, but they are not routinely aware to use HH due to they are too busy or because out of disinfect product or they forgot to do that. In addition, most of them, they did not remind other to disinfect their hands before touching the patient in case they forget to do that. Finally, the findings of the study also show there is no significant association between nurses’ knowledge regarding HH with gender, and years of experiences.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, University of Raparin, and all experiments were carried out in accordance with approved guidelines.

References


Risk Factors Increasing Prevalence of Type 2 Diabetes Under the Age of 40 Years attending Al-Diwanyia Teaching Hospital

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¹Department of Community and Family medicine, University of Al-Qadisiyah, College of Medicine, Iraq

Abstract

The present case control study of Iraqi people living in Aldiwanyia city to identify the attributable risk factors that leads to type 2 diabetes in younger age groups and the correlation of the most prevalence risk for developing diabetes. The study was designed to be a case control study includes 315 individuals, 150 is the number of controls, and the rest of the participants are patients that have been diagnosed by diabetes mellitus type 2 before or at age of 40 years. All these participants attended outpatient clinics at Al- Aldiwanyia teaching hospital in Al-Dewanyah province/ Iraq. The beginning of data collection was dated on the January 2019 and ended on June 2019. Mean age of patients with diabetes mellitus was significantly higher than that of control subjects. There was highly significant difference in the frequency distribution according to marital status, occupation, residency, level of education, economic status in patients and controls groups. It appears that the rate of overweight and obesity is comparable in patient and control group.

Key wards: diabetes mellitus; risk factors; prevalence; Iraq; young age.

Introduction

Type 2 diabetes mellitus (T2DM) is a complicated disease, the starting age of subjects is as early as 15 years old. Diabetes is widespread among people, ages and regions of the sphere due to changes in standard of standards of living, inheritance and environmental factors which all together in the disorder. Type 2 diabetes is a serious chronic disease resulting from a complex inheritance environment interaction along with different risk factors such as high BMI and sedentary lifestyle. Type 2 DM and its complications comprise a major worldwide public health trouble, affecting populations in both developed and developing countries with high rates of diabetes related morbidity and mortality. Diabetes mellitus is a clinical syndrome characterized by hyperglycemia caused by absolute or relative shortage of insulin. Lack of insulin affects the metabolism of carbohydrate, protein and fat, and can cause considerable disturbance of water and electrolyte homeostasis; fatality may result from acute metabolic decompensation. Long-standing metabolic derangement is associated with functional and structural changes in numerous organs, predominantly those of the vascular system, which guide to the clinical ‘complications’ of diabetes. In both of the common types of diabetes, environmental factors interrelate with genetic vulnerability to establish which group develop the clinical syndrome, and its onset. However, the fundamental genes, precipitating environmental factors and path physiology vary considerably between type 1 and type 2 diabetes. The prevalence of diabetes is rising in epidemic magnitude on a global basis. In the USA alone, it has been estimated that there are around 16 million people diagnosed with DM, representing about 6% of the population. Diabetes mellitus is becoming progressively more prevalent in developing countries, possibly due in part to alter in dietetic habits, diminished physical activity, and increase BMI.

The worldwide prevalence of DM has risen considerably over the last two decades, from an estimated 30 million cases in 1985 to 415 million in 2017. Based on current trends, the IDF projects that 642 million individuals will have diabetes by the year 2040. It is estimated that the greater part of patients with diabetes obtain their care from a family doctor. The complexity and chronicity of diabetes presents special challenges for family physicians, whose chief responsibility is the screening and avoidance of diabetes-related complications.
Patient and methods

The type of the design used in this study was a case control. This study was done in the Republic of Iraq, Aldiwayia governorate, in Aldiwayia general teaching hospital. Patient selected randomly throughout their attendance to outpatient clinic of diabetes in the former hospital. The study included period from January 2019 to July 2019. The patient introduce to this study were in the age under 40 years old or has been diagnosed with diabetes below 40 of age, and the study is only for type 2 DM. An arranged questionnaire was prepared by collecting any possible risk factors and Verbal consent was obtained from participant in the study. All diabetic patient with type two who were under the age of 40 or have been diagnosed with DM before that age we exclude patient that have been diagnosed after the age of 40 years and the patient with type 1 DM. The ethical approval of this study include the following:

- Acceptance of Scientific Committee of Community and Family medicine department in college of medicine/university of Al-Qadisiyah and acceptance of Committee of Ethical Scientific Researches in the college
- Verbal consent of individual participated in the study

Results

The Distribution of diabetic patients and control subjects according to age and gender is shown in table 1. Mean age of patients with diabetes mellitus was significantly higher than that of control subjects, 49.93 ± 9.95 years versus 34.68 ±12.56 years, respectively (P <0.001). No diabetic patient was under 20, 5 (3.1 %) were between 20-29 years, 15 (9.3 %) were between 30-39 years, 51 (31.5 %) were between 40-49 years, 32 (19.8 %) were between 60-69 years and 4 (2.5 %) were between 70-79 years, table 1. The mean age at diagnosis was 36.85 ± 4.48 years with a range of 20-40 years.

Table 1: Distribution of diabetic patients and control subjects according to age and gender

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Control group n = 149</th>
<th>DM n = 162</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>4 (2.7 %)</td>
<td>0 (0.0 %)</td>
<td></td>
</tr>
<tr>
<td>20-29, n (%)</td>
<td>61 (40.9 %)</td>
<td>5 (3.1 %)</td>
<td></td>
</tr>
<tr>
<td>30-39, n (%)</td>
<td>40 (26.8 %)</td>
<td>15 (9.3 %)</td>
<td></td>
</tr>
<tr>
<td>40-49, n (%)</td>
<td>22 (14.8 %)</td>
<td>51 (31.5 %)</td>
<td></td>
</tr>
<tr>
<td>50-59, n (%)</td>
<td>14 (9.4 %)</td>
<td>55 (34.0 %)</td>
<td></td>
</tr>
<tr>
<td>60-69, n (%)</td>
<td>4 (2.7 %)</td>
<td>32 (19.8 %)</td>
<td></td>
</tr>
<tr>
<td>70-79, n (%)</td>
<td>4 (2.7 %)</td>
<td>4 (2.5 %) &lt;0.001 †</td>
<td>HS</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>34.68 ±12.56</td>
<td>49.93 ±9.95</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>17 – 78</td>
<td>23 - 72</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>57 (38.3 %)</td>
<td>77 (51.7 %) 0.099 ¥</td>
<td>NS</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>92 (61.7 %)</td>
<td>85 (57.0 %)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; HS: highly significant difference at P ≤ 0.01; NS: not significant at P ≤ 0.05
There was also highly significant difference in the frequency distribution according to occupation between patients and control groups (P < 0.001) in such a way that employee was less frequent in patients than in control group, 32.2 % versus 51.7 %, respectively, whereas the frequency of housewives and retired was more in patients than in control group, 45 % versus 32.9 % and 16.8 % versus 4%, respectively.

In addition, there was highly significant difference in the frequency distribution according to residency between patients and control groups (P = 0.005) in such a way that rural residency is more frequent in patients than in control group, 29.5 % versus 14.1 %.

Moreover, there was highly significant difference in the frequency distribution according to level of education between patients and control groups (P = 0.001) in such a way that illiterate and primary and secondary levels were more in patients than in control group, 32.9 % and 28.2 % versus 16.8 % and 16.1 %, respectively. Regarding economic status, there was also highly significant difference in the frequency distribution between patients and control groups (P < 0.001) in such a way that very poor and poor categories were more frequent in patients than in control group, 13.4 % and 45 % versus 1.3 % and 14.8 %, respectively. The comparison of body mass index (BMI) and waste circumference between diabetic and control groups is shown in table 3. Patients and control subjects were categorized into underweight (< 18.5), normal (18.5 -24.9), Overweight (25-29.9), class I obesity (30-34.9), class II obesity (35-39.9) and class III obesity (≥40 kg/m2), as shown in table 3. It appears that the rate of overweight and obesity is comparable in patient and control group; in addition, there was no significant difference in mean BMI between patients and control groups, 27.02 ±2.83 kg/m2 and 28.01 ±6.85 kg/m2, respectively (P = 0.091); however, mean waste circumference was significantly higher in diabetic patients than in control group, 105.85 ±14.26 cm versus 99.21 ±16.62, respectively (P <0.001).

### Table 2: Body mass index and waste circumference of diabetic patients and control subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group n = 149</th>
<th>DM n = 162</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m2)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight (&lt; 18.5)</td>
<td>1 (0.7 %)</td>
<td>0 (0.0 %)</td>
<td></td>
</tr>
<tr>
<td>Normal (18.5 -24.9)</td>
<td>28 (18.8 %)</td>
<td>34 (22.8 %)</td>
<td></td>
</tr>
<tr>
<td>Overweight (25-29.9)</td>
<td>81 (54.4 %)</td>
<td>99 (66.4 %)</td>
<td></td>
</tr>
<tr>
<td>Class I obesity (30-34.9)</td>
<td>33 (22.1 %)</td>
<td>27 (18.1 %)</td>
<td></td>
</tr>
<tr>
<td>Class II obesity (35-39.9)</td>
<td>4 (2.7 %)</td>
<td>2 (1.3 %)</td>
<td></td>
</tr>
<tr>
<td>Class III obesity (≥40 kg/m2)</td>
<td>2 (1.3 %)</td>
<td>0 (0.0 %)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean ±SD</strong></td>
<td>28.01 ±6.85</td>
<td>27.02 ±2.83</td>
<td>0.091 †</td>
</tr>
<tr>
<td>Range</td>
<td>17 - 78</td>
<td>21 – 35</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Waste circumference</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean ±SD</strong></td>
<td>99.21 ±16.62</td>
<td>105.85 ±14.26</td>
<td>&lt;0.001 †</td>
</tr>
<tr>
<td>Range</td>
<td>23 - 150</td>
<td>78 – 150</td>
<td>HS</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; †: independent samples t-test; HS: highly significant difference at P ≤ 0.01; NS: not significant at P ≤ 0.05
Prevalence rate of smokers was significantly higher in patients with DM in comparison with control subjects, 19.5% versus 12.1%, respectively (P = 0.048). However, there was no significant difference in prevalence rate of alcoholism between the two groups (P = 0.171), as shown in table 3.

Sedentary life style and low activity are significantly more prevalent in patients with diabetes than in control subjects (P = 0.002); in addition, with respect to physical activity, bed ridden prevalence was significantly higher in DM patients than in control group, 24.2% versus 2.7%, respectively (P < 0.001), as shown in table 4.

**Table 3: Bad habits in diabetic patients and control subjects**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group n = 149</th>
<th>DM n = 162</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No smoker</td>
<td>120 (80.5 %)</td>
<td>111 (74.5 %)</td>
<td>0.048 ¥ S</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>11 (7.4 %)</td>
<td>22 (14.8 %)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>18 (12.1 %)</td>
<td>29 (19.5 %)</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not alcoholic</td>
<td>145 (97.3 %)</td>
<td>160 (107.4 %)</td>
<td>0.171 ¥ NS</td>
</tr>
<tr>
<td>Ex-Alcoholic</td>
<td>1 (0.7 %)</td>
<td>2 (1.3 %)</td>
<td></td>
</tr>
<tr>
<td>Alcoholic</td>
<td>3 (2.0 %)</td>
<td>0 (0.0 %)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; ¥: Chi-square test; S: significant difference at P ≤ 0.05; NS: not significant at P ≤ 0.05

**Table 4: Life style and physical activity in patients with DM and control subjects**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group n = 149</th>
<th>DM n = 162</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life style</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>4 (2.7 %)</td>
<td>20 (13.4 %)</td>
<td>0.002 ¥ HS</td>
</tr>
<tr>
<td>Low active</td>
<td>117 (78.5 %)</td>
<td>124 (83.2 %)</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>28 (18.8 %)</td>
<td>18 (12.1 %)</td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bed ridden</td>
<td>4 (2.7 %)</td>
<td>36 (24.2 %)</td>
<td>&lt;0.001 ¥ HS</td>
</tr>
<tr>
<td>Moderate</td>
<td>114 (76.5 %)</td>
<td>98 (65.8 %)</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>25 (16.8 %)</td>
<td>22 (14.8 %)</td>
<td></td>
</tr>
<tr>
<td>Very active</td>
<td>6 (4.0 %)</td>
<td>6 (4.0 %)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; ¥: Chi-square test; HS: highly significant difference at P ≤ 0.01
Discussion

In the current study the mean age of patients with diabetes mellitus was significantly higher than that of control subjects. The mean age at diagnosis was 36.85 ± 4.48 years with a range of 20-40 years, which indicate significant relationship between DM and aging. This result has been supported by many studies worldwide including study have been done in UK, which prove that type 2 diabetes is more common in the middle-aged and elderly. It affects 10% of the population over 65, and over 70% of all cases of diabetes occur after the age of 50 years. Gender differences arise from sociocultural processes, such as different behaviors of women and men, exposition to specific influences of the environment, different forms of nutrition, life styles or stress, or attitudes towards treatments and prevention. In this study there is no significant relationship between gender and DM, the result have been supported by USA study that found no difference between male and female, study done in Pakistan revealed that there’s no difference in prevalence of diabetes mellitus with the gender. In Iran the prevalence of DM 12.1, with no significant difference between male and female, while the prevalence of diabetes in Saudi Arabia as demonstrated by Al-Nozhal show a higher ratio in females than in males, with 42%, and 37.2%, respectively. There was highly significant difference in the frequency distribution according to marital status between patients and control groups in this study in such a way that the frequency of married is more in diabetic than control which may be attributed to alteration of habitual life style. Study was done throughout Iranian urban population found that marital status was not significantly related to diabetes mellitus. In our study rural residency is more frequent in patients with DM, which has a similar result conducted from a cross sectional study using data from US centers for Disease and Prevention’s (CDC’s) Behavioral Risk Factor Surveillance, has found the increasing prevalence of DM in rural area more than urban that may be attributed to increasing poverty and lower education. highly significant difference in the frequency distribution according to level of education between patients and control groups. A case-cohort study in eight Western European countries nested in the EPIC study demonstrates the inequalities in the risk of T2DM in Western European countries, with an inverse relationship between educational level and risk of T2DM that is only partially explained by variations in BMI. Individuals within the middle socio-economic level, who are physically inactive and do not consume large amounts of fruit are at greatest risk of developing type 2 diabetes mellitus as explained by study has been done in Ghana. Individuals within the middle socio-economic level, who are physically inactive and do not consume large amounts of fruit are at greatest risk of developing type 2 diabetes mellitus as explained by study has been done in Ghana as well as in this study.

Conclusion

This study has been focused on the most important risk factors for the development of type 2 diabetes in young age patients and has assimilate other studies conducted throughout the world which puts the BMI, family history, lifestyle, hyperlipidemia, hypertension, and psychological insults in the top of these risks which necessitates the screening for diabetes in earlier ages. other risk must have further evaluation

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Al-Qadisiyah, College of Medicine, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Psychosocial Consequences of Children with Idiopathic Growth Hormone Deficiency in Baghdad

Adraa H. Shawq¹, Zaid W. Ajil¹
¹Lecturer, Pediatric Nursing Department, College of Nursing, University of Baghdad, Iraq

Abstract

Study aim: to evaluate psychosocial problems of children with growth hormone deficiency.

Methodology: A cross sectional study, of (80) children from both gender, (4 – 15) years diagnosed with idiopathic growth hormone deficiency, attending endocrine clinics in Baghdad city. Consent form taken from children and their guardians to participate in the study. Pediatric Psychosocial Symptoms Inventory (mood, behavioural, somatic, learning, and personality problems) was used to evaluate children psychosocial consequences. SPSS programme version 23 used for data analyses.

Result: children with growth hormone deficiency experience moderate psychosocial problems, especially personality and behavioural problems.

Recommendation: The researchers recommended details psychological assessment for children with growth hormone deficiency during their routine physical examination and provide suitable psychological support for them and their parent.

Keywords: psychosocial consequences, growth hormone deficiency

Introduction

Children with growth hormone deficiency usually followed up endocrine clinics for treated their short stature and improve their quality of life (¹, ², ³), they aware about their physical growth and feel with embarrassment about body differences from peers ⁴. As reported in the pediatric studies that children with health disorders may suffer from different negative psychosocial consequences ⁵. Those children and their parents influence by children`s short stature, in addition, to the burden of treatment regime (¹, ⁶). In Baghdad a study conducted in 2009 showed the prevalence of short stature only was 18.7%, while with underweight and short stature was 13.5% ⁷. The prevalence of short stature related to growth hormone deficiency was 1/4000 child and 60-80% of them with idiopathic causes ¹⁷. Many previous literature documented adverse effects of the short stature on children`s psychologically and socially ⁸.

Method and Materials

Research design: cross sectional study used, data was collected from first of May tile the first of November 2018.

Setting: study was carried out in outpatient endocrine clinics at two pediatric teaching hospitals and two specialized centres for endocrine disorders in Baghdad city.

Instrument of the study and procedure: Pediatric Psychosocial Symptoms Inventory PEPSI, parents reported scale, Arabic and English version. It has good reliability, developed from two famous pediatric psychosocial scales: strength and difficulties scale and child behavioral checklist scale by Dr. Al -Ayed and Al-Haider in their study about screening of children`s psychosocial problems in Riyadh ⁹. The approval obtained from the ownerships by electronic mail. PEPSI consists of (38) items, with five subscales: Mood symptoms subscale scored from (0-24), Behavioral symptoms subscale scored from (0-27), Learning problems subscale scored from (0-18), Somatic
symptoms subscale scored from (0-24), and Personality characteristics subscale scored from (0-21). Rating from 0-3: no symptoms= 0, rarely= 1, occasionally= 2, and frequently= 3. The total score ranged from 0-114 (0-38 = mild, 39-76 = moderate, 77-114 = severe).

**Statistical Analysis**

SPSS programme version 23 was used, frequency, percentage, and mean used in data analysis.

**Results**

Figure (1) Child’s Age and Gender Distribution

In these figures, the mean age of children with growth hormone deficiency is (8.41±2.7), most of them (75%) at school age (6-12) years, and mostly are boys (65%).

Table (1) Pediatric Psychosocial Symptoms Inventory for children with growth hormone deficiency

<table>
<thead>
<tr>
<th>Levels of scales</th>
<th>Study Group</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>23</td>
<td>28.75</td>
</tr>
<tr>
<td>Moderate</td>
<td>39</td>
<td>48.75</td>
</tr>
<tr>
<td>Severe</td>
<td>18</td>
<td>22.5</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>100.0</td>
</tr>
</tbody>
</table>

This table reflects that (84.75%) of children with growth hormone deficiency experience moderate psychosocial problems when assessed by PEPSI.

**Discussion**

The results of the present study showed that (48.75% and 22.5%) of children with short stature related to growth hormone deficiency experience moderate and severe psychosocial problems respectively. Their physical growth (height) interferes with social competence and body differences from normal peers which caused psychological disturbances. A study about psychosocial problems of children with growth hormone deficiency by using the Visual Analogue Scale for Children, support the present findings that those children showed negative self perception concerning physical appearance. Another longitudinal study about terminated treatment of adolescents with growth hormone deficiency showed that, their shortness affects negatively on their psychosocial status and their quality of life. Children with growth hormone deficiency suffer from psychosocial disturbances and their psychosocial functioning depending on various factors especially family support and school adaptation. Short stature associated with psychosocial distress and emotional deprivation. While another study about reviewing the psychosocial consequences of children with short statured that, those children showed negative social experiences but they have not psychosocial problems. However some studies mentioned the benefit of growth hormone treatment on their quality of life and self esteem after one year of successful treatment.

The present findings showed the aspects of children psychosocial problems according to pediatric psychosocial symptoms inventory PEPSI (learning, somatic, emotional, behavioural, and personality problems), in all aspects of children psychosocial problems children with growth hormone deficiency scored such problems. Mostly they scored behavioural...
and personality problems (60.4%, 65%). As parent reported their children have mood changes, fight with others, fidgety, tell lies, do not listen to rules, feel down, no hobbies, no friends, spend time alone, depend on others, and seem shy.

Most of children experienced teasing or juvenalization, these negative psychosocial consequences related to their shortness. Those children experienced behavioral and emotional problems, low self esteem. They exposure to bullying and could not adopt with their condition, the psychosocial burdens may also play role in developing short stature among some children. Short stature in children can lead to adverse psychosocial impact on those children and affects on their school performance and development of their personality. Children with short stature scored behavioural problems as a result of negative social relationships especially with their peers. Another study to measured behavioral and social problems of (195) children with short stature compared with normal children found that children with short stature experience higher score of school difficulties and behavioral problems. Children with short stature experienced different barriers in their personality development which affects negatively on their school and sport competence. Children’s psychosocial problems and school performance are the most concern of their parent.

As parent reported children with short stature have low social competence when compared with normal children, because of their unsatisfied appearance which affects on the social relationships and development their personality. Those children may face bullying in school or playing with younger children. A study in Japan about psychosocial problems of children with growth hormone deficiency by using Child Behaviour Checklist (CBCL) scale, the result of the study showed those children have higher scored than normal children, they also showed high scored on subscale of Child Behaviour Checklist scale such social problems, anxiety and attention. Previous studies showed school and psychosocial problems due to their appearance, they experience internalize problems and behavioral problems, and showed low health related quality of life. By using child behavior checklist scale for children with short stature, the findings showed those children scored high score of behavioral problems than normal developing children in addition to the somatic, attention, and social problems. In a study about reviewing children with short stature reported that those children scored lower in academic functioning, while another American study reported no differences form normal children. More than half of children with short stature experienced bullying and showed low quality of life. Children with growth hormone deficiency scored low neuropsychological test and impaired social relation. Those children recorded poor academic level due to effects of social relationships in spite of IQ score comparing with normal children.

Kranzler and his colleague in their study about psychosocial function of children with short stature that, those children scored externalize problems and inadequate social relationships. Children with growth hormone deficiency showed aggressive behavioral problems, attention, social, emotional problems and maladaptive. Prepubertal short children developed more social, internal, and external problems than children in the same age group. Children with short stature experience behavioral problems. Children with short stature scored low self esteem, bullying, juvenalization, and stigmatization because of their shortness. Previous studies reported those children have academic, internal, external, and behavioural problems due to their shortness. Those children have low quality of life. Psychosocial assessment of children with short stature is important in diagnosis and treatment of those children and well being. Some studies reported those children experienced low quality of life, and internal problems. Child health problems affects negatively on their parents.

Early children diagnosis and treatment reduce the risk of psychosocial problems and financial burden of treatment comparing with untreated children, in addition to the psychosocial advantages. Before treatment regimen the psychological profile should be assessed. The psychosocial services should also provide during treatment of children with growth hormone deficiency, those children experience sad feelings because of their shortness and puberty development delayed the psychological assessment of short adult showed some psychological disturbances. The setting and cultural attribute play a role in developing the psychosocial problems associated with certain health problems. The psychological health services will decrease and children well-being improved. Psychosocial improvement is one of the important goals for treated children with short stature.
Conclusion

Children with growth hormone deficiency were experience psychosocial problems, especially behavioral and personality problems.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of nursing, University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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Prevalence of Hepatitis B and Hepatitis C Viruses in β-thalassemia Major Patient in AD-Diwanya province, Iraq

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¹Family medicine specialty, College of Medicine/ University of Al-Qadisiyah

Abstract

Background: transmission of infectious agent is still the most common cause of death and disability related to blood transfusion.

Objective: to estimate the prevalence of HBV and HCV in beta thalassemic major patients in Ad-Diwayah governatorate.

Method: The current study is a retrospective cross sectional study involving sample of 80 thalassemic major patients who regularly visit thalassemia center in Ad-diwuryah for treatment, data that required in the study had been collected from patients, their guardians and from the records.

Results: The prevalence rate of HBV was 2.5% (1 male and 1 Female ) which was significantly associated with family history of hepatitis ; while the prevalence rate of HCV was 3.8% (1 male and 2 female) which was significantly associated with age and family history of hepatitis.

Key words: prevalence; hepatitis; thalassemia; major.

Introduction

Thalassemia syndromes are characterized by varying degrees of ineffective hematopoiesis and increased hemolysis 1. Clinical syndromes are divided into α- and β – thalassemias, each with varying numbers of their respective globin genes mutated. There is a widen array of genetic defects and a corresponding diversity of clinical syndromes. Most –thalassemias are due to point mutation usually in both of the two β-globin genes (chromosome11), which can affect every step in the pathway of β- globin expression from initiation of transcription to messenger RNA synthesis to translation and post translation modification . A mutation in a single β- globin gene inherited along with triplicated alpha genes also may cause a β-thalassemia syndrome. Autosomal dominant forms of β–thalassemia also occur rarely 1 . β-Thalassemia major is caused by mutations that impair beta chain synthesis. Because of unbalanced synthesis of alpha and beta chains, alpha chains precipitate within the cells, resulting in RBC destruction either in the bone marrow or in the spleen . β -Thalassemia major is seen most commonly in individuals of Mediterranean or Asian descent. The clinical severity of the illness varies on the basis of the molecular defect. Signs and symptoms of β-thalassemia major result from the combination of chronic hemolytic disease, decreased or absent production of normal hemoglobin A, and ineffective erythropoiesis. The anemia is severe and leads to growth failure and high output heart failure. Ineffective erythropoiesis causes increased expenditure of energy and expansion of the bone marrow cavities of all bones, leading to osteopenia, pathological fractures, extra medullary erythropoiesis with resultant hepatosplenomegaly, and an increase in the rate of iron absorption. Treatment of β-thalassemia major is based on a hyper transfusion program that corrects the anemia and suppresses the patient’s own ineffective erythropoiesis, limiting the stimulus for increased iron absorption. This suppression permits the bones to heal, decreases metabolic expenditures, increases growth, and limits dietary iron absorption. Splenectomy may reduce the transfusion volume, but it adds to the risk of serious infection. Chelation therapy with deferoxamine or deferasirox should start when laboratory evidence of iron overload (hemochromatosis) is present an before there are clinical signs of iron overload (non immune diabetes mellitus, cirrhosis, heart failure, bronzing of the skin, and multiple endocrine
When it is certain that they require regular transfusion, they should be given washed red cell transfusions at monthly intervals; it is vital that the blood is screened for human immunodeficiency virus (HIV)/acquired immune deficiency syndrome, hepatitis B and C viruses. Most death and disability related to blood transfusion worldwide is still caused by the transmission of infectious agents. Despite the availability of a highly effective vaccine against hepatitis B, approximately 2 billion people worldwide are infected, 350 million with chronic active infection accounting for (600,000) attributable deaths annually worldwide. Hepatitis B is spread via blood and body fluid contact through heterosexual and homosexual relations, by sharing of needles by infected drug abusers, and by accidental needle sticks in the medical setting. In areas of high disease prevalence (e.g., Southeast Asia, China), transmission is primarily from mother to child during childbirth or in early childhood. The vaccination for hepatitis B uses recombinant DNA, requires three doses on a set schedule, and confers immunity in the majority of recipients. Patients with chronic infection can develop cirrhosis and end-stage liver disease. Hepatitis C affects more than 300 million people worldwide. At least six genotypes and 100 subtypes have been identified. The diagnosis is established with serum testing for HCVRNA antibodies; although an antibody is induced, it is not protective against disease contraction and progression. Transmission occurs via blood or body fluid contamination through IV and intranasal drug use, blood transfusions, and in health care workers (e.g., needle stick or skin disruption with contaminated instrument).

Patients and Method

The study has been designed as a retrospective cross-sectional study including a cohort of Iraqi patients having beta thalassemia major on regular transfusion therapy. No limitation for gender or age was proposed. An 80 patients were randomly selected in the study from the population of thalassemic patient regularly visit the thalassemia center in AD-Diwaniyah for transfusion therapy (at least once monthly). Any patient had been diagnosed with HBV or HCV infection before starting the first transfusion had been excluded from the study, also family history of HBV or HCV prior to infection of thalassemic persons was one of the exclusion criteria. The study had been done at AD-Diwanyah thalassemia center in AD-Diwanyah governorate in Iraq. The study had been started from first of April 2019 and ended on the third of June 2019. The study had been approved by the committee of ethical approval at Collage of Medicine university of Al-Qadisiyah. Verbal consent had been taken from the patients who included in the study if their age was more than 18 years or from their guardians if they were younger than 18 years old. The questionnaire had been designed to involve sociodemographic informations of patients included in the study like gender, age, residency; if it was rural or urban area and occupation. Socioeconomic state also was part of the questionnaire, the patients classified as high, moderate and low socioeconomic state; taking in consideration their level of education, income, residency, number of family member comparing with number of rooms and the house size, also if the house was renting or their own.

Results

Distribution of patients with beta thalassemia major according to age

The frequency distribution of patients with beta thalassemia major according to age is shown in table 4.1. Patients less than 10 years old accounted for 29 (36.2%), patients between 10 to 19 years old accounted for 38 (47.5%), patients between 20 to 29 years old accounted for 8 (10.0%) and patients between 30 to 39 years accounted for 5 (6.2%). The mean age of all participants was 13.38 ±8.26 years with a range of 2 to 39 years, table 1. Majority of patients were between 10 to 19 years and <10 years of age, as shown in figure 1.

Table 1: Distribution of patients with beta thalassemia major according to age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 years, n (%)</td>
<td>29 (36.2)</td>
</tr>
<tr>
<td>10-19 years, n (%)</td>
<td>38 (47.5)</td>
</tr>
<tr>
<td>20-29 years, n (%)</td>
<td>8 (10.0)</td>
</tr>
<tr>
<td>30-39 years, n (%)</td>
<td>5 (6.2)</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>13.38 ±8.26</td>
</tr>
<tr>
<td>Range</td>
<td>2 – 39</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation
Distribution of patients with beta thalassemia major according to gender

The frequency distribution of patients with beta thalassemia major according to gender is shown in figure 2. Male patients accounted for 41 out of 80 (51.2 %), whereas, female patients accounted for 39 out of 40 (48.8 %). The male to female ratio was 1.05:1.

Distribution of patients with beta thalassemia major according to residency

Distribution of patients with beta thalassemia major according to residency is shown in table 2. Patients from urban areas accounted for 27 out of 80 (33.8 %) and patients from rural areas accounted for 53 out of 80 (66.2 %), table 2.

Table 2: Distribution of patients with beta thalassemia major according to residency

<table>
<thead>
<tr>
<th>Residency</th>
<th>*N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>27</td>
<td>33.8</td>
</tr>
<tr>
<td>Rural</td>
<td>53</td>
<td>66.2</td>
</tr>
</tbody>
</table>

*n: number of cases

The prevalence rate of hepatitis B and C viral infection in patients with beta thalassemia major

The prevalence rate of hepatitis B and C viral infection in patients with beta thalassemia major is shown in table 3. Two patients out of 80 were infected with HBV accounting for 2.5 %, while 3 patients out of 80 were infected with HCV accounting for 3.8 %, table 3.

Table 3: Hepatitis B virus and hepatitis C virus infection, positive family history of HBV and HCV and history of hepatitis vaccination

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>HCV</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td>Family history of HBV</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Family history of HCV</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Vaccine</td>
<td>80</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*n: number of case

Correlation between HBV or HCV infection and age of patients with beta thalassemia major

Mean age of patients with HBV were older than patients who were free of HBV infection, 20.00 ±2.83 years versus 13.21 ±8.29 years, respectively; however, the difference did not reach statistical significance (P = 0.253). Mean age of patients with HCV were older than patients who were free of HCV infection, 24.33 ±12.74 years versus 12.95±7.86 years, respectively; and the difference was statistically significant (P = 0.018), as shown in table 4.

Table 4: Correlation between HBV or HCV infection and age of patients with beta thalassemia major

<table>
<thead>
<tr>
<th>Hepatitis virus</th>
<th>n</th>
<th>Mean age (years)</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>20.00</td>
<td>2.83</td>
<td>0.253†</td>
</tr>
<tr>
<td>Negative</td>
<td>78</td>
<td>13.21</td>
<td>8.29</td>
<td>NS</td>
</tr>
<tr>
<td>HCV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>24.33</td>
<td>12.74</td>
<td>0.018†</td>
</tr>
<tr>
<td>Negative</td>
<td>77</td>
<td>12.95</td>
<td>7.86</td>
<td>S</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; †: Mann Whitney U test; NS: not significant at

Discussion

The current study had been designated in order to highlight the prevalence of HBV and HCV infection beta thalassemic patients in Ad-Diwanyah province since those patient need multi transfusion therapy for the rest of their lives, the fact that make them at higher risk to have blood transfusion transmitted infections, including HBV and HCV, and these infections can be transmitted to healthy persons by many routes other than blood transfusion and causing dangerous morbidity that reach hepatocellular carcinoma or even death. Also there is no other published study concerning about this topic in the mentioned area. Patients with beta thalassemia major had been targeted in the current study because those patients needs blood transfusion in a very early life time mostly in the first year of life so, if those patients had been infected with HBV or HCV, that would be most likely due to transfusion of contaminated blood.
Regarding age distribution in thalassemic patients who involved in the study, it was ranging from 2 to 39 years old with 83.7% of them less than 20 years old which could be due to high fatality rate of thalassemic patients comparing with general population. This result had been agreed by many other studies in the vicinity like Kamal Dumaidi and co-worker study about prevalence of hepatitis C and B viruses among patients with B thalassemia in Palestine which reveal that (75%) of patients involved in the study were aging 24 years and less. The mean of age in the current study was 13.38±8.26 year, this result was near but less than the mean age in the studies that done in the vicinity, as in the study that had been done in Iran by silos Mohammadi and Mazaher Khodabandehloo about the prevalence of HCV antibodies among Beta thalassemia major patients which revealed that the mean age was 18±8.05 years. Male to female ratio was 1.05:1 since the inheritance of thalassemia is autosomal which was agreed by Al-Naamani study in Oman. 66.2 % of patients who included in the study were living in rural areas. Regarding occupations of patients who enrolled in the current study; most of patients were either so small to attend school, students or having no job apart from single patient who was a nurse. Most of patients involved in the current study was classified as low or moderate socioeconomic state (87.5%) and the other 12.5% was classified as high socioeconomic state. 85% of patients’ parents were relative since most of them live in rural areas in which there is high rate of marriage between relatives. The rate of blood transfusion was ranging from 1 to 4 times per month patients with small age and patients who had splenectomy usually need transfusion 1-2 times per month while patients who didn’t have splenectomy and reaching adulthood or teenage, they usually needs transfusion 3-4 times per month. The prevalence rate of hepatitis B virus infection in patients participating in this study was 2.5% (two patients out of 80) which was less than the result of similar study done by Widad Yazaji in Syria which is a neighboring country, the study involved 159 patients, the study reveal that 13.2% of patients were seropositive for hepatitis B infection (21patients out of 159). The prevalence rate of HCV infection in the current study was 3.8% (3 patients out of 80) which was slightly higher than the prevalence rate of HBV infection which might be due to the availability of HB vaccine in the national vaccine program while there is no vaccine for HCV till the current day in the entire world. The prevalence rate of HCV infection in patients with thalassaemia in Zabol city of Iran; another neighboring country, was 8.5% which was higher than the current study which might be due to higher prevalence in general population in Iran (0.5%) comparing with Ad-Diwanyah population (< 0.3%). Regarding to relation of gender with HBV and HCV infection, there was no significant relation between them, this result going with study of Ansari et al. In the current study, there is no association between residency and infection with HBV and HCV. Also, no significant association between socioeconomic state and infection with HBV and HCV. This result had been agreed by Ghufranud Din’s et al study in Pakistan.

Conclusions

Prevalence of HBV and HCV infection is more in multitransfused beta thalassemia major patient than in the general population. Measures taken in the blood preparation and checking still not enough to prevent infections transmitted by blood transfusion. Risk of having HBV and HCV infection in multitransfused patients increase with progression of age. There was highly significant association personal and family history of HBV and HCV infection.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine/University of Al-Qadisiyah and all experiments were carried out in accordance with approved guidelines.

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Deposition of TaN Film on Commercial Pure Titanium Disk by Modified Reactive Plasma Sputtering Technique

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Abstract

Background: The new trend of implants is to find materials which accelerate bone formation at bone implant interface and improve Osseo integration to provide immediate or early loading after placement and eliminate waiting period which is uncomfortable and disturbs patients. Titanium as an implant material still need some improvement of surface properties physically and chemically. Tantalum which is gaining more attention as a new metallic biomaterial. Coating layer over implant is an important way for improvement of surface properties of titanium. Plasma used for surface modification, has several advantages such as changing surface topography, increasing surface roughness and in increasing the wettability of the surface.

Aim of study: To evaluate the effect of TaN coating by modified plasma sputtering technique of commercially pure titanium disk on wettability, surface roughness, surface chemical composition in comparison to non-coated surface.

Materials and method: Two groups were tested in this study which include non-coated commercial pure titanium disks and coated commercial pure titanium with TaN. Modified reactive plasma sputtering apparatus was used to coat CpTi with TaN at 4, 6 & 8 h. Surface characterization by x-ray diffraction (XRD) analysis, scanning electron microscope (SEM), energy dispersive spectroscopy (EDS), contact angle measurement, were carried out for coated and uncoated disks.

Results: The result of coating specimen with TaN at times (4, 6 & 8) h showed that 8 h coating time was the best time. And this was according to the results of X-ray diffraction analysis which show a new peak formation of TaN coated CpTi disk which was not present in non-coated CpTi disk. The results of wettability test for CpTi disk coated with TaN disk was more than wettability of non-coated CpTi disk. Surface Roughness was more and better distributed in CpTi coated with TaN disk than non-coated one which appear clearly in electron microscope.

Keywords: TaN film, pure titanium disk, modified reactive plasma sputtering technique

Introduction

Engineering and surface modification of dental implant material become much advanced than it was earlier ¹. Titanium shows a favorable combination of intrinsic properties for the fabrication of dental implants such as low specific weight, high strength to weight ratio, low modulus of elasticity, very high corrosion resistance, easy surface coating and excellent general biocompatibility ² enhancing the osseointegration of CpTi results in reducing the non-functional time period of the implant, increase its applicability in alveolar bone with low quality, cause minimum discomfort to patient, and minimize the failure rates. ³ Surface coating was one of the methods used in accelerating the Osseointegration process and this by increasing roughness and change surface properties physically and chemically. Chemically and physically reactive plasma discharges are widely used to modify the surface properties of materials. ⁴ The stable chemical element Tantalum (Ta) can exist in the surface layers of base materials. Ta is one of the promising materials used in medical and dental fields.
6. The stable TAN protective film can provide better corrosion resistance and bioactive property than that of TiO film. 7. The Ta components offer a low modulus of elasticity, high surface frictional characteristics, and excellent Osseo integration properties (i.e. Bioactivity and biocompatibility) 8. Surface-coated implants are reported to have less failure and can support heavier and more dynamic forces 9 surface coating with TaN by using plasma sputtering techniques was applied in this study on Cp Ti disk. In dental field especially in dental implant, plasma coating constitutes a simple, dry technique, which does not harm the environment, of low cost and does not comprise the intrinsic properties of the biomaterial, affecting only its surface. Many types of gases can be used in such technique to modify surface properties physically and chemically. Aim of our study to coat CpTi with TAN by using coat plasma sputtering technique.

**Material and Method**

**Sample preparation**

Titanium disks were prepared (10×5mm) diameter and thickness respectively which were cut from Cp Ti rod by using Bench Nibbling machine (TAURUS 7000-W6 CNC, Italy). All specimens were abraded successively using Si C grinding paper with different grits started from 80 grit, and continued by 120, 230, 400, 600, 800 and 1000 grit to get flat and scratch free surface. These disks were polished until a smooth and mirror polished surface was obtained. After that all specimens cleaned by using ultrasonic cleaning device with ethanol 99.8%.

**Surface coating with TaN:**

The procedure of sputtering was performed by using modify Dc glow discharge reactive plasma sputtering system. The total number of samples were 10 disk which divided as 1 uncoated and 9 coated disk. These coated disks were divided into 3 groups according to the time of coating (4, 6, & 8) h. The procedure of sputtering started with placing the clean and polished disks on the center of anode base. Then evacuating process of chamber to high vacuum (≈1x10⁻⁵mbar) by using high vacuum system which consist of rotary and turbo molecular vacuum pumps to ensure the complete removing of the heavy gases like hydrocarbons. After that Power supply negatively charged voltage of 3.5 kV applied. For sputtering process, the voltage was gradually applied using variac until the required energy achieved (applied voltage and current), The pressure was 2x10⁻² to 7x10⁻² mbar and this pressure was achieved by feeding the bombardment and reactive gases. The appropriate voltage and amber were adjusted precisely by regulator until intended sputtering glow (purple color, which standardized for each gas) is achieved. One of the most important modification done to convert normal plasma system to sputtering plasma system include replacement of electrode position, so the cathode placed in upper part and anode electrode placed in lower part of chamber. A Target (cathode) and anode stainless disk. The cathode faced the anode with 7 cm distance between them, which provides electric field for the gas to be discharged. The electrical electrodes and the associated dc-power supply of 5kV. The bottom of the stainless steel disk cathode electrode are covered with tantalum sheet which regarded as target. The clean and polished samples were placed on the anode in the center of base which regarded as substrate. All the samples were cleaned by argon plasma sputtering for 15 min prior to the TaN coating process by applying a bios dc voltage of 100 Von the anode. Parameters used for reactive plasma sputtering procedure: argon was used as bombardment gas and nitrogen as reactive gas. The process was carried out at various sputtering times (namely 4, 6 and 8 hours). The reactive gas nitrogen was introduced into the evacuated chamber and the flow rate was adjusted until the pressure was stabilized to the required pressure (1x10⁻² mbar), then the argon gas introduced to the chamber until the sputtering pressure of 5x10⁻² mbar achieved. After the sputtering process completed, the samples were kept until ambient temperature in the vacuum chamber was reached.

**Phase Analysis by using X-Ray Diffraction**

Surface analysis for Phase’s distribution was performed for non-coated CpTi, and CpTi coated with TaN. It done by using X-ray Diffraction Facilities (SHIMADzu 6000, Japan) using Cu Kα radiation. XRD analysis were performed at room temperature in the 2θ range from 30˚- 80˚ with a 0.05˚ step and counting time of 5 secs per step. The indexing of the data and the diffraction peaks were identified according to the powder diffraction files. (PDF), received from ICDD (Intimations’ Center for Diffraction Data).
Wettability test

In this test, non-coated and coated disk (10×5) mm diameter and thickness respectively were used. An equal amounts of normal saline (0.25ml) from graduated container dropped on each disk. Then after 20 second from putting the drop on disk, surface measurement for the angle formed between titanium disk surface and drop of normal saline done by taking a picture obtained from a digital camera.

Scanning electron microscope examination

All tested group were examined by using SEM (JEOL-JSM-5600). Samples were prepared as cross section and then mounted in double face metal tape (electrically conductive) to make the sample holder electrically conductive before entering the samples into the SEM Chamber. VEGA3 TESCAN, SEM HV: 20 KV, SEM MAG:7.50kx, VIEW FIELD:27.7

Energy dispersive spectroscopy analysis

Chemical structures and relative concentrations for non-coated CpTi and coated CpTi with TAN discs were assessed via energy dispersive spectroscopy (EDS). EDS use of the X-ray spectrum radiate a solid sample with a focused beam of electrons to obtain a localized chemical analysis. All elements from atomic number 4 (Be) to 92 (U) can be detected in principle. Qualitative analysis involves the identification of the elements in the spectrum and is fairly straightforward owing to the simplicity of X-ray spectra. Quantitative analysis determination of the concentrations of the elements.

Atomic force microscopy examination

Atomic force microscope able to detect both conductive and nonconductive surfaces on the atomic scale. The AFM relied on the scanning technique and provided a high-resolution 3D image from the surface of the sample. A sharp tip at the end of the cantilever is in contact with the surface of the development and the sample displaced with piezoelectric scanners. The force on the tip causes deflection to measure with tunneling capacitive or optical detectors such as interferometer laser in this technique, the standard pressure applied to the joint is zero (to prevent any surface deformation).

Result

Surface characterization by using X-Ray Diffraction.

A: Before Heat treatment:

XRD patterns of the non-coated Cp Ti specimen and TaN coated by modified reactive plasma sputtering after different sputtering time (namely 4, 6 and 8 hours) are demonstrated in Figure (1). And these data were indexed according to the Powder Diffraction Files (PDF) for the hexagonal αTi (JCPDS-ICDD file # 44-1294), TaN (JCPDS-ICDD file # 25-0922, 33-1391and 33-1390), and TaN (JCPDS-ICDD file # 34-0977). The diffraction peaks of the uncoated CpTi were found to be corresponding to (100), (002), (101), (102), (110), (200), (112) and (201) α-Ti at 2θ values 35.00°, 38.30°, 40.05°, 52.9°, 62.8°,70.6°, 76.25°and 77.35° respectively. The patterns of the plasma sputtered specimen for 4 and 6 hours show wide peak in the range of 2θ 20-30° which seems to be due to the formation of not fully crystalline TaN, whereas the pattern of the specimen sputtered for 8 hours show clear and prominent TaN peaks corresponding to the reflections (111) at 2θ 35.14 and (200) at 2θ 41.24°. Also its very clear there are a shift in the 2θ position of the CpTi reflections towards the higher 2θ indicting the change in titanium crystal cell volume.

B: After Heat treatment:

The CpTi specimen’s coated with TaN for sputtering time 8 hours before and after heat treatment are shown in Figures (2). The pattern of the heat treated specimen show prominent new peaks of 111 TaN at 2θ 36.00 and
developed one assigned as 200 TaN at 2θ \( \text{o} \) 41.24. also The XRD pattern of the heat treated specimen shows the formation of 110 of tantalum peak TaN at 2θ \( \text{o} \) 27.23.

The XRD pattern of the heat treated specimen shows the formation of 110 of tantalum peak TaN at 2θ \( \text{o} \) 27.23.

Figure (2): XRD patterns of uncoated Cp Ti and coated with TaN for 8 hours before and after heat treatment.

Surface characterization using Wettability test

From the result of wettability test, 8 hr. coated disk with TaN give more hydrophilic surface feature with contact angle formed less than non-coated disk as shown in Figure (3).

<table>
<thead>
<tr>
<th>Material/Time</th>
<th>Cp Ti</th>
<th>TAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Non coated</td>
<td>60°</td>
<td>75°</td>
</tr>
<tr>
<td>B: 4h coating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C: 6h coating</td>
<td>65°</td>
<td></td>
</tr>
<tr>
<td>D: 8h coating</td>
<td>55°</td>
<td></td>
</tr>
</tbody>
</table>

Fig (3) Wettability test for CpTi and experimental groups at 4.6 and 8hr

Material/Time

- A: Non coated
- B: 4h coating
- C: 6h coating
- D: 8h coating

Surface characterization using SEM 3.3

A: Topographic

The scanning electron microscopy images of CpTi disk before coating in (figure 4a) revealed a relatively flat and smooth surface. While the surface morphology of CpTi plasma sputtering samples with TaN for 8 h showed a fully arranged nanochips and uniformly distributed in (figure 4b).

B: Chemical composition by EDS

Regarding the analysis of the chemical structure of CpTi figure (5, A) show two titanium peaks with these values (\( \kappa \alpha 4.512 \) and \( \lambda \alpha 0.452 \)), these values represent alpha phase of titanium. For coated disk with TaN, there were five peaks, two peaks for titanium which represent alpha phase, two tantalum peaks and one peak of nitrogen with following values percentage (\( \kappa \alpha 4.512 \) and \( \lambda \alpha 0.452 \) (\( \lambda \alpha 8.146 \) and \( \mu \alpha 1.712 \) (\( \kappa \alpha 0.392 \)) respectively as shown in figure (5, b). Elemental composition of tested group as follow in (fig a) titanium was 100%, and in (fig b) percentage of titanium was 3%, tantalum was 22% and nitrogen was 75%.

A: CpTi disk B: CpTi disk coated with TaN

Fig (5): EDS.A/ CpTi disk without coating B/CpTi disk coated with TaN for 8 hr.

Discussion

The new trend in implant research is to increase and accelerate the osseointegration of Cp Ti\(^{11}\). One of the methods used in accelerating the osseointegration process is to modify the surface properties of the implant\(^{12}\). Chemically and physically reactive plasma is widely
used to modify the surface properties of materials. In this research, tantalum is a material with specific properties which is used as a coat material with nitrogen gas to develop TaN surface on CpTi disk by using modified plasma sputtering technique which has several advantages such as changing surface topography, increasing surface roughness, wettability of the surface. Reactive plasma coating constitutes a simple, dry technique, which does not harm the environment, does not comprise the intrinsic properties of the biomaterial.

The results of X-ray diffraction analysis revealed a new peaks formation towards the 20 direction in coated disk as a result of deposition of TaN material on the surface of CpTi, these new peaks were more prominent after heat treatment in coated disk as evidence to the more crystallinity of TaN. Surface properties and roughness of uncoated CpTi and TaN coated disk for 8 h were examined by Scanning electron microscope, showed fully arranged nanochips with uniform shape in coated layers, this might be due to difference of chemical composition of coated layer of non-coated and TaN disk, this shape of nanochips due to the deposition of TaN on the surface of CpTi. Contact angle of fluid drop in 8 hr. CpTi coated disk with TaN less than non-coated CpTi disk, which mean the coated disk more hydrophilic than non-coated disk. This may be due to more affinity of tantalum and nitrogen to fluid.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of dentistry. University of Karbala and all experiments were carried out in accordance with approved guidelines.

References
Influence of Carcinogenic Substance (7, 12 Dimethylbenz [A] Anthracene (DMBA)) on Tissue, Hematology Character and Enzyme Activity in Rat

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Abstract

This study designed to investigate the effects of 7,12 dimethyl benz[a] anthracene (DMBA) on some blood parameters and hepatic histopathology in rats and antioxidant enzyme. twenty female Wistar albino rats, weighing 180–200 g, were randomly divided into two group 1- DMBA group (positive group) who received 20 mg DMBA/kg body weight/ (single Douse) 2-Control group (negative group) doesn’t receive any item. The animals in these groups were sacrificed at the end of the 90 days: The histological structure of the liver tissues in the control group was normal. the liver exhibited hydropic degeneration and coagulation necrosis in hepatocytes, severe dilation in the sinusoids, congestion in the central and portal regions, DMBA groups, was degenerative and necrotic changes were detected. MDA levels increased in the DMBA group, compared to the control group, Antioxidant activity CAT, SOD and GPX, the results showed increased enzyme activity in positive control compared with negative groups, the hematological parameter was increase in WBC, PLT, GRA, and LYM and decrease in RBC value.

Keywords: 7,12-dimethylbenz[a]anthracene (DMBA); Blood parameters; Antioxidant enzyme ; MDA ; Rat.

Introduction

Malignant neoplasm, is that diseases in which a crowd of cells illustrate abnormal growth, invasion and sometimes called metastasis 1. This disease begins in the moment that cells in body start to grow without control 2. More than 100 kinds of neoplasm are identified 3. The stimulation of tumors is named carcinogen, that have the capacity to change DNA in behavior that are inherited by daughter cells which called mutagens. Most of these tumors are associated to ecological, life, or expected behaviors 4. The statement “ecological”, as used by cancer investigators, means everything outside the body that reacts with humans 5. On the other hand some chemicals, like benzene, ketones, vinyl chloride, ethylene bromide, and dichlorodiphenyl-trichloroethane (DDT), known as carcinogens 6. When multiple factors are accumulated, altered genes and carcinogens change a normal cell into a cancerous one. Carcinogens which promote cancer have various precursors 7. exogenous and endogenous factor lead to cause Cancer development 8 such as nutritional habits (food preservation and preparation), socio-economic status, way of life, physical agents (ionising and non-ionising radiation), chemical compounds (natural and synthetic) and biological agents (Helicobacter pylori, Epstein Barr virus, human T lymphotropic viruses I and II, human papilloma virus and the hepatitis B virus 9.

Material and Method

Experimental animals and Tumor induction in rats:

Twenty female of Wistar albino rats and aged between (7-8) week were weighing between 150 – 250 g used in this study. All they were kept in ventilated cages, with temperature of 25±2°C. A 12:12 h light:dark cycle is regulated for the animals. Balanced rodent food and water is provided. The rats were randomly assigned to 2 groups, every group of 10 rats.
The following groups:

Group 1: Taking one dose of DMBA (positive control) 20 mg DMBA/kg body weight/ (single dose)

Group 2: Did not received any treatment (negative control).

DMBA administration: breast cancer that induced by 7, 12-

Dimethylbenz (a) anthracene (DMBA) \(^\text{10}\). One dose of DMBA dissolved within corn oil and given orally to each groups with the syringe and needles. DMBA was taken from Sigma Aldrich and melted in corn oil. The concentration of the mixture was 20 mg DMBA per 1 ml corn oil for each rat \(^\text{10}\).

**Preparation of tissue sample**

The liver was exteriorized and excised. All samples in the same time fixed in 10% formaldehyde. And then they processed in known method, and placed in paraffin for histopathological testing for liver. A scoring system (of no abnormalities, mild, moderate, severe) was used to classify the liver changes according to the severity of the damage and extent of histological changes. The histological sections were evaluated by a pathologist without prior knowledge of the treatment given to the animals \(^\text{11}\).

Blood parameters were determined in whole blood by the applying rat method of veterinary practice with a blood cell counter (Abocus Junior Vet-5, Austria). Measurements of biochemical parameters were made with a Modular PP autoanalyzer (Mindray BS800, China).

**Result**

Histopathological findings: In the DMBA group, the livers exhibited dilatation of the sinusoids, cholangiohepatitis in the portal region, and congestion in the sinusoidal and portal regions (Figure 1A). The control group showed normal histological structure in the liver tissue (Figure 1B).

Hematological parameters also change in negative group compared of positive group Table 1, in positive group: white blood cell (WBC), LYM, MON, GRA and PLT while other parameter such as RBC was decrease. The state of free radicals and antioxidants is given in Table 2. As seen in Table 2, the levels of MDA increased in liver groups in compare with control group, also SOD, GSH-Px, CAT, and GSH values decreased.

**Figure 1:** A: DMBA group showing cholangiohepatitis in portal region, mild dilation in sinusoids, hyperemia, and hydropic degeneration in hepatocytes;

B: manage group viewing liver tissue with a normal histological structure

**Table 1. Some hematological parameters in the groups (values are mean n=4)**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Negative control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WBC (10^3 /mm^3)</td>
<td>8.2</td>
<td>15.1</td>
</tr>
<tr>
<td>2</td>
<td>RBC (10^3 /mm^3)</td>
<td>8.8</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>LYM %</td>
<td>67%</td>
<td>78.5 %</td>
</tr>
<tr>
<td>4</td>
<td>MON %</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>GRA %</td>
<td>18.8 %</td>
<td>94 %</td>
</tr>
<tr>
<td>6</td>
<td>MCV (fl)</td>
<td>56.6</td>
<td>58.7</td>
</tr>
<tr>
<td>7</td>
<td>MCH (Pg)</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>MCHC (g/dl)</td>
<td>32.3</td>
<td>32.8</td>
</tr>
<tr>
<td>9</td>
<td>PLT (10^5 /mm^3)</td>
<td>338</td>
<td>737</td>
</tr>
</tbody>
</table>
Table 2. Some biochemical parameters in the groups (values are mean n=4)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Conc. of GPx(IU/mg)</th>
<th>Conc. Of SOD(IU/mg)</th>
<th>Conc. of CAT(IU/mg)</th>
<th>Conc. of MDA(nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative group</td>
<td>127</td>
<td>458</td>
<td>122</td>
<td>134</td>
</tr>
<tr>
<td>Positive group</td>
<td>312.3</td>
<td>567</td>
<td>187</td>
<td>267</td>
</tr>
</tbody>
</table>

Discussion

7, 12-dimethylbenz (α) anthracene (DMBA) is a famous carcinogen and immunosuppressor used in rodent model to learn tumor. DMBA is report to make mutations by creation DNA adducts, while, it is a well known skin carcinogen, however lots of researchers have reported the harmful result of DMBA in liver. Liver is the main site of metabolism and is frequently prone to injury by xenobiotics. obviously, liver cancer is the second most general source of cancer deaths universal.

a number of haematological and haematochemical parameters were altered when treated with DMBA and establish that DMBA caused hepatocellular carcinoma. new studies showed that DMBA-induced skin, oral, mammary and ovarian tumors.

The carcinogenic and mutagenic effect of DMBA needs to its metabolic activation by mixed gathering oxidases. The hydroxylation of DMBA at 7-methyl collection is a critical step towards its carcinogenesis. additional metabolism of DMBA leads to development of a large range of metabolites with changeable toxicity. along with these, trans-3,4-dihydrodiol-1,2-epoxide is the carcinogenic result of DMBA. The metabolic products of DMBA, when present within body, hampers ROS-antioxidant balance by overproduction of free radicals and the body go round reacts by modulating activities of antioxidant enzymes to control the destructive effects of an enlarged ROS.

Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, area, season, environmental heat, maintenance, and nutrition. a significant increase in the GPX, CAT, SOD level in rat treated with DMBA only was reported by. On other hand find MDA substance increase significantly with rat treated with DMBA only.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Warith Al-anbiya’a, Iraq, Iraq and all experiments were carried out in accordance with approved guidelines.

References
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Immunohistochemical Expression of CD68, P53 and Bcl2 in Thyroid Tumors

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Abstract

Background/aim: The aim of this study was to determine the expression of CD68, P53 and Bcl2 in thyroid tumors and correlation between them.

Materials and Method: The expression of CD68, P53 and Bcl2 was examined by immunohistochemistry on paraffin-embedded tissues obtained from patients with benign and malignant tumors between 2010 and 2015. Thirty three malignant thyroid tumors of variable subtypes and 10 benign tumors. The extents of staining and intensity were scored semi quantitatively.

Results: CD68 have shown strong positive score expression in (28.9%) in PTC while score was found in (18.5%). The higher percentage of strong weak positive score expression of P53 was in PTC (6.1%) Followed by (3.03%) strong score in FTC, (3.03%) moderate score in benign and (3.03%) in MTC. The expression of Bcl2 was higher in benign tumors (20%) than in malignant tumors were PTC (9.1%) followed by FTC (3.03%), the strong positive expression seen in MTC (9.1).

Keywords: Thyroid tumors, Immunohistochemistry, CD68, P53, Bcl2.

Introduction

The commonest type of endocrine malignancy is thyroid cancer, although it’s rare. Thyroid cancer accounting for about 1.5 of all newly diagnosed cancer in the United States, its occurrence being steadily increasing worldwide in the last three decades 1. It is categorized into three main histological types: differentiated including “papillary, follicular and hurthle» «medullary» and «anaplastic» (aggressive undifferentiated tumor) 12. Papillary thyroid carcinoma (PTC) represents 80.3% of malignant endocrine tumors and 65% of malignant thyroid tumors (12, 14) According to Iraqi cancer registry, it ranks the eight cancers from commonest ten cancers in general and the second cancer in female (Iraqi cancer registry, 2019). Combined with there are different cellular and molecular factors that plays a role in development and progression of thyroid cancer. One of the main cellular constituents in the stromal of many cancers is “tumor-associated macrophages” (TAM) 20. Advanced staging and poor prognosis in many human cancers including thyroid cancers related to tumorigenic role of “TAM” 17. TAMs have an essential role in tumor advancement at different levels, including stimulating genetic instability, nurturing cancer stem cells pavement the way to metastasis , taming defensive adaptive immunity 1. Transformations in the p53 gene are the most widely recognized in human cancer 11 and they express to the most frequent genetic changes in malignant transformation. P53 protein assumes an essential role in the regulation of the cell cycle. Wild sort p53 protein is capable of inhibiting cell proliferation and transformation and it has been observed to be latent in tumor cells 13. This gene mutation is the most prevalent genetic alterations in human tumors and has been found in over 15% of thyroid neoplasm. The p53 gene mutations are promoting more aggressive cancers. Bcl2 protein is a modular of programmed cell death and is involved in both lymphoid and epithelial malignancies. The reporting of bcl2 protein expression in thyroid cancer has been sporadic and some have shown a down regulation of bcl2 in papillary carcinomas 5. The present study aimed to evaluate level of CD68, P53 and Bcl2 expression in both malignant and benign thyroid
tumors, and to correlate the results with the variable clinicopathological parameters (taking in respect the age and sex of the patients combined with tumor types histopathological).

**Materials and Method**

Our study involved paraffin-embedded tissue blocks samples of 43 thyroid tumors patients, of which 33 thyroid carcinomas and 10 benign as control group. All these samples were collected from the Pathology Department of Teaching Laboratories /Medical city and Central Public Health Lab between the years 2010-2015. All Hematoxylin and Eosin stained tissue sections were reviewed by pathologist. Immunohistochemistry study was prepared on serial sections and the antibodies panel that has been used is shown in the table below (table 1).

**Table 1: Antibodies used for immunohistochemical study**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Code</th>
<th>Clone</th>
<th>Antigen retrieval</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD68</td>
<td>M 0814</td>
<td>KP-1</td>
<td>pH.9</td>
<td>1:50-1:100</td>
<td>Dako/Denmark</td>
</tr>
<tr>
<td>P53</td>
<td>M 7001</td>
<td>DO-7</td>
<td>pH.9</td>
<td>1:25-1:50</td>
<td>Dako/Denmark</td>
</tr>
<tr>
<td>Bcl2</td>
<td>M 0887</td>
<td>124 clone</td>
<td>pH.9</td>
<td>1:50-1:100</td>
<td>Dako/Denmark</td>
</tr>
</tbody>
</table>

CD68, P53 and Bcl2 immunohistochemical staining.

Immunohistochemistry: Four µm sections were cut from formalin-fixed, paraffin-embedded tissue blocks and placed on polylsine-coated glass slides. After overnight packing at 65°C, tissue sections were deparaffinized in xylene and rehydrated in descending grades of alcohol. Antigen retrieval was performed in a water bath for 15 min in 95 °C citrate buffer pH 9.0 for CD68, P53 and Bcl2. Endogenous peroxidase action was blocked by 3% hydrogen peroxide. The sections were incubated at room temperature for 1 hour with primary monoclonal antibodies.

Scoring of immunohistochemistry: The stained slides were observed microscopically by histopathologist using the p53, Bcl2 and semiquantitative criteria: 0 = negative; 1+ = <33% area of positive staining; 2+ =34-66% area of positive staining; 3+ = >67% area of positive staining. Positive staining was also graded on intensity, 0 to 2+. A combined score of 0 to 6 was allocated. Tumors assumed a score 0 to 1 were classified as negative; those given a score of 2 were ordered as weakly positive; a score of 3-4 was regarded as moderately positive; and a score of 5-6 was considered strongly positive.

Statistical Analysis

Data were analyzed by one -way analysis variance (ANOVA) followed by Fisher’s test for multiple comparison, using Statview version 5.0. Differences were considered “significant when p<0.05”. Reversion analysis was done by analysis of covariance (ANCOVA) likewise by Statview version 5.0.

Results and Discussion

**CD68**

In this study, the results of immunohistochemical analysis for CD68 have shown in table 2, 3 and 4. As shown in table 2 there significant correlation between CD68 with thyroid patients age less than (45 years) (P=0.01) and (P=0.0001) with thyroid patients age more than 45 years.

In table 3 and 4 the results recorded depended on scores weak positive and strong positive. There is significant difference between score of CD68 and gender (P. value: 0.058). (Table 3)

Score of CD68 showed associated with gender in table 2, from total 29 female were (20 cases, 98.9%) weak positive and (9 cases, 31%) strong positive while
from 9 male were (5 cases, 55.6%) weak and (4 cases, 44.4%) strong positive.

Table 4 showed CD68 score related with tumor type, PTC was appeared high associated with strong positive (11 cases, 28.9%) followed by MTC and Hurthle cell carcinoma (1 case, 2.6) in same score. While the weak score was found in (7 cases, 18.5%) in benign tumors, (10 cases, 26.4%) in PTC, (3 cases, 7.9%) in FTC and MTC and finally (1 case, 4.5) in ATC.

**Table 2: Distribution of thyroid carcinoma patients in relation with CD68 protein in immunohistochemical (IHC) method according to their age, gender and tumor type**

<table>
<thead>
<tr>
<th>factor</th>
<th>CD68 +</th>
<th>CD68 -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age total N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥45 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female 33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor type N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant 33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histotype 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary Ca 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular Ca 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medullary Ca 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaplastic Ca 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurthle cell 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Immunohistochemical score of CD68 related with gender**

<table>
<thead>
<tr>
<th>Sex (+ve n)</th>
<th>CD68 IHC score</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (+ve n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. %</td>
<td>No. %</td>
<td>Total</td>
</tr>
<tr>
<td>Female 29</td>
<td>20 69</td>
<td>9 31</td>
</tr>
<tr>
<td>Male 9</td>
<td>5 56</td>
<td>4 44</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>75</td>
</tr>
</tbody>
</table>
Table 4: Immunohistochemical score of CD68 related with tumor type

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>CD68 IHC score</th>
<th>POSITIVE N (38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;25 weak</td>
<td>≥25 strong</td>
</tr>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Benign 7</td>
<td>7 18.5</td>
<td>0 0</td>
</tr>
<tr>
<td>Malignant</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Papillary Ca 21</td>
<td>10 26.4</td>
<td>11 28.9</td>
</tr>
<tr>
<td>Follicular Ca 3</td>
<td>3 7.9</td>
<td>0 0</td>
</tr>
<tr>
<td>Medullary Ca 4</td>
<td>3 7.9</td>
<td>1 2.6</td>
</tr>
<tr>
<td>Anaplastic Ca 2</td>
<td>2 5.4</td>
<td>0 0</td>
</tr>
<tr>
<td>Hurthle cell 1</td>
<td>0 0</td>
<td>1 2.6</td>
</tr>
</tbody>
</table>

P53

Expression of P53 was related with age group, gender and tumor type summarized in table 5 Of 26 patients were less than 45 year, only (5 cases, 11.6%) were positive. These 5 cases were 4 female and 1 male which of them 1 benign tumor case, (2, 6.1%) PTC, (1, 3.03%) FTC and (1, 3.03%) MTC.

Table 6 showed significant difference between score of P53 and gender (p.value:0.001).

The results in table 7 show that high percentage of strong score was in PTC (2 cases, 6.1%), followed by 1 case 3.03% strong score in FTC, 1 case 3.03% moderate score in benign and 1 case 3.03% in MTC.

Table 5: Distribution of thyroid carcinoma patients in relation with P53 protein in immunohistochemical (IHC) method according to their age, gender and tumor type

<table>
<thead>
<tr>
<th>factor</th>
<th>P53+</th>
<th>CDP53 -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age total N</td>
<td>N %</td>
<td>N %</td>
</tr>
<tr>
<td>&lt;45 26</td>
<td>5 19.2</td>
<td>21 80.8</td>
</tr>
<tr>
<td>≥45 17</td>
<td>0 0</td>
<td>17 100</td>
</tr>
<tr>
<td>Sex N</td>
<td>N %</td>
<td>N %</td>
</tr>
<tr>
<td>Female 33</td>
<td>4 12.1</td>
<td>29 87.9</td>
</tr>
<tr>
<td>Male 10</td>
<td>1 10</td>
<td>9 90</td>
</tr>
<tr>
<td>tumor type N</td>
<td>N %</td>
<td>N %</td>
</tr>
<tr>
<td>benign 10</td>
<td>1 10</td>
<td>9 90</td>
</tr>
<tr>
<td>malignant 33</td>
<td>4 12.1</td>
<td>29 87.9</td>
</tr>
<tr>
<td>histotype 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Papillary Ca 22</td>
<td>2 6.1</td>
<td>20 60.6</td>
</tr>
<tr>
<td>follicular Ca 4</td>
<td>1 3.03</td>
<td>3 9.1</td>
</tr>
<tr>
<td>medullary Ca 4</td>
<td>1 3.03</td>
<td>3 9.1</td>
</tr>
<tr>
<td>Anaplastic Ca 2</td>
<td>0 0</td>
<td>2 6.1</td>
</tr>
<tr>
<td>Hurthle cell 1</td>
<td>0 0</td>
<td>1 3.01</td>
</tr>
</tbody>
</table>
### Table 6: Immunohistochemical score of P53 related with gender

<table>
<thead>
<tr>
<th>Sex</th>
<th>P53 IHC score</th>
<th></th>
<th></th>
<th></th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>score 0 (-ve)</td>
<td>2 weak</td>
<td>3 moderate</td>
<td>4 strong</td>
<td>Total</td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>---------</td>
<td>-------------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>28</td>
<td>84</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>9</td>
<td>90</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>13</td>
<td>6</td>
<td>6</td>
<td>200</td>
</tr>
</tbody>
</table>

### Discussion

The host’s immune response to the tumor is represented by macrophage infiltrates in the context of or surrounding a variety of malignancies (4,7). The “tumor-associated macrophages” (TAMs) had fundamental jobs in tumor development and metastasis of various cancers, including advanced thyroid malignant growth (17). This study demonstrated the presence of CD68+ in benign and malignant thyroid tumors. But all (7 cases, 18.5%) of CD68+ in benign were in weak score. While (11 out of 21) 28.9% from PTC were strong positive score which came in similar with Qing et al.,(2012) who investigated TAM density in both benign thyroid tumors and PTC by CD68 immunostaining. They establish that overall density of TAM was higher compared with “thyroid goiter” and “follicular adenoma”. On the contrary to previous studies, (17) pointed to the expression of TAM in 36 PTC Patient with L.N metastasis using immunohistochemical staining with anti-CD68 antibody. And Ryder et al.,(2008) who recorded a higher TAM thickness in ineffectively separated PTC and ATC This different was explained by the mixed cell populates of macrophage with CD136 ,CD68 and undifferentiated monocyte/macrophage which are for all positive for CD68 ,these kinds of macrophages play special roles in the advancement and progression HNSC (18). Other study by (10) using CD68 immunohistochemistry, observed that TAM anticipated metastasis in a number of patients. Of 121 patients with PTC, 15% had TAMs (CD68) that seemed to have a phagocytic capacity on cancerous cells. These patients had fundamentally less blood vessel attack and remote metastasis and largely supplementary invasion lymphocyte and dendritic cells, than patients without a CD68 cells. Further, the findings of the current study are in similar with other study by (21) who observed expression of p53 was more frequently in malignant than in benign lesions. Since p53 has been known to be rarely mutated in well differentiated tumors of the thyroid where an frequently expressed in poorly differentiated or undifferentiated tumors which are of different number, in our study, however there is a concern of tumor suppressor gene where it plays an important role in cancer progression,yet a serious studies should that it plays a role in early stages different types of cancers including thyroid cancers. We found a moderate immunoreactivity of Bcl2 in cytoplasm of epithelium of the benign and well-differentiated PTC and these findings are accordance with other studies by (26 , 22, 2 , 24).

### Conclusion

CD68 + macrophages are key players in thyroid tumors microenviroment leading to thyroid tumor development where its indirectly correlated with BCL2 family oncoproteins where antiapoptotic pathway is down regulated leading to increased aggregates of CD68+macrophages and that CD68+macrophages are positively correlated with p53 gene mutation expression where both markers can explain the biological behavior of tumor. BCL2&P53 have an inversed correlation since both have an opposite functions (p53 represent cell death gene while bcl2 is an antidote to a programmed cell death ,bcl2 was more expressed in benign tumor in our study than in malignant tumors unlike to p53 where its more expressed in WDT (PTC 6%followed by FTC3.03%) indicating that expression of p53 may be a late event in thyroid tumor genesis and that there’s a mutual expression between both bcl2 & p53 in thyroid tumors.
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry, Mustansiriyah University, Iraq, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Prevalence of Lung Cancer in Non Smoker Patients Attending Al-Diwaniyah Teaching Hospital

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² M.B.Ch.B/ Al-Diwaniyah Teaching Hospital / Al-Diwaniyah Province/ Iraq

Abstract

Background: Smoking is a well known risk factor that is implicated in a number of human malignant disorders.

Aim of the Study: To study the prevalence of lung cancer in a sample of Iraqi non smoker patients and the main risk factor implicated in such malignant tumor.

Patients and Method: In this hospital based study, in order to explore the prevalence rate of bronchogenic carcinoma in non smoker patients, it retrospectively collected data about those patients for the last four years, starting from January 2016 through June 2019. These information were retrieved from patients records that are already present in oncology and respiratory units in Al-Diwaniyah teaching hospital, Al-Diwaniyah province, Mid-Euphrates region of Iraq. The following variables were included in the questionnaire form: Age, gender, occupation, education level, residency, socioeconomic status, marital status, history of smoking, history of second hand smoking, number of household smokers, type of smoking, history of alcoholism, history of chronic illness, the main presenting clinical features and clinical features of carcinoma and finding in examination as well as those obtained from investigations.

Results: In the current study, the prevalence rate of all cases of lung cancer during the period of study was 61 out of 403 (15.1 %). the rate of lung cancer in none smokers, in the current study, was 39.3 %. Old age individuals were the main age group affected and there was female predilection. there was also no significant difference in the distribution of patients and control subjects according to residency, occupation, level of education marital status and socioeconomic status (P > 0.05). In the current study, the prevalence rate of passive smoking in patients was significantly higher than that in control subjects, 70.8 % versus 40 %. Moreover, the number of household smokers was significantly higher in patients group than in control group.

Conclusion: Lung cancer in non smokers appears to be significantly correlated with passive smoking in addition to increase incidence in old age and women.

Key words: Prevalence; Lung Cancer, Non Smoker, Iraq.

Introduction

The term, never smokers, refers to persons who have smoked fewer than 100 cigarettes in their lifetime, including lifetime nonsmokers. Most studies that track the trend of lung cancer rates often include both smokers and never smokers, and few studies independently study the trends over time for never smokers because of the limited longitudinal collection and the limited reliability of smoking information in population-based registries. From what is available, however, the overall global statistics estimate that 15% of lung cancers in men and up to 53% in women are not attributable to smoking, with never smokers accounting for 25% of all lung cancer cases worldwide ¹. If lung cancer in never smokers were considered separately, it would rank as the seventh most common cause of cancer death worldwide before cervical, pancreatic, and prostate cancer ². In countries in South Asia, up to 80% of women with lung cancer are never smokers ³. In the United States, one study estimated that 19% of lung cancer in women and 9% of
lung cancer in men occurs in never smokers\(^4\). The age-
adjusted rate for lung cancer in never smokers (ages 40–
79 years) ranged from 11.2 to 13.7 per 100,000 person-
years for men and from 15.2 to 20.8 per 100,000 person-
years for women. The rates are 12 to 30 times higher
in current smokers of the same age group. Lung cancer
included heterogeneous group of malignant epithelial
disorders that comprises squamous cell carcinoma,
adeno-carcinoma, small cell carcinoma and large cell
carcinoma as well as a number of rare histopathological
subtypes such as bronchoalveolar carcinoma. The most
common subtype by far is squamous carcinoma and is
mainly attributed to smoking but a number of cases have
been reported in life time non smokers. Because of the
shortage of Iraqi literatures dealing with lung cancer in
non smokers since most published Iraqi articles deal
with smoking related lung cancer, and because we are
interested in making an idea about the prevalence rate
of lung cancer in non smokers and to outline its possible
risk factors in our community we planned and conducted
the current study to one of leading articles in the Mid-
Euphrates region dealing with this important human
cancer.

Patients and Method

In this hospital based study, in order to explore
the prevalence rate of bronchogenic carcinoma in non
smoker patients we prospectively interviewed 5 cases
during 2019 and retrospectively collected data about
those patients for the last 3years, starting from January
2016 through June 2018. These information were
retrieved from patients records that are already present in
oncology and respiratory units in Al-Diwaniyah teaching
hospital, Al-Diwaniyah province, Mid-Euphrates region
of Iraq. However for purpose of comparison in order
to evaluate possible risk factors in association with
non smoker bronchogenic carcinoma we included in
the study 25 apparently healthy individuals serving as
control group. Records of all patients with established
diagnosis of bronchogenic carcinoma were included in
the present study. Variables were registered concerning
those patients who are never smokers. Although records
of smoker patients with bronchogenic carcinoma
were retrieved, information about them were used for
calculation of prevalence rate of lung cancer in non
smoker only, and the rest of information were excluded.
The total number of patients with all kinds of malignant
tumor was also obtained to calculate the prevalence
rate of broncogenic carcinoma out of all malignant
tumors. The following variables were included in the
questionnaire form: Age, gender, occupation, education
level, residency, socioeconomic status, marital status
history of smoking, history of second hand smoking,
number of household smokers, type of smoking, history
of alcoholism, history of chronic illness, the main
presenting clinical features and clinical features of
carcinoma and finding in examination as well as those
obtained from investigations. The study was approved by
the institutional ethical approval committee and formal
agreement was obtained from the directorate of Health
in Al-Diwaniyah province, the formal representative
of Iraqi Ministry of health. Data were collected and
transformed into a spread sheet of Microsoft Office
Excel 2010 and then into an SPSS (statistical package for
social sciences) version 23. Numeric quantitative data
were expressed as mean, range and standard deviation
(SD), whereas, qualitative data were expressed as
number and percentage. Comparison of mean between
any two groups was done according to independent
sample t-test, while chi-square test was used to evaluate
association between any two categorical variables. The
level of significance was considered at P ≤ 0.05.

Results

During that period the number of cases with
malignant tumors, including lung cancer, accounted
for 403. In addition, the total number of cases with
lung carcinoma during the period of study, irrespective
of smoking, was 61. Therefore, the prevalence rate of
all cases of lung cancer during the period of study was
61 out of 403 (15.1 %), out of which 24 cases were
associated with evidence of passive smoking (39.3 %).
Table .1 outlines the comparison of age between control
and patients group. There was no significant difference
in mean age between patient and control group, 61.63
±15.31 years versus 68.28 ±8.50 years, respectively (P
= 0.065). The age range of patients with bronchogenic
carcinoma was form 30 – 85 years. In addition the
frequency distribution of patients according to 10
years intervals was as following: 8.3 %, 16.7 %, 4.2
%, 41.7 %, 12.5 % and 16.7 % as 30-39 years, 40-49
years, 50-59 years, 60-69 years, 70-79 years and ≥ 80
years, respectively. Thus, the highest incidence rate was
observed at age interval of 60-69 years, table 1.
Table 1: Mean age and age range of patients and control subjects

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Control group n = 25</th>
<th>Patients group n = 24</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>0 (0.0)</td>
<td>2 (8.3)</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>0 (0.0)</td>
<td>4 (16.7)</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>2 (8.0)</td>
<td>1 (4.2)</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>13 (52.0)</td>
<td>10 (41.7)</td>
<td></td>
</tr>
<tr>
<td>70-79</td>
<td>6 (24.0)</td>
<td>3 (12.5)</td>
<td></td>
</tr>
<tr>
<td>≥ 80</td>
<td>4 (16.0)</td>
<td>4 (16.7)</td>
<td></td>
</tr>
</tbody>
</table>

Mean ±SD

<table>
<thead>
<tr>
<th>Range</th>
<th>68.28 ±8.50</th>
<th>61.63 ±15.31</th>
<th>0.065 †</th>
</tr>
</thead>
</table>

n: number; data were presented as mean standard deviation or number (%); †: independent samples t-test; NS: not significant at P ≤ 0.05

With respect to gender, women accounted to 15 out 24 patients with bronchogenic carcinoma thereby accounting for 62.5 %, making the male to female ratio 1:1.7. Control group included 12 men and 13 women; therefore there was no significant difference in the distribution of patients and control subjects according to gender, as shown in table 2. There was also no significant difference in the distribution of patients and control subjects according to residency, occupation, level of education marital status and socioeconomic status (P > 0.05), as shown in table 3. Regarding bad habits, none of patients or control subjects admit to be alcoholic of experience cigarette smoking.

Table 2: Frequency distribution of patients and control subjects according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Control group n = 25</th>
<th>Patients group n = 24</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>48</td>
<td>9</td>
<td>37.5</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>52</td>
<td>15</td>
<td>62.5</td>
</tr>
</tbody>
</table>

n: number of cases; ¥: Chi-square test; NS: not significant at P ≤ 0.05

Table 3: Demographic characteristics of control subjects and patients with bronchogenic carcinoma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group n = 25</th>
<th>Patients group n = 24</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residency</td>
<td>Urban</td>
<td>19</td>
<td>76.0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>6</td>
<td>24.0</td>
<td>10</td>
</tr>
</tbody>
</table>

Residency

1.738

0.187 ¥

NS
### Table 4: Chronic disorders in control and patients with bronchogenic carcinoma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group n = 25</th>
<th>Patients group n = 24</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>HT</td>
<td>14</td>
<td>56.0</td>
<td>9</td>
<td>37.5</td>
</tr>
<tr>
<td>DM</td>
<td>8</td>
<td>32.0</td>
<td>4</td>
<td>16.7</td>
</tr>
<tr>
<td>IHD</td>
<td>1</td>
<td>4.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Asthma</td>
<td>3</td>
<td>12.0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

n: number of cases; ¥: Chi-square test; F : Fischer exact test; NS: not significant; S: significant
Table 5: Prevalence rate of passive smoking in patients and control subjects

<table>
<thead>
<tr>
<th>Passive smoking</th>
<th>Control group n = 25</th>
<th>Patients group n = 24</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>40.0</td>
<td>17</td>
<td>70.8</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>60.0</td>
<td>7</td>
<td>29.2</td>
</tr>
</tbody>
</table>

n: number of cases; ¥: Chi-square test; S: significant at P ≤ 0.05

Table 6: Number of household smokers in patients and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>Median (IQR)</th>
<th>Range</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.64 ±0.86</td>
<td>0.00 (2.00)</td>
<td>0.00 - 2.00</td>
<td>0.027 †</td>
</tr>
<tr>
<td>Patients group</td>
<td>1.38 ±1.25</td>
<td>1.00 (3.00)</td>
<td>0.00 - 4.00</td>
<td>S</td>
</tr>
</tbody>
</table>

SD: standard deviation; IQR: inter-quartile range; †: Mann Whitney U test; S: significant at P ≤ 0.05

Discussion

In the current study, the prevalence rate of all cases of lung cancer during the period of study was 61 out of 403 (15.1 %). It has been estimated that lung cancer accounts for about 16 % of all cancers, a proportion that is nearly similar and in total agreement with our finding. On the other hand, the rate of lung cancer in none smokers, in the current study, was 39.3 %. Most published articles indicated that the proportion of non-smokers with lung cancer ranges between 10 to 25 % (6,7, 8,9). This indicates that a significant proportion of patients with bronchogenic carcinoma, in our study, have acquired the disease irrespective with cigarette smoking. Therefore, identifying other possible risk factors in association with lung cancer in those patients was the main aim of the current study. Indeed, we supposed that passive smoking may play a principal role in the development of lung cancer in patients who are not habitual smokers. Unfortunately lung cancer in never-smokers has been studied far less extensively than tobacco-related lung cancer. This indicates that age by itself may be a risk factor. Indeed, most published articles dealing with lung cancer in non smokers has linked age as a potential risk factor for such malignant disorder (10, 11,12,13). Older age is associated with cancer development due to biologic factors that include DNA damage over time and shortening telomeres. Accordingly, the median age of lung cancer diagnosis is 70 years for both men and women 14. On the other hand and with respect to gender, in the current study, women accounted to 15 out 24 patients with bronchogenic carcinoma thereby accounting for 62.5 %, making the male to female ratio 1:1.7. There are conflicting data regarding the possibility that women may be more susceptible to developing lung cancer 15. There is a higher rate of lung cancer in non-smoking women compared with non-smoking men, a higher proportion of epidermal growth factor receptor (EGFR) mutations in female NSCLC, and a higher incidence of adenocarcinoma with lepidic features in women (16, 17). Some genetic mutations found to be more common in females may predispose toward lung cancer development in women, including over-expression of the CYP1A1 gene, mutation of the glutathione S-transferase M1 enzyme, mutations of the p53 tumor suppressor gene, and over-expression of X-linked gastrin-releasing peptide receptor (15, 16,17). Moreover, in the current study, there was also no significant difference in the distribution of patients and control subjects according to residency, occupation, level of education marital status and socioeconomic status (P > 0.05). In agreement with our results, a number of authors have denied any association between residency, rural versus urban and lung cancer (18,19). Exposure to pollutant in association with occupation is the main hazard precipitating to lung cancer. The occupational
and environmental exposure to carcinogenic agents is an everyday phenomenon.  

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine/University of Al-Qadisiyah, Iraq and all experiments were carried out in accordance with approved guidelines.

References
Effects Hydrocortisone on the Body Weight of the Pregnant Rabbits and their Embryo with Histological Effects Skin, Stomach and Small Intestine of Embryos

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¹Dept. Anatomy and biology, College of Medicine. Univ. of Wasit – Iraq

Abstract

The aim of the study was to investigate the effects hydrocortisone of on the body weight of the pregnant rabbits and their embryo with histological effects pregnant skin, stomach and intestine of embryos for 45 days. The rabbit were randomly divided into two equal groups (control and one treated groups), and the animals were treated as follow: 1- Control group C. (n = 12) received Distilled water 1ml /kg b.w five times a week intramuscular for 26 days. 2- Treatment group. (n=12) treated daily with hydrocortisone 100mg/kg b.w/day/ intramuscular for 26 days. The body weight of the animals detected at the 1st, 7th, 14th, 21st and 26th days of the experimental period. At 26 days animals sacrificed and embryos weighted and skin, stomach, intestine were removed and taken for histopathological study. The present study showed that treatment with hydrocortisone causes a significant decrease in body weight of embryos and pregnant rabbits. As a conclusion: hydrocortisone cues decrease body weight of embryos and pregnant rabbits.

Keywords: Rabbits, hydrocortisone, skin, stomach, intestine

Introduction

Hydrocortisone belongs to a group of medicines called steroids. Their full name is Corticosteroids. These corticosteroids occur naturally in the body, and help to maintain health [1,2]. Hydrocortisone is an effective way to treat various illnesses involving inflammation in the body [3,4]. Indication and clinical use: Primary or secondary adrenocortical insufficiency [5]. Rheumatic disorders: As adjunctive therapy for short-term administration in: psoriatic arthritis, rheumatoid arthritis, including juvenile rheumatoid arthritis, ankylosing spondylitis, acute and subacute bursitis, acute non specific tenosynovitis, acute gouty arthritis, post traumatic osteoarthritis, synovitis of osteoarthritis and epicondylitis [6,7].

Collagen Diseases: During an exacerbation or as maintenance therapy in selected cases of systemic lupus erythematosus and acute rheumatic carditis [8].

Dermatologic Diseases: pemphigus, bullous dermatitis, herpetiformis, severe erythema multiforme, exfoliative dermatitis, mycosis fungoides and severe seborrheic dermatitis [9].

Allergic States: Control of severe or incapacitating allergic conditions intractable to adequate trials of conventional treatment: seasonal or perennial allergic rhinitis, bronchial asthma, contact dermatitis, atopic dermatitis, serum sickness and drug hypersensitivity reactions [10].

Ophthalmic Diseases: Severe acute and chronic allergic and inflammatory processes involving the eye and its adnexa such as: allergic conjunctivitis, keratitis, allergic corneal marginal ulcers, herpes zoster ophthalmicus, iritis and iridocyclitis, chorioretinitis, anterior segment inflammation, diffuse posterior uveitis and choroiditis, optic neuritis, sympathetic ophthalmia [11].

Respiratory Diseases: Symptomatic sarcoidosis, Löfgren’s syndrome not manageable by other means, berylliosis, fulminating or disseminated pulmonary tuberculosis when used concurrently with appropriate antituberculous chemotherapy, aspiration pneumonitis [12].

Hematologic Disorders: Idiopathic thrombocytopenic purpura in adults, secondary
thrombocytopenia in adults, acquired (autoimmune) hemolytic anemia, erythroblastopenia (RBC anemia), congenital (erythroid) hypoplastic anemia [13].

Neoplastic Diseases: For palliative management of: leukemias and lymphomas in adults, acute leukemia of childhood [14].

Edematous States: To induce a diuresis or remission of proteinuria in the nephrotic syndrome, without uremia, of the idiopathic type or that due to lupus erythematosus [15].

Gastrointestinal Diseases: To tide the patient over a critical period of the disease in: ulcerative colitis, regional enteritis [16].

CNS: Acute exacerbations of multiple sclerosis. Miscellaneous: Tuberculous meningitis with subarachnoid block or impending block when used concurrently with appropriate anti tuberculous chemotherapy, trichinosis with neurologic or myocardial involvement [17,18]. Hydrocortisone Injection can cause side effects although not everybody gets them. Steroids including hydrocortisone can cause severe mental health problems. These are common in both adults and children. They can affect about five in every 100 people taking medicines like hydrocortisone [19,20]. Feeling depressed, including thinking about suicide. Feeling high (mania) or having moods that go up and down. Feeling anxious, having problems sleeping, having difficulty in thinking or being confused and losing your memory. Feeling, seeing or hearing things which do not exist. Having strange and frightening thoughts, changing how you act or having feelings of being alone [21,22]. Corticosteroids have been shown to be teratogenic in many species when given in doses equivalent to the human dose. Animal studies in which corticosteroids have been given to pregnant mice, rats, and rabbits have yielded an increased incidence of cleft palate in the offspring [23]. There are no adequate and well-controlled studies in pregnant women. Corticosteroids should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Infants born to mothers who have received corticosteroids during pregnancy should be carefully observed for signs of hypoadrenalism [24].

The aim of the present study was to investigate the effects of hydrocortisone on the histological section of skin, stomach and intestine in embryo rabbits.

Materials and Method

Twenty for adult rabbit were obtained from market of Kut. Animals were placed at the animal house, College of science, Wasit University and fed with pellet during experimental periods, temperature was 25°C. Female was left in a separate cage with one male for the each cage ratio 2:1. Male and female couple were kept together in mating cage for six weeks.

The rabbit were randomly divided into two equal groups (control and one treated groups), and the animals were treated as follow:

1- Control group C. (n = 12) received Distilled water 1 ml/kg b.w five times week intramuscular for 26 days

2- Treatment group. (n=12) treated daily with hydrocortisone 100mg/kg b.w/day/ intramuscular for 26 days [25]. The body weight of the pregnant animals detected at the 1th, 7th, 14th, 21th and 26th days of the experimental period by electrical balance. At end of experiment period pregnant rabbit and embryo weighted and sampling skin, stomach, intestine of embryos preserved in 10% formalin buffer solution until preparation of histopathological section. Tissue was cut at 7-8µm and embedded in paraffin and takes sections of skin, stomach, intestine were stained with hematoxylin-Eiosin stain (H&E) for histopathological study [26].

Statistical Analysis:

Data were expressed as mean ± standard error of mean and were compared by one way ANOVA followed by LSD. P value more than 0.05 was considered as statistically significant [27].
Result and Discussion

**Table (1): Effect of Hydrocortisone on the body weight of the pregnant rabbit in the different periods**

<table>
<thead>
<tr>
<th>Groups Time</th>
<th>Control Weight rabbit Kg</th>
<th>Hydrocortisone Weight rabbit Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>1.78 ± 0.019 a</td>
<td>1.74 ± 0.017 a</td>
</tr>
<tr>
<td>7 day</td>
<td>2.4 ± 0.029 a</td>
<td>1.62 ± 0.044 b</td>
</tr>
<tr>
<td>14 day</td>
<td>2.8 ± 0.097 a</td>
<td>1.54 ± 0.056 b</td>
</tr>
<tr>
<td>21 day</td>
<td>2.12 ± 0.077 a</td>
<td>1.31 ± 0.023 b</td>
</tr>
<tr>
<td>Day 26</td>
<td>2.14 ± 0.08 a</td>
<td>1.22 ± 0.22 b</td>
</tr>
</tbody>
</table>

The value represent Mean(gram) + Standard Error-The different small letters show significant effect while the same small letters show insignificant effect between different groups.

Table (1) showed a significant decrease of body weight in hydrocortisone compared with the control along time of the experimental periods. Except one day non significant between hydrocortisone compared with the control. The decrease body weight because hydrocortisone therapy have included gastrointestinal upset, nausea, vomiting, and peptic ulcer disease, pancreatitis, ulcerative esophagitis [28,29]. Or may be due to hydrocortisone that impair carbohydrate, protein and lipid metabolism [30].

**Table (2): Effect of hydrocortisone on the body weight of the rabbits embryo in 26 day**

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control</th>
<th>Hydrocortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gram)of embryo In 26 day</td>
<td>1.60 + 0.066 a</td>
<td>1.30 + 0.033 b</td>
</tr>
</tbody>
</table>

-The value represent Mean(gram) ± Standard Error

-The different small letters show significant effect while the same small letters show insignificant effect between different groups.

When taken hydrocortisone in high doses and for long period of time can cross the placental parrier causing premature and low birth weight due to inhibition of metabolism and fetal glands [31,32].
Figure 1: histopathological examination of skin embryos (under fox 10x 0.25)

Histopathological examination of skin embryos in (fig. 1-A) control group no change in skin thickness (fig. 1-B) control group show normal dermal cell number and normal dermal collagenous fibres. (fig. 1-C) treatment group thinning of the skin. Hydrocortisone inhibition epidermis thickening and keratinization and remove a small amount of the outer layer of the skin which affect by passive diffusion. (fig. 1-D) decrease in dermal cell number and increase of dermal collagenous fibres [33, 34]. Hydrocortisone may cause primary irritant reactions which directly damage or kill epidermal cells and dermis [35, 36].

Figure 2: histopathological examination of stomach embryo (under fox 10x 0.25)
Histopathological examination of stomach embryo in (fig. 2-A) control group normal in the rate of epithelial cell formation (fig. 2-B) control group no reduction in stomach glandular epithelium (fig. 2-C) control group show normal size blood vessels (fig.2D) treated group reduction in the rate of epithelial cell formation due to hydrocortisone therapy had effect epithelial cell is rapidly turned over ,replaced with irritants leading to a stomach wall more susceptible to peptic disease(37,38). (fig.2-E)treated group a reduced in stomach glandular epithelium due to a reduced frequency of mitosis in glandular epithelium that incidence of gastric peptic ulcer is actually increased after hydrocortisone therapy [39,40]. (fig. 2-F treated group) hyperplasia blood vessels due to increase in mucosal blood flow secondary effect of hydrocortisone [41].

Histopathological examination of intestine embryo rabbits in (fig. 3-A) control group normal epithelial cell height with crypt height (fig. 3-B) control group increased in the number of goblet cells (fig. 3-C) control group increased in the number of the values with normal elongation of villi (fig. 3-D) treated group increased epithelial cell with crypt height and largamente mucosa. hydrocortisone therapy increases cell migration rate, thymidine kinase levels, and RNA and DNA content. The enhanced cell proliferation leads to mucosal hyperplasia and increases in intestinal weight ,crypt height which develops equally throughout the entire small intestine [42] (fig. 3-E)treated group decrease in the number of goblet cells . hydrocortisone inhibited the premature differentiation of goblet cells induced by thyroxine and lowered mitotic counts, as compared to control [43,44]. (fig. 3-F) treated group decrease in the number of the values with increased elongation of villi. Also higher doses of hydrocortisone had a marked suppressive effect on DNA , the values in the steroid-treated group being about one-half those of the control group . Also hydrocortisone acceleration villus maturation, including the formation of previllous ridges , elongation of villi, vascularization of villi [45].

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Medicine. Univ.
of Wasit – Iraq and all experiments were carried out in accordance with approved guidelines.

References


24. Weise, M. ; Drinkard B. ; Mehlinger L. ; Holzer,S and Eisenhofer,G. Stress dose of hydrocortisone


Comparison of Preoperative Ultrasound Report with Intraoperative Findings of Laparoscopic Cholecystectomy in Gallstone Disease in Al-Diwaniyah Teaching Hospital

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¹College of Medicine/Al-Qadisiyah University, Iraq

Abstract

Background: Gallstones constitute a significant health problem in developed societies, affecting 10% to 15% of the adult population, meaning 20 to 25 million Americans have (or will have) gallstones. The resultant direct and indirect cost of gallbladder disease represents a consumption of ~$6.2 billion annually in the U.S., constituting a major health burden that has increased more than 20% over the last 3 decades. The best diagnostic tool is ultrasound; however, several authors have raised the issue of inconsistency between preoperative ultrasound evaluation and laparoscopic operative findings.

Aim of the study: To compare preoperative ultrasound findings with operative findings in patients undergoing laparoscopic cholecystectomy in order to evaluate the accuracy of ultrasound in detecting gallbladder pathology.

Patients and Method: In this hospital based study, in order to evaluate the concordance and discrepancy between preoperative ultrasound findings and operative findings in patients subjected to laparoscopic cholecystectomy. At the end of study we were able to include 100 cases. The study started on January the 2nd 2019 and ended at June the 15th 2019. The study was carried out at laparoscopic unit in Al-Diwaniyah teaching hospital. Ultrasound findings were retrieved from available reports that are already kept with the case file sheet of each patient. Operative findings were obtained the surgery team and by the use of a special device which provide information about size of stone and gallbladder wall thickness.

Results: Regarding number of stones and for purpose of unification both ultrasound sound findings and operative findings were contrasted as single stone versus multiple stones, as shown in table 2. Indeed, there was almost complete agreement between ultrasound and operative findings since 22 patients were diagnosed as having single stone by both methods and 76 were diagnosed as having multiple stones by both methods. According to MeNemar test, there was no significant difference in distribution of patients into having single versus multiple stones (P = 1.000), a finding that was further supported by Kappa agreement statistic of 0.944 (P < 0.001), table 4.3.

Key word: Gallbladder Disease, Ultrasound, Gallstone, Laparoscopic Cholecystectomy.

Introduction

Gallstones constitute a significant health problem in developed societies, affecting 10% to 15% of the adult population, meaning 20 to 25 million Americans have (or will have) gallstones. The resultant direct and indirect cost of gallbladder disease represents a consumption of ~$6.2 billion annually in the U.S., constituting a major health burden that has increased more than 20% over the last 3 decades. With an estimated 1.8 million ambulatory care visits each year, gallstone disease is a leading cause for hospital admissions related to gastrointestinal problems. These numbers are likely an underestimate because laparoscopic cholecystectomy is often performed as a day procedure and thus not captured by hospital statistics that require overnight admission. Although the mortality rate for gallstones disease is relatively low at 0.6%, the high burden of disease imposes
troubling mortality figures, such as an estimated 1,092 gallstone-related deaths for 2004 in the U.S. Fortunately, case fatality rates have steadily diminished from over 5,000 deaths in 1950, falling >50% between the years 1979 and 2004. This decline represents the greatest decrease for any digestive disease. Gallstone disease per se also carries inherent risks. Prospective population-based surveys have revealed an increased overall mortality, particularly from cardiovascular disease and cancer, as seen in Americans and Pima Indians with cholelithiasis. Further, as the incidence of gallstone disease escalates, there is a concomitant increase in complications like gallstone-related pancreatitis. The best diagnostic test to confirm gallbladder disease is the abdominal ultrasound. It is noninvasive and is 90% to 95% accurate in detecting gallstones. Pericholic fluid and thickened gallbladder walls can also be identified as in acute cholecystitis. Gallbladder sludge and occasionally common bile duct stones can also be seen with abdominal ultrasounds. The gallbladder ultrasound may also be useful in detecting possible gallbladder neoplasms. The EUS or endoscopic ultrasound is not a first-line test for diagnosing gallbladder disease. There is no place for it as a diagnostic tool for cholecystitis. It is useful when evaluating and staging tumors of the gallbladder, pancreas, and bile ducts. Biopsies can also be done for tissue diagnosis. Ultrasound scanning has been the modality of choice for the clinical diagnosis of gallstone disease for the last 25 years. It is accepted to possess a higher sensitivity than oral cholecystography or computed tomography.

Patients and Method

In this hospital based study, in order to evaluate the concordance and discrepancy between preoperative ultrasound findings and operative findings in patients subjected to laparoscopic cholecystectomy. At the end of study we were able to include 100 cases. The study started on January the 2019 and ended at June the 2019. The study was carried out at laparoscopic unit in Al-Diwaniyah teaching hospital. The study was approved by the institutional ethical approval committee and formal agreement was obtained from the directorate of Health in Al-Diwaniyah province, the formal representative of Iraqi Ministry of health. Verbal consent was obtained from each patient after full illustration of the aim and procedures related to the current study.

Results

Patient’s demographics

The present comparative study included 100 patients who recently underwent laparoscopic cholecystectomy. The age range of those patients was form 14 to 85 years and the mean was 44.32± 14.53 years. With respect to gender, there was 19 male patients accounting for (19.0 %) and 81 female patients accounting for (81.0 %); therefore the male to female ratio was 1:4.26. Mean body mass index (BMI) was 29.28 ± 7.83 kg/m² and the range was from 24-33 kg/m² as shown in table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.32± 14.53</td>
</tr>
<tr>
<td>Gender</td>
<td>Male, n (%)</td>
</tr>
<tr>
<td></td>
<td>Female, n (%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.28 ± 7.83</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>24-33</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation

The degree of agreement between ultrasound findings and operative findings

The thickness of gall bladder wall

It is obvious that approximately more than half of cases that have been labeled as normal thickness had actually operative measures that are comparable to that regarded as thin by ultrasound examination. On the other hand, most of cases that have been thick wall, according to ultrasound examination, had indeed operative measures that are the same as those considered normal by ultrasound examination. Added to that, the value of R² was 0.206 which in terms of accuracy means that the accuracy of ultrasound in comparison with operative findings is only 20.6 %, as shown in figure 1. Therefore, one can conclude that ultrasound examination is very undependable in estimation of gallbladder wall thickness.
The mean thickness of gall bladder wall according to operative notes was 2.95 ±2.08 mm with a range of 0.8 to 15 mm. There was almost complete agreement between ultrasound and operative findings since 22 patients were diagnosed as having single stone by both methods and 76 were diagnosed as having multiple stones by both methods, table 2. The mean size of largest gall stone according to ultrasound was 10.02 ±5.94 mm and that according to operative findings was 9.93 ±6.56 mm; the difference was statistically insignificant (P = 0.770). On the other hand the range of sizes of gall stone was 2 -27 mm according to ultrasound and 2 -26 according to operative findings; therefore, there was fair agreement between ultrasound and operative findings regarding stone size, as shown in table 3. In 71 patients there was agreement between ultrasound and operative findings in the absence of peri-cholecystic fluid. In 13 patients there was also agreement between ultrasound and operative findings in the presence of peri-cholecystic fluid. However, disagreement was seen in 16 cases; but McNemar test showed no significant difference (P = 1.000) and Kappa statistic indicated fair agreement of 0.518 (P < 0.001), as shown in table 4. There was complete agreement between ultrasound and operative findings with respect to presence or absence of stone impaction, as shown in table 5, in which stone impaction was seen in 5 patients according to both ultrasound and operative notes. The level of agreement was supported by lack of significant variation according to McNemar test (P = 1.000) and a kappa statistic of (1.000) with high significant level of (P <0.001), as shown in table 5.

![Figure 1: Correlation between gall bladder thickness according to ultrasound and gall bladder thickness at operation](image)

**Table 2: Comparison of gall stone number according to ultrasound with that found during operation**

<table>
<thead>
<tr>
<th>Number of stones according to operative findings</th>
<th>Mc Nemar test</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of stones according to US</th>
<th>Single</th>
<th>Multiple</th>
<th>Total</th>
<th>P</th>
<th>Kappa</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>22</td>
<td>1</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td>1</td>
<td>76</td>
<td>77</td>
<td>1.000</td>
<td>0.944</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>77</td>
<td>100</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: not significant at P ≤ 0.05; HS: Highly significant at P ≤ 0.05
Table 3: Comparison of gall stone size according to ultrasound with that found during operation

<table>
<thead>
<tr>
<th>Size of stone (mm)</th>
<th>Ultrasound</th>
<th>Operation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean± SD</td>
<td>10.02 ±5.94</td>
<td>9.93 ±6.56</td>
<td>0.770 NS</td>
</tr>
<tr>
<td>Range</td>
<td>2 -27</td>
<td>2 -26</td>
<td></td>
</tr>
</tbody>
</table>

NS: not significant at P ≤ 0.05; HS: Highly significant at P ≤ 0.05

Table 4: The presence of peri-cholecystic fluid according to ultrasound and operative notes

<table>
<thead>
<tr>
<th>Pericholecystic fluid OP</th>
<th>Mc Nemar test</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pericholecystic fluid US</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Absent</td>
<td>71</td>
<td>8</td>
</tr>
<tr>
<td>Present</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>21</td>
</tr>
</tbody>
</table>

NS: not significant at P ≤ 0.05; HS: Highly significant at P ≤ 0.05

Table 5: Gall stone impaction according to ultrasound and operative findings

<table>
<thead>
<tr>
<th>GS impaction in OP</th>
<th>McNemar test</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS impaction in US</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Absent</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

NS: not significant at P ≤ 0.05; HS: Highly significant at P ≤ 0.05

Discussion

In the current study the degree of accuracy provided by US in measuring gallbladder wall thickness was extremely low (20.6 %). Therefore, one can conclude that ultrasound examination is very undependable in estimation of gallbladder wall thickness. Actually, as most patients are examined in the emergency setting, it has sometimes been questioned if the younger radiologists would perform as well as experienced radiologists, who are not always available at the time of examination7. prospectively evaluated the interobserver agreement in US examination of the gallbladder and the biliary tract performed by an experienced and a novice radiologist: they report that the novice radiologist’s expertise in the primary diagnosis of uncomplicated gallstone disease was as good as the one provided by the experienced colleague and the significant interobserver difference in the measurements of the thickness of the gallbladder wall and the common bile duct diameter might indicate that assessment of these parameters requires extensive practice. In the current study, there was almost complete agreement between ultrasound and operative findings since 22 patients were diagnosed as having single stone by both methods and 76 were diagnosed as having multiple stones by both methods and the Kappa agreement statistic highly significant 0.944 (P < 0.001). So the level of agreement in our
study was 98% and this is higher than that reported by\(^\text{11}\), who stated that the degree of agreement between preoperative US assessment of number of gallstones in comparison with operative findings was 82.7%. In another study, the degree of accuracy of preoperative ultrasound in estimating number of gallstones was 74%\(^\text{12}\) which is much lower than that of the current study. In the current study, ultrasound was able to agree with operative findings in 84% of cases; however, false results were seen in 16% of cases; therefore the degree of agreement was fair. Despite thorough search in available published articles, we failed to find an article that compares the accuracy of pre-operative ultrasound in the detection of pericholecystic fluid with that of operative findings as most of published articles have assessed gallbladder wall thickness and stone characteristic, compare ultrasound with other imaging modalities or evaluate the accuracy of pericholecystic fluid in predicting conversion to open surgery or difficult operation or in association with accurate detection of acute cholecystitis\(^\text{13,14,15,16,17,18}\). Therefore, this study may be regarded as the first one to raise the issue of ultrasound accuracy in detecting pericholecystic fluid in comparison with operative findings. In the current study, there was complete agreement between ultrasound and operative findings with respect to presence or absence of stone impaction and stone impaction was observed in 5% of cases. Despite thorough search in available published articles, we failed to find an article that compares the accuracy of pre-operative ultrasound in the detection of gallstone impaction with that of operative findings as most of published articles have assessed gallbladder wall thickness and stone characteristic, compare ultrasound with other imaging modalities or evaluate the accuracy of pericholecystic fluid in predicting conversion to open surgery or difficult operation or in association with accurate detection of acute cholecystitis\(^\text{13,14,15,16,17,18}\). Therefore, this study may be regarded as the first one to raise the issue of ultrasound accuracy in detecting impacted gallstone in comparison with operative findings. In the current study, adhesion was seen in 34 (34.0%) patients and operation was difficult in 12 (12.0%). Adhesion was significantly correlated to thick gall bladder wall, presence of pericholecystic fluid, dilated gall bladder and stone impaction; whereas, difficult operation was significantly correlated to female gender, biliary tree dilatation, presence of pericholecystic fluid and stone impaction. Actually these findings are in agreement with the vast majority of articles dealing with how to predict difficult laparoscopic cholecystectomy and may alter the decision of the surgeon to change into open surgery to avoid unnecessary complications\(^\text{19,20,21,22,23,24}\).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Medicine/ Al-Qadisiyah University, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Frequency of Arrhythmia after Acute Myocardial Infarction During Admission to the Coronary Care Unit in Ad_diwaniyah Teaching Hospital

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¹Al-Qadisiyah University/ College of Medicine, Iraq

Abstract

Aims of the Study: To study the incidence of arrhythmias after Acute Myocardial Infarction (AMI) with respect to type of arrhythmia, risk factors, age and sex distribution, and location of infarction in a sample of patients during their admission in the coronary care unit in Ad Diwaniyah teaching hospital.

Patients and Method: This is a cross sectional study involving a cohort of 97 Iraqi patients diagnosed with acute myocardial infarction and admitted to the coronary care unit of Ad Diwaniyah teaching hospital in Ad Diwaniyah governorate / Iraq. All patients who admitted in the ccu for the period from January 2019 to July 2019 were followed by ECG records for any arrhythmia detected.

Result: Out of 97 patients diagnosed with AMI, 61 patients (62.88%) developed one type or more of arrhythmia, including 35 male (57.3%) and 26 female (42.6%), with the highest incidence (40.98%) above 70 years of age.

Reviewing the main risk factors in the past Medical History of those patients: hypertension was the most significant with 39 hypertensive patients (63.93%). The types of arrhythmias found in the patients included in this study were 27 cases with Premature Ventricular Contraction (27.8% of all patients with AML), 13 cases of Atrial Fibrillation (13.4%), 18 cases of Sinus Tachycardia (18.5%), 7 cases of premature Atrial Contraction (7.2%), 6 cases of Ventricular Tachycardia (6.2%), 3 cases with Ventricular Fibrillation (3.09%), 3 cases of Sinus Bradycardia (3.09%), 5 cases with accelerated junctional rhythm (5.1%), 3 cases of first degree A-V Block (3.09%), 2 cases with Complete Heart Block (2.06%), 7 cases of Right Bundle Branch Block (7.2%) and 5 cases with Left Bundle Branch Block (5.11%).

Key words: arrhythmia, acute myocardial infarction, coronary care unit

Introduction

Acute myocardial infarction (AMI) is myocardial necrosis in a clinical setting consistent with acute myocardial ischemia and detection of elevated values of cardiac biomarkers (CK-MB/troponin-I) above the 99th centile of the upper reference limit 4 hours after starting of symptom. Major risk factors for AMI can be classified as non-modifiable (e.g. genetic makeup) or modifiable (e.g. diet, cholesterol). In order to minimise the risk of AMI, modifiable risk factors should be identified and managed appropriately. In practice, the disorder is diagnosed and assessed on the basis of clinical evaluation, the electrocardiogram (ECG), biochemical testing, invasive and noninvasive imaging, and pathological evaluation. Diagnosis and treatment of patients with the hope of improving clinical outcomes depend on precise classification of infarction. In patients with myocardial infarction, 30-day mortality rates are between 7.8 - 11.4 percent (data reported by the American Heart Association in 2015). Of these, 18% men and 23% women (≥45 years of age) it is known that myocardial ischemia and infarction leads to severe metabolic and electrophysiological changes that induce silent or symptomatic life-threatening arrhythmias. Sudden cardiac death is most often attributed to this pathophysiology. Both atrial and ventricular arrhythmias may occur in the setting of ACS and sustained ventricular tachyarrhythmia’s (VAs) may be associated with circulatory collapse and require immediate treatment.
Atrial fibrillation (AF) may also warrant urgent treatment when a fast ventricular rate is associated with hemodynamic deterioration \(^7\). The incidence of sustained VT and VF occurring within 48 h of the onset of an ACS seems to have decreased over the past decades \(^8-10\). This is most likely due to the widespread availability of revascularization therapy, limiting the size of infarction and to an increased use of beta-blockers \(^11\). However, in a recent retrospective analysis of two randomized trials sustained VA occurred in almost 6% of patients in the very early phase of acute MI indicating the significance of VT/VF in this situation \(^12\).

**Patients and Method**

The study was designed to be a cross-sectional study involving a cohort of 97 Iraqi patients diagnosed with acute myocardial infarction and admitted to the coronary care unit of Ad Diwaniyah teaching hospital in Ad Diwaniyah governorate / Iraq. Patients were selected randomly through their admission in the ccu for the period from January 2019 to July 2019. ECGs used to follow the patients during their stay in the ccu to detect any arrhythmia and know the type of MI (STEMI or NSTEMI) and site of infarction.

The questionnaire form was based on the following:

- Sociodemographic characteristics of patients: Mainly age, gender and smoking.
- Past medical history of the patients regarding hypertension, diabetes and previous ischemic heart disease.

Data were collected, summarized, analyzed and presented using two software programs; these were the Statistical package for social sciences (SPSS) version 23 and Microsoft Office excel 2013. Numeric variables were presented as mean, standard deviation (SD) and range, whereas, categorical variables were expressed as number and percentage. Association between categorical variables was assessed using either Chi-Square test or Yates correction for continuity when more than 20% of cells have expected counts less than 5. The level of significance was considered at \( P \leq 0.05 \).

**Results**

Ninety seven patients admitted to the CCU with AMI included in this study: 57 of them were males (58.7%), and 40 of them were females (41.2%). Their ages ranged from 23 to 90 years with the mean age of the participants was 62.5 (±16.54 SD) years (\( p \) value <0.01 HS). Age distribution of patients was (3.09%) in the age group 20–29 years, (5.15%) 30–39 years, (11.34%) 40–49 years, (18.56%) 50–59 years, (27.84%) 60–69 years and (34.02%) above 70 years as shown in (Table 1).

### Table (1) Age and sex distribution of patients with Acute Myocardial Infarction

<table>
<thead>
<tr>
<th>Age group</th>
<th>Gender</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>30–39</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>40–49</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>50–59</td>
<td>15</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>60–69</td>
<td>14</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>≥70</td>
<td>13</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>40</td>
<td>97</td>
</tr>
</tbody>
</table>

Regarding the risk factors in the Medical History of those 97 patients, 61 Patients (62.8%) were hypertensive, 55 patients (56.70%) diabetic, 38 patients (39.71%) were smoker, 31 cases of Ischemic Heart Disease (31.95%), and 14 (14.43%) ex-smoker as shown in (Table 2).

### Table (2) Risk factors for all cases with AMI

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Gender</th>
<th>Total</th>
<th>%</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>33</td>
<td>28</td>
<td>61</td>
<td>62.88%</td>
</tr>
<tr>
<td>D.M</td>
<td>29</td>
<td>26</td>
<td>55</td>
<td>56.70%</td>
</tr>
<tr>
<td>SMOKING</td>
<td>36</td>
<td>2</td>
<td>38</td>
<td>39.71%</td>
</tr>
<tr>
<td>IHD</td>
<td>20</td>
<td>11</td>
<td>31</td>
<td>31.95%</td>
</tr>
<tr>
<td>EX.SMOKING</td>
<td>12</td>
<td>2</td>
<td>14</td>
<td>14.43%</td>
</tr>
</tbody>
</table>

\( \gamma \): Chi-square test; HS: highly significant difference at \( P \leq 0.01 \)

Out of the 97 patients, 61 patients (62.88%) developed one type or more of arrhythmia, including 35 male (57.3%) and 26 female (42.6%), with age...
distribution (0%) in the age group 20-29, (4.92%) 30-39 years, (9.84%) 40-49 years, (16.39%) 50-59 years, (27.87%) 60-69 and (40.98%) above 70 years as shown in (Table 3).

Table (3) Age and sex distribution of patients with arrhythmia following Acute Myocardial Infarction

<table>
<thead>
<tr>
<th>Age group</th>
<th>Gender</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30-39</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>40-49</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>50-59</td>
<td>8</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>60-69</td>
<td>9</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>≥70</td>
<td>11</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>26</td>
<td>61</td>
</tr>
</tbody>
</table>

; ¥: Chi-square test; NS: not significant at P ≤ 0.05

Reviewing the main risk factors in the past Medical History of the 61 patients who developed arrhythmia following AMI, regarding Hypertension, diabetes, IHD and smoking, the results were as the following for both sexes, 39 hypertensive (63.93%), 32 diabetic (52.45%), 20 cases with previous history of IHD (32.78%), 20 (32.78%) smokers, and 6 (9.83%) ex-smokers as shown in (Table 4, Figure 3).

Table (4) Risk factors of 61 cases with AMI who had developed Arrhythmia.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Gender</th>
<th>Total</th>
<th>%</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>21</td>
<td>18</td>
<td>39</td>
<td>63.93%</td>
</tr>
<tr>
<td>D.M</td>
<td>18</td>
<td>14</td>
<td>32</td>
<td>52.45%</td>
</tr>
<tr>
<td>SMOKING</td>
<td>19</td>
<td>1</td>
<td>20</td>
<td>32.78%</td>
</tr>
<tr>
<td>IHD</td>
<td>13</td>
<td>7</td>
<td>20</td>
<td>32.78%</td>
</tr>
<tr>
<td>EX_SMOKING</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>9.83%</td>
</tr>
</tbody>
</table>

; ¥: Chi-square test; HS: highly significant difference at P ≤ 0.01; NS: not significant at P ≤ 0.05

The types of arrhythmias found in the patients included in this study were as the following: 27 cases with Premature Ventricular Contraction (27.8% of all patients with AML), 13 cases of Atrial Fibrillation (13.4%), 18 cases of Sinus Tachycardia (18.5%), 7 cases of premature Atrial Contraction (7.2%), 6 cases of Ventricular Tachycardia (6.2%) , 3 cases with Ventricular Fibrillation (3.09%), 3 cases of Sinus Bradycardia (3.09%), 5 cases with accelerated junctional rhythm (5.1% ) , 3 cases of first degree A-V Block

Studying the site of infarction for cases with AMI demonstrated that there are 12 patients with Anterior MI 8 of them developed Arrhythmia (66.6%), 15 patients with Anteroseptal MI 11 patients of them developed arrhythmia (73.33%), 8 patients with Anterolateral MI (5 of them had arrhythmia 62.5%), 5 patients with septal MI, 2 of them developed arrhythmia (40%), 13 patients with Inferior MI (10 of them developed arrhythmia 76.92%), 9 patients with extensive MI (including 8 patients with arrhythmia 88.8%) and 29 patients with NSTEMI (including 12 patients with arrhythmia 41.3%) and no case recorded with posterior MI AS shown in (Table 5).

Table (5) Distribution of patients according to the site of infarction.

<table>
<thead>
<tr>
<th>Site of Infarction</th>
<th>Total Number of MI Patients</th>
<th>Pts who developed Arrhythmia</th>
<th>Percentage</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>12</td>
<td>8</td>
<td>66.66%</td>
<td></td>
</tr>
<tr>
<td>Anteroseptal</td>
<td>15</td>
<td>11</td>
<td>73.33%</td>
<td></td>
</tr>
<tr>
<td>Anterolateral</td>
<td>8</td>
<td>5</td>
<td>62.5%</td>
<td></td>
</tr>
<tr>
<td>septal</td>
<td>5</td>
<td>2</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>Inferior</td>
<td>13</td>
<td>10</td>
<td>76.92%</td>
<td>0.9 NS</td>
</tr>
<tr>
<td>inferolateral</td>
<td>7</td>
<td>4</td>
<td>57.14%</td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>0</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td>8</td>
<td>8</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>29</td>
<td>13</td>
<td>44.82%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>61</td>
<td>62.88%</td>
<td></td>
</tr>
</tbody>
</table>
(3.09%), 2 cases with Complete Heart Block (2.06%), 7 cases of Right Bundle Branch Block (7.2%) and 5 cases with Left Bundle Branch Block (5.11%) as in (Table 6, Figure 4).

Table (6) Frequency and percentages of Arrhythmias recorded in patients with AMI.

<table>
<thead>
<tr>
<th>Type of Arrhythmia</th>
<th>Number</th>
<th>Percentage out of the 61 Patients who developed arrhythmia</th>
<th>Percentage out of the Total Number of Patients with AMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC</td>
<td>27</td>
<td>44.2%</td>
<td>27.8%</td>
</tr>
<tr>
<td>AF</td>
<td>13</td>
<td>21.3%</td>
<td>13.4%</td>
</tr>
<tr>
<td>Sinus Tachycardia</td>
<td>18</td>
<td>29.5%</td>
<td>18.5%</td>
</tr>
<tr>
<td>PAC</td>
<td>7</td>
<td>11.4%</td>
<td>7.2%</td>
</tr>
<tr>
<td>VT</td>
<td>6</td>
<td>9.8%</td>
<td>6.1%</td>
</tr>
<tr>
<td>VF</td>
<td>3</td>
<td>4.9%</td>
<td>3.09%</td>
</tr>
<tr>
<td>Sinus Bradycardia</td>
<td>3</td>
<td>4.9%</td>
<td>3.09%</td>
</tr>
<tr>
<td>AiVR</td>
<td>5</td>
<td>8.1%</td>
<td>5.1%</td>
</tr>
<tr>
<td>1st AV Block</td>
<td>3</td>
<td>4.9%</td>
<td>3.09%</td>
</tr>
<tr>
<td>3rd AV Block</td>
<td>2</td>
<td>3.2%</td>
<td>2.06%</td>
</tr>
<tr>
<td>RBBB</td>
<td>7</td>
<td>11.4%</td>
<td>7.2%</td>
</tr>
<tr>
<td>LBBB</td>
<td>5</td>
<td>8.1%</td>
<td>5.11%</td>
</tr>
</tbody>
</table>

Discussion

This study included 97 Patients admitted with AMI, 57 (58.7%), males patients and 40 (41.2%) females patients, so there is a clear male predominance. Acharya LD et al, also reported similar findings in accordance with the findings of this study. Siddique MB et al, also observed males (67%) predominance over the females (33%) 14. Overall incidence of myocardial infarction in this study was more common with the age group above 70 years (34.02%). Deshpande JD et al, in their study observed that, the incidence of AMI was highest i.e. 31% among the study subjects with 51 to 60 years. 17 Regarding risk factors In this study, we have concentrated on four main risk factors (Diabetes Mellitus, Hypertension, IHD and smoking) for precipitating AMI and its arrhythmic complications, Hypertension was a major risk factor for 62.88% of overall patients with AMI and 63.93% of those who developed arrhythmia following AMI, it is well known that uncontrolled and prolonged elevation of BP can lead to a variety of changes in the myocardial structure, coronary vasculature, and conduction system of the heart. 18. The second significant risk factor was DM, 55 patients (56.70%) of the total number of cases were diabetic and 32 patients (52.45%) of them developed arrhythmia, since Diabetes causes endothelial dysfunction, decreases endothelial thromboresistance, and increases platelet activity, thus accelerating atherosclerosis 19,20. Because there is a strong association between cigarette smoke exposure and heart disease, in the present study out of all AMI cases 38 patients (39.71%) were smoker, 14 (14.43%) ex-smoker, because Nicotine in Cigarette smoke produces a marked elevation in serum catecholamine concentration that is potentially arrhythmogenic, 22, smoking was found in 32.78% of patients and EX smoking 9.83% of AMI patients who develop arrhythmia. The maximum incidence (40.98%) of arrhythmias in this study was found in patients above 70 years of age (despite of p value 0.217 which is not significant statistically). The results of the study were in accordance with those of the American Heart Association, which showed 42% in age group of 60 or more. This is due to high prevalence of DM, dyslipidemia, atherosclerosis in age group of 60 or more. According to gender wise distribution incidence was more in males (57%) as compared to females (42%).

Conclusion

The frequency of arrhythmias and particularly premature ventricular contractions and sinus tachycardia following acute myocardial infarction was significantly high in this study patients sample. Increasing with age in both sexes. More common in extensive infarctions, while Hypertension, Diabetes Mellitus, previous ischemic heart disease and smoking are the commonest associated risk.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Qadisiyah University/ College of Medicine and all experiments were carried
out in accordance with approved guidelines.

References


19. Paynter NP, Mazer NA, Pradhan AD. Cardiovascular risk prediction in diabetic men and women using hemoglobin A1c vs diabetes as a


Sex Determination of in Vitro Produced Goat Embryos by Epididymal Spermatozoa Selected by Swim up Technique

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Department of Surgery and Obstetrics, College of Veterinary Medicine/Baghdad University

Abstract
The study was conducted to determine the sex of the male and female goat embryos produced in vitro fertilization by Epididymal spermatozoa of local buck and separated by swim-up technique. The sex of produced embryo was identified by polymerase chain reaction (PCR). Results indicate that the fertilization by Epididymal spermatozoa selected using Swim-up technique at 200×g centrifugation showed that 48.70 ±0.62 % of spermatozoa were detected in the supernatant while the precipitate contained 42.1±0.70. Spermatozoa in the supernatant were used for IVF of matured oocytes. The sex of goat embryos produced in vitro fertilization was determined by polymerase chain reaction (PCR) using specific primers to detect the SRY gene. The percentage of male embryos recorded 71.73% while female embryos recorded only 28.26% from the total goat embryos obtained after IVF by sperms selected using swim-up at centrifugation force of 200×g. It was concluded that the use of swim-up technique up at centrifugation force of 200×g on Epididymal spermatozoa showed the ability of selection male embryos in caprine.

Key words: Swim up, Gender, Goat, Cauda, Epididymis, Embryo, SRY Gene.

Introduction
The animal manufacture depends upon increase in the number of productive livestock and a major concern is to increase the efficiency of offspring from them. The goat plays a significant role in socioeconomic development because of its contribution of milk, meat, skin and fur to humans and plant nutrition as farmyard manure, being small in size and having a short gestation period 1. Predetermining the sex of animals has been a central goal of producers for generations because of its economic advantage. The current development of state of the Assistant Reproductive Techniques (ART) has made it possible to predetermine the sex, involving the separation of X- from Y- chromosomes bearing sperms, used in artificial insemination (AI), in vitro fertilization, and embryo transfer 2. Sperm sexing has progressed from research to commercial application for humans and cattle 3. Sperm separation methods are capable to significantly improving sperm quality with a high rate of progressive motility and morphological normal spermatozoa 4. Several investigators have attempted to separate X- and Y spermatozoa using diverse techniques based on principles of differing mass and motility, swimming method, surface changes, centrifugal countercurrent distribution, immunologically relevant resources and volumetric differences (2,5). The potency of sex preselection in semen has been demonstrated for multiple species, including endangered species in zoos and aquarium animals 6. The objective of this research is the separation of X- from Y-bearing Epididymal spermatozoa of local goat by swim-up technique which with can be simply appropriated in small laboratory and with least laboratory appliances.

Materials and Method
Gonads of local goat were collected from Al-shu’alah abattoir and transported in a normal saline by cool box within 30 minutes to the laboratory. The ovaries were removed and washing three times with fresh normal saline and twice with a collecting media (TCM-199, TALP and MEM) and gentamycin to get out of contamination and were sliced into small pieces in fresh normal saline with a surgical blade. The oocytes have been collected in the Hood cabinet by aspiration, slicing or puncture from the normal saline solution to MEM according to procedures of Wani et al10. Aspiration of 2-8 mm size follicles were aspirated with 18 gauge needle attached with a sterile 3 ml disposable syringe containing 2 ml of collecting medium and the
slicing was performed by placing the ovaries in a sterile Petri dish containing 10 ml of collecting medium, held with forceps and the ovarian surface were incised with a scalpel blade while puncture visible follicles on the surface of the ovaries ranging from 2-6 mm in diameter with 18- gauge syringe needle. (7,8,9) The media with harvested oocytes were transferred to one petri dish (10,11). The wells of the dish were examined under inverted microscope and then the total number of oocytes was counted. Collected oocytes were examined and graded according to 12 as grade A, grade B and grade C on the foundation of cumulus cells and uniformity of cytoplasm.

Only grade A, and grade B oocytes were selected under a stereomicroscope, washed twice with cultured medium, and incubated in appropriate maturation medium at 39 °C, 5% CO₂ and 90% relative humidity for 24 hrs, the numbers of matured oocytes were calculated. Every 5 oocytes were placed in a well out of 24 wells dish containing 2 ml of the medium MEM containing HEPES buffer, sodium Bicarbonate, crystalline penicillin, streptomycin, fetal calf serum 10%, was used as maturation medium, presence of first polar body was the criteria of oocytes maturation. 13.

**Sperm separation by Swim-up technique:**

The procedure of the modified swim-up technique is essentially the same as that described by 12, Ham’s F10 (Euro-Lone, Italy) was used as an substitute media to estimate the effectiveness of the swim-up technique in separating X and Y chromosome bearing sperms. Sperms sample transferred to 10 ml centrifuge tube. Sperms washed twice using 0.5 ml Ham’s F-10 medium then centrifuged at 300×g or 200×g for 10 minutes. Supernatant removed and overly sperm pellets with 0.5ml of Ham’s F-10 medium in each tube. The tubes were put in the incubator, inclined at an angle around 45° and incubated at 37°C and 5% CO₂ for 60 minutes. By inclining the tubes at 45 to ameliorate the capability of the sperms to swim out of the sample and reaching the medium. Following incubation, the first 0.25ml was discarded and the final 0.25ml was used for IVF after sperm evaluation 12.

Groups of matured oocytes were kept in of 5 in petri dish containing media MEM supplemented with 10% heat inactivated estrus goat serum (EGS), BSA and antibiotics (Penicillin and Streptomycin) for fertilization with sperms and incubated at 39 °C, 5% CO₂ and 90% relative humidity for 24-27 hrs 13. After fertilization at 24-27 hrs, oocytes have 2nd polar body or oocyte with head of sperm in the cytoplasm was evaluated as fertilized oocyte. 13.

Cultures of fertilized oocytes were performed; embryos were cultured in MEM at 38.5-39 °C, 5% CO₂, and 90% humidity. Every 24 hrs, developments of embryos were perceived, 50% of the media volume was replaced with fresh medium at 24 hrs intervals according to 14 procedure, and then DNA extraction from embryos was applied.

**DNA extraction from cultured embryos:**

The primers (Promega, Germany) were used to detection SRY gene, they prepared according to the details of the producer. The sequences and product size of the primers are:

The forward primer sequence was: ATGAATAGAACGTTGCAATCG

(OD-260: 12.9, Microgram: 382, Pico moles: 58704)

The reverse primer sequence was: GAAGAGGTTTTTCCCAAAGGC

(OD-260: 11.7, Microgram: 363, Pico moles: 58842)

The Statistical Analysis System- SAS (2012) program was used to influence of difference factors in parameters of study. Chi-square test was used to significant comparison between percentage and least significant difference –LSD test (ANOVA) was used to significant comparison between means in this study.

**Results and Discussion**

Several techniques were used for the collection of oocytes from the ovaries in goats. The effect of collection methods on the recovery rate of oocyte is shown in table (1). Aspiration technique showed oocytes recovery rate of 62% (81/131) with a mean of oocytes per ovary and Perforation (puncture) showed oocytes recovery rate of 57% (71/125) while slicing showed oocytes recovery of 48% (55/114). There was a significant difference (p‹0.05) between different collecting techniques. Recovered ova via aspiration and puncture are statistically different than ova recovered by slicing. Similar observation was shown by 15 while 16 and 17 in the sheep. The results disagreed with 9 in goat and 12 in sheep. The aspiration
and puncture methods recorded a high recovery rate due to the aspiration and puncture considered as the applicable technique for obtaining perfect oocytes quality and quantity production, while the presence of the ovarian tissue debris in the slicing due to destruction the ova during the examination 13.

**Table (1): Effect of collection methods on recovery rate of ova**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Ovaries numbers</th>
<th>Ova numbers</th>
<th>No. of Recovered oocytes</th>
<th>Recovery rate percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspiration</td>
<td>50</td>
<td>131</td>
<td>81</td>
<td>62%a</td>
</tr>
<tr>
<td>Slicing</td>
<td>50</td>
<td>114</td>
<td>55</td>
<td>48%b</td>
</tr>
<tr>
<td>Perforation(puncture)</td>
<td>50</td>
<td>125</td>
<td>71</td>
<td>57%a</td>
</tr>
</tbody>
</table>

Different superscripts showed significant difference (P<0.05).

Table (2) showed the type of collection methods on the grade of the ova recovered. Results showed that higher recovery rates were obtained from grade B in all collection methods as compared with grade (A) or grade (C). Corresponding observation was shown by 15 in goats. Wani et al 12 have made comparable observation in sheep and 18 in goats. The low quality grade oocyte recovered could be due to slaughters of low-quality does. There were statistically significant differences (P<0.05) in oocytes quality between aspiration and puncture as compared to the slicing method.

**Table-2-: Effect of collection methods on the grade of oocytes (oocyte quality).**

<table>
<thead>
<tr>
<th>Type of collection method</th>
<th>No. of ovaries</th>
<th>Grade (A) %</th>
<th>grade (B) %</th>
<th>Grade (C) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspiration</td>
<td>50</td>
<td>40a</td>
<td>65a</td>
<td>26b</td>
</tr>
<tr>
<td>Slicing</td>
<td>50</td>
<td>29b</td>
<td>51a</td>
<td>34b</td>
</tr>
<tr>
<td>Puncture</td>
<td>50</td>
<td>42a</td>
<td>61a</td>
<td>22b</td>
</tr>
</tbody>
</table>

Different letters reveal a statistical variation (P<0.05).

Table (3) at centrifugation 200 ×g there was significantly higher (P<0.05) in sperm number after washing, sperm number at the top of the tube and sperm number lost than in centrifugation 300 ×g, while the number sperms in the bottom part of centrifugation 300 ×g were significantly higher than centrifugation 200 ×g (P<0.05). The mean of sperms lost through the centrifugation procedure at 200×g and 300 × g was 6.06 ± 0.41 % and 3.18±0.86 % respectively and there was a significant difference between two groups (P<0.05).
Table 3: Number of sperms used in swim-up technique after centrifugation at 200 × g or 300 × g

<table>
<thead>
<tr>
<th>Centrifugation</th>
<th>After washing 2 times</th>
<th>% of sperms in the upper part of the tube</th>
<th>% of sperms in the lower part of the tube</th>
<th>% of sperms lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>(at 200 × g)</td>
<td>90.8±0.38 a</td>
<td>48.70±0.62a</td>
<td>42.1±0.70b</td>
<td>6.06±0.41 a</td>
</tr>
<tr>
<td>(at 300 × g)</td>
<td>87.82±0.55b</td>
<td>42.32±0.51b</td>
<td>45.50±0.91a</td>
<td>3.18±0.86b</td>
</tr>
</tbody>
</table>

Table 4: Maturation and fertilization rate of grade A and B oocytes using sperms obtained by swim-up technique in goat:

<table>
<thead>
<tr>
<th>Cultured oocytes</th>
<th>Total maturation rate No and %</th>
<th>Total Fertilized oocytes No and %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total oocyte no</td>
<td>Grade A No and %</td>
<td>Grade B No and %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>243</td>
<td>142 (58.43%)</td>
<td>101 (41.56%)</td>
</tr>
<tr>
<td></td>
<td>125/243 (51.44%)</td>
<td>78/125 (62.4%)</td>
</tr>
</tbody>
</table>

In this research, for amplification genomic DNA of in vitro produced embryos of local goat, we were used the primers for SRY gene of caprine, and the PCR products were separated on 2% agarose gels. The result showed that amplification fragment of size 116 bp, the embryos were successfully amplified and when one band dyed by red gel the embryos recorded as male embryos while the result which showed the nonappearance of such bands recorded for the produced embryos as female goat embryos (Figure 2).

The caudal spermatozoa selected from the upper layer of swim up technique, gave 71.73% male embryos and 28.26% female embryos for determination of the sex of IVF produced local goat embryos. Because X sperm has more DNA than Y sperm, the Y chromosome...
is faster than the X chromosome which results in different movement rate. Some authors described that supernatant of swim-up procedure contained more Y sperms. In this study we used accurate protocol for embryos sexing by PCR, it based on detection of the presence of SRY gene. The Y-specific target sequence amplification only was adequate to identify the gender as has been done effectively in bovine and caprine embryos sexing studies.

Table (4) shows the percentage of male and female goat embryo after IVF by spermatozoa and sexing by using PCR the result disclose that 33 (71.73%) from 46 sexed caprine embryos were male, while female embryos revealed only 13 (28.26%) with a significant difference between the two detected sex (P<0.01).

Table 4: Number and percentage of male and female local goat embryos after obtained by IVF using caudal sperms selected by swim-up technique and detection of sex by PCR.

<table>
<thead>
<tr>
<th>Technique</th>
<th>No of embryos</th>
<th>No of sexed embryos</th>
<th>No and % of Male embryos</th>
<th>No and % of Female embryos</th>
<th>Chi-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swim-up (200×g)</td>
<td>78</td>
<td>46</td>
<td>33 (71.73% )</td>
<td>13 (28.26% )</td>
<td>12.644 **</td>
</tr>
</tbody>
</table>

** (P<0.01).

After used swim up technique, the percentage of male embryos evaluated high compared with the results of Marco-Jiménez and Vicente using swim up technique in ovine, and that of Shnawa, (2013) who obtained 81.80 % of male embryos after swim up technique in ovine.

**Conclusion**

From these data it can conclude that it is feasible to reaping Y bearing spermatozoa from epididymis cauda of local buck by swim up technique (200×g) and use these sperms for IVF to get a high percentage of male embryos successfully.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Veterinary Medicine/Baghdad University and all experiments were carried out in accordance with approved guidelines.

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Associations System for Breast Cancer Microarray Data

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University of Information Technology and Communications, Iraq

Abstract
The aim of this paper is to give biologists a tool to explore reasons and impacts of the breast cancer as patients with the same stage of illness can have different treatment responses. This paper proposes a Breast Cancer Associations system (BCA) to discover and interpret the associations among the breast cancer patient’s gene expressions data. The data used in this paper is the array data of 24,483 gene expression measurements recorded for 19 breast cancer patients. BCA consists of: data preprocessing, and data mining. In the first process in BCA, the data is carried out four preprocessing steps to be suitable and enhance the second process in BCA. These four steps are data filtration, normalization, discretization, and data adaptation. The mining process stage uses a new algorithm called Row Intersection Support Starting (RISS), which traverse the row enumeration space using the user-defined mines up threshold as a starting point deploying a new data format called Row Set (RS). The last stage in the system concerns the production of the association rules based on the user defined minimum confidence threshold. Fifteen different experiments have been conducted with different parameters. The results of the experiments are recorded and compared

Keywords: Breast cancer association’s data, microarray data, data adaptation and Row Intersection Support Starting.

Introduction
Gene expression dataset provides information on the variation of gene activity across conditions. It contains numbers which characterize the expression level of a particular sample. It allows the identification of genes that are differentially expressed linked to given condition which help in understanding the response to treatments as well as provide deep insight into the nature of many diseases and lead in the development of new drugs. For example, indicates which genes will be over expressed and which will be under expressed when a breast cancer treatment is given to the cells. Since the number of samples is usually limited and the number of genes is measured in thousands, such dataset are characterized as very high dimensional ones. Association rule mining is the task of finding useful correlation between items in a dataset. They are rules of the form LHS $\leftrightarrow$ RHS, where LHS and RHS are item sets and LHS $\cap$ RHS= $\emptyset$. A brief survey for association rule mining algorithms found in. While frequent Item set are all item sets whose support is at least equal to the minus threshold, closed frequent item set is a condensed representation of frequent item sets. A frequent item set X is closed if there is no superset Y such that Y $\supseteq$ X with $\text{sup}(X) = \text{sup}(Y)$. Since the set of all frequent item sets and their exact support can be extracted from the closed frequent item set, new researches mines closed frequent item sets. The very high dimensional data like the gene expression needs special data mining techniques to discover closed frequent item sets. IRG are a set of rules that are generated from the same group of rows and meet user interestingness constrains including minus, mincing, and minimum chi-square (Minch) threshold. Mine Top-K focuses on the same problem of FARMER but generates most significant Top-K covering rule groups rather than generating IRG. Top-K covering rule groups are defined by first setting criterion for ranking and applying it to the resulted rules in the dataset. All these works performed their experiments on the same data with different minus and different row lengths. In most cases, COBBLER and FARMER outperform CARPENTER. The second category algorithms are IIMA, charm, and RERII. IIMA mines all frequent item sets separately in parallel mode and then joins the results which are returned in a compressed Set Enumeration (SE) and finally generates the rules.
BCA System

BCA is a proposed system that discovers the associations among breast cancer patients’ data. BCA consists of data preprocessing and data mining process, Fig. 1.

Data Preprocessing

a) Filtration

Data filtration task is done in two steps according to the recommendations of a domain specific specialist. The first step is removing redundancy in the data while the second step is a data domain requirement that filters data according to a threshold. Biologists specify a certain threshold to get closer to certain data values which give more insight on the disease under study. Genes expression values greater than the threshold are excluded from the mining process.

b) Normalization

The dataset is normalized using a Mat Lab Built in function called manorm. Manorm scales the values in each column by dividing it by the column mean.

C) Discretization

The dataset is discretized using the mid-range-based cutoff method. The mid-range value for each gene is the mean value for its corresponding column. Values below or equal to its mid-range are set to 0 otherwise is set to 1. Gene expressions with zero value indicate under expressed while those with value one indicates over expressed.

d) Adaptation

In this task, each gene is presented in two columns; one column is for the over expressed state of the gene and the other is for the under expressed state. This adaptation is used to produce rules that specify the state of the genes in addition to their associations. For example, the rule GI $\rightarrow$ G2 — implies that there is an association between GI and G2, when GI is over expressed, G2 becomes under expressed.

Data Mining Process

a) Closed Frequent Item sets Generation

This task produces closed frequent item sets by implementing RISS algorithm Fig.4. RISS algorithm uses the Row Set data format and the minimal bottom-up search space.

Row Set Data Format (RS)

RS is a new vertical format. Let table 1, be a discretized gene expression table of biological Samples B. \{b1, b2, .........., b6\} in rows, and genes G-{g1, g2, .........., g6} in columns. Table 1 is a triple (B, G, R), where R B*G is a relation. (bi, gj) R denotes that gene gj is over expressed or under expressed in bi. Fig.2-a shows b1 has genes g1, g2, g4, and g5 are over expressed while g3 and g6 is under expressed.

<table>
<thead>
<tr>
<th>Biological Samples</th>
<th>g1</th>
<th>g2</th>
<th>g3</th>
<th>g4</th>
<th>g5</th>
<th>g6</th>
</tr>
</thead>
<tbody>
<tr>
<td>b1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>b2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>b3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>b4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>b5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>b6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 3. Comparison between the VTL and RS presentation of table 1.
The difference between VTL and RS is that the second uses the Tad (biological samples) instead of using the items (Genes) to build the VTL with condition that infrequent genes are deleted from the dataset. Fig.3 (a and b) shows RS and VTL of the data, in Fig.2 with mines up equals 30% of the samples.

The common genes among given samples is denoted as $G (B')$. Given a set of biological samples $B' \subseteq B$, the row support set, denoted $G (B) \subseteq G$ is defined as the maximal set of genes common to all the rows in $B'$ and the support of these genes denoted $sup(G(B'))$ is the count of Biological samples in $B'$. As an example, let $B'=\{b1, b3\}$ in Fig.2, then $G (B') = 2$.

**Minimal Bottom-up Search Strategy**

It checks row combination from the smallest to the largest to traverse the search space, Fig.3-a, for example, 1-russets, then 2-rowsets, ..., and finally n-russets. It is a modification from the traditional one (5, 6, 7, 8) as it starts the search from the items up ‘russets rather than the 1- russets. This is valid as the maximum support for the K-russets, for example if the mines up =3 the enumeration tree starts from level 3 as shown in Fig.3-b. Each node in the enumeration tree is represented by RS format. For example, the first node in Fig.3-b tree is represented by $B'=\{1, 2, 3\}$ and $G(B') = \{g2, g5\}$ as shown in Fig.3-c.

**RISS Algorithm**

RISS traverses the Minimal Bottom-up row enumeration search space using breadth first search (BPS). It has two pruning actions to eliminate unnecessary searches. RISS performs recursive generation of the Russets to present a node during traversing the row enumeration tree, Fig.4.

The first pruning action is performed in step 2 of the procedure intersection rows, it checks if the $G(B')$ is empty. This implies that there is no maximal genes common to the biological samples $B$, so no further enumeration will be required on the branch of this node.

The second pruning action is performed in step 3. It checks if the $G (B')$ exists in CFI, if it is true, current and further enumeration of this node is truncated. In other words, $G(B')$ does not discover any new closed frequent item sets. At $B'=\{1 2 3\}$, then $G (B') = \{g2, g5\}$ and results in $\{g2 g5\}$ with frequency $\{1 2 3\} \in CFI$ and no further enumeration for node $\{1 2 4\}$. At $B'=\{1 2 5\}$, then $G (B') = \{g2, g5\}$ which already exists in CFI and results in updating it to $\{g2, g5\}$ with frequency $\{1 2 3 4\} \in CFI$ and no further enumeration for node $\{1 2 4\}$. The same for $B'. \{2 3 5\}$. RISS does not need to perform the closure check among the discovered Item sets since step 1 in Intersection. Rows procedure extracts only the closed item sets. The proof is that $G (B')$ cannot be a maximal gene set that is common to all biological samples $B'$ unless it is a closed item sets 10. For example, in Fig. 2, it is not possible for the item set $\{g1 g2\}$ to be enumerated although both $\{g2\}$ and $\{g1 g2 g5\}$ are closed item sets. This is unlike the column enumeration algorithms which enumerates both $\{g1 g2\}$ and its superset $\{g1 g2 g5\}$, then checks for equal support (= 4) and same frequency ($\{b1 b3 b4 b5\}$) between them. Thus, discarding the subset $\{g1 g2\}$ and denoting the superset as a closed item set.

**b) Rule Discovery**

Rule discovery task is achieved by implementing the faster algorithm in 18. It first generates all the rules with one item in the LHS. Then, recursively use the LHS with K items of the discovered rules to generate all
possible consequents with K+1 items that can appear in the further rules. All resulted rules with the associated parameters are stored in a database to ease querying and maintenance.

**Experimental Study**

**Data**

Array data is used of 24,483 gene measurements recorded for 19 breast cancer patients. It contains: Systematic name given to each gene or sequence and a description of what is known about gene’s function. Also, it contains three values for each tumor sample profiled: Log 10 (Intensity), Log10 (ratio), and P-value. According to the biology specialists’ opinion, the P-value is used in mining process.

**Experiments**

Fifteen experiments are conducted with different filtration and minimum support thresholds. In the first step of filtration, removes the continues P-values keeping only the overall gene P-value. The second step uses three thresholds for the P-values which are 0.04, 0.05, and 0.06. Each filtered data is experimented by four mines up values (15.7%, 26.2%, 42.1%, 52.63%, and 63.15%) and mincing equals to 0.05. Experiments are performed on a PC with Core Duo 2 GHz CPU, 1GB RAM and a 120GB hard disc and the algorithm is coded in MATLAB.

**Results**

The results recorded for each experiment are shown in Table 1. For all experiments, the drawing charts exhibit decaying exponential function with different decaying parameter. Also, the drawn points collapse at higher support level for all datasets. In an attempt to carry the analysis of results one step further, a comparison for CFI and rules count is conducted. A General observation is that whenever the minus increases the common CFI and rule count among the sets increase except for one case AB in rule count at minus=8, the intersection reaches it.1 maximum then started to decrease with increasing mines up. Also, the percent of increase in intersection varies descending from AB to BC to AC.

<table>
<thead>
<tr>
<th>P value</th>
<th>Gene</th>
<th>Mines up</th>
<th>Processing Time (last three tasks)</th>
<th>CFI count</th>
<th>CFI Processing Time</th>
<th>Rules Count</th>
<th>Rules Processing Time</th>
<th>Total Processing Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04</td>
<td>74</td>
<td>3</td>
<td>0.047</td>
<td>10663</td>
<td>1034.563</td>
<td>1804</td>
<td>2.125</td>
<td>1036.734</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.047</td>
<td>7974</td>
<td>859.953</td>
<td>638</td>
<td>0.703</td>
<td>860.703</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>0.063</td>
<td>2094</td>
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<td>484</td>
<td>0.375</td>
<td>701.922</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.063</td>
<td>624</td>
<td>624.609</td>
<td>448</td>
<td>0.297</td>
<td>624.969</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>0.063</td>
<td>168</td>
<td>564.125</td>
<td>407</td>
<td>0.297</td>
<td>564.484</td>
</tr>
<tr>
<td>0.05</td>
<td>78</td>
<td>3</td>
<td>0.031</td>
<td>12303</td>
<td>1289.781</td>
<td>3796</td>
<td>2.313</td>
<td>1292.125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.047</td>
<td>9324</td>
<td>1045.422</td>
<td>801</td>
<td>0.656</td>
<td>1046.125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>0.031</td>
<td>2304</td>
<td>757.297</td>
<td>481</td>
<td>0.406</td>
<td>757.735</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.047</td>
<td>656</td>
<td>655.172</td>
<td>434</td>
<td>0.313</td>
<td>655.531</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>0.016</td>
<td>172</td>
<td>588.344</td>
<td>399</td>
<td>0.313</td>
<td>588.672</td>
</tr>
<tr>
<td>0.06</td>
<td>86</td>
<td>3</td>
<td>0.125</td>
<td>15197</td>
<td>1764.563</td>
<td>2158</td>
<td>2.906</td>
<td>1767.594</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.078</td>
<td>12027</td>
<td>1468.609</td>
<td>1070</td>
<td>1.016</td>
<td>1469.703</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>0.094</td>
<td>3301</td>
<td>1029.453</td>
<td>538</td>
<td>0.391</td>
<td>1029.938</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.063</td>
<td>980</td>
<td>767.766</td>
<td>437</td>
<td>0.313</td>
<td>768.141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>0.063</td>
<td>253</td>
<td>616.594</td>
<td>524</td>
<td>0.313</td>
<td>616.969</td>
</tr>
</tbody>
</table>
Discussion

The proposed BCA system discovers the relationships among breast cancer patients’ gene expressions. The first set of BCA advantages exists in the different preprocessing tasks. Data filtering is an important preprocessing task since it reduces the amount of data genes under study which speeds up the mining process, and gives more insight into specific data values. The first step in the data filtration removes data redundancy existing in the continues of the genes listed in the data file. Moreover, the second filtering step reduces the amount of the data which greatly enhances the performance in terms of processing time and memory space. The second task in the preprocessing, data adaptation is a necessary one in order to indicate the state of the gene in the rules. If not applied, the discovered ‘rules specify only the over expressed associations. The discretization task converts the continuous data into binary one, thus speeds the processing cycle and reduces the memory requirement. This step did not affect the aim of the study since the interest is the differentiation between over and under expressed genes without using quantified measures.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Information Technology and Communications, Iraq and all experiments were carried out in accordance with approved guidelines.

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Physico-Mechanical Behavior of Room Temperature Vulcanized Maxillofacial Silicon after Addition of Glass Flakes

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Abstract

The aim of this study was to assess the effect of glass flakes addition on some physical and mechanical properties of a maxillofacial silicone material. Material and method: A room temperature vulcanized (RTV) maxillofacial silicone (VST-50, Factor II Inc., USA) and Micronized glass flake, surface pre-treated with silane coupling agent (GF002, Glass flake Ltd, Leeds, UK) were used in this study. Two hundred (200) specimens were prepared and divided into five main groups based on the tests conducted (tear strength, tensile strength, elongation percentage, surface hardness and surface roughness). Then, each group was further divided into four subgroups according to weight percentage of glass flakes as follow; control group (0%), other groups contain 0.5%, 1% and 1.5% of glass flake micro-particles (10 specimen from each group). Results: The collected data were analyzed with a one-way ANOVA and LSD multiple comparison test were utilized to show the differences among four studied groups. The 1% glass flakes-incorporated specimens exhibited the highest mean values of tear strength, tensile strength and elongation percentage. While, the highest mean values of hardness and roughness were obtained with 1.5% specimens.

Keywords: Glass flakes, Maxillofacial silicone material (VST-50), Tear strength, Tensile strength, elongation percentage, Hardness, Roughness.

Introduction

Prosthetic devices have been widely used to compensate for defects either from congenital or acquired origin (such as disease or trauma), the need for prosthetic rehabilitation has proportionally increased since surgical intervention may not always be possible because of the location and size of the defect 1. Various types of polymeric materials such as polyvinyl chloride, polyurethanes, poly (methyl methacrylate), chlorinated polyethylene and silicones (poly(dimethyl siloxane); PDMS) elastomers are well-known materials for the production of maxillofacial prosthesis 2. However, given their low surface-free energy (hence poor wettability), they can cause adverse reactions such as tissue irritation, abrasion and ulceration 3. The ideal mechanical and physical characteristics of the maxillofacial materials should be comparable to that of the tissue to be substituted. These materials should be non-toxic and tissue-compatible, should be colored with intrinsic and extrinsic pigments, should have easy handling and processing and should not deteriorate during clinical use 4. Regarding the vulcanizing process using heat or not, silicones are available as heat vulcanized (HTV) or room temperature vulcanized (RTV). In comparison to other materials, both HTV and RTV have high tear resistance, because the specimens do not tear but expand, as in tensile elongation and high percent elongation ranging from 422% to 445% 2. Medical grade silicone adhesives have been combined with RTV silicone based in various ratios to control the elastic properties 5. Due to hydrophobic nature, these have low adhesion to non-silicone adhesive material and suffers from limited working time 6. Surface-treated silica with small particle size fillers had increase the surface area and significantly enhance the physical and mechanical properties of
silicone elastomers. Lately, researchers have found even stronger enhancement through the use of nano silica powder, which has an even larger surface area than micrometer-size silica powder. Glass flake has been utilized as a reinforcing agent in many industrial and commercial applications, their manufacturers proved that its addition to some thermoplastics has resulted in a significant improvement in flexural properties and planar reinforcement. The efficacy of the glass flakes reinforcement is strongly based on the interfacial adhesion between the glass and the surrounding polymer matrix.

Materials and Method

Materials

In the present study, a room temperature vulcanized (RTV) maxillofacial silicone (VST-50, Factor II Inc., USA) and Micronized glass flake, surface pre-treated with silane coupling agent (GF002, Glassflake Ltd, Leeds, UK) were used. The glass flake consists of flake particles of 1.3-2.3 μm thick with a range of diameters mostly below 50μm.

Specimen grouping

A total of two hundred (200) specimens were prepared and divided into five main groups based on the tests conducted (tear strength, tensile strength, elongation percentage, surface hardness and surface roughness) with 40 specimens for each test. Then, each group was farther divided into four subgroups according to weight percentage of glass flakes as follow; control group (0%), other groups contain 0.5%, 1% and 1.5% of glass flake micro-particles (10 specimen from each group).

Specimen preparation

Four plastic mold were fabricated using laser cutting machine (JL-1612, Jinan Link Manufacture and Trading Co., Ltd., China). Each mold was fabricated into the specified dimension for each test. The thickness of the plastic sheet used for tear, tensile and percentage elongation tests was 2 mm, while the sheet used for hardness and roughness tests was 6 mm. Around 40 mm × 40 mm square test specimen was used for hardness and roughness tests in accordance with ISO 7619-1:2010 (10). Whilst, an angle test specimen without nick with dimensions in accordance with ISO 34-1:2010 was selected for tear strength test (11). While, a dumb-bell specimen was chosen for tensile strength and elongation at break test according to ISO 37:2011 (12). For each plastic mold, two sheets one on top and other glued sheet on the bottom were fabricated with the same outer dimensions of the mold to sandwich the mold between them (Figure 1).

Figure 1. Metal molds for A) Hardness and roughness tests, B) Tear strength test, C) Tensile strength and elongation tests

The mixing of RTV silicone was done according to manufacturer’s instructions. For control groups, part A (base) of silicone and part B (crosslinker) was weighed with a ratio of 10:1 and mixed by a vacuum mixer (Multivac 3, Degussa, Germany) for 5 minutes.

Testing method

Tear strength test was done by adjusting the test specimen on a universal testing machine (WDW-20, Laryee Technology Co. Ltd., China) and stretching...
it with a speed of 500 mm/min until the break. The thickness of the specimen was measured at the area of the right angle by a digital caliper before testing. The tear strength was measured by dividing the maximum force obtained from the universal testing machine by the thickness of the specimen.

For tensile strength test, the thickness and the width of the narrow portion of the specimen were measured by a digital caliper at 3 areas; at the two ends and in the middle. The average of the 3 readings was considered as the thickness and the width of the specimen. Then the specimen was fixed on a universal testing machine and stretched at a crosshead speed of 500 mm/min until it breaks. The tensile strength was calculated using the following equation: recorded for each specimen, and the transverse strength was calculated using the following equation:

$$T_S = \frac{F_m}{W_t},$$

Where $F_m$ is the maximum force in Newton, $W$ is the average width of the narrow portion of the specimen in millimeters, and $t$ is the average thickness of the specimen over the narrow portion in millimeters.

On the other hand, elongation percentage was assessed using an extensometer for each tensile specimen and percentage of elongation was calculated using the following equation:

$$E_{\%} = \frac{L_b - L_o}{L_o} \times 100$$

Where $L_o$ is the initial test length in millimeters, $L_b$ is the test length at the break in millimeters.

Statistical analysis

Statistical analyses were performed using SPSS (statistical package for social science – version 24) computer software. Descriptive statistics were made which include Means and Standard deviation. The homogeneity of variances was confirmed by Levene test and also inferential statistics includes; One-way analysis of variance (ANOVA) was used to compare means among all groups and LSD multiple comparisons test was utilized to show the significance among different groups.

Results

The results of this study agree with the hypothesis that the silica filler-treated will increase the surface area of silicone elastomers and is an important factor to enhance the physical and mechanical properties.

Table 1. Descriptive statistics, one-way ANOVA and multiple comparisons test of tear strength values among different concentrations of incorporated glass flakes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Descriptive statistics</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Tear strength Mean ± S.D. (N/mm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>0% of glass flakes</td>
<td>10</td>
<td>19.45±2.70</td>
</tr>
<tr>
<td>0.5% of glass flakes</td>
<td>10</td>
<td>21.65±1.70</td>
</tr>
<tr>
<td>1% of glass flakes</td>
<td>10</td>
<td>22.80±2.12</td>
</tr>
<tr>
<td>1.5% of glass flakes</td>
<td>10</td>
<td>20.75±1.08</td>
</tr>
</tbody>
</table>
**Table 1.** Descriptive statistics, one-way ANOVA and multiple comparisons test of tear strength values among different concentrations of incorporated glass flakes.

<table>
<thead>
<tr>
<th>Groups Comparison</th>
<th>Mean Difference</th>
<th>p-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% of glass flakes</td>
<td>-2.20</td>
<td>0.018</td>
<td>*</td>
</tr>
<tr>
<td>1%</td>
<td>-3.35</td>
<td>0.001</td>
<td>***</td>
</tr>
<tr>
<td>1.5%</td>
<td>-1.30</td>
<td>0.153</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.5% of glass flakes</td>
<td>-1.15</td>
<td>0.205</td>
<td>N.S.</td>
</tr>
<tr>
<td>1%</td>
<td>0.90</td>
<td>0.319</td>
<td>N.S.</td>
</tr>
<tr>
<td>1.5%</td>
<td>2.05</td>
<td>0.027</td>
<td>*</td>
</tr>
</tbody>
</table>

**Table 2.** Descriptive statistics, one-way ANOVA and multiple comparisons test of tensile strength values among different concentrations of incorporated glass flakes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Descriptive statistics</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Tensile strength Mean ± S.D. (MPa)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>0% of glass flakes</td>
<td>10</td>
<td>4.41±0.53</td>
</tr>
<tr>
<td>0.5% of glass flakes</td>
<td>10</td>
<td>5.53±0.75</td>
</tr>
<tr>
<td>1% of glass flakes</td>
<td>10</td>
<td>5.88±0.59</td>
</tr>
<tr>
<td>1.5% of glass flakes</td>
<td>10</td>
<td>5.24±0.54</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups Comparison</th>
<th>Mean Difference</th>
<th>p-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% of glass flakes</td>
<td>-1.12</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td>1%</td>
<td>-1.47</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td>1.5%</td>
<td>-0.83</td>
<td>0.005</td>
<td>**</td>
</tr>
<tr>
<td>0.5% of glass flakes</td>
<td>-0.35</td>
<td>0.211</td>
<td>N.S.</td>
</tr>
<tr>
<td>1.5%</td>
<td>0.29</td>
<td>0.290</td>
<td>N.S.</td>
</tr>
<tr>
<td>1% of glass flakes</td>
<td>0.64</td>
<td>0.024</td>
<td>*</td>
</tr>
</tbody>
</table>

**Table 3.** Descriptive statistics, one-way ANOVA and multiple comparisons test of elongation percentage among different concentrations of incorporated glass flakes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Descriptive statistics</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Elongation % Mean ± S.D.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>0% of glass flakes</td>
<td>10</td>
<td>361.56±50.67</td>
</tr>
<tr>
<td>0.5% of glass flakes</td>
<td>10</td>
<td>488.63±35.03</td>
</tr>
<tr>
<td>1% of glass flakes</td>
<td>10</td>
<td>546.26±40.03</td>
</tr>
<tr>
<td>1.5% of glass flakes</td>
<td>10</td>
<td>446.59±27.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups Comparison</th>
<th>Mean Difference</th>
<th>p-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% of glass flakes</td>
<td>-127.07</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td>1%</td>
<td>-184.70</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td>1.5%</td>
<td>-85.03</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td>0.5% of glass flakes</td>
<td>-57.63</td>
<td>0.002</td>
<td>***</td>
</tr>
<tr>
<td>1.5%</td>
<td>-42.03</td>
<td>0.022</td>
<td>*</td>
</tr>
<tr>
<td>1% of glass flakes</td>
<td>99.67</td>
<td>0.000</td>
<td>***</td>
</tr>
</tbody>
</table>
Concerning the results of the present in vitro study, the superior tensile strength, tear strength and elongation percentage of the micro-particles glass flakes treated groups in comparison to the non-treated control group were likely attributed to the presence of high molecular weight Polydimethylsiloxane (PDMS) chains combined with glass micro-particles. Cross-linking of a high molecular weight polymer produces a highly elastic material, thus increasing the viscosity of the resulting polymer as well increase the length of PDMS chains and produce a very elastic cross-linked network and this may considered for the high mechanical properties, thus the resulting polymer matrix is able to withstand greater deformation without rupturing or tearing. Tear strength is the most important property of maxillofacial prosthesis which indicates the thin margin integrity and durability of maxillofacial prosthesis. The results of tear strength test indicated that the tear strength was significantly increased (p > 0.005) when the glass flakes were incorporated to the silicone (Table 1). In all experimental groups, the flakes mechanically interacted with the silicone matrix and associated strongly with the polymer chains thus increasing tear strength values. Tear strength of a material depends on the ability of the polymer to scatter energy at the area of the crack as tearing propagates. Micro-sized fillers dissipate strain energy within the matrix of the polymer, thus making it more resistant to tearing and a higher applied force is needed to completely break the polymer chains. This explains the increase in tear strength, as explained by Kraus in 1978 and Sun et al. (2009). For the control group, the interaction between the –OH groups and PDMS chains is not strong enough to prevent the material from rupturing under an applied force. Generally, the tensile strength depends greatly on the crosslinking between the silicone chains. It seems that the results of tensile strength was significantly increased after incorporation of the glass flakes into the RTV silicone specimens (p > 0.000) (Table 2). The silicate groups act as multifunctional crosslinks through the generation of new bonds with silicone chains. These multifunctional crosslinks increase the overall crosslinking density of the cured silicone and make it stiffer and stronger. When the polymer is subjected to tensional forces, these crosslinks inhibit the chains from sliding over each another as well prevent the breakage, thus increasing the tensile strength. The elongation of cured silicone elastomer depend greatly on the cross-linking system. One of the reinforcement mechanisms of silica is that glass micro-particles act as multifunctional cross-links via formation of strong hydrogen bond between its surface hydroxyl group and PDMS chains; these multifunctional cross-links increase the overall cross-linking density of the polymer and make it more stiff and strong. In this study, the experimental groups revealed reduction in tear strength, tensile strength and percentage of elongation when the concentration of glass flakes was increased to 1.5% might be due to the agglomeration of flakes particles within the matrix. These agglomerates are formed when two or more filler particle aggregates bind together by weak electrostatic Van der Waals forces. These agglomerates act as stress concentration

### Table 4. Descriptive statistics, one-way ANOVA and multiple comparisons test of shore A hardness values among different concentrations of incorporated glass flakes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Descriptive statistics</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Hardness Mean ± S.D. (units)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>0% of glass flakes</td>
<td>10</td>
<td>32.46±2.53</td>
</tr>
<tr>
<td>0.5% of glass flakes</td>
<td>10</td>
<td>35.16±1.41</td>
</tr>
<tr>
<td>1% of glass flakes</td>
<td>10</td>
<td>36.32±1.58</td>
</tr>
<tr>
<td>1.5% of glass flakes</td>
<td>10</td>
<td>37.47±1.38</td>
</tr>
</tbody>
</table>

### Groups Comparison

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Difference</th>
<th>p-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% of glass flakes</td>
<td>-2.704</td>
<td>0.002</td>
<td>**</td>
</tr>
<tr>
<td>1% of glass flakes</td>
<td>-3.856</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td>1.5% of glass flakes</td>
<td>-5.007</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td>0.5% of glass flakes</td>
<td>-1.152</td>
<td>0.159</td>
<td>N.S.</td>
</tr>
<tr>
<td>1% of glass flakes</td>
<td>-2.303</td>
<td>0.007</td>
<td>*</td>
</tr>
<tr>
<td>1.5% of glass flakes</td>
<td>-1.151</td>
<td>0.160</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
areas within the polymer matrix. When external forces are applied to the polymer, the agglomerates break and weaken the matrix leading to crack propagation, as explained by 13.

**Conclusion**

The addition of glass micro-particle is necessary for achieving certain degree of reinforcement that leads to significant improvement of the mechanical properties. Within the limitations of this in vitro study, the following conclusions can be obtained that the addition of various concentration of glass flake to RTV silicone material considerably improved the mechanical and physical properties of the material. The 1% glass flakes-incorporated specimens revealed the highest mean values of tear strength, tensile strength and elongation percentage. While, the highest mean values of hardness and roughness were obtained with 1.5% specimens.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Dentistry/ University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Investigation the Anti-Sterility Role of Ubiquinone-10 Against Procarbazine-Induced Infertility in Male Rats

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College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Abstract

Procarbazine is cytotoxic chemotherapy drug with obvious deleterious effects on male fertility. The present study aimed to investigate protective method via co-administration of Ubiquinone-10 with procarbazine to reduce harmful effects of procarbazine. Twenty-eight adult healthy male rats (3 months in age and weighing 250-300g) were randomly divided into four equal groups as following: animals of first group were received propylene glycol 20% orally and phosphate-buffered saline solution (PBS) intraperitoneally as control group while of second , third and fourth group were received procarbazine (30mg/kg), Ubiqunone-10 (10 mg/kg) and combination of Ubiqunone-10 with procarbazine respectively, all treatments were lasted for 9 weeks.

Results revealed that procarbazine induces significant increase in serum FSH and LH level while Testosterone level was significantly decreased. Similar decrease were also noticed in levels of glutathione (GSH) as well as activity of catalase (CAT) and superoxide dismutase (SOD) in testis. Moreover, procarbazine caused significant decrease in sperm count, motility and viability and significant increase in sperm abnormality. On the other hand, Co-administration of Ubiqunone-10 with procarbazine lead to ameliorate levels of hormone as well as to improve testicular catalase activity in addition to glutathione and superoxide dismutase levels.

Key words: sperm count, Ubiquinone-10, male fertility

Introduction

There are many categories of causative agents that included in male infertility, drugs related infertility, need to be investigated. Impact of anticancer agents on fertility are well-known. Antineoplastic drugs possess deleterious effects on germ and Sertoli cells in men, resulting severe oligozoospermia or azoospermia after chemotherapy courses. Spermatogonia are more affected by these drugs than that in other developing-stage due to differences in rate of cell division between them. Most alkylating chemotherapy agents (procarbazine) are toxic to spermatogenic cells and caused long lasting azoospermia due to their ability to cross-link DNA. In addition, there are patients within their reproductive age, therefore investigation a method to protect spermatogenesis and fertility is very important. It is obvious that cytotoxic drugs damaged mainly rapid dividing cells such as spermatogonia but the mechanism that govern ‘spermatogenic arrest’ to decline damage of testis is still unclear. About 30% to 80% of subfertility cases in male resultant from sperm damaging caused by oxidative stress. Susceptibility of spermatozoa to oxidative stress and reactive oxygen species (ROS) was documented by many previous studies that considered (ROS) involved in sperm damage as well as male infertility. Ubiquinone-10 is a molecule possess antioxidant ability, contribute in the respiratory chain. Light thrown on the ability of antioxidant to reduce male infertility and investigate whether antioxidant supplementation to infertile men with can enhances seminal indices. Ubiquinone-10 was one of various compound that tested due to its role in energy metabolism and antioxidant status via its function as a liposoluble chain-breaking for lipoproteins and cell membranes. Considerable amount of Ubiquinone-10 present in seminal plasma and spermatozoa to reduces oxidative stress and protect sperm viability. Seminal plasma Ubiquinone-10 concentration is significantly related with sperm number and motility. The present study aimed to assessment ability of Ubiquinone-10 to achieve recovery of procarbazine-induced infertility in a male rat.
Materials and Method

Animals and Experimental Design

Twenty-eight adult male albino rats, aged three months and their weight range 250-300 gm, were used in the present study. Rats were reared in metal cages and food and water were ad libitum. Male rats were distributed into four groups (7 rats each group), as following: Vehicle control group, rats were firstly gavaged with propylene glycol 20% then intraperitoneally injected with 0.25 ml of PBS after one hour of gavage. Ubiquinone-10 group, rats were orally administered 10 mg/kg BW of ubiquinone-10 (dissolved in propylene glycol 20%) via gavage. Procarbazine group, rats were injected intraperitoneally with 30 mg/kg BW of procarbazine (NATULAN® 50 mg, Sigma-Tau Pharmaceuticals Company, France) according to. Ubiqunone-10 plus procarbazine group, rats were gavaged with ubiquinone-10 then injected with procarbazine within one hour interval. Ubiquinone-10 (NOW Health group Inc. USA). All treatments were performed daily for 9 weeks. At the end of the experiment, blood samples were collected via cardiac puncture. Serum separated and frozen at –20°C till perform the biochemical and hormonal measurements. Animals were anesthetized by chloroform the sacrificed in order to harvest epididymis of each rat and right testis to perform epididymal sperm analysis and processed for enzymatic antioxidant assessment respectively.

Hormone Assay

Serum Testosterone, FSH and LH concentrations were measured by use commercial ELISA kits (American Laboratory Products Company (ALPCO), USA) according to the instructions of manufacture.

Antioxidant Indices Assessment

The right testis of each rat was homogenized in (1-4 v/v) of PBS (pH 7.4) thereafter, the homogenate was centrifuged (10 000×g for fifteen minutes at 4°C). The resultant supernatant used to perform enzymatic antioxidants measurement. Estimation the testicular SOD and CAT activity as well as GSH level were performed according to procedures described by Misra and Fridovich, Clairborne and Jollow et al respectively.

Epididymal Sperm Analysis

The cauda epididymis was excised and sperm suspension obtained by minicing it to release spermatoza onto a Petri dish to mixed with 2 ml of physiological saline (0.9% NaCl) at 37 °C. sperm concentration and progressive motility of sperm were performed by using methods described by Belsey et al while Sperm viability and morphological abnormalities of sperm were assayed according to Wells and Awa.

Statistical Analysis

All data were statistically analyzed by using Statistical Package for Social Sciences (SPSS, version 19). One-way analysis of variance (ANOVA) were carried out to compare between groups of study and least significant differences (LSD) used to identify significance of the differences between means and P value less than 0.05 were considered significant. Obtained results were expressed as the mean plus minus standard error.

Results and Discussion

The means of serum testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels are present in Table 1. The LH and FSH levels were significantly (P≤0.05) increased while testosterone levels decreased in rat treated procarbazine as compared with all other groups. Procarbazine produce toxic effects on rodent spermatogenic epithelium and induces sterility in male rodents largely by killing stem spermatogonia.. Primary gonadal dysfunction is resultant from testicular damage lead to deleterious effect on spermatogenesis and/or Leydig cells dysfunction. According to above mention effect of procarbazine, the elevation of FSH in the present study may be resulted from impairment of spermatogenesis, whereas decrease of testosterone levels that associated with LH elevation may happen as a result of Leydig cell dysfunction.

The exposure to cytotoxic chemotherapy and radiotherapy causes rise in FSH levels co-incident with low sperm counts, therefore, the of suppressive effects of testosterone and analogs of gonadotrophin on spermatogonial furthermore, chemotherapy and radiotherapy are often used in combination associated with greater testicular dysfunction and germinal epithelial damage. To lead to an availability of therapy on male infertility after cancer treatment.
Qu et al., were conclude that the therapy on male infertility after chemo- and radiotherapy is an available because sever testicular dysfunction and damage of germinal epithelia resulted post cancer treatment.

Results in table 1 also revealed that ubiquinone 10 reduces the toxic effect of procarbazine represented by elevation in testosterone level and reduce in levels of FSH and LH in ubiquinone-10 plus procarbazine group although their value still less than that recorded in control group. Ubiquinone 10 also called coenzyme Q10 supplementation was found to ameliorate the reduction in testosterone induced by chemical reproductive toxicants, mainly by neutralizing the damaging effect of the generated free radicals. The mechanism by which ubiquinone 10 could counteract testicular toxicity is associated with its potent antioxidant properties and with ability to enhance transcription of steroidogenic enzymes. Therefore, the enhancement of hormone levels in the present study may resulted from reduce damage of germinal epithelia by anti-oxidant effects exerted by ubiquinone.

According testicular antioxidant status, The changes in some enzymatic antioxidants levels in testis were shown in Table 2. Significant decline in the activity of SOD, CAT, GSH levels was observed in rats treated with procarbazine as compare to other groups. Co-administration of ubiquinone 10 to procarbazine treated rats caused elevation in activity of testicular SOD, CAT, and GSH in compare to procarbazine group. However, above mentioned antioxidants exhibit non-significant differences between rats received ubicoinone 10 alone and rats of control group. Procarbazine is one of Alkylating agents that produce reactive oxygen species (ROS) causing decrease cell capability to detoxifying thiols and depletion of antioxidant enzymes.

Table 1: Effect of Ubiquinone-10 on serum hormone level in Procarbazine- treated male rat. (Mean ± SE)

<table>
<thead>
<tr>
<th>Hormones Group</th>
<th>Testosterone (ng/mL)</th>
<th>LH (ng/mL)</th>
<th>FSH (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.52 ± 0.63</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.13 ± 0.13</td>
<td>6.52 ± 1.23</td>
</tr>
<tr>
<td>Procarbazine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.68 ± 0.09</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.41 ± 0.05</td>
<td>16.46 ± 3.42</td>
</tr>
<tr>
<td>Ubiquinone-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.85 ± 0.91</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.21 ± 0.12</td>
<td>5.79 ± 1.08</td>
</tr>
<tr>
<td>Ubiquinone-10 Plus Procarbazine</td>
<td></td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>B</td>
<td>1.73 ± 0.46</td>
<td>2.71 ± 0.08</td>
<td>9.33 ± 2.91</td>
</tr>
</tbody>
</table>

Different letters represent significant difference between groups (p≤0.05).

Superoxide dismutase stimulates the radical of superoxide peroxide dismutation into plus oxygen molecule plus H2O2 ,the later converted into H2O and molecular oxygen by CAT. GSH, have an important role in cellular redox balance and participate in free radicals scavenging inside cells and considered the first guard against oxidation. In the present study, decline in levels and activity of these antioxidants due to procarbazine treatment was corroborates with result of previous studies on testis and liver which may be resultant from testicular oxidative damage.

In the present study, ubiquinone significantly emulates the redox balance in the rat’s testis which also in coordinate with previous findings in human and rats. We suppose that the improvement of antioxidant capacity and the reduction of oxidative stress caused by procarbazine due to co-admenstration of ubiquinone could be associated with the improvement of sperm indices that observed in table 3.
Table 2: Effect of Ubiqunone-10 on testicular SOD and CAT activity as well as GSH level in Procarbazine-treated male rat. (Mean ± SE)

<table>
<thead>
<tr>
<th>Antioxidant Group</th>
<th>SOD (U /mg protein)</th>
<th>CAT (µmole / min/ mg protein)</th>
<th>GSH (µg /g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>10.17 ±2.14</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>C</td>
<td>4.09 ± 1.05</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Ubiqunone-10</td>
<td>A</td>
<td>10.55 ± 2.23</td>
<td>0.28 ± 0.12</td>
</tr>
<tr>
<td>Ubiqunone-10 Plus Procarbazine</td>
<td>B</td>
<td>7.63 ±1.91</td>
<td>0.19 ± 0.08</td>
</tr>
</tbody>
</table>

Different letter denote presence difference between groups (p≤0.05).

Results of epididymal sperm analysis in table (3) revealed that treatment with procarbazine for 9 weeks lead to a significant decrease in sperm count, studies (33,34) reported the ability of a single dose of procarbazine to cause toxic effect on spermatogenic epithelia of rodent. Moreover, Gould et al., 18 found that the procarbazine may cause most harmful effect on spermatogenic cells throughout other agents included in the MOPP chemotherapy protocol. The exact mechanism by which procarbazine can resulted the toxicity remains unknown. Conversion of procarbazine to an alkylating intermediate via bioactivation is necessary in both the anticancer activity and spermatotoxicity of the drug 35. The antioxidant properties of ubiquinone 10 make it involved in biochemistry of sperm and male infertility. Sperm number and motility are correlated with concentration of ubiquinone 10 in seminal fluid 39. However, ubiquinone 10 in the present study improve sperm count, motility and viability as well as reduces abnormal sperm percentage may be via elevation in the coenzyme Q10 level or due to improve the antioxidant status in the testis

Table 3: Effect of Ubiqunone-10 on sperm count, motility, viability and abnormality in Procarbazine-treated male rat. (Mean ± SE)

<table>
<thead>
<tr>
<th>Antioxidant Group</th>
<th>Sperm count (× 10⁶/ml)</th>
<th>Motility (%)</th>
<th>Viability (%)</th>
<th>Abnormality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>A</td>
<td>62.53 ±3.24</td>
<td>58.40 ± 9.10</td>
<td>74.23 ± 13.12</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>C</td>
<td>2.15 ± 0.76</td>
<td>18.17 ± 2.19</td>
<td>14.16± 3.25</td>
</tr>
<tr>
<td>Ubiqunone-10</td>
<td>A</td>
<td>67.21 ± 4.93</td>
<td>61.73 ± 11.02</td>
<td>78.18 ± 16.62</td>
</tr>
<tr>
<td>Ubiqunone-10 Plus Procarbazine</td>
<td>B</td>
<td>18.18 ±2.47</td>
<td>33.17 ± 3.94</td>
<td>27.36 ± 7.13</td>
</tr>
</tbody>
</table>

Different letter denote presence difference between groups (p≤0.05).
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under College of Veterinary Medicine, University of Kerbala and all experiments were carried out in accordance with approved guidelines.

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Association of BRCA1 Epigenetic, with Breast Cancer

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Abstract

Breast cancer is the most frequent malignancy among Iraqi women cancer. Epigenetic alterations such as DNA methylation were involved in the earliest phases of tumorigenesis and help for early diagnoses of breast cancer.

In this study, the methylation and unmethylation BRACA1 gene have been detected of Iraqi women patients having breast cancer.

DNA extraction kit and DNA modification kits as well as polymerase chain reaction (PCR) have been used. The samples of 80 women patients’ blood with breast cancer and 20 apparently healthy individual’s blood sample are collected during the period of February 2018- April 2018 from Oncology Hospital in the Medical City - Baghdad, Iraq.

On using PCR(24) out of (80) patients having methylated BRACA1 and (56) having unmethylated BRACA1.

As discussed in this thesis, one can recommend by using BRACA1 gene as biomarker for prediction of early diagnoses.

Keywords: methylation, unmethylation, BRACA1, modification, CPG

Introduction

Epigenetic mechanisms in normal cells

Many types of human cancers and diseases are often induced by miss regulation of gene expression. Epigenetic modifications and genetic mutations are the mechanisms for this miss regulation (genetic instability) that disrupt the function of genes, including oncogenes and tumor suppressor genes, as well as other cancer-related genes ¹.

Chromatin is made of repeating units of nucleosomes, which made of ~146 base pairs of DNA wrapped around an octamer of four core histone proteins (H4, H3, H2B and H2A) ². The main epigenetic mechanisms that modify chromatin structure are: histone modifications, DNA methylation. These modifications work together to regulate the functioning of the genome by changing the local structural dynamics of chromatin, primarily regulating its accessibility and compactness. The interplay of these modifications creates an ‘epigenetic landscape’ that regulates the way the mammalian genome manifests itself in different cell types, developmental stages and disease states, including cancer (³, ⁴, ⁵).

DNA methylation

Vertebrate DNA is modified by addition of methyl residues at the 5’ position of cytosine’s residing mostly in CG (also known as CpG) dinucleotides ⁶. Not all CGs are methylated in vertebrate genomes, and the distribution of unmethylated and methylated CGs in the genome is tissue-specific, resulting in a cell specific DNA methylation pattern ⁷. The DNA methylation reaction is catalysed by DNA methyltransferases (DNMTs) ⁸, ⁹. The C-terminal catalytic domain of DNMTs transfers methyl groups onto cytosine residues within the DNA, thus methyltransferases represent the critical enzyme class responsible for hypermethylation of tumor suppressor genes. In mammals, five members of the DNMT protein
family have been discovered (Dnmt1, Dnmt2, Dnmt3a, Dnmt3b, and Dnmt3L), of which only three were shown to possess catalytic methyltransferase activity (Dnmt1, Dnmt3a, and Dnmt3b) 10.

Cancer was the first group of diseases to be related with DNA methylation alterations and to be considered for DNA methylation targeted therapeutics 15. Aberrant expression of DNMT1 and other DNMTs, hypermethylation of tumor suppressor genes, and hypomethylation of repetitive sequences and unique genes are common epigenetic features of many types of cancers (16, 17, 18).

Silencing of tumor suppressor genes by DNA methylation provides a powerful molecular mechanism by which DNA methylation can provide a rationale for therapeutics aimed at inhibition of DNA methylation trigger cancer and re-expression of silenced tumor suppressor genes 19.

The original idea driving the study of DNA methylation changes in diseased states that limited sets of candidate genes are critical for disease progression and initiation. However, unbiased approaches could potentially detect new genes and new functional gene networks that are linked with a disease, whereas candidate approaches essentially allow proof of genes that are already known to be involved. Early studies attempting to take benefit of the emerging role of methylation of promoters of tumor suppressor genes in cancer examined whether methylation of specific CGs in tumor suppressor genes associates with different breast cancer clinical states 19.

BRCA1 is directly involved in preventing cells from growing and dividing in a controlled manner and repairing damaged DNA. BRCA1 (breast cancer) gene encodes a multifunctional protein involved in DNA repair, control of cell-cycle checkpoints, protein ubiquitination and chromatin remoulding 20. It was basically detected as a gene responsible for familial breast cancer 21.

Material and Method

The study of this thesis include 20 healthy female and 80 female patients with breast cancer where their ages ranged between (30-60 years), who are suffering from breast cancer disease. The blood are collected from Medical city in Baghdad, Iraq during the period from February 2018 to April 2018.

DNA extraction kits and DNA modification kits have been used to extract and modify the DNA structural. Also nano drop techniques have been used to measure the DNA concentration before modify it. Then Polymerase chain reaction have been used with special primers designed for BRACA1 gene Table (1).

**Table (1) Primers Sequences and Their Size of Amplicon**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1-M-F</td>
<td>5'-GAGAGGTGTGGTTTATAGCGG-3'</td>
<td></td>
</tr>
<tr>
<td>BRCA1-M-R</td>
<td>5'-CGCGCAATCGCAATTATATA-3'</td>
<td></td>
</tr>
<tr>
<td>BRCA1-U-F</td>
<td>5'-ACTCAGTTAGCGGTGAATC-3'</td>
<td></td>
</tr>
<tr>
<td>BRCA1-U-R</td>
<td>5'-CCCATCCTGCAAATCACAACCA-3'</td>
<td></td>
</tr>
</tbody>
</table>

Result and Discussion

The results show that out of 80 patients with breast cancer disease 24 women’s have methylation BRCA1 gene. And the remain 56 women’s have un methylated BRCA1 gene Table (2). BRCA1 promoter methylation is associated with the early onset of breast cancer other Saudi Arabia studies also show that 13 women with breast cancer have methylation BRCA1 out of 47, and 34 out of 47 have un methylated BRCA1.
The association between BRCA1 promoter methylation and the clinicopathological features of breast tumors was assessed. A strong association ($p<0.001$) was found between BRCA1 methylation and young age ($\leq 40$ years) at diagnosis (1875% figure (1). Table (2) the proportion of methylated BRCA1 within the age of 40 and younger was 75% which confirm the important role for BRCA1 gene in the initiation and progression of breast cancer. While only 25% of the breast cancer patients aged 40 years and older have shown BRCA1 DNA methylation in from peripheral blood samples.-for more details one can see appendix (B.1) .

In Al-Moghrabi, studies show strong association between BRCA1 methylation and young age ($\leq 40$ years)

<table>
<thead>
<tr>
<th>BRCA1 gene types</th>
<th>Women’s with breast cancer disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylated BRCA1 gene</td>
<td>24</td>
</tr>
<tr>
<td>Un methylated BRCA1 gene</td>
<td>56</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
</tr>
</tbody>
</table>

Table (2) : BRCA1 * Age at diagnosis Cross tabulation

<table>
<thead>
<tr>
<th>Age at diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;= 40</td>
<td>&gt;= 40</td>
</tr>
<tr>
<td>Methylated</td>
<td>Count</td>
</tr>
<tr>
<td>% within BRCA1</td>
<td>75.0%</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>Count</td>
</tr>
<tr>
<td>% within BRCA1</td>
<td>25.0%</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
</tr>
<tr>
<td>% within BRCA1</td>
<td>40.0%</td>
</tr>
</tbody>
</table>

Figure (1) : Distribution of Patients According to Age at Diagnosis and the BRCA1
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under College of Science, Mustinusiryah University, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References
Evaluation of Hematological Parameters And DHEA-S Hormone Association with Acne In The Province Of Thi-Qar

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Abstract
This study was conducted in the labs of the College of Education for Pure Sciences, Al-Hussein Educational Hospital and Al-Nahrain Specialized Laboratory of the Health Department of Thi-Qar province, during the period from October 2018 to March 2019. The study included of 100 blood sample of patients with acne (39 males) and (61 females) and their age between 10-35 years. The aim of the study was to assess the level of DHEA-S hormone in acne vulgaris patients in the serum using a technique enzyme-linked immunosorbert Assay (ELISA). The study included the test of the susceptibility of phagocytic cells on phagocytosis (coefficient of phagocytosis) and measurement of hematological parameters (Red blood cell count, hemoglobin ratio, total and differential white blood cells count).

The results showed the presence elevate in the level of DHEA-S for 21 sample out of 70 patients with acne. Results showed that there was no association between disease incidence and DHEA-S when compared with the healthy control in the population of Thi-Qar province. Increase coefficient phagocytosis was significant (P≤ 0.05) in all patients with acne compared with a group of control. As for the blood parameters, the results of the present study showed no significant differences (P>0.05) in the rates of red blood cells and hemoglobin in all acne patients compared to the control group. It also showed increased rate of counting the total of WBCs and differential cell neutrophils and lymphocytes (P≤0.004), (P≤0.004), (P≤0.025), respectively in acne patients compared to healthy controls group.

Keywords: Acne vulgaris, Hematological parameters, DHEA-S.

Introduction
Acne vulgaris is the most common a chronic inflammatory disease of the pilosebaceous unit and characterized by non- inflammatory lesion (comedones with black and white heads), inflammatory lesions like (papules, pustules and nodules) (1,2). Resulting from increased sebum production, inflammatory process, androgen excess states, abnormal follicular epithelial differentiation, insulin resistance, obesity and the proliferation of Propionibacterium acnes 3.

Acne can be classified according to its severity into: mild, moderate and severe using the Global acne assessment scale 4. It occurs primarily in the oily (seborrheic) areas of the skin that involved face, neck, back and chest (5,6).

Androgen enhances sebum production and follicular keratosis plays important role in pathogenesis of acne. Therefore, testosterone, androstenedion, dehydropiandrosterone sulphat are involved in the evolution of acne 7.

The circulating androgen dehydropiandrosterone sulphat (DHEA-S) is mostly produced in the adrenal glands. DHEA-S is the highest androgen concentration in the serum in both sexes and is considered the most important regulator of sebum secretion (8,9). Elevated DHEA-S levels in male and female with acne have been repeatedly demonstrated in many studies 6. Acne is a common feature in the path of endocrine diseases distinguished by elevated levels of androgens 10.

The aim of the current study was to assessment of some blood parameters (WBCs, RBCs, HB), phagocytosis and knowledge of their relationship with acne and study of dehydropiandrosterone sulphat (DHEA-S) and its relationship with acne.
Materials and Method

Subjects :- The study groups have the following included:

This study was conducted in the labs of the College of Education for Pure Sciences, Al Hussein Educational Hospital and Al-Nahrain Specialized Laboratory of the Health Department of Thi-Qar province, during the period from October 2018 to March. The patients in this study were divided into three groups According to age :

1-First group : comprised 10 acne patients less than 20 years old.

2-Second group : comprised 10 acne patients whose ages between 21-30 years old.

3-Third group : comprised 6 acne patients whose were 31 years old and above.

In addition, the study included 30 people as a control group, with no history or clinical evidence of common acne or any other chronic disease, and have no obvious abnormalities, divided into three groups according to their age:

1-First group : comprised 10 acne patients less than 20 years old.

2-Second group : comprised 10 acne patients whose ages between 21-30 years old.

3-Third group : comprised 6 acne patients whose were 31 years old and above.

Blood sample collection: -

Blood samples were collected by venipuncture from 100 patients (39 males, 61 females) and 30 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. each blood sample was divided into two parts:

a- Two milliliters were placed in a sterile tube containing EDTA for WBC deferential count and phagocytosis processes ( during 2 hour).

b- Three milliliters were put directly in a sterile Gel tube and allowed to clot , then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -20 C˚ freezing. These sera 70 acne patients (30 male, 40 female) and 20 controls ( 10 male,10 female) were used for estimating the concentration of DHEA-S hormone.

Phagocytosis Procedure :

The procedure was carried out according 11 as follow: 0.025 ml of the collected blood was put in plane tube , then added for it 0.05 ml from Killed yeast suspension which was prepared by soluble 10 grams of Saccharomyces cerversiae yeast made in Turkish pakamaya company in 150 milliliters of normal saline and put suspension in water bathe for 60 minutes , then this suspension was filtered after cooling.

0.025 ml of HBSS were added to the mixture and incubated at 37 C˚for 30 minutes . One drop of the mixture was placed on a slid and smeared, then left to dry , fixed by methyl alcohol (99%) for min and stained for 20 min with Wright stain. then, examined under oil immersion.

No. of phagocytic cells

Phagocytosis index = × 100%

Total number of cells

Hematological assay:

The hematological tests including (total and differential WBCs counts ,RBC and Hb ) were done by using Genux Auto Hematology Analyzer in which the results read and printed automatically.

Serological assay: -

A number 1 kit was used for DHEA-S hormone, where DHEA-S hormone concentration were measured for control group (non-infected) and infected group by using the Enzyme-linked Immunosorbent Assay (ELISA) method and using the ELISA device and a measurement kit designed for this device for hormone.

Statistical Analysis

The analyses of data were expressed as mean ± SD. The comparisons between each Acne patients group with age matched healthy control were performed with T-test using computerized Minitab program. The comparisons among the three age group of Acne patients were performed with analysis of variance ( Chi – square, Odds Ratio) by using computerized Minitab 14 program. P< 0.01 was considered to be the least limit of significance . All the statistical analysis were done by
Results

Hematological parameters

RBCs, Hb count:

This doesn’t show the results of the current study in Table (1), no significant differences (p≤0.05) in blood picture of patients and healthy control group. The red blood cells (RBCs) count, hemoglobin concentration (Hb) in Acne compared with the healthy control group, where the RBCs mean are (4.89 ± 0.58) X10 cell/ml for patients and (4.98 ± 0.55) X10 cell/ml for healthy control group respectively, while the hemoglobin concentration are (13.40 ± 2.54) g/dl for patients and (13.52 ± 1.64) g/dl for healthy control group respectively, as the table (1).

Phagocytic index:

The results of the current study show significant differences (p≤0.05) in the rate of phagocytosis, as it increased the rate of phagocytosis in Acne patients to (45.89 ± 1.07) compared with the healthy control groups, and that the rate of phagocytosis (29.58 ± 1.34), as in the Table (1) and figure (1).

Table (1): some hematological parameters of Acne patients and healthy control

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient (N=100), Mean ± SD</th>
<th>Healthy (N=30), Mean ± SD</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>4.89 ± 0.58</td>
<td>4.98 ± 0.55</td>
<td>0.423</td>
</tr>
<tr>
<td>Hb(g/dl)</td>
<td>13.40 ± 2.54</td>
<td>13.52 ± 1.64</td>
<td>0.808</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>45.89 ± 1.07</td>
<td>29.58 ± 1.34</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Degree freedom (df) = 128 * P.value ≤ 0.05

Figure (1): Phagocytosis of yeast cell by Phagocytic cell 1000x
WBCs, Neutrophil, Lymphocytes, Monocytes, Eosinophil and Basophil count:

The results of the current study show significant differences between the two groups of patients compared with healthy controls that has been shown in Table (2). The WBCs count, Neutrophil, Lymphocytes, Monocytes, Eosinophil and Basophil in Acne vulgaris compared with the healthy control group, WBCs count that means are (9.98 ± 3.30) for patients and (8.14 ± 1.73) for healthy control group respectively, neutrophils means are (6.04 ± 2.57) for patients and (4.61 ± 1.32) for healthy control group respectively, lymphocytes means are (2.95 ± 1.08) for patients and (2.60 ± 0.60) for healthy control group respectively, while monocytes means are (3.80 ± 1.80) for patients and (3.85 ± 1.05) for healthy control group respectively, Eosinophil means are (2.05 ± 1.04) for patients and (4.65 ± 1.57) for healthy control group respectively, eventually the basophils there would have no significant difference, too and the means are(0.94 ± 0.80) for patients, and (0.78 ± 0.74) for healthy control group, as in Table (2).

Table (2): Hematological parameters in Acne patients and healthy control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient (N=100), Mean ± SD</th>
<th>Healthy(N=30), Mean ± SD</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>9.98 ± 3.30</td>
<td>8.14 ± 1.73</td>
<td>0.004*</td>
</tr>
<tr>
<td>NEU</td>
<td>6.04 ± 2.57</td>
<td>4.61 ± 1.32</td>
<td>0.004*</td>
</tr>
<tr>
<td>LYM</td>
<td>2.95 ± 1.08</td>
<td>2.60 ± 0.60</td>
<td>0.025*</td>
</tr>
<tr>
<td>MONO</td>
<td>3.80 ± 1.80</td>
<td>3.85 ± 1.05</td>
<td>0.931</td>
</tr>
<tr>
<td>ESO</td>
<td>2.05 ± 1.04</td>
<td>4.65 ± 1.57</td>
<td>0.104</td>
</tr>
<tr>
<td>BASO</td>
<td>0.94 ± 0.80</td>
<td>0.78 ± 0.74</td>
<td>0.319</td>
</tr>
</tbody>
</table>

Degree freedom (df) = 128 *P.value ≤ 0.05

Serological parameters

Serum DHEA-S hormone concentration:

The results of the current study show no significant difference (p>0.05) concentration of DHEA-S hormone in a group of patients compared with a group of control as concentration as follow: DHEA-S concentration (3.71 ± 1.51) for male patients compared with a group of control (1.96 ± 0.48), while DHEA-S concentration (3.31 ± 1.16) for female patients compared with a group of control (3.28 ± 1.21) with a significant difference (0.05) as seen in Table (3).

Table(3) : Comparison of serum DHEA-S hormone concentration (µg/ml) of the patient groups with healthy control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject</th>
<th>No. of cases</th>
<th>Mean ± SD</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S</td>
<td>Male patient</td>
<td>30</td>
<td>3.71 ± 1.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male control</td>
<td>10</td>
<td>1.96 ± 0.48</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Female patient</td>
<td>40</td>
<td>3.31 ± 1.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female control</td>
<td>10</td>
<td>3.28 ± 1.21</td>
<td></td>
</tr>
</tbody>
</table>

Χ²= 0.66 degree freedom (df)=3 P.value > 0.05
Figure (2): Standard curve of DHEA-S

\[ y = 0.073x - 0.0354 \]
\[ R^2 = 0.932 \]

Figure (3): Acne lesions, including comedones (white arrows) and pustule (black arrow) on the facial skin.

Figure (4): Acne lesions, including papule on the facial skin.
Discussion

Acne vulgaris belong to a complex and multifactorial disease of pilosebaceous unit. Acne pathogenesis cannot viewed as impartial since it is etiologies influence each other. Pathogenesis of acne vulgaris recently emphasizes inflammatory process. The latest finding indicates role of P. acnes as a triggering factor of inflammatory responses which exerts an influence on severity of acne vulgaris. Pathways underlying the formation of papules, pustules, and nodulocystic acne which involve cytokines were detected. The results of the current study showed a lack of significant difference (p>0.05) in each of the concentration of RBC between the two groups of patients (4.89 ± 0.58) and healthy control group (4.98 ± 0.55), and the percentage of the hemoglobin in patients (13.40 ± 2.54) and healthy control (13.52 ± 1.64) and attribute the lack of significant differences to that the inflammation of acne doesn’t affect the effectiveness of red blood cells and its ability , as well as RBC membrane not effective and nor old and this is reflected in the level of HB ,there has been no change in rate.

Phagocytosis is considered the first line of defense against injury and carried out by Neutrophil and Macrophage and Dendritic cells, which should migrate to the places of injuries or infections in order to do its job and be the migration process in response to some of the attraction of chemical and phagocytosis one of Nonspecific immune mechanism and motivated by the entry of foreign bodies.

The current results showed significant difference in percentage of phagocytosis (P<0.05) patients with Acne (45.89 ± 1.07) and healthy control (29.58 ± 1.34). The reason of this result due to increasing the numerous of Neutrophil and Macrophage during the inflammation, and due to high levels of cytokines such IL-1α and IL-10 which belong to cellular compound inflammation that leads to the increase of phagocytosis especially in patients with acne vulgaris. This study agree with.

On the other hand there was a significant variation (p<0.05) in the WBCs count between Acne patients and the control group, the increase in WBCs may be due to the response of the immune system to face the inflammatory effects in the tissues and increase is the rise in the number of neutrophils in this study are agree with.

The results of our study showed that the proportion of hormone in males with acne (3.71 ± 1.51) compared with healthy males (1.96 ± 0.48) is higher than women where the proportion of women infected (3.31 ± 1.16) compared to healthy women (3.28 ± 1.21) Where the rise in males significantly while in females is not.
significant. The reason is probably due to the amount of DHEA-S hormone produced in the body is related to age and sex, in addition to its concentration in the blood may undergo changes during the day. As well as, the physiological role of androgens in women during adulthood is unclear. Women who suffer from hyperandro-genic disorders may develop acne, hirsutism, deepening of the voice, androgenic alopecia. This study agree with (16,17,18,19).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under University of Thi-Qar- College of Education for Pure Sciences, Biology Department and all experiments were carried out in accordance with approved guidelines.

**References**


Evaluation of Humoral Immunological Profile in Infertile Women after IVF Failure in Baghdad City

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1College of Health and Medical Technology, Middle Technical University

Abstract

**Background:** An (in Vitro) Fertilization (IVF), means fertilizing an ovum with a spermatozoon outside the body in a culture dish under controlled culture conditions. And It was taken a principle option to treat infertility, which is define as a disease of the reproductive system can be diagnosis by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. The cause of infertility may be immunological or genetic. A progress was made an in vitro fertilization (IVF) techniques, However the majority of transferred embryos fail to implant. Morphology embryo scoring is the standard procedure for most IVF centers for choosing the best embryo, but remains limited since even the embryos classified as (top quality) may not implant. The initial investigation on the cause recurrent (in vitro) fertilization failure ignites the attention on the reproductive immunology. The production of antibodies is an immunomodulatory factor that causes failure of embryo implantation, and the most commonly studied antibodies in women with implantation failure after IVF procedure, include antiphospholipid (aPL), antisperm antibodies (ASA), and antinuclear antibodies (ANA).

**Objectives:** The aim of this study is to determine the immunological aspects of patients after IVF failure, such as ; antiphospholipid antibodies (aPLs), antinuclear antibodies (ANAs), and anti-sperm antibodies (ASA).

**Patients, Materials and Method:** This prospective study was undertaken on ninety infertile women undergoing in vitro fertilization (IVF) programme. Their age was from 20 years to 49 years. Blood specimens were collected from all women, on the day of egg pick up, and screened for all studied parameters by ELISA, and were statistically analyzed.

**Results:** In the present study clinical pregnancy rate was 27.8%. And the present study showed a highly significant difference (P<0.01) in aPL (IgM, IgG) between patients and control group. The data in the present study, demonstrated a highly significant difference (P<0.01) in Antinuclear antibodies (ANAs) between the studied groups. Also a highly significant difference (P<0.01) in Antisperm antibodies (ASAs) between the studied groups.

**Conclusions:** In vitro fertilization (IVF) failure, precisely failed embryo implantation, associated with produced auto-antibodies (anti-phospholipid antibodies, anti-sperm antibodies, and anti-nuclear antibodies), in the sera of the female.

**Key words:** In vitro fertilization, immunological profile and infertility.

**Introduction**

Infertility is usually defined as the disappointment of a couple to conceive after one year unprotected regular intercourse. It has been assessed that 10 –15% of couples look for medical help for fertility assessment, and the problem is obviously equally shared between male and female associates, most of the time, childlessness is some level of subfertility where 1 in 7 couples need specialist help to conceive. The infertility is divided in to; primary infertility, when never the couple have had a live birth, and secondary infertility, which means a failure to achieve live birth after having a live birth or an abortion.
The etiology of infertility is believed to be multifactorial. The realized hazard components incorporate genetic abnormalities, ovulatory disorders, tubal damage, uterine or peritoneal issues, and male factors. Unexplained infertility is characterized as absence of conception notwithstanding a year of unprotected intercourse, not clarified by anovulation, poor sperm quality, tubal pathology, or any known reason for infertility. The most applied treatments for unexplained infertility is (in vitro) fertilization, includes using standard protocols for controlled ovarian stimulation, oocyte retrieval with ultrasound management, insemination, embryo culture and trans-cervical replacement of embryos at cleavage or blastocyst-stage. Repeated (in vitro) fertilization (IVF) failure has been characterized as the lack of implantation after transfer of an embryo, which can cause physical, emotional, and financial distress for couples looking to begin family. For a significant number of ‘unexplained’ failures of IVF treatment, an immunologic basis has been suspected for a long time, and thinking about a few distinctive in vitro immune parameters, the abnormalities are related with reproductive failure in clinical cases. One of the most unexplained infertility issue in female is recurrent implantation failure (RIF). RIF is determined when transferred embryos fail to implant following several (in vitro) fertilization (IVF) treatment cycles.

There are several autoimmune factors have been associated with implantation failure result. And certain investigations concentrated on relationship between the autoimmune system and the IVF/ICSI result highlighting the role of autoantibodies during treatmen. The predominance of antiphospholipid antibodies (aPLs), and antinuclear antibodies (ANAs) was increased in unexplained infertility women. That the aPL have been described with increased frequency in women with recurrent implantation failure after (in vitro) fertilization (IVF). If aPL contained in the serum, it will have a significant impact on ovulation, fertilization and/or early embryonic development. The aPL have been presented to interact in the maternal-fetal interface causing a defective endovascular trophoblastic invasion, That aPL may induce infertility through their negative effects on implantation.

Immune or immunological infertility is identified when produced antibodies bind to the antigens spontaneously, which is occurring on either the male or female gametocytes. Antibodies bind to seminal proteins or structures present on the sperm or oocyte. So, anti-sperm antibodies (ASA) have been recognized more frequently than anti-oocyte antibodies. And the presence of ANAs is associated with immunologically estimated infertility, that the female, with high levels of ANA in their sera have also higher ANA levels in their follicular fluids, and these levels will be negatively correlated with the number of good quality embryos obtained in IVF/ICSI cycles. That ANAs could directly impact oocyte maturation and embryo development resulting in infertility. found that approximately 50% of spontaneous abortion women express ANAs, so that ANAs could be related to infertility, premature ovarian failure, and embryo transfer failure. The presence of ANAs consider a risk factor for infertile women and can be included in the mechanism, resulting in embryo implantation failure, and could impair the fertilization rate and the number of good quality embryos and thus could lead to IVF/ICSI failure.

The pathogenic mechanisms for antiphospholipid antibodies (aPL)-mediated pregnancy loss

1- aPL and intraplacental thrombotic phenomena

placental thrombosis and infarction were described. It was suggested that such an effect might be cause by the in vitro capacity of aPL, mostly anti-β2 glycoprotein I antibodies (anti-β2GP1), to induce a pro-coagulant state by disruption of annexin A5 shield on trophoblast and endothelial cell monolayers.

2- aPL and direct placental damage

Numerous evidence showed that alternative aPL, mediated pathogenic mechanisms which is directly affect placental tissue. The observation of β2GPI reactivity with trophoblast cell membranes, human stromal decidual cells, and human endometrial endothelial cells (HEECs), suggested the placental tropism of anti-β2GPI antibodies. aPL Inhibit trophoblast differentiation, which exposed via the reduced secretion of women chorionic gonadotrophin (hCG), aPL estimate trophoblast damage and apoptosis.

aPL and inflammation-mediated damage

Acute inflammatory events can be result in a negative pregnancy outcome by the activity of pro-inflammatory mediators, for example, complement, tumor necrosis factor-α [TNF-α], and chemokines that have been shown to have a role in animal models of aPL.
result in fetal loss\textsuperscript{12}

The pathogenic mechanisms for antisperm antibodies mediated pregnancy loss:

It was established that antispermatozoal antibodies impairment fertility by different mechanisms; The antispermatozoal antibodies may perhaps mask some antigens which are essential for the penetration of the spermatozoa into the ovule, Spermatozoa and antibody, can procedure complexes on uterine tissue, which triggers the excretion of histamine and makes the exclusion of the implanted embryo. It is assessed that around 5% of infertility cases are of immunological origin and related to the presence of ASAs in both women or/and men\textsuperscript{6}

The pathogenic mechanisms for antinuclear antibodies - mediated pregnancy loss

In the early stages after fertilization, the determining whether the fertilized egg would successfully develop into an embryo done by stability of nucleus, which consider the key factor in this process. In the process of mitosis, new components of the cells will be produced. Some of these components, as well as proteins, polysaccharides, and glycoproteins, may get exposed at the surface of the cells. In normal conditions, these components would not be known by the immune system. However, in an imbalanced immune system, these components may trigger the activation of autoantibodies. These autoantibodies generally associated with the components of nucleus, and ANAs have been supposed as an important immune cause of the implantation failure\textsuperscript{10}

Subjects, materials and methods

Ninety infertile couples undergoing (in vitro) fertilization (IVF) program from the Kamal Al-Samarraee IVF Center, Ministry-of Health in Baghdad-Iraq and The Institute for Infertility Diagnosis and Assisted Reproductive Technologies Al-Nahrain University in Baghdad-Iraq, enrolled in this study through the period from November / 2018 - June /2019. All couples were subjected to the basic fertility work-up of the fertility center which consists of history- taking, physical examination, ovulation detection, evaluation of tubal patency and uterine cavity, and semen analysis. The patients were divided into two groups according to success or failure IVF, into sixty five infertile women have implantation failure and didn’t get pregnant , and twenty five infertile women managed to get pregnant after where the embryos is placed in the uterine cavity in order to implant(control group). Venous blood samples were collected from all patients and healthy controls. Chemical and biological materials used in this study (to estimate to measure serum markers as ASA, aPL, and ANA) , including ; Phospholipid screen IgG/IgM ELISA, ANA Screen ELISA, and Anti-Spermatozoa Antibody (ASA) ELISA.

Results

The current study showed a highly significant difference (P<0.01) between patients ( IgM +v) and control group (aPL IgM -ve), That the mean level of of non-pregnant women and control group was (A 15.76±0.15 and B 3.5±0.31 u/ml respectively) . Also result of estimation aPL(IgG) in sera of another patients and control group show that the mean of non-pregnant women with (IgG +ve ) was (A19.7±3.22)u/ml, and for pregnant women (control IgG) was (B 0.85±0.25)u/ml. The statistical analysis shows a highly significant difference ( P<0.01) between pregnant (control group) and non-pregnant with positive result for aPL IgG (Table 1). These results were in agreement with study done by \textsuperscript{17}, that they compared with fertile control women, significant differences in the prevalences of aPLs in the IgG and IgM. All all of the IgG aPLs studied were significantly elevated over fertile control values. These results were in agreement with study done \textsuperscript{2}, who found that the results consistently showed significantly higher levels of aPL among women experiencing IVF failure.

<table>
<thead>
<tr>
<th>Test groups</th>
<th>Statistic</th>
<th>Range</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Patients group (IgM +ve)</td>
<td>A 15.76± 0.15</td>
<td>15.63</td>
<td>0.08</td>
</tr>
</tbody>
</table>
**Cont... Table (1) Estimation of aPL in the sera of patients and control group**

<table>
<thead>
<tr>
<th>Study groups (s/co)</th>
<th>Statistic</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>NO(%)</th>
<th>Std Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive samples</td>
<td>A 1.67 ± 0.3</td>
<td>1.28</td>
<td>2.21</td>
<td>14(15.5)</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative samples</td>
<td>B 0.57 ± 0.06</td>
<td>0.41</td>
<td>0.69</td>
<td>51(56.7)</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>B 0.55 ± 0.08</td>
<td>0.43</td>
<td>0.69</td>
<td>25(27.8)</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Two valid default values were computed to complete the statistical analysis for the comparison purpose, noting that the number of positive samples is only one and its value 15.73.

The data demonstrated in table (2) showed that The mean in non-pregnant with ANA+ve, and control group was (A 1.67±0.3) s/co, (B 0.57±0.08)s/co respectively. This data demonstrated a highly significant difference (P<0.01) between the studied groups. The current study was in agreement with study done by 10, which found, the ANA expression in the infertile patients was higher than that in the fertile group. Another Study showed reveals the presence of ANA may exert a detrimental effect on the outcome of IVF impaired oocyte and embryo development and decreased pregnancy and implantation rate 21.

**Table (2): Estimation of ANA in the sera of patients and control group.**

<table>
<thead>
<tr>
<th>Study groups (s/co)</th>
<th>Statistic</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group</td>
<td>Mean ± SD</td>
<td>Minimum</td>
</tr>
<tr>
<td>Positive samples</td>
<td>A 1.67 ± 0.3</td>
<td>1.28</td>
</tr>
<tr>
<td>Negative samples</td>
<td>B 0.57 ± 0.06</td>
<td>0.41</td>
</tr>
<tr>
<td>Control</td>
<td>B 0.55 ± 0.08</td>
<td>0.43</td>
</tr>
</tbody>
</table>

And the data demonstrated in Table (3) showed that The Mean in non-pregnant with ASA+ve was (A 121.26±13.98)U/mL, and the Mean for the pregnant (control group), was (B 31.00±4.34) U/mL. This data demonstrated a highly significant difference (P<0.01) between the studied groups. The current study was in agreement with study done by 7 in Baghdad, suggested that detections of antisperm antibodies in the serum of infertile women were which is significantly (p<0.05) higher from control group, the conclusions of this study was These higher levels of complement components may be due to activation of classical pathway by ASA that directed against sperm antigens ending in defect in function and motility of the sperms.
Table (3): Estimation of level of ASA in the sera of patients and control group.

<table>
<thead>
<tr>
<th>Study groups (U/ML)</th>
<th>Statistic</th>
<th>Range</th>
<th>NO(%)</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group</td>
<td>Mean ± SD</td>
<td>Minimum</td>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>Positive samples</td>
<td>A 121.26±13.98</td>
<td>108</td>
<td>155</td>
<td>12(13.3)</td>
</tr>
<tr>
<td>Negative samples</td>
<td>B 31.87±4.67</td>
<td>23</td>
<td>40</td>
<td>53(58.9)</td>
</tr>
<tr>
<td>Control</td>
<td>B 31.00±4.34</td>
<td>23</td>
<td>38</td>
<td>25(27.8)</td>
</tr>
</tbody>
</table>

F ratio(P value) 49 (0.000)**

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under College of Health and Medical Technology, Middle Technical University and all experiments were carried out in accordance with approved guidelines.

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The Socio-Psychological Variables in the Meaning of Self-Consciousness and Movement Satisfaction of Physical Education and Sport Sciences Students

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Abstract

This research aims at determine socio-psychological variables, self-consciousness and movement satisfaction of Physical Education and Sport Sciences students. Then, identify the relationship among the socio-psychological variable, self-consciousness and movement satisfaction of Physical Education and Sport Sciences students and predicting the socio-psychological variables in the meaning of self-consciousness and movement satisfaction of Physical Education and Sport Sciences students. In order to achieve the aims of the research, the researchers used the descriptive method by using the surveying method to recognize the variables of the research, the relationships between them and the predictions studies in order to find out how much self-consciousness and movement satisfaction contribute to the psychosocial variable. The community of the research made of Physical Education and Sport Sciences students/ University of Babylon / Fourth grade- morning classes, they were (140) students, the sample of the research made up of (15) students in order to represent the research sample by (11%), the researchers distributed the questionnaires for the research variables to the sample of the research, after analyzing and discussing the results they found out the following:

1. Physical Education and Sport Sciences students enjoy high degrees in movement satisfaction, self-consciousness and the socio-psychological variables.

2. The increasing of socio-psychological variables degrees has a relationship with the increasing of degrees in movement satisfaction, self-consciousness.

3. There is an ability of predicting the psychosocial variables, depending on the reality of movement satisfaction, self-consciousness.

Keywords: Movement Satisfaction, Self-Consciousness and the Socio-Psychological Variables.

Introduction

The Sport is considered one of the main pillars of the overall advancement of civilized societies, which explain the behavior and activates of persons in community through a set of rules and foundations, which have a direct effect in guiding the relationship among athletes in a social form that expresses the spirit of Sports and its role in creating an opportunity to race, held and work together. The Socio-Psychological variables are considered as one of the most important requirements of sport, which athlete lives in, it’s also contained things, persons and thoughts that communicated with the athlete. The Socio-Psychological variables also considered as an important factor of the psychological health of the athletes, their directions towards the team and their performance. Modern trends have confirmed that the Socio-Psychological variables in which athletes live in and a group of friends participates in it, to achieve more and better production, which makes players fell of comfortable and confidence, then it gives them the ability to adaptation and satisfaction of performance and all that is related to indications, which contains lots of Abstract factors such as feelings and emotions.
of person and team members. So, it may be say that the socio-psychological variables have a significance of player or student who face a difficult environmental situations under certain circumstances. Then, the player or the student should has a high awareness of the socio-psychological variables, because it has a great role in improving the teams’ score, because of that all athletes in the sports teams try to recognize their abilities together in order to achieve the best performance. So, athlete or student who has a motivation to achieve his maximum physical abilities, skills, knowledge and plans in order to achieve the aims of the team, which has a big role in the growth of the athlete physically, psychologically and socially. The psychological awareness is more important than all the awareness of modern life, because it studies human as the most important in the world. The self-knowledge, feeling and awareness begin from human’s observation of his/herself, with full recognition of his/her feelings as he see. Through some of starting points in which the researchers begin with, it was clear to him a clear vision in his feeling of the present research problem through the reality of the studied sample, the students of the university, the study period within the framework of the student community, in which the element of effect and effectiveness, with the varying level of awareness, culture and socialization. The university students are not immune from different circumstances and extreme life situations and conflicts and contradictions and problems that increase the requirements of life, so that there are many requirements that need to be satisfied and desires that may not be achieved, which leads them to surrender to temptations life and its charms which become urgent needs that lead the person to satisfy them in ways that may be socially unacceptable. Success in motor activity is connected with satisfaction and enjoyment, which considered as motivational elements that encourage to interesting in motor activity, the sport activities have a big and positive role to the participants in it, whether physically, mentally, socially or psychologically. Movement satisfaction is the person’s feeling of ability to achieve motor skills with his/her feeling of satisfaction and happiness as a result of performance. As Ratib mentioned “The positive previous experiences in which the player achieves success and satisfaction in any activity lead to increased willingness and desire to continue to practice the activity, which provides a better opportunity to improve the sports skills and the feeling of happiness and satisfaction as a result of achieving the aims”.

Statement of the Research Problem

One of the most important factors that contribute to the achievement of sports success is the availability of an ideal psychological and social situation for the athlete, the education is received by members of the family and society may contribute to the acquisition of many values, habits and experiences, the formulation of some rules may also contribute of behaviors that it should be directed in the light of the self-awareness of the individual and his self-building as well as the individual’s sense of motor building about the level of performance, as its measurement gives us an indication of the state of satisfaction of the learner or the athlete, so, there is no study in such a topic, thus the researchers decided to build measures for these variables to recognize the importance of the three variables and the relationship between them.

Aims of the Research

This research aims at the following:

1. Identify the changing psychosocial variables, self-awareness and motor satisfaction of students of physical education and sports science.

2. Learn about the relationship between the psychosocial variables and both self-awareness and motor satisfaction for students of physical education and sports science.

3. Predicting the psychosocial variables in the meaning of self-awareness and motor satisfaction for students of physical education and sports science.

The Research Hypothesis

The researchers hypothesized the following:

1. There is a real relationship social-psychology variables and both the self-awareness and movement satisfaction

2. There is a possibility to predict the social-psychosocial variables in the meaning of self-awareness and movement satisfaction for the students of physical education and sports science.

Limitations

1. Human limit: the students of the fourth grade in the college of physical education and sports science at
University of Babylon for the academic year 2018-2019.


3. Place limit: Classrooms of the college of Physical Education and Sports Science at the University of Babylon.

**The Basic Terms**

The social-psychosocial variable: is the qualitative aspect of interpersonal relationships, which is manifested as a sum total of psychological conditions and facilitates or hinders the productive activities and the overall development of the individual in the group, (al-Hayali: 2011, 12).

Self-awareness: identifying the individual’s feelings and vocabulary system, monitoring and deriving self-assessing results, as well as monitoring one’s own self through his/her actions and trying to influence the results of his/her actions to become more effective.

Movement satisfaction: “is the outcome of emotional feelings felt by the individual towards a particular activity, and express the extent of satisfying the appropriate needs and achieve the aims for which he/she joins this activity”.

The Methodology and Practical Procedures of the Research

The methodology of the research: the description method was used by a survey, correlation relationships and predictive studies to suit its relevance and the nature of this research.

**The Sample and Community of the Research:**

The community of the research consists of the students of college of physical education and sport science/ University of Babylon/ Fourth grade/ morning classes that made up of (140) students, the sample of the research contained (15) students to present the sample of the research with percentage of (11%).

**Tab No. (1): Shows the SD and X of the sample**

<table>
<thead>
<tr>
<th>Variables</th>
<th>X</th>
<th>SD</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychological variable</td>
<td>119.4000</td>
<td>12.87744</td>
<td>15</td>
</tr>
<tr>
<td>Movement Satisfaction</td>
<td>104.9333</td>
<td>11.94910</td>
<td>15</td>
</tr>
<tr>
<td>Self-awareness</td>
<td>130.2000</td>
<td>10.83117</td>
<td>15</td>
</tr>
</tbody>
</table>

The previous table (1) shows that they are distinguished by a good social-psychosocial variables compared to the hypothetical mean which is (90) for having an arithmetic mean of (119,400) that means they are in a good health. This may be due to the fact that they are fourth stage students in the college of Physical Education and Sport Sciences and the positive effects of its lessons. While the degree of standard deviation, which reached (12,877), indicates a slight variation in the homogeneity of the mentioned sample of the psychiatric climate variable, movement satisfaction and self-awareness, especially if we know that the highest hypothesis of movement satisfaction and psychological variables is (150), while the highest hypothesis of self-awareness is (160) Degrees.

**Tab No. (2) Shows the value of Correlation Coefficient between Psychological variable and Movement Satisfaction**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean X</th>
<th>SD</th>
<th>Value of Correlation Coefficient</th>
<th>Significance Level</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychological variable</td>
<td>119.40</td>
<td>12.87</td>
<td>.569*</td>
<td>0.01</td>
<td>Sig.</td>
</tr>
<tr>
<td>Movement Satisfaction</td>
<td>104.93</td>
<td>11.94</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The previous table (2) shows that there is a correlation between psychological variable and movement satisfaction where the correlation coefficient reached (0.569) at the level of significance (0.01), after conducting correlations to achieve the second objective of the research, the results proved above in the table, and through the examination of the fact of correlation (Sig.) shows that the relation is strong and real, which indicates the credibility of the first hypothesis of the research, which is a real correlation between both movement satisfaction and self-awareness of the social-psychosocial variables of students of the college of Physical Education and Sports Science. This indicates that the satisfaction of the movement satisfaction a very significant contribution to the imposition of psycho-social variables, and can be explained by the results that the student who has a positive psychological variable is shown by his behavior and positive behaviors that make the student self-confidence which leads to the feeling of the student Satisfaction with his/her behavior and movements, which promotes a sense of movement satisfaction, this will achieve good results, cheers to positive self-talk, which helps the player in the formulation of goals well and work to achieve them and look at all the positive things, which makes him confident of success in his tasks and performance because of the high confidence needed, as motor satisfaction is one of the most important determinants in the development of confidence if the student’s self-image is positive, he is certainly confident and high. First in the competition.

Tab No. (3) Shows the Value of Pearson Correlation between the psychological variables and self-awareness

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean X</th>
<th>SD</th>
<th>Value of Correlation Coefficient</th>
<th>Significance Level</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychological variable</td>
<td>119.40</td>
<td>12.87</td>
<td>.799**</td>
<td>0.01</td>
<td>Sig.</td>
</tr>
<tr>
<td>Self-awareness</td>
<td>130.200</td>
<td>10.83117</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to Table (3), there is a strong correlation between psychological variable and self-awareness in the research sample. The correlation coefficient was (0.799). Students who have a good self-awareness helps the student to formulate aims well and work to achieve them and look at all the positive things which makes him/her confident of success in his/her tasks and performance due to the high confidence needed. Self-awareness is an important determinant in the development of confidence and enhance self-esteem. If the student’s image of himself is positive, he certainly has high confidence and good, and therefore can be said that the more self-awareness when the player positive, the higher self-esteem leading to achieve good sports results It is the top spot in the competition.

**Predicting the Psychological Variable in the Meaning of the Variables Studied**

Tests and measures may be used for the purpose of forecasting, meaning to anticipate a future outcome in the light of the results of tests and measures, and therefore use some appropriate statistical methods such as regression so that individuals who can predict their success according to a particular test can be selected.

**Array Data:**

The aim of this array is to obtain one value that reflects the psychological variables of all the sample members, as well as one value for the variable of self-awareness and the value of movement satisfaction. This measure was applied to the students in the colleges of physical education and sports sciences at the University of Babylon, which number (140) students, The data were then extracted and processed statistically by extracting the arithmetic media and standard deviations. The table below shows that.
Tab No. (4) Shows Means X and SD of the sample vocabulary at the three scales

<table>
<thead>
<tr>
<th>No.</th>
<th>Variables</th>
<th>Sample</th>
<th>Mean X</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Social-psychological variables</td>
<td>15</td>
<td>119.40</td>
<td>12.87</td>
</tr>
<tr>
<td>2</td>
<td>Self-awareness</td>
<td>15</td>
<td>130.2000</td>
<td>10.83117</td>
</tr>
<tr>
<td>3</td>
<td>Movement satisfaction</td>
<td>15</td>
<td>104.93</td>
<td>11.94</td>
</tr>
</tbody>
</table>

Predicting the psychological variables in the meaning of self-awareness and movement satisfaction among students of the colleges of physical education and sports sciences:

**Array of Simple Correlations**

The main aim of this research is to predict the social-psychosocial variables in the meaning of self-awareness and movement satisfaction. This can only be achieved by knowing the simple correlations between these variables by using the Pearson equation to calculate the array of inter-correlations and Table (5) shows that.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean X</th>
<th>SD</th>
<th>Value of Correlation Coefficient</th>
<th>Significance Level</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychological variable</td>
<td>119.40</td>
<td>12.87</td>
<td>.569*</td>
<td>0.01</td>
<td>Sig.</td>
</tr>
<tr>
<td>Movement satisfaction</td>
<td>104.93</td>
<td>11.94</td>
<td>.799**</td>
<td>0.01</td>
<td>Sig.</td>
</tr>
</tbody>
</table>

The above table shows that there is a correlation between psychological variables, movement satisfaction and self-awareness. Social and self-awareness has reached a value of 0.799 which is also an acceptable correlation, which gives an indication that the social-psychosocial variables of the student is moving in the same direction movement satisfaction, while the relationship between the social-psychosocial variables and self-awareness has a value (0.799), which is also an acceptable correlation relationship.

The predictive value of social-psychosocial variables in the meaning of self-awareness among students of the colleges of physical education and sports sciences:

The researchers used linear regression equations and table (6) shows that.
Tab (6) Shows the Linear Regression Equations of the Research Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>N. of C.</th>
<th>Value of C.</th>
<th>Correlation Coefficient</th>
<th>Nature of Correlation</th>
<th>Calculated T-Value</th>
<th>Level of Sig. on 0.05</th>
<th>Calculated F-Value</th>
<th>Sig. Level of 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social-Psychological Variables of the meaning of Self-awareness</td>
<td>F. A</td>
<td>4.420</td>
<td>.799**</td>
<td>Simple</td>
<td>-2.486</td>
<td>.019</td>
<td>1.046</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>0.714</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26.391</td>
<td>.000</td>
</tr>
<tr>
<td>Social-Psychological Variables of the meaning of Movement Satisfaction</td>
<td>F. A</td>
<td>5.004-</td>
<td>.569*</td>
<td>Simple</td>
<td>26.391</td>
<td>0.000</td>
<td>8.466</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>0.318</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.932</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>0.408</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.743</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Through Table (6), the researcher can deduce depending on the coefficients referred to, for the purpose of constructing the equation of the contribution of each of the self-awareness and movement satisfaction of the members of the research sample as follows:

Linear Regression ($y_i = \beta_0 + \beta_1 x_{i1} + \cdots + \beta_p x_{ip} + \varepsilon_i = x_i^T \beta + \varepsilon_i, \quad i = 1, \ldots, n$)

Psychological Variables = 4.420 + 0.799 X 82 (self-awareness) = 62.968

Psychological Variables = 5.004- + (0.318 X 82) + (0.408 X 101) = 62.28

**Conclusions**

Investing the present values of the studied variables in achieving the aims of physical education and sports sciences. Making attention of the social-psychosocial variables of college students through the formation of social relations between the students themselves and the teaching staff. The need to organize courses and scientific lectures for students of the college and teachers by specialists in sports psychology to promote positive values and organize the social-psychological variables when students to achieve the desired aims. The need for teachers and psychologists to hold periodic meetings with students in order to achieve the ideal social-psychosocial variables to express their feelings in a constructive manner and guide them.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under University of Babylon, College of Physical Education and Sport Sciences, Iraq and all experiments were carried out in accordance with approved guidelines.

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Evaluation of Efficacy and Safety of Oral Cyclosporine in the Treatment of Severe Alopecia Areata

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Abstract

Background: One of common forms of scaring hair loss is alopecia. Alopecia is an autoimmune disorder that is characterized by relapsing and remitting episodes of non scaring hair loss. Several treatment options are nowadays available to be prescribed to patients with alopecia areata including local and systemic corticosteroid therapy, immune suppressant agents and contact immunotherapy. Nonetheless, none of these agents have been 100 % effective in eradication of the disease totally and some agents are associated with intolerable side effects. Therefore, dermatologists are continuously searching for new agents and modalities in order to get better response and less sided effects.

Aim of the Study: The current study was planned and conducted to evaluate the efficacy and safety of oral cyclosporine in treating alopecia areata.

Patients and Method: This study included 35 patients with alopecia areata. The study was conducted at the dermatology unit, Al-Diwaniyah Teaching Hospital, Al-Diwaniyah Province, Mid-Euphrates Region of Iraq. The study started on June the 2nd 2018 and extended to June the 2nd 2019. The study was approved by the institutional ethical approval committee and included a verbal consent was obtained from each participant following full demonstration of the aim and procedures of the study. Every patient has received a daily dose of oral cyclosporine (3 mg/kg) for a period in the range of 2 to 12 months. Variables included in the current study were age, gender, type of alopecia areata, duration of disease, duration of treatment and treatment response. Outcome in the end of the study included clinical response and main side effects.

Results: Following treatment with oral cyclosporine, 28 patients (80 %) had satisfactory response and 7 patients (20 %) had unsatisfactory response. Treatment response was not correlated to age, gender, disease duration or type of disease (P > 0.05). There were unremarkable adverse effects in association with oral cyclosporine use.

Conclusion: oral cyclosporine is effective and safe mode of treatment in patients with alopecia areata

Key words: Cyclosporine, alopecia areata, Iraq

Introduction

In modern dermatological practice, hair loss may be broadly classified into scaring and non scaring hair loss ¹. Scarring hair loss is characterized by loss of hair follicles, whereas, non scaring hair loss is characterized by preservation of hair follicles ¹,². One of common forms of scaring hair loss is alopecia ³,⁴. Alopecia is an autoimmune disorder that is characterized by relapsing and remitting episodes of non scaring hair loss ⁵,⁶. Clinically it is classified into several forms according to the distribution of hair loss ⁷. Patchy involvement of scalp is commonly referred to as multifocal alopecia, the one which involves all scalp hair is called alopecia totalis, while the type that involves all body hair is called alopecia universalis ⁸,⁹.

The immunological basis of disease was made clear during the last 60 years owing to the discovery of several immune cells and humeral agents in close association with the disease ¹. The prevalence rate of alopecia areata has been estimated to fall between 0.1% and 0.2 %,
whereas, the lifetime incidence has been claimed to be approximately 2 % \(^1\). The data about gender variation in disease incidence are conflicting with some reports referring to a higher incidence among females \(^12\), while others have reported higher incidence rate in association with male gender \(^5\). There are no reported suggestions about ethnic predilection \(^13\). The onset of the disease can happen at any age; however, the majority of cases are seen before 40 and the mean age of onset has ranged from 25 to 36 \(^14\). The disease is often severe when being encountered in child age group \(^15\).

Familial clustering and the occurrence of disease in patients with positive family history and twins more often than general population suggest a strong genetic contribution in the causation of this dermatological disorder \(^1\). Moreover, the disease happens in association with a number of autoimmune disorders such as systemic lupus erythematosus, thyroid disorders and rheumatoid arthritis \(^14\). A physical environmental trigger has rarely been identified, but a number of psychological triggers have been linked to the onset of disease such as emotional or physical stress \(^14\).

Several treatment options are nowadays available to be prescribed to patients with alopecia areata including local and systemic corticosteroid therapy \(^16\), immune suppressant agents \(^17\) and contact immunotherapy \(^1\). Nonetheless, none of these agents have been 100 % effective in eradication of the disease totally and some agents are associated with intolerable side effects \(^17\). Therefore, dermatologists are continuously searching for new agents and modalities in order to get better response and less sided effects.

Oral cyclosporine has been regularly tested as a mode of treatment of alopecia areata by a number of researchers during the last three decades but the results were controversial \(^18-20\). Due to lack of consensus in the available published articles regarding the efficacy and safety of oral cyclosporine in treating alopecia areata and because of the rarity of national data with regard, the current study was planned and conducted in Al-Diwaniyah province, Mid-Euphrates region of Iraq to evaluate the efficacy and safety of oral cyclosporine in treating alopecia areata.

Patients and Method

This cohort study included 35 patients with alopecia areata. The study was conducted at the dermatology unit, Al-Diwaniyah Teaching Hospital, Al-Diwaniyah Province, Mid-Euphrates Region of Iraq. The study started on June the 2\(^{nd}\) 2018 and extended to June the 2\(^{nd}\) 2019. The study was approved by the institutional ethical approval committee and included a verbal consent was obtained from each participant following full demonstration of the aim and procedures of the study. Every patient has received a daily dose of oral cyclosporine (3 mg/kg) for a period in the range of 2 to 12 months. Variables included in the current study were age, gender, type of alopecia areata, duration of disease, duration of treatment and treatment response. Outcome in the end of the study included clinical response and main side effects.

Data were transferred into an SPSS (version 23) spread sheet and presented as mean, standard deviation, range, number and percentage. Mann Whitney U test was used to study difference in mean between two groups, while, Chi-square or Yates correction were carried out to assess association between any two categorical variables. The level of significance was considered at P ≤ 0.05.

Results

The currents cohort study was based on the inclusion of 35 Iraqi patients with alopecia areata. The mean age of enrolled patients was 23.69 ±10.03 years and the age range was from 10 to 40 years; most of the cases were above 15 years of age, as shown in table 1. The study included child age group cases that accounted for 10 (28.6 %), but none of patients was above 40. According to gender, the study included 24 (68.6 %) males and 11 (31.4 %) females with a male to female ratio of 1:2.18. According to type of alopecia, patients were categorized into 12 (34.3 %), 20 (57.1 %) and 3 (8.6 %) as multifocal, totalis and universalis, respectively, as shown in table 1.

Following treatment with oral cyclosporine, 28 patients (80 %) had satisfactory response and 7 patients (20 %) had unsatisfactory response. Treatment response was not correlated to age, gender, disease duration or type of disease (P > 0.05), as shown in table 2. There were unremarkable adverse effects in association with oral cyclosporine use.
Discussion

The pathophysiology of alopecia areata has been linked to immune basis a long time ago and anti-immune treatment whether local of systemic steroids or immune suppressant agents have extensively tested in treating the disease; however, complete satisfactory response has been never reached in daily dermatological practice. Therefore, there is always a need to search for new modalities in treatment approach especially if one takes into consideration the psychological trauma and poor quality of life experienced by victims who never get improved.

Cyclosporine is unusually practiced in our country when dealing with alopecia areata; however, reports from all over the world have encouraged its use in such dermatologic autoimmune disease. Nevertheless, the rate of successful remission and safety of the drug has been frequently questioned, despite the bulk of data supporting its efficient and safe use. For that reason, we in the current study included 35 patients with alopecia areata aiming at exploring the efficacy and safety of oral cyclosporine in alopecia areata and found satisfactory response in 80% of cases. Side effects were negligible especially when one takes into consideration the relatively low dose of the drug (3 mg/kg/day).

Several previous studies have found comparables results. For instance Açıkgöz et al., described significant hair growth in about 50% of enrolled cases; however, they mentioned that 3 patients have discontinued the drug because of intolerable side effects. Açıkgöz et al., have stated that they reach a dose of 6 mg/kg in some patients which is probably the cause behind intolerable side effects. Jang et al., have shown that the response of patients to oral cyclosporine was significantly better than that of oral betamethasone and that side effects were much less in association with cyclosporine use. Indeed, the findings of the latter study support the findings of the current study in that oral cyclosporine if an efficient safe treatment modality for alopecia areata. In one review it has been stated that the success rate of oral cyclosporine was in the range of 25 to 76.6% and that side effects may limit its use.

In conclusion, it is better to wait for future more research work to make clear consensus about the value
of oral cyclosporine in patients with alopecia areata; nonetheless and based on our observation and previous reports oral cyclosporine appears to be effective and safe mode of treatment in patients with alopecia areata

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**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under Department of pharmacology and therapeutics, Faculty of medicine, University of Kufa, Najaf, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

Removing Methods of Ultracide Residues in Imported Apple Peels in Iraqi Local Markets

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Abstract

The residues of ultracide insecticide used in controlling apple insects may have public health problems. This work was designed to find much effective method that capable of removing as much as possible of such residues from imported apple peels available in local markets. Imported apple samples were collected during winter 2018 from different local markets and divided into four subsamples as physical treatments where the first was left unwashed apple, the second was washed by tap water, the third was washed with saline solution and finally apple sample washed by tap water with acetic acid. Apple peels of all examined samples were left to dry in an oven at 85°C for 24 h and then powdered. 2.5 g from each sample was received 12.5 ml distil water to estimate the insecticide residues by using gas chromatography. It has been found that unwashed apple peels had the highest ultracide insecticide level followed by that which washed by tap water while the peels washed by saline water was obviously free from the insecticide residues.

Key words: Ultracide residues, apple peels, tap water, saline water, acidic water.

Introduction

Crop plants may severely suffer from many diseases that are induced by different kinds of organisms such as viruses, fungous, herbs, insects, rodents and other organisms. Such crop diseases would cause significant reduction and economic losses. From early ages, farmers have tried to prevent such diseases and improve crop growth by using other biological species that can control plant pathogens [1-3].

In modern agriculture, biocides in general and pesticides in particular are very efficient materials to control crop diseases and enhancing agricultural production and therefore it is used worldwide. However, such insecticides particularly those contain phosphorous and intensively used with vegetables and fruits are concerned the most toxic material that may cause severe health problems to infants and teenagers [4-6].

The randomly use of various insecticides by farmers without scientific and official surveillance in terms of trade name, type and recommended doses suggested by producing companies may lead to real public health troubles since most of these insecticides are very toxic for both the health and the environment [7,8] where such disaster related to the fungicide was occurred in Iraq during seventeenth of last century with wheat grains treated with organ mercury [9].

Organophosphorus compounds are very toxic (LD50-25) especially ultracidine pesticide which affects cholinesterase due to increasing exposure and causes reducing of the enzyme level in the blood resulting in syndromes of muscarinic, nicotinic and central nervous system damage [10,11].

The application of such compounds as agricultural insecticides being very effective in protecting various edible plant crops from different insect species [12,13] where such edible plants have large surface area capable of taking significant quantities of these insecticides [14] such as peach, apple, cherry, ground berries, grape, spinach, potato, lattice and other vegetables.

Most of these oregano phosphorene insecticides such as parathion, malathion, dipterex and ultracide may form significant public health and environmental threats where several studies have reported the inhibition of choline enzyme at higher doses [15,16].
However, handling and removing such pesticide residues was received much attention being very significant public phenomena [17-21] which needs to be investigated thoroughly and to find a proper removing method that should be accessible particularly at every home.

This work was designed to find accusable way to remove the residues of ultracide insecticide from imported apple where apple samples were collected from local markets and subjected to lab tests.

**Material and Method**

Imported apple samples were collected from local markets during winter 2018 and these samples were divided into four subsamples and subjected residues removing test where the first sample was left unwashed while the second was washed thoroughly by only tap water. The third sample was washed by diluted saline water at concentration of 1% for one minute and the forth sample was by acidic water which was tap water mixed with acetic acid at concentration of 1%. However, each treatment was replicated three times.

All apple samples after being treated as explained above were peeled and the peels placed in electric oven at 85 C° for few minutes then moved out and left to cool under room temperature. Dried peel apple samples were grinded by electric grinder and 2.5 g from each peel powder was placed in 250 volumetric flasks and received 12.5 ml distil water (1:5) and shacked for 30 min. Ultracidine pesticide residues was isolated by using chromatography/Mass Spectrometry-GC/MS following the method of previous study [22].

**Results and Discussion**

All obtained results were presented for unwashed, tap water, saline water and acetic acid water washed respectively of examined apple samples (Figures of analysis not shown).

It has been found that the residues of ultracide insecticide in apple peels of unwashed sample had highest sharp peak by the value of 87.376 among other treatments. Also, there is another peak with a value of 62.621 due to reactions of unknown materials.

The residues of organophosphorin compounds in apple peels may be accumulated in human body up to lethal dose causing severe damage of various tissues and organs and also affect cell divisions [18].

In case of apple sampled washed by tap water, it was found the ultracide insecticide residues were less than those left unwashed and had two peaks where the first peak was highest sharp peak was 12.23 while the second peak was 86.77

Again, the residues of such phosphoric compounds may be accumulated up to the lethal dose having severe effects as explained previously, the ultracide insecticide residues in apple peel after being washed by saline water.

Apparently, there was no insecticide residues in the apple samples washed by saline water but there was a small peak due to unknown material reaction. However, it seems that saline water has totally destroyed all toxic phosphorene compounds.

Finally, the ultracide insecticide residues were again totally removed from apple sample washed by acidic water (acetic acid and tap water)

The obtained results suggest that the better effective methods of removing any quantity of residues of the ultracide insecticide are washing such apples by using either saline water or acidic water that are available at all homes.

Several previous works examining similar pesticide residues removing methods were carried out worldwide. Previous work [23] has used dissolved ozone to get rid of four pesticides from vegetable which were methyl-parathion, parathion, diazinon and cypermethrin and found that low level (1.4 mg/l) of dissolved ozone was capable to oxidize up to 90% of all these pesticides except parathion within 5 minutes. Similar method (dissolved ozone) was followed by a work [24] carried on by using low concentration of dissolved ozone to remove four residues of four pesticide species and recorded a removing rate up to 99% within 30 minutes but it has reported that such removing rate was affected by temperature, concentration of applied ozone gas and preparing dissolved ozone concentration. In another study [25], five species of solutions were applied to remove blended organophosphorus pesticide residues from chines cabbage such as glycyrrhiza-mung bean, garlic juice, sods-salt, tap water and washed rice water and reported that soda-salt solution was the best removing solution while washed rice water showed very
poor effects. Another study [26] has examined the impacts of using four washing methods (tap and ozone waters, ultrasonic cleaning and boiling) on 16 pesticide residues from raw strawberries and found that within less than 5 minutes, boiling technique had the highest removing rate followed by ultrasonic cleaning and ozonized water while tap water had the lowest removing rate which were 92.9%, 91.2%, 75.1% and 68.1% respectively.

Apparently, it seems that several solutions can be used to remove pesticide residues from fresh fruits and vegetables where some of them such as ozonated, saline and acidic waters are capable of removing rate up to 90%. Nevertheless, such ability may be affected by certain variable such temperature, solution concentration and pesticide residues quantity as suggested by previous study [24]. So, the current results are supported by those of other works mentioned above but it necessary to examine other variables particularly temperature that may have significant effect on these methods used in this study.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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**References**


Perception of Roles as Peer Educators in High Schools to Prevent Drug Abuse among Adolescents

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Abstract

Adolescence is a transition period between childhood and adulthood, adolescent tends to have high curiosity and seek identity. Adolescent is a vulnerable group as a target for drug dealer. The objective of this study was to determine the perception of students in carrying out their duties as peer educators. This study used qualitative study approach with a case study design which intended to define the perceptions of the research subjects through in-depth interviews, observations, and also document studies. The students’ perception of their roles as peer educators such as a keeper of confidential informations, motivators, sources of information, and discussion partners. The conclusion was that the students who were selected as peer educators in their school environment had a positive perception of the application of the method to prevent drug abuse among their peers. Therefore, the application of peer educator method in high schools in North Surabaya can be used as a reference for other schools to implement peer educator method to their students.

Keywords: Student, Peer Education, Drug Abuse Prevention, School

Introduction

Adolescence is a transition period between childhood and adulthood which is characterized by changes in physical, psychological and psychosocial aspects (1). The process of independent for these adolescents are easily affected by their environment and it can lead to drug abuse (2).

Drug abuse that occurs in adolescents is caused by many factors that lead to addiction. Several factors causing drug abuse include personality, family, environment, education, and social and community, and vulnerable population factors (3).

A report from the United Nation Office on Drugs and Crime (UNODC, 2015) stated that drug abuse at 15-64 years old was as much as 3.4-6.6% of the total world population. The increase number of drug abuse also occurred in several ASEAN countries, especially Indonesia. Of the 100% narcotics transactions in the ASEAN region, 40% were in Indonesia (5).

East Java Narcotics Agency explained that in 2010 there were 2,986 cases, and then in 2011 there was an increase of 3,008 cases. The highest drug abuse occurred in adolescents between 15-19 years old with a percentage of 37.88% in 2015. Social reinforcement among students must be carried out as the basis of national development.

A research explained how to fund youth-based school programs. The article presents the rationale and content of PASS, a Danish school-based prevention program targeting cannabis-culture-related beliefs among high school students (i.e. typically 15–19 years old). The objective of the program was to prevent or delay initiation of cannabis use and limit use among students who already initiated (7).

The implementation of the peer educator was developed based on the assumption that teens will feel more open in expressing their problems to their peers. Therefore, the establishment of peer educators in the school environment is a strategy that can minimize the occurrence of drug abuse in adolescents.
The senior high schools in North Surabaya that formed peer educator method empowered their students as peer educators with the aim that students can play an active role in activities in the school environment as well as outside school activities. In carrying out the peer educator strategy, the students were required to be able to play an active and sensitive role in the social environment in their schools especially as facilitators for their friends in providing solutions and bridging the problems experienced by them. The aim of this study was to understand the students’ perception of their role as peer educators.

Material and Method

This study used qualitative study approach with a case study design which intended to define the perceptions of the research subjects. A Case Study Research was conducted to gain in depth/detail/complete information to obtain a complete picture of an event, in the sense that the data collected in the research was studied as a whole, complete and integrated.

The key informants of this study were students who appointed as peer educators. The informants were chosen to explore information according to the purpose of the study. The number of informant in this study was 10 student identified as peer-educator by the teachers.

In a qualitative case study approach, the researcher becomes the main instrument in the data collection because the aim of the research was to explore the students’ perceptions to obtain comprehensive information. Other data collection tools used to support this research were interview guidance, observations and tape recorders.

The interview guidance was prepared based on the objectives of the study, which was also guidance in the form of questions to explore the students’ perception in carrying out their roles as peer educators. The observation was done by observing the students’ access to health promotion media in the school environment such as the presence of drug abuse prevention posters. The researcher conducted an interview by using open questions. The researcher tried to create a comfortable atmosphere, so that the informants could freely convey information that the researcher wanted to obtain.

The data analysis in this study was done through some procedures, namely data reduction, data presentation and conclusion drawing. After collecting the data, the recording of the interview with each informant was transcribed and the key words related to this study was identified.

There are four forms of data analysis along with the interpretation in the case study research, namely collection of categories, direct interpretation, researcher formed pattern and found comparability between two or more categories, and later the researcher developed the result of the data obtained to infer the result of the research.

Regarding the explanation of peer educator about the program that has been running at each school, the program has varied activities. The informants explained that the existing program was considered to be less effective because of the lack of activities, so the school have to make a lot of programs that could build the students’ enthusiasm in avoiding drug abuse.

Findings

Role as a Keeper of Confidential Information

In carrying out the role as peer educators, the informants explained that their interaction with their peers was spontaneous and informal. This means that the interaction can occur anytime and anywhere (inside or outside school area). However, the principle of confidentiality should be upheld.

Seven students revealed their reason for being peer educators was because they wanted to help their peers to solve their problem. According to informants, the students tend to feel reluctant or afraid if they had to consult their problems with teachers. For example, as SK (Student 1) explained:

I want to help my friends. When they get into problems and go to the guidance counselor, they certainly will not feel comfortable. They will be less open. If they have friends to share what the feel, they will be more opened.

Although not all of the problems can be solved properly, peer educators are committed to help their peers. Peer educators also sometimes ask for help or input from the Teachers regarding problems experienced by their peers to find solutions for the problems. SK (Student 1) explained:
The problem is that there are students who tell us about their problems with their significant others, but then I cannot give them solution because I have no experience in that field. But, they said, what kind of counselor you are. That is what I experienced.

**Role as Motivators**

Based on the result of the interview, the peer educators also revealed that they have a role as motivator. They have ability to help their peers solve the problems become a motivation for them.

The peer educators encouraged their peers to solve problems without having to do negative things like drug abuse. By motivating their peers, it is hoped that this program will be able to help students to solve their problems, and motivate them to be more productive at school activities. As DH (Student 2) pointed out:

> When my friends seemed not in a good mood, I must be sensitive. I would ask them why. Maybe they are having problems or feared of something, like fear of falling into negative things. So I should motivate them.

The role of peer educators as motivators is an attitude that can be an example for other young people that as the next generation of the nation they must stay away from drug abuse by participating in positive activities at school and outside of school.

**Role as a Sources of Information**

The lack of knowledge of adolescents about the consequences of drug abuse is caused by the limited access to obtain information regarding the impact that will happen. Peer educators are students who become resources or counselors for their peer groups (11).

Peer education is a program that is implemented as a useful source of information for adolescents regarding the dangers of drug abuse so that they can avoid all kinds of drug abuse forms.

In conveying information on the dangers of drug abuse, peer educators must have good knowledge. At present, trainings are provided for the peer educators, but are still not as intensive as they once were, so the peer educators need support from the schools and the government in providing guidance and trainings for them so that they can give information to their peers optimally. The following comment was from RH (Student 3):

> [...] (The training) from the school is still minimal. Although the school gives us support but it is not maximal. There is also a funding problem.

**Role as Discussion Partners**

To achieve the goal to make students understand the danger of drug abuse, peer educators can provide peer counseling through formal and non-formal situations. Formal situation counseling can be carried out in counseling events and non-formal counseling can be done by holding a discussion (11).

The role of the peer educators as friends of discussion can also be seen from how often their friends at the school use the peer educators as facilities to exchange thoughts about information in preventing drug abuse. The following comments were from DH and KN:

> Almost every day I get questions from my friends (DH, Student 2).

Yes, (I) often (experience the same thing), it is just not as often as every day. The problem is that we are now in different classes, XII class. We often had discussion when we were still classmates. (KN, Student 5).

Students who become peer educators have the ability to understand the main tasks and roles as peer educators. However, sometimes, some cases were not well understood because the peer educators have never experienced the same things.

Actually, this can be anticipated by conducting regular joint discussion that is accompanied by teachers. The discussion can be a media to exchange views on the possibility of cases that occur in adolescents and discussion of actions to be taken so that the peer educators can carry out their role to the fullest.

**Discussion**

Perception is a process of how stimulus that influences responses is selected and interpreted the perceptions of each person towards an object is different\(^{(13)}\). Therefore, perceptions have a subjective nature. Stimulus is any physical form or verbal communication that can affect individual responses.

The previous research explained that peers are friends who can influence each other towards goodness or toward risky behavior\(^{(14)}\). In a study regarding
strategies for prevention and intervention of drug abuse among students in secondary schools in Kenya, it was showed that drug abuse was widespread among students, regardless of gender, and that there was a strong relationship between drug abuse and family members abusing drugs \(^{(15)}\).

According to the psychological abilities, adolescents, including students, have mental characteristics that are still unstable. This condition makes them vulnerable to the effects of negative interactions such as drug abuse. Students must have strong and independent attitudes and characters with the spirit to build the nation by staying away from drug abuse.

One program that has been running in several high schools in North Surabaya, is peer educator as a facility to share stories, experiences, and problems faced by students. Peer educators thought peer education to be an effective method to prevent drug abuse \(^{(16)}\). To be effective, the program is recommended to be started during middle school instead of high school, that families and local institutions should be involved in the program, and the students should be directed to participate in arts, sports, and social activities.

Goodlad (1998) explained that there are seven regulations as criteria for implementing peer educator strategy, one of which is giving trainings to students who are peer educators with assignments and explaining techniques in teaching the possibility to solve problems that are often experienced among adolescents. The Goodlad Theory provides an explanation that the school should be represented by accompanying teachers or Teachers to hold trainings so that the peer educators can carry out their duties in a good way \(^{(17)}\).

The facilities provided by the school are in the form of support to the peer educators. If there is an event outside the school that asks the peer educators to contribute, the peer educators should get a dispensation to leave the class. In addition, the guidance counselor’s room can be used as a discussion room, unless this discussion activity has not been conducted regularly.

Based on the data obtained from the peer educators and also from the teachers, there was no funding assistance from the school. The funding was intended to be developed by the peer educators in making an activity in the school environment involving the students at the school with the aim of getting to know the danger of drugs and preventing drug abuse.

From the results obtained by the informants regarding the perception of the peer educators in carrying their duties, the researcher reviewed the need to improve the support for the peer educators to be more maximal in carrying out their roles.

Peer educator prgram needs to be organized, starting from appointing a chairman of the management. The point is that it will ease the peer educators to hold meetings or discussions with other peer educators and teachers.

In addition, the guidance counselor, as a companion to the peer educators, must routinely control the development and notice obstacles faced by the peer educators. If there are problems that are difficult to solve by the peer educators, the accompanying teachers can provide input or solution.

In addition to the assistance that can be provided by the guidance counselor, the person in charge for the health unit must also routinely provide information to the peer educators. The information provided is specifically related to health impacts that will occur if adolescents commit drug abuse. Thus, in conveying the danger of drug abuse to the students, the peer educators can link it to the health problem that might occur.

The school must also involve the peer educators with activities outside the school that are collaborated with stakeholders to provide trainings for the peer educators. The activities can be in the form of counseling from the National Narcotics Agency or from the police related to the prevention of drug abuse. In addition, the school must also provide facilities to the peer educators to hold regular discussions.

**Conclusion**

The conclusions of this study are about the perceptions that arise in students who are motivated to become peer educators because they want to contribute to prevent their peers from committing drug abuse. Most students who are peer educators felt changes in themselves such as getting new friends from various classes, understanding life problems and gaining new knowledge when they were invited to the counseling. Only a few students who just felt neutral or stated that they did not feel any change in their individual self.
The constraint that was still felt by the peer educators was the lack of guidance, so that when they faced new problems that they had never experienced before, their role as peer educators could not function optimally.

Conflicts of Interest: The authors declare that there is no conflict of interests.

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Identification of Recurrent Laryngeal Nerve and Parathyroid Glands Intraoperatively by Methylene Blue Spraying Technique

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Abstract

Background: Thyroid surgery has been associated with complications ranging from recurrent laryngeal nerve injury, inadvertent parathyroidectomies to death. Improvement in thyroidectomy techniques have reduced the mortality rate to minimum, while morbidity remained a significant concern. Aims: This study aims to assess a method to identify recurrent laryngeal nerve and localize the parathyroid glands during thyroid surgery by using methylene blue stain which improve outcomes following thyroidectomy.

Materials and methods: This a prospective cross-sectional study was performed on 48 adult patients of both sexes who presented to the surgical ward at Salah Al-Deen general hospital in Tikrit city between October 2017 and July 2018. Full medical history was taken and clinical examination was done to those who were suitable for surgery. After indirect larngyoscopic confirmation of normal vocal cord mobility, in addition hospital medical records were obtained including patient’s name, age, gender, residence, nature of thyroid disease, duration of thyroid disease, previous thyroid surgery, family history of thyroid disease and past medical history, relevant investigations like final histopathology; all were chosen to undergo thyroidectomy, following superior but before inferior thyroid pedicles ligation, methylene blue dye, in a concentration of 2:8 and 1:10 of Methylene Blue: Normal Saline was sprayed over the thyroid lobe and perilobar area, in the region where recurrent laryngeal nerve is predictable to be found. Recurrent laryngeal nerve and parathyroid glands were identified and safe dissection of the thyroid without injuring these structures were ensured. Results: Recurrent laryngeal nerve was not stained in all cases, in contrast to the blue stained adjacent tissue. Within three minutes parathyroid glands washed out the dye and the yellow color was regained. Conclusions: Several techniques of recurrent laryngeal nerve and parathyroid glands identification were established, our study demonstrate that visual delineation of these structures by methylene blue dye spraying during dissection of thyroid is a simple, safe and effective method for localization and injury avoidance to both recurrent laryngeal nerve and parathyroid glands during thyroid surgery.

Keywords: Recurrent Laryngeal Nerve; Parathyroid Glands; Intraoperatively; Methylene Blue Spraying Technique

Introduction

Disorders of the thyroid gland constitute the second most common endocrine disease following diabetes mellitus. The burden of thyroid disease is in an increment, therefor thyroidectomy is a very common operation, although the most frequent indications for surgery are goitre due to iodine-deficiency, other indications are: uncertainty about the nature of the thyroid mass or treatment of large goitre causing compressive symptoms, thyroid cancer and thyrotoxicosis that may or may not be refractory to medical treatment. Total thyroidectomy(TT)provides the advantages of complete removal of suspicious malignant thyroid tissue from a point and eliminating the risk of recurrence of benign conditions from another point, this resulted in increasing the number of TT.

The significant complications associated with thyroidectomy include: hemorrhage, respiratory obstruction, infection, transient or permanent
recurrent laryngeal nerve (RLN) palsy, and parathyroid insufficiency. 

A number of investigational methods for identification of RLN have been used, including visual and palpatory approach, intraoperative neuromonitoring, laryngeal nerve electromyographic activity recording, electrophysiologic nerve monitoring and staining. These techniques are accompanied with differing degrees of accuracy as well as safety to the patient and are of little help in the dissection of the tissue planes at the time of surgery, thus most surgeons nowadays prefer accurate dissection to preserve the RLN and delicate parathyroid vasculature, in order to avoid nerve injury and postoperative hypocalcaemia.

**Materials and Method**

This prospective, cross-sectional study was conducted from October 2017 to July 2018 on forty eight adult patients, belonging to both genders, with thyroid swelling who underwent thyroidectomy in Salah Al-Deen General Hospital, Iraq. The study sample involving patients with different thyroid lesions were undergoing thyroidectomy. The patients classified into 16 patients their lobar and perilobar areas stained by syringe group (A); the remaining 32 patients their lobar and perilobar areas stained by spraying pump group (B).

After positioning the patient on the operating table and induction of general anesthesia, surgical field was painted with 10% povidone iodine solution and draped. After the standard Kocher incision, total or partial thyroidectomy was performed according to the need. Following the elevation of flaps, lateral thyroid vein ligation and dissection of thyroid lobes were done. The superior pole was ligated and cut. Before ligation the inferior pedicle , the thyroid lobe rotated medially, normally at this stage the identification of RLN or the parathyroid gland is difficult ,so we spray the area with methylene blue aqueous staining solution (CDH Co. India)with the following concentrations and techniques.

In group A1: A concentration of 2:8 of (MB: NS) was sprayed over the thyroid lobe and perilobar area of 6 patients , requiring 1 min. to be stained and 2 times washing with normal saline and In group A2: A concentration of 1:10 of (MB: NS) was sprayed over the previously mentioned area in 10 patients, requiring 2 min. to be stained and rinse one time with normal saline, so In group B1:A concentration of (2:8) MB: NS was sprayed in over the lobe and perilobar area of 12 patients , the time needed for staining the tissue was 1 min. and 2 times washing for washing out the stain, In group B2:A concentration of ( 1:10 ) MB:NS respectively was sprayed over the same area in the remaining 20 patients of this group, the tissue took 2 min. to be stained without washing.

Different thyroid procedures were done for the cases of the study sample.

Statistical analysis: The Statistical Package for Social Sciences (SPSS, version 18) was used for data entry and analysis. One way ANOVA- test was used to compare mean of different factors among different groups of study sample. P value of ≤ 0.05 was regarded as statistically significant. Bar charts, and Pie graphs used to present the data.

**Finding**

The sex distribution show that 44(91.7%) of the patient were females versus 4 (8.3%) males.

The patient distribution according to the address show that 37 (77.1%) of the patient from rural versus11 (22.9%) from urban . The histopathological type was : FA 1(2.1%), HT 3(6.3%), STN 3(6.3%), MNG 26(54.2%), CG 15 (31.3%), as shown in table(1).

<table>
<thead>
<tr>
<th>Histopathological type</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>HT</td>
<td>3</td>
<td>6.3</td>
<td>6.3</td>
<td>8.3</td>
</tr>
<tr>
<td>STN</td>
<td>3</td>
<td>6.3</td>
<td>6.3</td>
<td>14.6</td>
</tr>
<tr>
<td>MNG</td>
<td>26</td>
<td>54.2</td>
<td>54.2</td>
<td>68.8</td>
</tr>
<tr>
<td>CG</td>
<td>15</td>
<td>31.3</td>
<td>31.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
The nature of thyroid disease was diffuse thyroid enlargement (DTE) 20(41.7%), multi nodular goitre (MNG) 28(58.3%),

Forty six (95.8%) were not previously undergone thyroid surgery versus 2(4.2%) had previous thyroid surgery.

The mean No. of washes was lower among group B2 0.1±0.3, versus 1.2±0.4 for groups A2 and B1, and A1 2.2±0.4 this relation was statistically significant as shown in table (2).

**Table (2): The mean Number of washes among study groups**

<table>
<thead>
<tr>
<th>Number of washes</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>2.2</td>
<td>0.4</td>
<td>2</td>
<td>3</td>
<td>&lt;0.001 (59.9) significant</td>
</tr>
<tr>
<td>A2</td>
<td>10</td>
<td>1.2</td>
<td>0.4</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>12</td>
<td>1.2</td>
<td>0.4</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>20</td>
<td>0.1</td>
<td>0.30</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>0.9</td>
<td>0.8</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

The mean volume for staining was lower among group B2 0.4±0.1, versus 2.3±0.4, 2.15±0.6, 0.3±0.1 for groups A1, A2 and B1 respectively, this relation was statistically significant as shown in table (3).

**Table (3): The mean volume of staining among study groups**

<table>
<thead>
<tr>
<th>Volume for staining</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>2.3</td>
<td>0.4</td>
<td>2</td>
<td>3</td>
<td>&lt;0.001(112) significant</td>
</tr>
<tr>
<td>A2</td>
<td>10</td>
<td>2.15</td>
<td>0.6</td>
<td>1.5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>12</td>
<td>0.3</td>
<td>0.1</td>
<td>0.25</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>20</td>
<td>0.4</td>
<td>0.1</td>
<td>0.25</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>1.01</td>
<td>0.9</td>
<td>0.25</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

The mean Time for parathyroid identification was lower among group B2 0.6±0.3, versus 1.5±0.3, 1.8±0.6, 0.8±0.4 for groups A1, A2 and B1 respectively, this relation was statistically significant as shown in table (4).

**Table (4): The mean time for parathyroid identification among study groups**

<table>
<thead>
<tr>
<th>Time for parathyroid identification</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>1.5</td>
<td>0.3</td>
<td>1.3</td>
<td>2</td>
<td>&lt;0.001 (30.9) significant</td>
</tr>
<tr>
<td>A2</td>
<td>10</td>
<td>1.8</td>
<td>0.6</td>
<td>1.45</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>12</td>
<td>0.8</td>
<td>0.4</td>
<td>0.4</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>20</td>
<td>0.6</td>
<td>0.3</td>
<td>0.45</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>0.99</td>
<td>0.6</td>
<td>0.4</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

The mean Time for RLN identification was lower among group B2 1.1±0.2, versus 1.9±0.3, 2.08±0.5,1.2±0.2 for groups A1, A2 and B1 respectively, this relation was statistically significant as shown in table (5).
Table (5): The mean time for RLN identification among the study groups

<table>
<thead>
<tr>
<th>Time for RLN identification</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>1.9</td>
<td>0.3</td>
<td>1.45</td>
<td>2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A2</td>
<td>10</td>
<td>2.08</td>
<td>0.5</td>
<td>1.4</td>
<td>3</td>
<td>(43.6)</td>
</tr>
<tr>
<td>B1</td>
<td>12</td>
<td>1.2</td>
<td>0.2</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>20</td>
<td>1.0</td>
<td>0.2</td>
<td>0.5</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>1.4</td>
<td>0.5</td>
<td>0.5</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Intraoperatively, RLN could be identified in all the patients, as an unstained structure in the tracheoesophageal groove. The parathyroid glands washing out methylene blue stain within approximately 3 minutes and then become visible in their original yellow colour, whereas it took nearly 15 minutes for the thyroid tissue to wash out the stain in all cases. The perithyroid muscles, tendons and lipoid structures retained the blue colour for more than 30 minutes.

**Discussion**

Complications associated with thyroid surgery is comparatively reduced now a days. Standardization of the thyroidectomy technique and advances in perioperative management have led to significant decrease in the overall mortality and morbidity over the past decades (2,4,19). The most important and serious complications following thyroidectomy are RLN injury and parathyroid insufficiency (transient and permanent), when they occur, they cause catastrophic lifelong handicap (12).

Among the various risk factors that results in injury to these structures are reoperation, malignancy, retrosternal goitre, graves’ disease and surgeon inexperience (13).

Another important factor is the extent of resection i.e. TT in opposition to partial thyroidectomies. Permanent post-operative RLN palsy occurs in approximately 0.3–3% of cases and transient palsies in 3–8% of cases (Hayward 2013), while temporary hypocalcemia occurs in 50–68% of post-TT patients and permanent hypocalcemia occurs in 3% of post-TT patients (13).

The key method to protect RLN and parathyroid glands during thyroid surgery is meticulous dissection, this can be achieved by various means including capsular dissection, protection of parathyroids arterial branches and avoidance of excessive manipulation (14,15).

Several methods are used to identify the RLN intraoperatively including visual inspection first described by Lahey and Hoover in1938, laryngeal palpation, finger palpation of cricoarytenoid during nerve stimulation, intraoperative neuromonitoring (IONM), direct or fibreoptic laryngoscopy for observation of vocal cords and the use of intramuscular vocal cord electrodes (16,17).

In a current multicenter trial of 16,448 thyroidectomies, concluded that visual nerve localization, in respect to RLN, is emphasized as the “gold standard” of maintenance (18).

The technique of using dyes in identification of parathyroid glands and prevention of hypoparathyroidism, was first described by Klopper PJ and Moe RE. Initial studies were conducted using dyes like toluidine blue and tryptan blue, however, these were replaced by methylene blue as their teratogenic effects were discovered. All the studies in the literature which are associated with thyroid surgery and parathyroid staining was performed by injecting the dye via intravenous or intra-arterial route. Dudley NE applied an intravenous infusion method on 17 patients and revealed one or more parathyroid glands by this technique. Elias D et al., had
applied the similar technique over 59 cases and was able to precisely localise the parathyroid glands in 87% cases (9).

The technique above ensure the visualization of the parathyroid glands only. This is because of the rich vascularity of the glands, while RLN cannot be identified.

In our study forty eight adult patients were divided into group (A) where the spraying technique was done by a syringe in 16 patients, while in group (B) the spraying technique was achieved by a spraying pump in the remaining 32 patients.

In group A1: with the use of concentration of (2:8) MB : NS, both the concentration and the time required for staining the tissue was agree with Nofal A A ,while differ in the volume of solution needed for staining. Inasmuch we found 2ml of solution is required for adequate staining of the whole perilobar area for better identification of RLN and parathyroid glands. The number of washings required to rinse the tissue are variable with a mean 2.2 ± 0.4 while are not mentioned in any previous study.

In groups B1 and B2 the spraying technique was performed by a spraying pump with both concentrations of (2:8) and (1:10) respectively. MB: NS. We observe that the mean time for staining, number of washings and the stain volume required for staining was much less than that with a syringe insofar the staining by a pump with the concentration (1:10) did not required washing with normal saline. In addition, the resolution was better than the staining with a syringe with both concentration used. We did not find in literatures the usage of spraying pump in the staining of thyroid lobe and perilobar area for identification of both RLN and parathyroid glands.

Conclusions

- intraoperative identification of RLN and parathyroid glands by Methylene Blue Spraying is safe, cheap and readily available method which allow the surgeon especially junior one to perform safe thyroidectomy with lower level of stress.

- Both concentration of (1:10) and (2:8) mL of MB: NS by either a syringe or spraying pump helps in the identification of both RLN and parathyroid glands in all patients with disparity in resolution. The technique of spraying by a spraying pump had the superiority to that with a syringe for both concentrations ultimately for that of (1:10) mL of MB: NS in rapid and easier identification of RLN and parathyroid glands.

Conflict of Interest: None

Source of Findings: Self

Ethical Clearance: Nil

References

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Evaluation of the Mexiletine Effects on Normal Cats’ Electrocardiogram

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2Department of Pharmacy, Islamic Azad University Pharmaceutical Sciences Branch

Abstract

Mexiletine has been suggested in various studies for the treatment of ventricular arrhythmias. Extremely low toxicity of this drug has been reported in these literatures; however, there is no reasonable report on the effects and function of this drug on the cat’s heart processes in different phases of cardiac contraction. This article evaluated the effects of different dosages of mexiletine on cat’s electrocardiography.

Seven cats were used for this study and incremental dosage method with oral capsule has been used. Before starting the study, each cat were evaluated by an electrocardiogram sample, which was considered as a normal electrocardiogram (ECG). To evaluate the effects of mexiletine, doses of 4, 10, 15, 20 and 40 mg/kg were adjusted and prescribed. For each cat, the doses were prescribed sequentially every 24 hours and after prescription of the drug, the cats were examined clinically and electrocardiographically. This evaluation was done at the peak of the drug, about 2 hours after prescription. Finally, all clinical observations and ECGs were evaluated.

In the clinical study, any clinic symptoms was considered and rhythm, heart rate, and determination of heart electrical functions such as P amplitude and duration, QRS duration, R amplitude, ventricular contraction time (QT interval) and PR interval were evaluated in electrocardiographic experiments.

According to the results of this study, it can be concluded that the drug is quite safe for cats and no cardiac complication and electrophysiological disorder was observed even with the use of high doses or even incremental doses, so this indicates that the drug can be safe in terms of cardiac effects.

Key words: mexiletine, electrocardiography, cats, small animal, veterinary,

Introduction

Heart diseases are one of the most common and important diseases in human being and animals, especially dogs and cats. Among heart diseases, heart arrhythmias are assigned as a special and common group, almost (1, 2). Heart arrhythmias may generate by different origins and can produced from sino-atrial node, atrial-ventricular node and even due to electrolytes disorders. In some guidelines, treatments based on medical advices are not appropriate for animals because of a different understanding of the fundamental alterations (such as coronary heart disease versus non-coronary heart disease). Unfortunately, vet-clinicians do not know what is happening in dogs and cats with arrhythmia for various reasons and it is not possible to compare arrhythmia with different causes regardless of the outcome and the medication results (1,2).

Various classes of medications are available and used to treat cardiac arrhythmias. Antiarrhythmic drugs are mainly divided into four categories, given their potential effects on action potential. For example, selective drugs in group I, block fast sodium channels and reduce sodium inversion during the 0-depolarization step. This group is divided into 3 subgroups A, B and C with different drugs in these categories (3,4).
Due to the effects of the antiarrhythmic drug class Ib which blocks the fast sodium channel in the heart, the drug group therefore affects the zero-phase of cardiac contraction and has beneficial effects on ventricular tachy-arrhythmias. Given the performance of this class of drugs at stage zero of cardiac contraction, minimal pharmacological effects can be observed in the sinoatrial node, atrial and ventricular nodes and atrial muscles (5).

Mexiletine is one of the drugs recently proposed for veterinary use, which is an oral analogue of lidocaine. It appears to be more effective and healthier than lidocaine. Mexiletine, which belongs to group Ib, blocks fast sodium channels and has been suggested in various sources for the treatment of ventricular arrhythmias (6, 7).

Mexiletine is orally absorbed and less than 10% of this drug eliminated by the first passage of the liver. Side effects vary in dogs and include nausea, anorexia and tremor. Sinus tachycardia, imbalances, confusion and thrombocytopenia are other potential problems (1). No information is available on its use in cats (1), but in some cases it has been used to treat dogs.

For example, in a group of dogs of the Boxer breed with familial arrhythmia, mexiletine continuously tested with atenolol as one of four forms of treatment. This anti-arrhythmia treatment has been shown to decrease the frequency and degree of ventricular arrhythmia, as well as a decrease in the peak heart rate. However, the frequency of syncope has not decreased with this understanding of treatment or other methods studied. Both Mexiletine and Tocainide have synergistic properties in combination with drugs of class IA and II. These drugs are mainly used to treat ventricular arrhythmias and cardiac glycoside poisoning arrhythmias (lidocaine, Phenytoin). Tocainide is a closely related lidocaine (1, 2, 8-12).

According to the available literature and sources, no comprehensive study of the function of this drug has been evaluated in cats (2). This study, performed on 7 healthy and adult cats, studied the effects of Mexiletine on the cardiac electrophysiology of cats.

Materials and Method

Seven cats were used for this study. The cats selected were all healthy and stray. They were all short haired cats and tried to be selected from a weight class. The selected cats were also of different ages (1-3 years) and sex. All of the selected cats were clinically healthy and none showed cardiac disease at the time of examination. In addition to clinical examinations and blood tests, cardiac function was evaluated by electrocardiography to assess cardiac health in these cats.

The cats adapted to the new environment before the study and used the same commercial food during the study. The studied cats were repeatedly placed on the same examination table and trained to adapt to the environment and how to place the electrocardiogram. After adjustment with the examination table, only physical restraint was applied for electrocardiography to minimize stress at studied time.

Hexagonal electrocardiogram was obtained by Siemens single channel electrocardiography in each cat and selected as primary and control electrocardiogram.

The ECG obtained from cats at the first time (no-prescription conditions) was introduced as zero-dose (D0) and control ECG. Mexiletine is available as a 100 mg oral capsule. The drug was administered daily to 7 cats for 10 days to evaluate the drug’s effects. The doses used in this study were 4, 10, 15, 20 and 40 mg / kg. Capsules were administered orally (daily) and electrocardiograms were taken at least two hours after drug prescription in the time of drug peak effect. After 10 days of prescription of mexiletine, the cats were in rest for two weeks as drug wash out time and then, higher doses were administered to the same cats.

At the time of drug prescription, if any neurological symptoms (incoordination, ataxia, etc.) as well as gastrointestinal symptoms (vomiting and diarrhea) were observed, all cases were recorded and images and videos were obtained. Finally, in electrocardiographic examination, heart rhythm, heart rate and cardiac coordinates including P amplitude and duration, QRS duration, R amplitude, QT and PR interval were investigated. Finally, evaluation of results in this study was adjusted to mean ± SE, and the evaluation list of the results of different doses was analyzed by one-way ANOVA followed by Tukey test.

Results

All results have been evaluated for lead 2 standardization in this lead and it should be noted that no apparent abnormality was observed in any ECG and all ECGs were without arrhythmia.
In evaluating the P wave amplitude in the normal state and after administering the drug at different doses, the same results as detected in table 1 (A) were observed. According to the results, it appears that this drug has no positive or negative effect on P wave amplitude. In cats, the Pamp should not exceed 0.2 mV.

**Table 1. P wave amplitude in (mV) (A), P wave duration (B), R wave amplitude (C), QRS duration (D), PR interval (E), QT interval (F) (per second) and the number of heartbeats (in minute) in cats.**

<table>
<thead>
<tr>
<th></th>
<th>Before prescription of drug</th>
<th>Dose 4 mg/kg</th>
<th>Dose 10 mg/kg</th>
<th>Dose 15 mg/kg</th>
<th>Dose 20 mg/kg</th>
<th>Dose 40 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>P wave amplitude (Mean ± SE)</td>
<td>0.114±0.009</td>
<td>0.107±0.007</td>
<td>0.107±0.007</td>
<td>0.107±0.007</td>
<td>0.114±0.009</td>
</tr>
<tr>
<td>B</td>
<td>P wave duration (Mean ± SE)</td>
<td>0.024±0.002</td>
<td>0.024±0.002</td>
<td>0.025±0.002</td>
<td>0.025±0.002</td>
<td>0.022±0.002</td>
</tr>
<tr>
<td>C</td>
<td>R wave amplitude (Mean ± SE)</td>
<td>0.63±0.098</td>
<td>0.74±0.065</td>
<td>0.60±0.058</td>
<td>0.62±0.074</td>
<td>0.67±0.032</td>
</tr>
<tr>
<td>D</td>
<td>QRS wave duration (Mean ± SE)</td>
<td>0.028±0.002</td>
<td>0.031±0.002</td>
<td>0.032±0.002</td>
<td>0.028±0.003</td>
<td>0.032±0.003</td>
</tr>
<tr>
<td>E</td>
<td>PR int duration (Mean ± SE)</td>
<td>0.067±0.004</td>
<td>0.068±0.005</td>
<td>0.070±0.002</td>
<td>0.068±0.002</td>
<td>0.057±0.003</td>
</tr>
<tr>
<td>F</td>
<td>QT int duration (Mean ± SE)</td>
<td>0.128±0.001</td>
<td>0.147±0.007</td>
<td>0.145±0.006</td>
<td>0.132±0.004</td>
<td>0.131±0.007</td>
</tr>
<tr>
<td>G</td>
<td>Number of heartbeats (Mean ± SE)</td>
<td>220±23.62</td>
<td>206±15.91</td>
<td>209±12.26</td>
<td>209±16.74</td>
<td>199±23.63</td>
</tr>
</tbody>
</table>

**Figure 1.** Normal ECG of one cat (50 mm/s, 1 mv) (A), and ECG of the same cat after prescription of the drug at a dose15 mg/kg (50 mm/s, 1 mv) (B).
The same results were observed in the P duration evaluation after the administration of drugs at different doses, as shown in table 1 (B). According to the results, it seems that this drug does not have a significant effect on P wave duration. In normal cats, the normal P duration is less than 0.04 seconds.

The following results regarding the R wave amplitude obtained before and after drug administration suggest that different doses have no significant effects on the R wave amplitude. However, in some cases, some decrease in the amplitude of the R wave is observed. The amplitude of R wave varies in different references but should not be less than 0.5 mV. However, in this study, the amplitude of the R wave decreases at some doses (table 1 (C)).
There appears to be little difference in the QRS duration with different drug doses as shown in table 1 (D). It should be noted that the QRS duration should be less than 0.04 in cats.

![Figure 4. ECG of one cat before prescription of mexiletine (A), and ECG of the same cat after prescription of the mexiletine at a dose 15 mg/kg (50 mm/s, 1 mv).](image)

The PR interval time is one of the best coordinates for evaluating heart function, the results of which are measured in table 1 (E). According to our results, there is no significant difference in PR interval change. The PR interval in normal cats ranges from 0.05 to 0.09 seconds.

![Figure 5. ECG of one cat under before prescription of the drug (A), ECG of the same cat after prescription of the drug at a dose 10 mg/kg (B), and ECG of the same cat after prescription of the mexiletine with a dose 40 mg/kg in (50 mm/s, 1 mv) (C).](image)
In evaluating the ventricular contraction time (QT interval), after evaluating the ECGs obtained, the results are presented in Table 6. Due to the duration of this wave, which is typically from 0.11 to 0.18 seconds, no significant change has been achieved following the administration of different doses of this drug (table 1 (F)).

Table 1 (G) shows the heart rate in the ECG assessment of the tested cats in terms of heart rate. The results show that the heart rate varied from 111 to 333 heart beat per minutes. The heart rate appears to be highly variable both due to stress in cats and drug administration as well as the development of compensatory processes in the heart.

The results in Fig. 15 can be due to stress during ECG use or due to the heart compensatory processes due to drug administration.

In this study, a number of neurological and gastrointestinal complications have been observed due to large doses. These include temporary anorexia, tremor, obsession and incoordinance. In most cases, doses of 20 and 40 mg / kg cause symptoms of unwanted medication.

Discussion

Due to the lack of a reliable report on the effects and function of mexiletine on the electrical function of cats’ heart, the effects of this drug were evaluated in this study (2).

According to the results of this study, no increase or decrease in P wave amplitude and duration was observed and all results were normal and no significant difference was observed between different doses. So it can be concluded, this drug had no major effect on atrial function and contraction. This finding is in similar with the results of other researchers (1, 2). Therefore, it can be concluded that this drug is safe for atrial function even at very high doses.

By measuring the function of incremental doses on PR interval according to the results of this study, this drug had no adverse effects on PR interval and there was no significant difference before and after drug prescription even with the use of incremental doses (P <0.05).

According to the reports of different literatures on the absence of adverse drug effects on the dogs’ heart function (2) and also according to the findings of the present study, it was found that on R amplitude, QRS duration and ventricular contraction time (QT interval), this drug had no adverse effects on ventricular contractions (P<0.05). Therefore, mexiletine had no obvious effects on ventricular contraction strength (especially left ventricle). The evaluation of QRS duration also showed that there was no significant difference between the groups in increasing doses, so that the mexiletine had no abnormal effects on ventricular contraction time (P<0.05). But despite a slight increase
in QT interval, this increase was in normal range. Evaluation of ventricular contraction time (QTint) also revealed no significant difference between normal ECG and after different doses (P<0.05). Therefore, increasing doses of this drug did not increase the duration of ventricular contraction.

Therefore, it can be concluded that mexiletine has no adverse effects on the severity and duration of ventricular contraction even at increasing doses. It seems that mexiletine is completely safe for heart function in normal cats, indicating its safe effects on ventricular function. This seems to be the reason for choosing of mexiletine as one of the drugs of choice for the treatment of ventricular tachyarrhythmia.

Evaluation of heart rate also showed that mexiletine was able to reduce heart rate, but this change was not associated with bradycardia and there was not a significant difference in the increase or decrease of heart rate when using incremental doses (P <0.05). In only one case at doses of 20 and 40 mg / kg was observed a decrease in heart rate, which is not very abnormal at these doses.

Therefore, according to the results of this study, it can be concluded that the drug is quite beneficial and safe for cats and no cardiac complication and electrophysiological disorder was observed even with the use of high doses or even incremental doses, so this indicates that the drug can be safe in terms of cardiac effects.

Various studies have reported unwanted gastrointestinal and neurological complications while using this drug (6, 7). These studies show that the side effects are dose-related and reversible. Common complications observed in various reports include nausea, anorexia, head tilt and limb tremor, imbalance and incoordinance (2).

In this study, some of the complications mentioned in the tested cats were observed. However, these complications are individual and are usually observed at very high doses, such as 20 and 40 mg / kg. The most common complications observed in this study include temporary anorexia, tremor, obsession and imbalance. However, all of these effects were temporary and no side effects were observed when the next dose was administered or in drug wash out time. It is also suggested that the drug be used with foods to minimize the risk of drug side effects, as suggested by various studies to minimize the side effects.

However, in order to minimize drug complications in cats, a review of safe doses with a greater number of cats is needed. It seems that investigating different drug doses, as well as measuring the left ventricular ejection fraction in cats, may be helpful in adjusting the dose required in cats.

According to the results of this study, it is recommended to re-evaluate the appropriate pharmacological effects of this drug for treatment of cats with supra or ventricular tachyarrhythmia and the results to be reviewed. In addition, it is recommended to determine the necessary and safe doses for cats and it is necessary to determine the proper and therapeutic doses by HPLC.

Ethical Clearance: The ethical permission was taken from the ethic committee of faculty of veterinary medicine, Islamic Azad University Tabriz Branch.

Source of Funding: The project was co-funded through faculty of veterinary medicine and Veterinary Organization of Iran.

Conflict of Interest: There is no conflict of interests in this project.

References

The Role of BRAF Mutation (V600E) in Papillary Thyroid Carcinoma (PTC)

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Abstract

Introduction. Thyroid cancer is usually associated with genetic alterations. Papillary thyroid carcinoma (PTC) was found to be accompanied by point mutation occurs in a part of exon 15 in specific gene called BRAF which is a part of pathway that responsible for cell main functions such as division. The role of BRAF gene point mutation (V600E) in the occurrence of PTC was investigated in this study.

Study designs and subjects: 70 patients previously diagnosed with PTC in addition to 35 controls were recruited in this control-based study. Patients were grouped according to their ages and gender. Blood samples were collected for DNA extraction. PCR was run to detect V600E point mutation in BRAF gene.

Results: PTC incidence was found to be higher in patients aged from 40-49 years. The percentage decreased as ages increased. Females had higher PTC frequency than males, 66% to 34%, respectively. The V600E point mutation was detected in 35 patients out of 70 (50%).

Conclusion. Genetic studies have to be a part of thyroid cancer investigations. The study confirmed that PTC is usually associated with molecular defect. Other studies such as thyroid profile tests (TSH, T3 or T4) have to be done along with genetic studies for more confirmation and more understanding of this molecular defect.

Key words: PTC, BRAF, V600E and point mutation.

Introduction

Thyroid cancer is considered one of the most prevalent endocrine tumor with higher incidence rates (three-fold) in women than in men (¹). In 2008, thyroid cancer was the sixth most diagnosed cancer in women (¹,²). The frequency of thyroid cancer has increased in the recent decades due to the improvement in the diagnostic procedures and increased use of therapeutic irradiation but it still constitutes about 1% of all human malignant tumors (³). Thyroid tumors are derived primarily (90%) from thyroid follicular cells while the remainder is arising from calcitonin-producing cells or other thyroid cells (⁴). Papillary thyroid carcinoma (PTC) comprises about 90% of all thyroid cancers with incidence ages ranges from 20-50 years (⁴). Somatic mutations in BRAF gene; which is a part of signaling pathway known as RAS/MAPK pathway that responsible for controlling cell main functions such as proliferation, differentiation, migration and apoptosis, has been associated with many types of cancer including thyroid cancers (⁵). PTC is usually related to molecular defect resulted from an activating point mutation in BRAF gene (²). This point mutation is a result of thymine to adenine transversion at position 1799 (T1977A) in exon 15, leading to a replacement of valine amino acid with glutamic acid at residue 600 (V600E) which terminating with constitutive activation of BRAF kinase (²). The active kinase is believed to be produced constitutively in the presence of mutation due to the disruption in the hydrophobic interaction between the activation loop and residues in the ATP binding sites that allow the kinase to be in an inactive conformation (⁶). The mutation leads to more
interaction to be generated rendering the kinase into a catalytically competent structure (6). Mutant BRAF correspondingly exhibits higher level of kinase activity (6). The prevalence of BRAF mutation was reported by some studies in 29-83% of PTC cases as the most common detected oncogene (7-10). Poor prognosis and high aggressiveness are both related with PTCs with BRAF mutation (11). The activating point mutation in BRAF gene leads to overexpression of BRAF protein which was found in some studies to be significantly higher than that in other thyroid disorders such as multinodular goiter (MNG) (2). It is also higher in PTC with lymph nodes metastasis (LNM) than that without metastasis (2).

Keeping into mind the increased occurrence of PTC nowadays, this study was aimed to point out the frequency of BRAF point mutation (V600E) in PTC cases. The frequency of PTC among specific age groups or gender was studied as well.

**Material and Method**

**Patients and controls**

A total of 70 histologically confirmed patients with thyroid cancer admitted to Al-Sadder Teaching Hospital in Basra province/oncology unit where they prospectively considered. Patients with other types of cancer, patients undergone radiotherapy, or chemotherapy in the last three weeks; and patients with other active medical conditions such as (benign thyroid tumor, heart failure, hepatic disorder, renal failure, uncontrolled diabetes and infections) were excluded. Additional 35 persons with benign thyroid tumors were enrolled as a control group. The Ethics Committee at our institute has approved the study. The stages of PTC were classified according to the TNM staging system and related information were taken from saved patients records (12).

**Sample collection/ storage**

70 blood samples were collected from all patients in EDTA tubes for DNA extraction. PCR was run to determine the presences of BRAF (T1799A) mutation. Genomic DNA was extracted from EDTA anticoagulant peripheral blood leukocytes using Accupower® Genomic DNA extraction kit (Bioneer. Korea), and then stored at -20 C till the day of use.

**Polymerase chain reaction**

Primers used for amplification of the BRAF gene and BRAF mutant genes were designed using Primer Premier 7.0 (Bioneer. Korea ). The upstream and downstream primers; respectively, were: 5’-TCATAATGCTTGCTCTGATAGGA-3’, and 5’-GGCCAAAAATTTAATCAGTGA-3’. The amplified fragment was 224 bp in length. The reaction mix was done in 25 µl volumes including 5µl of template DNA, 12.5 µl of GoTaq®Promega Green Master Mix 2X, 2µl of primers (foreword and reverse) and 3.5µl of nuclease Free water (Applied PCR system, USA).

PCR conditions were as following: initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 30s, annealing at 60°C for 1 min and 1 min of extension at 72°C, with a final extension of 7 min at 72°C. The fragments were visualized by electrophoresis on a 1% agarose gel stained with 5% ethidium bromide. The gel was then analyzed under UV light using Cleaver Gel Documentation System (Cleaver Scientific Ltd, UK) for the presence or absence of an allele specific band.

**Statistical Analysis**

Graf pad prism 5 computer software was used to statically analyze the results. The strength of association between 2 categorical variables, such as the presence of certain grade, stage, sex and disease status, also the differences of genotype between thyroid cancer patients and control groups, the odds ratio (OR) and 95% confidence intervals (CIs) were assessed by a special χ² formula. The difference was considered significant, if \( p < 0.05 \).

**Results**

**1-Demography study**

The present study showed that maximum cases; where V600E point mutation presented, were observed in the age group (40-49 yrs) that recorded 47%, ages range between (50-59 yrs) recorded 27%, followed by 16% in (60-69 yrs) and finally 10% (70-79 yrs), Figure 1-A. The results also showed that the prevalence of mutation was higher in females ((23) 65.72%) than in males ((12) 34.28%), Figure 1-B.

2-Distribution of patients according to Staging and Grading

By returning to the saved patients’ records and as shown in table 2, the patients were classified according
to the size of tumor (T), the spread of cancer to lymph nodes (+ve for spreading and –ve for not) as well as if there is a metastasis (M1) or not (M0) were all clarified (Table 1). A significant difference (P≤0.05) was seen among different stages.

The results also showed that 14 cases (20%) were classified as grade I, while 25 cases (33.7%) and 31 cases (44.3%) were classified as grade II and III, respectively. The difference among different grades was significant (P ≤0.05).

Results of DNA sequencing

Part of exon 15 of BRAF gene was amplified using specific primers which ensures yielding a product where the length of the wild-type BRAF is 97bp while the mutant BRAF is 224bp. The results of electrophoresis of the PCR reaction products are shown in Figure 2. Nine samples were included, eight samples with PTC and one with benign thyroid tumor. The product of the eight samples were between 200 and 300 bp, while the wild-type showed a product of about 97bp. In total, BRAF V600E mutation was detected in 35 out of 70 cases (50%). No BRAF V600E mutations were detected in 35 benign thyroid cancers controls.

<table>
<thead>
<tr>
<th>Category</th>
<th>thyroid cancer No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor stage</td>
<td></td>
</tr>
<tr>
<td>T 1-2</td>
<td>32 (45.7%)</td>
</tr>
<tr>
<td>T 3-4</td>
<td>38 (54.3%)</td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
</tr>
<tr>
<td>N +ve</td>
<td>40 (57.2%)</td>
</tr>
<tr>
<td>N -ve</td>
<td>30 (42.8%)</td>
</tr>
<tr>
<td>Metastatic</td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>24 (34.3%)</td>
</tr>
<tr>
<td>M1</td>
<td>46 (65.7%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14 (20%)</td>
</tr>
<tr>
<td>11</td>
<td>25 (35.7%)</td>
</tr>
<tr>
<td>111</td>
<td>31 (44.3%)</td>
</tr>
</tbody>
</table>

X2 = 16.060 , df = 3, P value≤0.01)
Discussion

Papillary thyroid cancer (PTC) is considered the most frequent malignancy that is usually associated with somatic mutations in oncogene BRAF gene \(^{(13)}\). Because of its importance in the risk prognosis and management of PTC, this gene has attracted the attention of researchers through the world in the recent years. Our study’s aim was to point out the association between the inherited BRAF variants and PTC based on a case–control study. The study showed that 35 out of 70 patients with PTC were diagnosed with V600E point mutation with a majority observed in the age group (40-49 years). The chance of having the mutation decreases gradually after the age of 50. The results also showed that gender could be a determination factor of V600E occurrence where 23 females (65.72%) were diagnosed with PTC, comparing to 12 males (34.28%). V600E has been described by many studies as the most common mutation in the BRAF which occurs in about 29%-83% of PTC cases, where classical PTC and worse patients’ outcomes can be associated. \(^{(13)}\) Importantly, research found that BRAF could play an important role in the tumor development and evolution in addition to its main role in the tumorigenesis \(^{(14)}\). Kim et al. reported that metastasis in the lymph nodes of PTC could be predicted from the BRAF V600E mutation in the primary lesion, and could be more valuable than other PTC-related factors such as age, clinical stage, and tumor size \(^{(15)}\).

Another study confirmed that BRAF gene alterations are not restricted to thyroid cancers, but they also could be seen in various cancers like malignant melanoma (27-70%), colorectal cancer (5–22%), and serous ovarian cancer (<30%) \(^{(2)}\). The progression and aggressiveness in the carcinogenesis of thyroid tissue can be indicated by the BRAF mutation and over-expression. Many other studies have focused on the association between Thyroid stimulating hormone (TSH) and PTC. A study conducted by Huang et al. found that serum TSH level below normal ranges associated with high risk of PTC among women in contrast to the risk of PTC in men where high TSH level was associated \(^{(16)}\). Boelaert et al. found high aggressiveness of malignancy in patients with solitary nodules and elevated TSH levels \(^{(17)}\). It is highly recommended that in patients at stage III or IV, the TSH level must be targeted at the undetectable level until further investigations on BRAF mutation become available \(^{(18)}\).

In conclusion, in this study we tried to point out the role of BRAF point mutation (V600E) in PTC patients especially in the ages above 40 years. Patients aged 40 to 49 years were at higher risk of having V600E mutation. As observed by many other studies, our study found that V600E is highly presented in females. The mutation was found in 50% of our study’s patients which reflect the role of this mutation in the occurrence of PTC. However, the study had many limitations such as the accessibility to only 70 patients which is considered a small sample size, and the lack of other tests results such as TSH or other thyroid hormones which could have drawn a conclusion on the relationship between any of these hormones and PTC.
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Reference


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Forensic and Legal Victimology Education: The Actualization of Ethical Values in Law

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Abstract
Ethics need to be redeveloped as an effort to build the excellent forensic and legal victimology education, and law-abiding human beings with moral value. Being ethical means, therefore the awareness of the importance of ethics must always be a basic requirement in the forensic and legal victimology education order. The relation between ethics and educations is indeed close. There are many links which make both share similarities and differences, but the difference is not meant to separate them one from another. In addition, ethics and legal norms both have the same social aims, namely humans to do deeds according to the norms of society. Without a strong ethical principle, the awareness of forensic and legal victimology education may not grow well, the education may lose its substance. Even, the forensic and legal victimology is used as a tool to merely invigorate greed and selfishness. But by sticking to ethical principles, the awareness of forensic and legal victimology might grow well, as it may deliver and elevate human standards may realize social justice in Indonesia.

Keywords: Values, Ethics, Forensic, Victimology, Law

Introduction
The awareness of legal ethics is one of the most important components in forensic education and legal victimology. Even the ethics within the said education is deemed to be more challenging to establish than building either education facilities and infrastructure or legal victimology. The awareness of legal ethics is tantamount to questioning the human characters as an object or subject of a teaching related to forensic and victimology. Therefore, the awareness of legal ethics needs to be redeveloped as an effort to build the character of students who are currently studying forensic education and legal victimology.

Forensic science reveals legal issues—specifically in criminal acts, to investigate the cause of one’s death case allegedly by poisoning in particular, the investigators will use Forensic Science and will ask for help from forensic experts. Forensics is commonly used to assist investigations in a crime case. Forensics is a field of science which is used to assist law enforcement processes through the application of science. Forensic science is applied for legal purposes by providing scientific evidence which may be used in court in solving crimes, as explained by Soekanto and Mamudji. (1)

Terminologically, according to Gosita(2) victimology means a legal study which studies the victims, the causes of victims and the consequences of victims which then become human issues as a social reality. The condition of Indonesians today shows that instead of growing further into improvement, it is evidently experiencing a setback. One of them is how the norms of decency is gradually blurred. Wiguno(3) said that there are numerous cases of rape, harassment, and crime which run rampant to other moral issues. The said norm of decency is one of the norms which applies in the society both as a barrier and reminder for each individual to not fall into such despicable act.

Legal victimology education is deemed equal as rebuilding human characters in order to learn and be more outspoken regarding existing issues, hence the
most fundamental issue in victimology is its relation to ethics.

Legal victimology issues is closely related to the existence of ethics, whereas it may be proven that when human beings are exposed to the importance of respect to others, and take care of each other’s dignity. For that reason, the actualization of ethics in human life requires such processes in a long period, in the sense of ethical education to guide human life. Ethics does not question human circumstances, yet rather on how humans should act properly, as said by Djuneaidi. (4)

It’s undeniable how humans constantly search for good things. And when humans distinguish whether something is good or not, or something should be done or not, those things happen as the humans understand those of which are deemed to be good, proper, and appropriate in order to reach the supposedly right direction, said Poespoprodjo(5) It’s the consequence of the ethics, which questions how humans act, their behavior upon their existence as human being. Basically, legal awareness will be well formed, if human individuals are aware of the importance of the ethical noble values as a guide to be expressed in various dimensions of life. Therefore, according to Keller(6) community obedience upon the existing norms might be developed by itself, which is why legal awareness is of collective awareness which needs to be supported simultaneously by individuals in order to achieve a common goal that the order of life would be more directed and run dynamically.

The legal ethics awareness issue emerges when the collective awareness of the importance of ethical values is no longer discussed in forensic education and legal victimology. Ethical values began to be fragmented of cultural influences of hedonism, pragmatism, materialism, and ego of power. So, it is crystal clear, that such influences would gradually erode the morality of forensic experts and legal victim. The impact of ethical awareness in forensic and victimology examination efforts is extremely low, the law enforcement also loses its substance if the crime is unsolved.

Method

This research used juridical normative methods and a library research about ethics and moral on the Forensic and Legal Victimology educations in Indonesia.

Findings and Discussion

In its literal meaning, the word ethics is deprived from the word ethos (singular, Greek) which then became ethics (English). Bartens(7) explained that the word ethics is rooted from ancient Greek; ethos in singular form which means customs, or good morals. The plural form of ethos is ta etha which means customs or good morals. This plural form then birthed the term of ethics which, by the Greek philosopher, Aristotle (384-322 BC) had been used to denote moral philosophy.

The word “ethics” has a number of meanings. Generally, ethics is seen as a branch of value philosophy (axiology). In this value philosophy, aside from ethics (behavior or moral philosophy). On daily basis, ethics may be interpreted as product, which then emerged the terminology of professional code of ethics, which a collection of norms which govern “the do’s and the don’ts” of a profession. Norms in a professional code of ethics are set independently (self-regulation) by those with the profession including forensic and victimology experts. It contains the vision and mission of the profession, including all traditions which involve the forensic and victimology professions. Shidarta(8) explains that the aim is nothing but to maintain the honor of the said profession.

Meanwhile, according to James J. Sphilane SJ in Muhammad(9), he considers ethics to always pay attention or consider human behavior in moral decision making. Ethics directs or connects the use of individual reason under the objectivity to determine “truth” or “mistakes” and one’s behavior toward others. While in the ethical aspects in Immanuel Kant’s view of moral values is an act which depends on the intention of the person who carries out the particular act, and such act has a moral value only if it was enacted due to obligation. This means that such act may not only have to be in accordance with what is mandated by the obligation, but it also has to be carried out in order to fulfill the obligations of the doer. According to Acton(10) an act may be considered as an obligation if it were enacted for concern of punishment or to gain advantages for others, yet said act has no moral value.

Ethics in general is to equip oneself with noble character, commendable attitude in associating and interacting with humans, or circumstances and proper approach, what might help in prevent from conducting
mistakes, or nature which may keep someone from matters which is potential to humiliation, as said by Aliyah.\textsuperscript{(11)} Hence, it can be said that the word “ethic” (manner), according to the previous Muslim writers is not indicated on the commitment to morals only, but it is also indicated on the commitment to sharia and law, the qualities that must be possessed by the authorities in power, and so forth, as mentioned by Al-Qasimi.\textsuperscript{(12)}

The Chief Justice of the United States of America, Earl Warren (1953-1969) in Asshiddiqie \textsuperscript{(13)} once said, “In civilized life, law floats in a sea of ethics”. Earl Warren called forensic science as something that can only be upheld, sailing, moving on ethics. Ethics is the foundation for the law to float on the sea. He further stated that the law could not be upheld in a fair manner if the ethical oceanic water did not flow or did not function well.

Therefore, in order for the law to be upheld and properly maintained, the development of ethical awareness in the community is very urgent. Ethics is basically very urgent. In every violation of forensic procedures and victimology provision, most of them are also a violation of ethics. Forensic officers and victimologists can let go of the criminal by changing the forensic test results or the victimology examinations if they ignore ethics.

Thus, ethical values must be explicitly stated in the law enforcement efforts that are in line with the current development. So that the education of ethical values which is the result of crystallization of human behavior must always be realized in forensics and victimology. Without ethical values, forensic science and victimology can also be controlled by crime to exploit other humans by producing false reports. For this reason, there are some basic ethical values that must be used as guidelines in building an education system of forensics and victimology to make law enforcement more authoritative, protective and giving a sense of justice.

Forensic test and victimology examination in law enforcement are vital things that must prioritize principles and ethics and must not compartmentalize humans, discredit humans, discriminate them, let alone castrate human rights. Forensic expert and legal victimologists must conduct honest examinations and in accordance with the actual fact because it is part of the ethics that must be fulfilled in the profession, said Harahap.\textsuperscript{(14)}

Thus, ethical values in the education of forensics and legal victimology are the main means that must be achieved to develop knowledge in forensics and victimology. Therefore, ethical values are the moral foundation in the development of forensic science and victimology to uphold the law in line with the empathy for humanity and the sense of justice.

According to Fernard Brundel, the current tendency for forensic experts and victimologists to betray their country and colleagues by falsifying the reports and or being half-hearted in conducting examinations, so that the mutual trust fades, the law or the institution are ineffective in maintaining order and peace, the hard work and the integrity are made into enemies, the laziness and the corruption are glorified. Ethical policies are destroyed and replaced by violence and greed.

The destroyed virtue of ethics has led to the emergence of negative connotations in the society related to the profession of forensic expert and legal victimologist such as: unethical, immoral, or uncultured. The connotations of ‘uncultured’, ‘unethical’, ‘immoral’ are people who are considered lowly civilized. This connotation is often attached to many people in the community who are considered to do the act, according to Imran.\textsuperscript{(15)}

Ethics needs to be redeveloped in an effort to build the existence of forensic expert and victimologist that are civilized and moralized. The Grand Indonesian Dictionary (KBBI-Kamus Besar Bahasa Indonesia) defines ethics as the science of what is good and what is bad, and about the obligation and rights of moral or value and the moral norm that become a guideline for a person or a group in adjusting their behavior.

Ethics and science (forensics and victimology) cannot be separated from each other, when one of them is questioned, then ethics becomes a problem. Ethics questions the problem of good and bad of human actions, ethics need to be upheld, actualized and realized in the law, with the hope to develop a legal awareness for every forensic expert and legal victimologist as a collective awareness that must be developed together in order to achieve the basic objective of the law namely justice.
Although some of these experts in the country are experiencing a moral crisis, with various unethical, immoral, and uncivilized acts, such as the misuse of authority, receiving bribes on forensic and victimology tests and other unethical actions. To realize all that, it needs to be fought by promoting ethical values as the basic rules of education that underlie it, as said by Harahap.\(^{(14)}\) Indonesia has made a policy to develop the education of forensics and legal victimology by incorporating ethical elements since 1983 to the present. Where the policy is directed toward achieving the purpose of forming forensic experts and victimologists who meet professional ethics, as mentioned by Imran.\(^{(15)}\)

Back to the ethical issue in its relation to legal awareness, in line with that, according to Franz Magnis Suseno, one of the main ethical functions is to help critically seek orientation when dealing with confusing morality. Ethics is systematic thinking, and what it produces directly is not goodness, but a more basic and critical understanding. That way, ethics can be interpreted as the attitude, the habit, the belief of a person or group with another person or group that becomes a guide for them in adjusting their behavior, said Suseno.\(^{(16)}\)

Thus, there are several main ethical functions in developing; first, ethics oriented on the behavior or the action that prioritize the values of courage, honesty and sincerity, with those values awakening the attitude and the mentality of forensic experts and victimologist to always uphold ethical values in every action and in every behavior in daily life as well as in carrying out their profession.

**Conclusion**

Education of forensics and legal victimology is not only *an sich* substantially in its field but also discusses the professional ethics, even further, because it also teaches forensic expert and legal victimologist to carry out the test based on the actual fact and present the examination result seriously and avoid all bad behavior in daily life and in carrying out the profession. Pramudya and Ananto\(^{(17)}\) said that there are many links that make the two sciences and ethics have similarities, such as both has normative nature and contain ethical norm as well as are binding.

Without a strong ethical basis, forensic experts and victimologists will not develop well, and will lose their substance, even forensic science and victimology are used as tools to strengthen greed and selfishness. However, by sticking to an ethical foundation, the awareness will develop well, because it can deliver and elevate the degree of people who work as forensic expert and victimologist, its enforcement can realize the human values that are just and civilized.

**Ethical Clearence:** Yes

**Conflict of Interest:** No

**Source of Funding:** Author

**References**

Risk Factor based on the Type of Stroke at RSUD Dr. Soetomo, Surabaya, Indonesia

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Abstract

Introduction: Stroke defined as a disease due to the trouble in the blood circulation which affected by several risk factors. It consists of fixed factors and unfixed factors. The fixed factor such as age, and gender, while unfixed factors including hypertension, blood glucose level, dyslipidemia, and job. Aims: This research aims to identify the distribution type of stroke, and risk factor which affecting the stroke patients in the RSUD Dr. Soetomo, Surabaya. Method: Research was designed as descriptive research to the stroke patient in the RSUD Dr. Soetomo, Surabaya. Various data was collected including age, gender, job, blood pressure, blood glucose level, and lipid profile when patient involved in the hospital at the first time, history of smoking, history of stroke in family. All of stroke patients in the RSUD Dr. Soetomo, Surabaya within October-December 2018 was addressed as subject in this research. Result: Result showed that ischemic stroke had the highest incidence rate in the hospital (72.6%), most of patient of stroke are male (56.2%) with age in less 65 years old (72.6%). Both hypertension 37(50.7%) respondent and smoking 29 (39.73%) respondent are the highest modified risk factor in this research. In the ischemic stroke, hypertension (62.3 %) is the highest modified risk factor. Whilst, in the hemorrhagic stroke, smoking (35 %) is the highest modified risk factor.. Moreover, the highest risk factor in all type of stroke is in age less than 65 years old (72.6%). Conclusion: Number of ischemic stroke case is higher than hemorrhagic stroke. Hypertension is defined as the highest risk factor in hemorrhagic stroke, while smoking is the highest risk factor of ischemic stroke.

Keywords: Hemorrhagic stroke, ischemic stroke, smoking, risk factor

Introduction

Non-communicable diseases (NCDs) such as heart disease, stroke, cancer, diabetes mellitus, chronic injury, and obstructive pulmonary constitute 68% (38 million) of deaths worldwide by killing 56 million people per year (1). According to the WHO, deaths of NCDs are expected to continue to increase throughout the world which the biggest increase will occur in middle and poor countries. More than two thirds or 70% of the global population will die from NCDs such as cancer, heart disease, stroke and diabetes (2). The prevalence of NCDs in Indonesia, such as strokes increased from 2007 to 2013 which was 8.3 per mile to 12.1 per mile. It increases by age. The highest stroke cases diagnosed with health workers are age 75 years and above (43.1%) and the lowest in the age group 15-24 years that is equal to (0.2%). The prevalence of stroke based on sex is more male (7.1%) compared to women (6.8%). The prevalence of stroke in cities is higher than in the villages, both based on the diagnosis of health workers (8.2 ‰) and based on the diagnosis of health care or symptoms (12.7 ‰). Prevalence is higher in people who are not working either diagnosed with health (11.4 ‰) or diagnosed with health or symptoms (18%). East Java is a province that ranks fourth and has increased from 2007 to 2013 after South Sulawesi, DI Yogyakarta and Central Sulawesi in stroke. The prevalence of stroke at the age of ≥15 years by province, East Java ranks sixth (9.1 ‰) based on health diagnosis and fourth (16.0 ‰) based on the
diagnosis of professionals or students (3). The prevalence of stroke patients in Surabaya has increased during 2007-2013. It started from 0.7% in 2007 to 16.2% in 2013 (2).

Stroke is caused by an ischemic or hemorrhagic process that is often preceded by lesions or injuries to the arteries. Of all stroke events, two-thirds are ischemic and one third are hemorrhagic. It is called ischemic stroke because of thromboembolic blood vessel blockage which results in an ischemic area under the blockage. This is different from the hemorrhagic stroke that occurs due to ruptured microaneurysm (4,5). Several factors that can cause strokes are distinguished into risk factors that cannot be changed and risk factors that can be changed. Risk factors that cannot be changed including age and male sex. Risk factors that can be changed including hypertension, diabetes mellitus, and dyslipidemia. Hypertension is defined as a condition where a person’s blood pressure exceeds the normal blood pressure limit. Hypertension is a potential risk factor for the incidence of stroke because hypertension can cause rupture of the blood vessels of the brain or cause narrowing of the blood vessels of the brain. Rupture of cerebral blood vessels will result in cerebral hemorrhage, whereas if there is a narrowing of the blood vessels of the brain it will interfere with blood flow to the brain which ultimately causes the death of brain cells. This research aims to identify the distribution type of stroke, and risk factor which affecting the stroke patients in the RSUD Dr. Soetomo, Surabaya.

Method

Research design. This research was cross-sectional design. The sample was collected by systematic random sampling method. The sample had to attain selected inclusion and exclusion criteria. The inclusion criteria were patient with Stroke diseases, willing to participate in this research, and have a complete medical history. Whilst, exclusion criteria were Stroke patients with uncompleted medical history, patients with complicated condition.

Study population and study periode. The subject in this research was inpatient and outpatient Stroke disease at RSUD Dr. Soetomo during Januari–Oktober 2018. The research sample was calculated by the formula of the cross-sectional research which included 73 respondents.

Data source and data variable. The data source in this research is primary data obtained by distributed questionnaire to Stroke patients. Whilst, secondary data obtained from medical history. Research variable in this research including dependent variable and independent variable. Dependent variable was selected as a medical history with complete information, such as age, gender, blood pressure, blood glucose level, lipid profile, Smoking history and job description. Data on age, gender and job description were obtained from primary data, whereas blood pressure, glucose level, and lipid profile were collected from secondary data. While variable of hypertension was collected from the blood test. Respondent was included into hypertension group if they have blood pressure ≥140/90 mmHg in Stroke patients without another chronic diseases and blood pressure ≥130/80 mmHg in Stroke patients with another chronic diseases. Then, variable of hypercholesterolemia was also obtained from blood test. Respondents were determined into hypercholesterolemia group if they have high density of lipoprotein ≥100 mg/dl. Meanwhile, respondents were addressed into diabetes mellitus group if they have blood glucose level of fasting about ≥110 mg/dl. Then, variable of physical activity was obtained from interview. Respondent was acknowledged into bad physical activity group if they did not have physical activity in 30 minutes for 5 times in a week.

Data analysis. Data were analyzed by univariate method. We describe all variable independent and ranked it.

Ethical Clearance. Before collecting data, This Study approved Ethical Clearance by Ethic Commision of Government Hospital Dr. Soetomo, Surabaya (Number: 0727/KEPK/X/2018). All participants were provided with written informed consent approved by the Ethics Commission of Government Hospital Dr. Soetomo, Surabaya.

<table>
<thead>
<tr>
<th>Type of stroke</th>
<th>Number</th>
<th>Porsentase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic</td>
<td>53</td>
<td>72,60</td>
</tr>
<tr>
<td>Hemorrhaghi</td>
<td>20</td>
<td>27,39</td>
</tr>
</tbody>
</table>

Table 1 : Distribution type of stroke respondent
Table 2: Distribution patient stroke base on modified risk factor

<table>
<thead>
<tr>
<th>Age</th>
<th>Ischemic stroke</th>
<th>Hemorrhagic stroke</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 65 years</td>
<td>35 (66.0)</td>
<td>17 (85.0)</td>
<td>52 (72.6)</td>
</tr>
<tr>
<td>≥ 65 years</td>
<td>18 (34.0)</td>
<td>3 (15.0)</td>
<td>21 (27.4)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29 (54.7)</td>
<td>12 (60.0)</td>
<td>41 (56.2)</td>
</tr>
<tr>
<td>Female</td>
<td>24 (45.3)</td>
<td>8 (40.0)</td>
<td>32 (43.8)</td>
</tr>
</tbody>
</table>

Table 3: Distribution patient stroke base on modified risk factor

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Ischemic stroke</th>
<th>Hemorrhagic stroke</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>22 (41.5)</td>
<td>7 (35.0)</td>
<td>29 (39.7)</td>
</tr>
<tr>
<td>No</td>
<td>31 (58.5)</td>
<td>13 (65.0)</td>
<td>44 (60.3)</td>
</tr>
<tr>
<td>History of family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (20.8)</td>
<td>6 (30.0)</td>
<td>17 (56.2)</td>
</tr>
<tr>
<td>No</td>
<td>42 (79.2)</td>
<td>14 (70.0)</td>
<td>56 (43.8)</td>
</tr>
<tr>
<td>History of hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33 (62.3)</td>
<td>4 (20.0)</td>
<td>37 (50.7)</td>
</tr>
<tr>
<td>No</td>
<td>20 (37.7)</td>
<td>16 (80.0)</td>
<td>36 (49.3)</td>
</tr>
<tr>
<td>History of diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 (22.6)</td>
<td>5 (25.0)</td>
<td>17 (56.2)</td>
</tr>
<tr>
<td>No</td>
<td>41 (77.4)</td>
<td>15 (75.0)</td>
<td>56 (43.8)</td>
</tr>
</tbody>
</table>

Table 4: Rank of Distribution risk factor by type of Stroke

<table>
<thead>
<tr>
<th></th>
<th>Ischemic stroke</th>
<th>Hemorrhagic stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Porsentase</td>
<td>Rank</td>
</tr>
<tr>
<td>age &lt; 65 year</td>
<td>35 (66.0)</td>
<td>1</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>33 (62.3)</td>
<td>2</td>
</tr>
<tr>
<td>Sex (man)</td>
<td>29 (54.7)</td>
<td>3</td>
</tr>
<tr>
<td>Smoker</td>
<td>22 (41.5)</td>
<td>4</td>
</tr>
<tr>
<td>History of diabetes mellitus</td>
<td>12 (22.6)</td>
<td>5</td>
</tr>
<tr>
<td>History of Family</td>
<td>11 (20.8)</td>
<td>6</td>
</tr>
</tbody>
</table>

Discussion

A. Characteristic of respondent. From these data, it is known that ischemic stroke types (72.60%) are higher than hemorrhagic strokes (27.39%). This result is similar to the research conducted by Mahdi Habibi-koolae (6), which also stated that ischemic stroke patients are higher than hemorrhagic stroke patients. However, it is different from the research conducted by Derrel V. Barahama (2) which exhibited more hemorrhagic stroke patients than ischemic stroke sufferers. Overall, ischemic stroke patients (66%) and hemorrhagic strokes (85%) were at most age less than 65 years old. This is similar to the research conducted by Barahama (2) which showed that more patients <65 years old who come to the hospital. More stroke patients are male in ischemic stroke and hemorrhagic stroke. In subjects with hemorrhagic strokes, 60% of the subjects were male. In subjects with ischemic stroke, 54% were male. Another study conducted by Mahdi Habibi-koolae (6) showed that male sex is more in each type of
stroke. This is supported because there is no vascular protection from endogenous estrogen in men so that it will increase the risk of stroke. In addition, lifestyle such as smoking, and drinking alcohol in men can increase the risk of hemorrhagic stroke (7).

B. Risk factor that can be change.

1. Smoking. In ischemic stroke patients (58.5%), it found that they were non-smokers. In patients with hemorrhagic strokes, 65% are non-smokers. Some study stated that smoking is a factor in determining stroke (5,8). This can happen because the majority of subjects are passive smokers. Passive smoker is a person who breathes smoke from the burning of cigarette tobacco and smoke exhaled by an active smoker (4).

2. History family of stroke. In ischemic stroke patients, only 20.8% have a history of stroke in their family members. In patients with hemorrhagic stroke, 30% of patients have a history of stroke in their family members. Some study found that there are positive associations between family history of stroke and functional status at discharge (9) lacunar stroke (10,11) atherothrombotic stroke (10,11) and the severity of early neurological deficit (12). Family history of stroke is a complex profile that includes the types of affected relatives (ie, paternal, maternal, or sibling), the ages of relatives at stroke onset (early onset versus late onset), and other characteristics. In addition, a family history of stroke may have different effects on the risk of stroke recurrence, depending on the patient’s age of stroke onset (13,14).

3. Hypertension. There are about 62.3% of patients with ischemic stroke have a history of hypertension. In patients with hemorrhagic stroke, it is only 20% who have a history of hypertension. Other study found that Blood Pressure in stroke patient are higher than in control patient without a diagnosis of stroke. Sistolic Blood Pressure on arrival significantly are higher in ischemic stroke than in controls (15). Other studies conducted by Laily and Imanda et al (5,16) also stated that there is a relationship between hypertension and ischemic stroke.

4. Diabetes Mellitus. About 22.6% of ischemic stroke patients have a history of diabetes mellitus. In hemorrhagic stroke patients, as many as 25% of patients have a history of diabetes mellitus. The prevalence of stroke with diabetes for more than 20 years in DM Type 2 patients is 7.9%, while DM Type 1 is 2.7% (17). Stroke deaths in people with DM Type 2 (13.4%) were higher than those in DM Type 2 (12.2%) (17). Diabetes mellitus is a risk factor for stroke that can be modified. In someone with diabetes mellitus, the risk of stroke increases two-fold compared with people without diabetes (18). It happens because an increase in blood sugar can increase the risk of atherosclerosis as well as other stroke risks such as hypertension, obesity, and hyperlipidemia (6). Patient with diabetes have a higher proportion of ischaemic stroke compared to haemorrhagic stroke. Microvascular disease disease dan co existence of hypertension can caused it (19–22).

C. The Rank of Risk factor

The most risk factors that are owned by ischemic stroke patients and hemorrhagic strokes are the same to those aged less than 65 years. However, second rank in ischemic strokes is a history of hypertension while ranking second in hemorrhagic stroke is male sex.

Conclusion

Based on this research, it concluded that: 1) Ischemic stroke had the highest prevalence among patients (72.6%), 2) Most of patient were 65 years old (56.2%), 3) Age of less 65 years old patient had the highest risk of any type of ischemic stroke. Moreover, the highest controlled factors among patients with ischemic stroke is hypertension history (62.3%), while among patients with hemorrhagic stroke is smoking (35%).

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request and restricted by the ethics of Local Government Hospital Dr. Soetomo in order to protect subject privacy.

Conflicts of Interest: The authors confirm that there are no conflicts of interest.

Acknowledgment: We would like to thank all participants in this study including the patient as respondent. We also thank all investigators who collected and analyzed these data. It is research and publication of this article was funded by Universitas Airlangga through Faculty Public Health Research Grant in 2018.

References

1. WHO. Global status report on noncommunicable


Risk Factors for Diabetes Mellitus Occurrence in the Elderly at the Griya Antapani Public Health Center

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Abstract

Diabetes Mellitus is a health disorder that is caused by an increase in insulin levels. Diabetes Mellitus, including disease, is also a risk factor for heart and blood vessel disease. Risk factors for Diabetes Mellitus itself from Abdominal/Central Obesity, Hypertension, Dyslipidemia, and smoking. This study aims to see whether there are factors associated with the place of Diabetes Mellitus in the work area of the Griya Antapani Community Health Center. The research method used is Analytical Epidemiology with a Cross-Sectional approach, Sampling many as 84 respondents. The results of study showed that there was a relationship between Abdominal/Central Obesity, Hypertension, dyslipidemia and smoking the relationship between the incidence of Diabetes Mellitus in the Working Area of the Griya Antapani Public Health Center in Bandung. Based on the results of this study, it is expected that the community will find an unhealthy lifestyle to be healthy.

Keywords: Diabetes Mellitus, Risk Factors, Elderly

Introduction

Health is a human right and one of the elements of well-being that must be realized in accordance with the ideals of the Indonesian people as referred to in the Pancasila and the 1945 Constitution of the Republic of Indonesia. Anything that causes health problems in Indonesian society will result in economic losses big for the country, and every effort to improve the degree of public health also means investment in the country’s development.1

Non-communicable diseases are one of the world’s health problems and Indonesia, which is still a concern in the world of health, non-communicable diseases are one of the causes of death.2 Non-communicable diseases, also known as chronic diseases, are not transmitted from person to person, they have a long duration and generally develop slowly.3

The Ministry of Health of the Republic of Indonesia has established a national policy to control non-communicable diseases since 2005, one of which is the “PROLANIS” or Chronic Disease Control Program implemented by the Health Social Insurance Organizing Agency.4.5

One non-communicable disease that is still a global public health problem is Diabetes Mellitus. Diabetes Mellitus is a health disorder in the form of a collection of symptoms caused by increased blood glucose levels due to deficiency or insulin resistance.6

Diabetes mellitus, besides being known as a disease, is also a risk factor (FR) for heart and blood vessel disease. Diabetes Mellitus risk factors themselves are divided into 2 (two) parts, namely: 1) Factors that cannot be modified such as Race/Ethnicity, Age, Family History, birth history > 4 kg and birth history <2.5 kg. 2) Modifiable factors such as excess body weight, central obesity, lack of physical activity, high blood pressure, cholesterol, unhealthy diet, history of impaired sugar tolerance and smoking. For this study, the researcher reviewed risk factors that could be modified or
Material and Method

This research uses a cross-sectional study. The population in this study were the elderly in the Antapani Wetan Village in the work area of Griya Antapani Health Center as many as 5938 people. So in this study the samples taken were as many as 84 people.

Findings

Table 1. Analysis of Risk Factors for Diabetes Mellitus

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Bivariate</th>
<th>Diabetes</th>
<th>Total</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Not Diabetes</td>
<td>Mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Obesitas Abdominal/Central</td>
<td>26 (31%)</td>
<td>19 (73,1%)</td>
<td>7 (26,9%)</td>
<td>26 (100%)</td>
<td>0,043</td>
</tr>
<tr>
<td>Obesitas Abdominal/Central</td>
<td>58 (69%)</td>
<td>27 (46,6%)</td>
<td>31 (53,4%)</td>
<td>58 (100%)</td>
<td></td>
</tr>
<tr>
<td>Not Hypertension</td>
<td>32 (38,1%)</td>
<td>23 (71,9%)</td>
<td>9 (28,1%)</td>
<td>32 (100%)</td>
<td>0,025</td>
</tr>
<tr>
<td>Hypertension</td>
<td>52 (61,9%)</td>
<td>23 (44,2%)</td>
<td>29 (55,8%)</td>
<td>52 (100%)</td>
<td></td>
</tr>
<tr>
<td>Not Dyslipidemia</td>
<td>26 (31%)</td>
<td>21 (80,8%)</td>
<td>5 (19,2%)</td>
<td>26 (100%)</td>
<td>0,003</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>58 (69%)</td>
<td>25 (43,1%)</td>
<td>33 (56,9%)</td>
<td>58 (100%)</td>
<td></td>
</tr>
<tr>
<td>Not Smoke</td>
<td>71 (84,5%)</td>
<td>33 (46,5%)</td>
<td>38 (53,5%)</td>
<td>71 (100%)</td>
<td>0,001</td>
</tr>
<tr>
<td>Smoke</td>
<td>13 (15,5%)</td>
<td>13 (100%)</td>
<td>0 (0%)</td>
<td>13 (100%)</td>
<td></td>
</tr>
<tr>
<td>Not Diabetes Mellitus</td>
<td>46 (54,8%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>38 (45,2%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Abdominal/Central Obesity

From the results of research in the field, researchers found that most of the respondents experienced Abdominal/ Central Obesity, i.e. as many as 58 respondents or 69% of the total respondents. From the results of statistical tests, the P-value is smaller than α 0.05, which means there is a relationship between Abdominal / Central Obesity and the incidence of Diabetes Mellitus.

The impact of Abdominal / Central Obesity is a higher risk to health. Abdominal /Central obesity can cause health problems such as Diabetes Mellitus and other metabolic syndromes. Metabolic syndrome is a condition in which a person experiences hypertension, Abdominal/ Central Obesity, Dyslipidemia, and insulin resistance at the same time.

A person who has Abdominal/Central Obesity (a man’s circumference> 90 cm while in a woman> 80 cm) is 5.19 times the risk of suffering from Diabetes Mellitus. It can be explained that Abdominal/Central
Obesity which is described by waist circumference can predict disorders due to insulin resistance in Diabetes Mellitus. An increase in the amount of abdominal fat has a positive correlation with hyper insulin and is negatively correlated with insulin sensitivity.\textsuperscript{10}

A person who has abdominal circumference above normal has more risk for Diabetes Mellitus. The fat stored in that part is visceral fat or visceral fat. In addition to its position in the abdominal cavity so that it covers the vital organs of the body inside it, such as the pancreas and liver, visceral fat also produces cytokine compounds and Free Fatty Acid (FFA) which is dangerous. FFA compounds cause inflammation in the body which increases the risk of developing cancer, increases the potential for coronary heart disease, and causes the body’s resistance to insulin, as the main cause of Diabetes mellitus.\textsuperscript{11,12}

**Hypertension**

From the results of the study respondents who experienced high blood pressure or hypertension as many as 52 respondents or 61.9\% of all respondents. From the results of statistical tests, p-value \( \leq \alpha 0.05 \) was obtained, which means there is a relationship between hypertension and the incidence of diabetes mellitus.

The effect of hypertension on the incidence of Diabetes Mellitus is caused by thickening of the arteries which causes the diameter of the blood vessels to narrow. This will cause the process of transporting glucose from the blood to cells to be disrupted. A person who has hypertension has 2.3 times the risk of developing Diabetes Mellitus. Increased insulin concentrations that cause hypertension occur due to increased sodium retention in the kidneys and increased sympathetic nervous system activity.\textsuperscript{13}

In addition, insulin acts like a growth hormone that can stimulate hypertrophy of the smooth muscle cells of the vessels. Insulin can also increase blood pressure by increasing intracellular calcium concentration, which will lead to increased resistance from vessels.\textsuperscript{12}

**Dyslipidemia**

From the results of the study respondents who experienced high blood cholesterol levels amounted to 58 Respondents or 51.2\% of the total Respondents. From the results of statistical tests, p-value \( \leq \alpha 0.05 \) was obtained, which means there is a relationship between Dyslipidemia and the incidence of Diabetes Mellitus.

Dyslipidemia is an abnormality of lipids (fat) in the bloodstream. These lipids include cholesterol, cholesterol esters (compounds), phospholipids and triglycerides. These materials are transported in the blood as part of a large molecule called a lipoprotein. Circulating lipoproteins that only depend on insulin and only exist in plasma glucose. Thus, the performance of the pancreas is inhibited in the spread of insulin in the body and makes blood sugar levels accumulate so that it can cause the incidence of diabetes mellitus.\textsuperscript{13}

**Smoke**

From the results of the study, a small proportion of respondents who consumed tobacco (smoking) were 13 respondents or 15.5\% of the total respondents. From the results of statistical tests, the P-value is smaller than 0.05, which means there is a relationship between smoking and the incidence of diabetes mellitus.

Nicotine in cigarettes has been shown to cause insulin receptor resistance and can reduce insulin secretion in the pancreas \( \beta \) cells. Insulin receptor resistance occurs through the nicotine process that stimulates mTOR. mTOR is responsible for cell growth, where if the activity of mTOR is excessive there will be abnormal cell growth and proliferation of insulin receptors so that the receptor does not recognize insulin anymore.\textsuperscript{14,13}

Nicotine intake can increase levels of hormones such as cortisol, which can interfere with the effects of insulin, so the risk of diabetes mellitus is higher. Respondents with active smokers are fewer in number than respondents who do not smoke (15.5\%). However, passive smokers have a higher risk of active smokers in the vicinity, which is smoking 75\% of the smoke emitted by active smokers and only smokes 25\%, so that respondents who do not smoke have a higher risk of Diabetes Mellitus.\textsuperscript{15,2,14}

**Diabetes Mellitus**

From the results of research in the field, researchers found that most of the respondents who had Diabetes Mellitus were 38 respondents or 45.2\% of the total respondents.

Diabetes Mellitus itself is defined as a disease in which the patient’s body cannot automatically control
the level of sugar (glucose) in his blood. Diabetics cannot produce insulin in sufficient quantities, resulting in excess sugar in the body. Chronic excess sugar in the blood (hyperglycemia) is toxic to the body. Diabetes Mellitus can be reduced, especially if both parents have severe diabetes, but the emergence of Diabetes Mellitus is more influenced by a bad lifestyle, even in couples who one of them is a Diabetes Mellitus sufferer, then a partner who previously did not suffer from Diabetes Mellitus, in the end, can also have it, because it follows or is influenced by their partner’s lifestyle.  

Conclusions
1. Most of the respondents experienced Abdominal/Central Obesity, high blood pressure, cholesterol Working Area of Griya Antapani Health Center, Bandung.

2. A small proportion of respondents consume tobacco (smoking) in the working area of the work area of Puskesmas Griya Antapani, Bandung.

3. Nearly some respondents have Diabetes Mellitus in the Work Area of the Griya Antapani Health Center in Bandung.

4. There is a relationship between Abdominal/Central Obesity, Hypertension, Dyslipidemia and smoking with the incidence of Diabetes Mellitus in the working area of Puskesmas Griya Antapani, Bandung.

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: The study was approved by the institutional Ethical Board of The Bhakti Kencana University.

All subjects were fully informed about the procedures and objectives of this study each subject prior to the study signed an informed consent form.

References


2018;14:59–68.


Medical Environment of Traditional Market Quality in A Business Competition

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Abstract

There are two emerging markets in Indonesia, namely traditional markets and modern markets. Traditional market reform is one of the government’s programs to improve the performance of market traders. The existence of medicine environment aspects as the development of the quality of traditional markets is important to be discussed. The greater the health and medicine environment that occur shows a reflection of the level of public welfare. its development Traditional markets are beginning to be marginalized, this is due to the quality of commodities and un health environment that are not guaranteed.

Keywords: Medicine, Environment, Traditional markets, Constitutions, Law

Introduction

In general, business competition law is the law that regulates everything related to business competition, while the terms used for this field of law, in addition to the term business competition law, namely antimonopoly law, antitrust law, as argued by Nugroho.¹ Martadisastra opines that the business competition includes all actions of business actors in the market; therefore competition law is a law that regulates the market (law regulating the market or the set of laws and regulations governing market behaviour).² Based on the above understanding, the understanding of business competition is very broad and covers all actions of business actors in the market, including unfair competition actions.

Competition law is a legal instrument that determines how competition must be conducted. Next Christopher Pass and Bryan Lowes in Hermansyah.³ Stated what is meant by competition laws are parts of legislation governing monopoly, merger and acquisition, restrictive trade agreements and anti-competitive practices. Boner dan Krueger⁴ states that competition policy includes all government policies to maintain and protect competition between buyer and seller. The aim is to improve consumer welfare by encouraging the efficient allocation of community resources, which is based on the fundamental principle that the company is competitive in conditions.

According to Boner and Krueger, quoted by Thee Kian Wie⁴ states that competition policy includes all government policies to maintain and protect competition between buyers and sellers in a free market that is relatively unconstrained. The aim is to improve consumer welfare by encouraging efficient allocation of community resources, based on the fundamental principle that in The company’s competitive conditions give consumers good prices and quality.⁶

The existence of health and medicine environment as the development of the quality of traditional markets is important to be discussed, Noor argues that the greater the volume of transactions that occur shows a stable economy and is a reflection of the level of public welfare.⁷ There are two developing markets in Indonesia, namely traditional markets and modern markets. Traditional markets are built and managed
by the Regional Government, whereas modern/central markets are sold or leased to businesses or managed by themselves to carry out trading activities of goods.\(^7\)

Modern markets provide product prices and have an health environment that is more able to answer customer demand. Compared to traditional markets that are synonymous with slums, chaos, dirty, high crime, uncomfortable, minimal facilities such as parking, toilets, trash, electricity, water, muddy and narrow streets, modern markets provide more value in the convenience of shopping. The added value offered by the modern market is what makes most customers more interested in shopping in the modern market than in the traditional market, according to Tambunan.\(^9\)

Bintoro argues that traditional markets in addition to functioning economically\(^10\) even more, important is as a place to interact with individuals in the community. Unlike the Modern Market, everything is fixed price and served by machines. this difference ultimately affects the behaviour of people’s lives, namely from a social society to an individual society. The existence of modern markets has an impact on the existence of traditional markets whose existence has decreased, it is understandable that there are striking differences between traditional markets and modern markets, especially in terms of health aspects.

This health aspect covers environmental hygiene in traditional markets. For example, not littering, packaging, cigarette butts, candy wrappings carelessly in the market environment, as well as the hygienic quality of the commodities traded. Problems arise when modern markets begin, entering the realm of traditional markets. Tambunan opines that this aggressive expansion of the establishment of a modern market shopping centre has already been granted by the Regional Government, where the process of granting permits for local authorities is not done transparently and often clashes with various personal interests therein.\(^9\)

**Findings and Discussion**

The emergence of a modern market is indeed beneficial for consumers, but a threat to the existence of traditional markets. The rapid growth of modern markets with hygienic conditions, not only influences the number of traditional markets but also greatly affects the income of traders in traditional markets. According to Boeke in Sutrisno, there is a dichotomy between modern markets and traditional markets in Indonesia\(^12\) The two economic systems are not transitional economic systems in which the nature of one is getting weaker and the other is getting stronger, but both are side by side with different systems.

The existence of markets, especially traditional markets, is one of the most tangible indicators in the economic activities of a region’s community. The government must pay attention to the existence of traditional markets as one of the public facilities that support the economic activities of the society. The development of the era makes the existence of traditional markets threatened. However, traditional markets are still able to survive and compete amid the rush of modern markets in various forms, as argued by Indrakh.\(^13\) For people who think positively will most likely behave, that “traditional markets have lost competitiveness” because infrastructure is far behind amid modern markets and poor health aspects in traditional markets.

In business, activity can be ensured that competition occurs between business actors. One important essence for the implementation of market mechanisms is the competition of market participants in meeting consumer needs. In the health aspect of the business will endeavour to create, package, and market the products that are owned by the goods or services as well and as attractive as possible so that they are in demand and are bought by consumers.

The problem of traditional market competition is a logical consequence arising from the presence of modern markets. Problems arise when modern markets begin, entering the realm of traditional markets. According to Tambunan, this aggressive expansion of the establishment of a opping centre or modern market has received permission from the relevant Regional Government where the process of issuing permits by local authorities is not done transparently and often clashes with various personal interests in it.\(^9\)

**Method**

The research method used descriptive study. This research was at the same time prescriptive research, namely research aimed at providing solutions to problems rather than theoretical testing. This research was research that connects the essence and reality of developing medicine environment in the traditional market.
Competition in business can have positive implications, on the contrary, it can become negative if it is carried out with negative behaviour and an economic system that causes it to be uncompetitive. Business competition which is carried out negatively or is often termed unfair competition, according to Hikmahanto Juwoana, according to Nugroho. Will result in a) death or reduced competition between business actors; b) the emergence of monopolistic practices, where the market is controlled only by the business actor; and c) even the tendency of business actors to exploit consumers by selling expensive goods without paying attention to adequate quality.

The increasingly unavoidable investment liberalization has made traditional markets increasingly pushed by the presence of modern markets that offer more commodity advantages, price and convenience. Conditions like this, of course, very influential on the level of growth of traditional markets. On the one hand the emergence of modern markets is indeed beneficial for consumers, but it is a threat to the existence of traditional markets, as argued by Malono.

Law No.5 of 1999 is in line with the spirit of the Pancasila and the 1945 Constitution, particularly Article 27 Paragraph (2) and Article 33. This can be seen from the sound of Article 2 and Article 3 of Law Number 5 the Year 1999 concerning the principles and objectives of establishing Law Number 5 of 1999. Article 2 states, “Business actors in Indonesia in carrying out their business activities are based on economic democracy by taking into account the balance between the interests of business actors and the public interest”. for the rapid growth of the modern market in Indonesia. Whereas Article 3 states the purpose of establishing this law is to: a) safeguard the public interest and improve national economic efficiency as an effort to improve the welfare of the people; b) create a conducive business climate through the regulation of fair business competition so as to ensure the certainty of equal business opportunities for large businesses, medium business actors, and small business actors; c) prevent monopolistic practices and or unfair business competition arising from business actors.

The market mechanism turns out to lead to a dualism of economic activities, especially trade, which in turn will also refer to dualism of other aspects such as land use distribution, environmental health, and socio-cultural conditions. In trade, the superior group usually appears that dominates the inferior group. Emerging modern markets amid the existence of traditional markets, different and adjoining economic systems, more wide open as the times. a condition where “superior” coexists with “inferior” so that there is a gap between “superior” and “inferior”.

Dualism (modern markets with traditional markets), one of the consequences in the development of trade areas The differences in zoning management and regulation are often not counted in providing spatial patterns. Planned which eventually lead to fiction and the pros and cons of his presence. This phenomenon makes us take into account the development of an area from the planning period so that the coexistence of the two situations is not opposite or antagonist, but as complementary or inter-dependency. Reformulation of business competition law as an effort to realize integration of traditional and modern markets requires various policies such as spatial planning policies, zoning regulations, development plans for the production sectors, regulation of economic infrastructure (including regulation of functions and determination of market locations), licensing, fiscal and monetary, etc. So that there is no mutual domination, modern markets cannot dominate traditional markets, otherwise traditional markets cannot dominate modern markets. So that the reformulation of business competition law as an effort to realize the integration of traditional markets and modern markets can be done through three aspects: regulatory aspects; structuring aspects and market cementation aspects.

Traditional markets that are spread both in cities and villages have distinctive features in the buying and selling process, not only as a meeting place between sellers and buyers, but also a place for very personal relations, a place of information resources, and an important means of power support the economy of cities and villages. The market is a social institution that is regulated by norms and sanctions and is formed through social interaction. This opinion confirms that the market is not just an economic space, but also as a social space. It is in this space (the market) that social capital is activated which ties social relations together and allows for lasting economic transactions.

Traditional markets have the potential that cannot be ignored both economically and socially. First, economically, the traditional market is able to support
thousands of people or is an arena to meet the needs of life or space for the empowerment of the people’s economy. Second, the traditional market as a public space is an arena to form relationships of socio-economic relations, in which the values of mutual trust, mutual respect, and feelings of empathy for each other are built. Third, naturally, in the traditional market a community of various social groups has been built, ranging from large traders, small traders, foster workers, and buyers.

There are things that traditional markets have that don’t exist in the modern market. The development of modern markets in various categories (minimarkets, supermarkets, hypermarkets, department stores) will become competitors that will be able to threaten the presence of traders in traditional markets, because of the existing reality, buyer segments for certain types of goods (nine staples) between traditional markets and modern markets are not much different (the same), this is because there is almost no significant price difference for the types of staple goods between modern markets and traditional markets, even for certain types of commodities such as sugar and cooking oil prices in Modern markets are cheaper than prices in traditional markets.

Considering the important role of traditional markets from the socio-economic aspects of society, protection of the existence of traditional markets is absolutely necessary by making efforts to synergize the strengths of modern markets with the weaknesses of traditional markets. The existence of modern markets must be able to maintain the existence of traditional markets and not vice versa. One way that can be done by the government is by issuing several laws and regulations governing the pattern of relations and structuring between traditional markets and modern markets. So, the laws in the form of laws and regulations are able to realize the protection of traditional markets. Through reformulation of regulations regarding the types of commodity commodities that can be traded in the modern market, and the types of commodity commodities for the traditional market share, there will be hormonal or synergy (mutual symbiosis) between traditional markets and modern markets. The intended integration can be in the form of physical integration, distribution channel integration, and regulation integration.

Market segmentation is an attempt to classify markets, from heterogeneous markets to market parts that have a homogeneous nature, according to Gitosudarmo. Market segmentation can also mean the division of markets into different groups of buyers who may require separate marketing or products, as argued by Kotler and Armstrong. So market segmentation is a comprehensive process where companies must pay attention to the purchase of each segment, at least the business will be more economical if the purchase units are grouped into several groups. This all can not be separated from efforts to achieve maximum profit.

The formulation of target markets in market segmentation can be done on the basis of regional criteria, regional density, population criteria such as age, gender, education, income and so on, psychiatric criteria (psychographic criteria) such as lifestyle, personality, product use behavior criteria (behavioral criteria) and various other criteria, such as the criteria for the benefits sought by consumers in selecting / consuming products (benefit segmentation). If the quality of traditional market health is improved, it will lead to an increase in consumers. The influence of the health aspects of both goods sold and the environment causes an increase in trader income. So that a positive and significant effect on the welfare of traditional market traders.

Conclusion

Reformulation of business competition law in the medicine environment is carried out to integrate traditional markets and modern markets so that one is not superior and dominates the inferior group. So that the modern market cannot dominate the traditional market otherwise the traditional market cannot dominate the modern market. Integration can be done through three aspects: regulatory aspects; structuring aspects and health aspect. Every citizen and state operator must obey the law with the principle of justice. the government must also pay attention to environmental medicine as a guarantee of the quality of traditional markets.

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Source of Funding- Authors
Ethical Clearance- Yes

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Triability or Observability That Influences The Youth Decision Using Mobile Application “Remaja Sehat”?

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Abstract

Reproductive health of adolescents in Indonesia has become an unresolved priority problem and the number of its cases keep increasing from year to year. It caused by access to information and adolescent health programs that are haven’t been distributed evenly. In fact, not all programs use the media that is in line with the current characteristics of adolescents. Media is an adolescent health promotion strategy that has to be considered in order to increase knowledge. There have been many kinds of media related to adolescent reproductive health in the form of posters, leaflets, public service announcements and so on. According to the previous research, during all this time the media tend to be patronizing and boring. The adolescents nowadays will not be far from gadgets, so researchers have developed “Remaja Sehat” application as a media for adolescent health education. The purpose of this study was to analyze the effect of media on the variables of triability and observability on the decision to use “Remaja Sehat” application.

This research was conducted to high school students in Surabaya. The method used in this research is the Quasi Experiment and uses post only design. Samples in this study were 302 conducted by using simple random sampling. The students were given a number of questions in the questionnaire after be given intervention in the form of exposure to a healthy adolescent application.

The results showed that there was a significant effect on triability (p value = 0.000) and obsevability (p value = 0.042) on the decision to use “Remaja Sehat” application. Based on this research, it can be concluded that the “Remaja Sehat” application can be used as a media for health promotion which matches the characteristics of adolescents.

Keywords: application, decision, adolescents, observability, triability

Introduction

Indonesia is one of the countries that still have problems in the field of health, especially in adolescent reproductive health. Health problems in Indonesia still become one of the things that have to be considered both in terms of the community itself as well as the health sector. Perpetrators of the community one of which must be considered are the adolescent. The adolescent is the age of transition to maturity. This age forms the identity of a person and will go through a process of instability both in psychological or feelings. The physical organ, psychological and intellectual of one’s health will be more productive and develop rapidly in adolescence because at the age of adolescence have a great curiosity and the behavior of wanting to try new things is high.

The age of adolescents according to WHO are 10-24 years old and geographically adolescents are divided into two groups, namely the 10-14 year age group and the 15-19 year age group. While the age of adolescents according to the BKKBN are 10-24 years old and unmarried. Adolescence is a transition period that has challenges of risky sexual behavior.

Adolescent sexual risk behavior can be described through one of the previous studies. Adolescents whose sexual behavior not at risk and the one who at risk were exposed through almost the same media, where adolescents whose sexual behavior not at risk using the internet as many as 25 people (32.1%). Likewise, adolescents with risky sexual behavior using the internet as many as 53 people (67.9%). The results of the bivariate analysis showed there’s no significant
relationship between electronic media and adolescent sexual risk behavior\(^2\).

Chi-square test results show there is a relationship between smartphone usage and adolescent sexual behavior in senior high school “X” Jember (p-value = 0.004; CI = 95\%). Exposure to pornography when the adolescents access the internet and social media through smartphones raises the desire of adolescents to engage in sexual behavior by their self (onani or masturbation) or with their partners (holding hands, hugging, kissing the forehead, kissing the cheeks and kissing the lips)\(^3\). It can be seen that adolescent sexual behavior can be affected by exposure to the media used so there need to be a media that can provide knowledge about adolescent reproductive health issues.

Knowledge of adolescent reproductive health is very important in the role of adolescent health. The higher knowledge of adolescents about adolescent reproductive health will cause a decrease in adolescent sexual behavior and vice versa\(^4\). One of the ways to increase knowledge is through the media as learning media. Media is all forms of intermediaries used by humans aiming to convey or spread ideas, thoughts or opinions\(^5\). The media as the means to deliver the information or messages whether it’s ideas or opinions to get thoughts, feelings, attention and to make effective and efficient communication. Media can also be a learning method in order to increase knowledge.

There are three types of exposure to mass media such as printed media (newspapers and magazines), radio, and television other than that, regarding exposure to the internet\(^6\). There are fifteen percent of women and 20 percent are not exposed to all three information media in a week. So that the three types of mass media can develop along based on their level of education. But the internet nowadays is not only for an adult age, but even adolescents can’t get far from the internet.

One of the ways to measure the effectiveness of media is by using the Diffusion of Innovations. Diffusion is one type of communication where the characteristic of the main message being communicated is new or can be called innovation. Diffusion is also an innovation communication process between citizens using a certain channel and a certain time\(^7\).

The Remaja Sehat application is one of the educational innovations regarding reproductive health knowledge that will be given to adolescents. The Android-based application will be used as an educational medium for adolescents. Adolescents are more enthusiastic about new things, including the development of information and technology. Adolescents use an android smartphone because it has excellence over other types of mobile phones. The excellence in applications and appearance is more attractive. The easiness of downloading applications through Google Play\(^8\). This application was designed based on the results of previous studies. The results showed that during all this time, adolescent health program methods still tend to be patronizing and boring so that it requires methods that are matched to the current characteristics of adolescents\(^9\).

Diffusion of Innovations has various characteristics in innovation including Triability and Observability. Triability is the possibility to be tested from an idea or innovation. While Observability is the level of results of an innovation that can be seen easily from the technical-economic advantages so it can accelerate the adoption process \(^10\). An innovation in order to reach the goals that want to be achieved it must be right on target at its usefulness. Therefore, this study will analyze the effect of triability and observability on the decision to use Remaja Sehat application.

**Material and Method**

This study uses a pre-experimental method. Researchers will provide intervention in the form of exposure to Remaja Sehat application and then will be given a questionnaire about Triability and Observability and the decision to use Remaja Sehat application. This research refers to the theory of diffusion of innovation to analyze the effect of triability and observability on the decision to use Remaja Sehat application. This study uses a post only design.

This research was conducted on senior high school students in Surabaya. The sample in this study was conducted using simple random sampling technique. There are 302 students in total as the sample. The sample was given action in the form of health education about reproductive health by using the Remaja Sehat application. The respondents study about reproductive health material and play games in the application. The respondents are given 1 week to study and operate the application. Then it will be measured according to the variables studied. The variables include adolescent
characteristics as well as triability and observability towards the decision to use Remaja Sehat application.

**Findings**

1. **Characteristic of Respondent**

   **Table 1. Characteristic of Respondent based on Age, Residence, Gender**

<table>
<thead>
<tr>
<th>Characteristic of Respondent</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondent’s Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Years old</td>
<td>124</td>
<td>41.1</td>
</tr>
<tr>
<td>17 Years old</td>
<td>161</td>
<td>53.3</td>
</tr>
<tr>
<td>18 Years old</td>
<td>17</td>
<td>5.6</td>
</tr>
<tr>
<td>Respondent’s Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>House</td>
<td>293</td>
<td>97</td>
</tr>
<tr>
<td>Boarding house/dormitory</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Respondent’s Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>121</td>
<td>40.1</td>
</tr>
<tr>
<td>Female</td>
<td>181</td>
<td>59.9</td>
</tr>
</tbody>
</table>

   The frequency distribution generated according to the age of the respondents is based on the table above, at the age of 17 years old, there are 161 students or 53.3% while the age of 16 years old, there are 124 students or 41.1%, and the age of 18 years old, there are 17 students or 5.6%. The frequency distribution according to most respondents’ residence based on the table above showed that 293 students live in the house or 97% while respondents live at the boarding house/dormitory are as much as 9 students or 3%. The frequency distribution according to the gender of the most respondents shown on the table above. There are 121 male students or 40.1%, while female respondents are as much as 181 students or 59.9%.

2. **Triability and Observability categories based on the results of respondents**

   **Table 2. Triability and Observability categories**

<table>
<thead>
<tr>
<th>Triability</th>
<th>Frequency</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>232</td>
<td>76.8</td>
</tr>
<tr>
<td>Enough</td>
<td>52</td>
<td>172.2</td>
</tr>
<tr>
<td>Less</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Observability</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>245</td>
<td>81.1</td>
</tr>
<tr>
<td>Enough</td>
<td>39</td>
<td>12.9</td>
</tr>
<tr>
<td>Less</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

   Table 2 shows that the most categories in Triability are categorized as good with the total number 232 or 76.8%, while there are 52 students categorized as enough or 172.2% and categorized as less as much as 18 students or 6%.

   Most observability variables are categorized as good with the number of students as much as 245 or 81.1% while in enough category, there are 39 students or 12.9 and as much as 18 students or 6% are categorized as less.

3. **Cross-tabulation Result of Characteristic Respondent (Age and Gender) with Triability, Observability**

   **Table 3. Cross-tabulation Result of Characteristic Respondent (Age and Gender) with Triability, Observability**

<table>
<thead>
<tr>
<th>Age</th>
<th>Characteristic Respondent</th>
<th>Triability</th>
<th></th>
<th>Observability</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Good</td>
<td>%</td>
<td>Enough</td>
<td>%</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>N</td>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88</td>
<td>29.1</td>
<td>25</td>
<td>8.3</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>134</td>
<td>44.4</td>
<td>23</td>
<td>7.6</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>10</td>
<td>3.3</td>
<td>4</td>
<td>1.3</td>
</tr>
</tbody>
</table>
## Table 3

Table 3 show that Triability (44.4%), observability (43.7%) most widely categorized as good at the age of 17 years. Tables 3 show that Triability (47.4%), observability (50%), most widely categorized as good at female adolescents.

### 4. Cross-tabulation Result of Characteristic Respondent (Age and Gender) with Decision

#### Table 4. Cross-tabulation Result of Characteristic Respondent (Age and Gender) with

<table>
<thead>
<tr>
<th>Gender</th>
<th>Decision</th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Male</td>
<td>100</td>
<td>33.1</td>
<td>21</td>
</tr>
<tr>
<td>Female</td>
<td>1147</td>
<td>48.7</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 4 show that high decisions (48.7%) most widely categorized as good at female adolescents. Table 4 show that high decisions (45.4%) most widely categorized as good at the age of 17 years.

### 5. Cross-tabulation Result of Characteristic Respondent (Residence) with Triability and Observability

<table>
<thead>
<tr>
<th>Gender</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Male</td>
<td>100</td>
</tr>
<tr>
<td>Female</td>
<td>1147</td>
</tr>
</tbody>
</table>

Table 4 show that high decisions (48.7%) most widely categorized as good at female adolescents. Table 4 show that high decisions (45.4%) most widely categorized as good at the age of 17 years.
Table 5. The result of cross-tabulation of Recidence with triability, observability

<table>
<thead>
<tr>
<th>Recidence</th>
<th>Triability</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Enough</td>
<td>Less</td>
<td>Good</td>
<td>Enough</td>
<td>Less</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>House</td>
<td>226</td>
<td>74.8</td>
<td>50</td>
<td>16.6</td>
<td>17</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>Boarding house/dormitory</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0.7</td>
<td>1</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Observability</td>
<td>House</td>
<td>293</td>
<td>79.1</td>
<td>36</td>
<td>11.9</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Boarding house/dormitory</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6. The result of cross-tabulation of Recidence with decision

<table>
<thead>
<tr>
<th>Recidence</th>
<th>Decision</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>House</td>
<td>240</td>
<td>79.5</td>
<td>53</td>
<td>17.5</td>
</tr>
<tr>
<td>Boarding house/dormitory</td>
<td>7</td>
<td>2.3</td>
<td>2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Tables 5 and 6 show that Triability (74.8%), observability (79.1%), and high decisions (79.5%) most widely categorized as good at the adolescents residing at house.

6. The Effect of Triability and Observability with Decisions on the use of Remaja Sehat application

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Independent</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triability</td>
<td>Decision</td>
<td>0.000</td>
</tr>
<tr>
<td>Observability</td>
<td>Decision</td>
<td>0.042</td>
</tr>
</tbody>
</table>

There is a significant effect of triability and observability on the decision to use Remaja Sehat application. These results indicate that Remaja Sehat application is possible to be retested and the results of this application can be seen easily as good from the technical-economic advantages so it can accelerate the adoption process.

Discussion

The Effect of Triability on the Decisions to Use Remaja Sehat Application

This study uses several indicators to measure how much the triability is. These indicators are able to decide to maintain reproductive health, to feel happy to try Remaja Sehat application, and feel that Remaja Sehat application provides information that can be accessed easily.

Triability is the possibility to be tested from an innovation. According to Kurniawan, an innovation that can be tested can be adopted faster than an innovation that can’t be tested. Innovation must also show how excellence and usability match the innovation users so that innovation can be adopted faster. In this study, triability is included as good categories. In line with the results of research on the usage of android smartphones are widely used in accessing various education needed. The easiness of downloading educational applications on Google Play(8). In this study, application access has been available on Google Play as well as the easiness in operating Remaja Sehat education application.

In addition, innovation has to be maximally promoted to users to explain its usefulness, excellence until it can be tested. This can cause an increase in the user’s confidence in using an innovation. Sholahudin’s research shows there is no influence between triability and intention to adopt Solopos epaper because most of the respondents still don’t know that the innovation can’t be tested(11).

This research is also supported by research from Kurniadi. The results show that there is a significant effect of triability on Blackberry product purchase decision making(12).

The intensity of using a smartphone influences communication patterns and behavior. The results of other studies conducted have provided information that smartphone users use high duration and frequency. The content viewed is games, videos, music, social media(13). In the results of this study can be found that the adolescents use and try the application that have been made.
The effect of Observability on the Decision to Use Remaja Sehat Application

Observability is the results level of an innovation that can be seen easily as good from an economic technical advantage. The easier the user sees the results of an innovation, the greater the user will decide to adopt an innovation\(^{(5)}\). This study uses an indicator as an observability measurement tool listed in the questionnaire. These indicators are other people or friends interested in the Remaja Sehat application, friends have liked the innovation of Remaja Sehat application, feeling easy when friends or other people ask about the innovation of Remaja Sehat application.

Based on the results of the study, it shows that the more other people recommend or suggest to use Remaja Sehat application, the faster students will make decisions to use or download Remaja Sehat application. Supporting research is on Sholahudin research that showed there is a significant influence between observability on decision making to adopt innovative e-paper sloops\(^{(11)}\). In addition, on the research of Pertiiwi that showed there is an influence of observability on the decision of purchasing Blackberry products and the most influential factor is the Observability\(^{(12)}\).

Remaja Sehat application that is developed can be effective as an educational media. Remaja Sehat media application can affect the Triability and Observability, it means the adolescents can try and feel that the application can be applied easily after observing and trying the media. This is in line with the research on using Android-based games that encourage the adolescents to change healthy lifestyles, that games can improve healthy lifestyles\(^{(14)}\).

Conclusion

The effect of Triability and Observability in this study effect on the decision making on the use of Remaja Sehat application. Therefore the Remaja Sehat application can be used as an effective medium to provide education about adolescent reproductive health. The Android-based application that is developed can be obtained easily and downloaded on the Google Play application. Aside from there are educational materials about reproductive health, there are also games that relevant to increase adolescent knowledge about reproductive health. The Remaja Sehat application that can be used easily can be one of the factors of Triability and Observability variables categorized as good.

Conflicts of Interest: None declared.

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Ethical Clearance: Ethics approval was received from the Health Research Ethics Committee, Faculty of Nursing, Universitas Airlangga.

References


Biochemical Analyses of Male Rat’s Serum Administered Dexamethasone and Green Synthesis Cerium Oxide Nanoparticles Treatment

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Abstract

Current research involves preparing cerium oxide nanoparticles using simple, environmental- friendly green synthesis approach via fresh green tea leaf extract (Camellia sinensis) as a capping and reducing agent, Ultraviolet-visible spectroscopy (UV-Vis.) is used to check the formation of nanoparticles by investigate the absorption peak and energy gap ($E_g$); Maximum absorption peak was around 295nm and $E_g$ was 4.2eV calculated using Planck’s equation, X-Ray diffraction (XRD) analysis of Nano powder stated that Cerium Oxide has a cubic fluorite structure with face centered cubic (FCC) structure, blood serum glucose levels, liver functions after CeO₂ Nanoparticles treatment for rats were reported in this paper. Forty eight adult male rats were used and divided randomly into six groups each group has eight rats (n=8), Results stated that blood glucose levels, liver enzymes were increased in groups that injected by dexamethasone while considerably modified in rats administered by Cerium Oxide Nanoparticles.

Keywords: green synthesis, dexamethasone, XRD, Cerium Oxide NPs, AST.

Introduction

Nanomaterials are the forms of matter at nanoscale. The “Building blocks” for nanomaterials consist of organics, semiconductors, metals and metal oxides. Nanomaterials with structural features at the nanoscale can be found in the form of clusters, thin films, multilayer and Nano-crystalline materials which are often expressed by the dimensionality of 0, 1, 2 and 3 (1)(2), The synthesis of nanomaterials is of present concern due to their huge applications in medicine and many other fields (3), commonly, most of these applications are due to the reality that the Nanometer scale has significantly different characteristics compared to the bulk state. the nanoparticles have strong surface bonds, the high surface-volume ratio in Nanoparticles is the main reason for a structural deformation that can change the physical properties of nanoparticles (4).

The most popular complications of hyperglycemia are the generation of (ROS) reactive oxygen species (5). Reactive oxygen species can cause serious harm to β-cells and insulin resistance (6). Hyperglycemia characterized by increases the percentage of metabolism and enzyme failure of elevated oxidative stress caused by beta cells failure in the pancreas (7). Using plants extract is one of the latest techniques used to synthesize nanoparticles. Many metal nanoparticles were produced this way (8), Biosynthetic pathways can provide Nanoparticles of a better defined size and morphology than some of the physicochemical preparation techniques. Plant extracts can behave both as reducing agents and as stabilizing agents in the synthesis of Nanoparticles (9).

Many researchers have defined the capacity of CeO₂ Nanoparticles to scavenge free radical species to behave as an antioxidant. Multiple scientists have indicated that cerium NPs behave as a mimetic of superoxide dismutase, the primary catalyst that reduces the production and damage caused by ROS to mammalian cells by converting superoxide into oxygen and hydrogen peroxide (10).

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The objective of this research is to investigate the capacity of CeO$_2$ NPs to decrease BGL, improvement liver function using biochemical assays and histological examinations.

**Materials and Method**

8.68 grams of Ce(NO$_3$)$_3$.6H$_2$O (0.09M) was added to 200 ml of deionized water in a flask and they stirred for 5 minutes on 1,500 rpm magnetic stirrer, then gradually 20 ml of previously prepared *Camellia Sinensis* leaf extract was added to the flask. At 40°C magnetic hotplate, the mixture was constantly stirred for two hours; the solution was cooled at room temperature. Blackish brown CeO$_2$ Nanoparticles were collected by centrifugation (4,000 rpm) for 15 minutes. The NPs were washed repeatedly with deionized water and dried it with hot air oven then calcined for ~5 hours at 400°C.

**Experimental animals**

Forty eight adult male Wister albino rats weighing (175-225g) and average (10-14) weeks old, the rats were housed in polypropylene cages under controlled conditions of temperatures (25±5)°C and 12/12 hours light/dark cycles, Diet and drinking water were given *ad libitum*, the animals were reared and treated in animal house of college of science, University of Baghdad.

Laboratory animals were randomly divided into six groups, each group contained eight rats (n=8) treated for 30 days, and intended as follows: the first group (G1) was served as negative control, the second group (G2) was injected intraperitoneally with (0.5 mg/kg) dexamethasone sodium phosphate, (G3 and G4) were injected intraperitoneally with (34 and 84) mg/kg CeO$_2$ Nanoparticles and (0.5 mg/kg) of dexamethasone respectively, the fifth group (G5) was injected intraperitoneally with 34 mg/kg CeO$_2$ Nanoparticles and (G6) was injected intraperitoneal with 84 mg/kg CeO$_2$ Nanoparticles.

The experimental animals in this model was fast for 24-hours, the rats were weighed and anesthetized by their inhalation in a glass dome. Animals were injected dexamethasone sodium phosphate (0.5 mg/kg) for 30 days in a single dose daily (11). Only 30 minutes after drug administration, food and water were provided to the animals. Within 15 days of dexamethasone injection; blood glucose elevated and insulin secretion reduced these consequences of dexamethasone were agreed with Makoto Ohneda’s outcomes (12), animals have been received (34 and 84 mg/kg) Cerium dioxide nanoparticles (13).

**Biochemical analysis**

After the exposure period end, blood sample collected from animals by heart puncture, Collected blood was centrifuged at 4000 rpm for 10 min, serum was separated for assessment of various biochemical parameter such as blood glucose level (BGL) measured by commercially available kit (Biosystem, Germany), cholesterol (TC), Triglyceride (TG), High Density Lipoprotein Cholesterol (HDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) were estimated using a commercially available kit (Linear kit, Spain), serum levels Aspartate Amina transferase (AST) Alanine Amino transferase (ALT), Alkaline Phosphate (ALP) and Total Bilirubin (TB) were estimated by commercially available kit (Reflotron kit, Germany).

**Histological Examinations**

Pancreatic Samples were collected from animals after organs separation and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70%), cleared in xylene, and embedded in paraffin. 5 µm thick paraffin sections were prepared and then regularly stained with Hematoxylin and Eosin (H&E) (14) and then examined microscopically.

**Statistical Analysis**

The statistical analysis was performed using analysis of one way (ANOVA) variance and t-test analysis, carried out in complete randomized design. Different between means have analyzed by Least Significant differences (LSD) at (P≤0.05) and expressed as (Mean ± SE) with letters to explain the significant using the Statistical Package for Prism 8.1.2 (Version 8.1.2, Graph Pad, San Diego, CA, USA) software.

**Results and Discussion**

Nano-powder structural characterization was registered by Pro Penalty CAL with Cu-Kα radiation (1.5406 Å). XRD models for prepared Cerium Nano-powder showed that the structure is polycrystalline; as synthesized CeO$_2$ showed no pure intense peaks Fig. (1a), which is caused by amorphous state. While eight diffraction peaks existed when calcined in 400°C. Eight diffraction peaks were recorded in tab. (1) proved the
cubic fluorite structure with face centered cubic (FCC) structure of CeO$_2$ with standard data (no: 89-8436 of JCPDS card) Fig. (1b&c). No peak of any other phase was identified the sample’s high purity. The diffraction peaks of CeO$_2$ particles are quite broad, indicating very small crystalline sizes of sample. The pure CeO$_2$ nanoparticle’s lattice parameter is 5.412Å which matches well with the bulk CeO$_2$ lattice parameter a=5.411. Using Debye-Scherrer formula, the calculated average particle size was around 14.29 nm, these findings were agreed with (15)(16).

Table (1): The structural XRD parameters of the calcined synthesized CeO$_2$ Nanoparticles

<table>
<thead>
<tr>
<th>2θ (Deg.)</th>
<th>G.S (nm)</th>
<th>FWHM (Deg.)</th>
<th>d$_{hkl}$ Exp.(Å)</th>
<th>d$_{hkl}$ standard (Å)</th>
<th>(hkl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.575</td>
<td>5.77</td>
<td>1.412</td>
<td>3.124</td>
<td>3.018</td>
<td>(111)</td>
</tr>
<tr>
<td>33.134</td>
<td>5.77</td>
<td>1.412</td>
<td>2.706</td>
<td>2.701</td>
<td>(200)</td>
</tr>
<tr>
<td>47.5</td>
<td>10.4</td>
<td>0.760</td>
<td>1.913</td>
<td>1.912</td>
<td>(202)</td>
</tr>
<tr>
<td>56.80</td>
<td>7.2</td>
<td>1.094</td>
<td>1.631</td>
<td>1.619</td>
<td>(311)</td>
</tr>
<tr>
<td>59.5</td>
<td>15.8</td>
<td>0.500</td>
<td>1.562</td>
<td>1.551</td>
<td>(222)</td>
</tr>
<tr>
<td>69.1</td>
<td>15.8</td>
<td>0.500</td>
<td>1.352</td>
<td>1.358</td>
<td>(400)</td>
</tr>
<tr>
<td>77.08</td>
<td>39.7</td>
<td>0.200</td>
<td>1.241</td>
<td>1.236</td>
<td>(313)</td>
</tr>
<tr>
<td>79.5</td>
<td>13.9</td>
<td>0.570</td>
<td>1.209</td>
<td>1.204</td>
<td>(402)</td>
</tr>
</tbody>
</table>

The optical behavior recorded by VARIAN spectrophotometer (Cary 5000 Scan), Fig. (2) Shows the appearance of single peak validates the sample purity and shows a maximum absorption peak at 295 nm of green synthesized CeO$_2$ NPs. Primarily absorption peaks were formed due to the electronic transitions and excitation of electrons from valance band to conduction band between outer energy, The calculated Energy gap of CeO$_2$ NPs was 4.2 eV evaluated by following relation (17), From the obtained result, the band gap energy was blue shifted which happened by the quantum confinement effect. This effect may create by oxygen vacancies present in
the inter-granular areas. Typical UV absorption peaks were also seen in previously studies \(^{(18)}\).

![Absorption Spectrum of CeO\(_2\) nanoparticles](Image)

**Figure (2): Absorption Spectrum of CeO\(_2\) nanoparticles**

The biochemical results in tab. (2) presented a significant increase in blood glucose level (BGL) in group which treated by dexamethasone (G2) compared with control group (G1), these results similar to previous study \(^{(19)}\) they reported that dexamethasone cause tissue damage in pancreas which leads to decrease insulin secretion from Langerhans cells. After animals treating with CeO\(_2\) NPs for 30 days, there was a significant decrease (\(p\leq0.05\)) with blood glucose level for infected animals in G3 and G4 compared to G2. CeO\(_2\) nanoparticles had the ability to reduce BGL for many reasons such as delaying or inhibiting the absorption of glucose in the intestines \(^{(20)}\), Stimulate insulin secretion from pancreas cells \(^{(21)}\) or facilitate the entry of glucose into cells in fatty and muscular tissues \(^{(22)}\), G5 and G6 were found to be near the normal range of BGL.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGL (mg/dL)</td>
<td>113.2±6.4</td>
<td>395.2±29.7</td>
<td>131.7±6**</td>
<td>168.5±4.7**</td>
<td>107.5±3.9</td>
<td>118.7±4.6</td>
</tr>
<tr>
<td>ALT(IU/L)</td>
<td>35.7±1.6</td>
<td>66.2±4.7</td>
<td>41.5±1.3*</td>
<td>43.2±0.8*</td>
<td>40.7±2.7</td>
<td>46.7±4.2</td>
</tr>
<tr>
<td>AST(IU/L)</td>
<td>27.2±4.4</td>
<td>48.2±3.5</td>
<td>34±1.7*</td>
<td>38.5±4.4*</td>
<td>40.5±1.5*</td>
<td>43.5±0.6*</td>
</tr>
<tr>
<td>ALP(IU/L)</td>
<td>151±17.8</td>
<td>383.2±30.7</td>
<td>180.2±24.9*</td>
<td>206.7±22.3**</td>
<td>149.2±26.6</td>
<td>206.5±40.7</td>
</tr>
<tr>
<td>TB(mg/dL)</td>
<td>0.33±0.01</td>
<td>1.42±0.08</td>
<td>0.36±0.02***</td>
<td>0.47±0.13**</td>
<td>0.54±0.07</td>
<td>0.45±0.08</td>
</tr>
</tbody>
</table>

Also; the results showed there was a significant increase in liver functions parameters such as ALT, AST and ALP in experimental animals compared with control group (G1), liver enzymes indicate the extent of liver diseases \(^{(23)}\), this study indicates increases of liver enzymes levels possibly due to hepatic damage, increases the activity of liver enzymes in experimental animals may reveal possible leakage of these cytoplasmic enzymes into the blood stream across damaged plasma membrane or increase synthesis of these enzymes in the liver \(^{(24)}\). Results of this study showed that there was a significant decreases (\(P<0.05\)) of liver enzymes in treated animals (G3 and G4), the administration of CeO\(_2\) Nanoparticles may reduce dexa-induced hepatotoxicity in rats, the effective of CeO\(_2\) Nanoparticles as an antioxidant agent by inhibiting oxidative stress formation and Reactive Oxygen Spices (ROS) due to the large surface area to volume ratio that creates reactive sites to scavenge free radicals \(^{(25)}\), increase of Bilirubin levels may be attributed to the changes in cell membrane permeability with change of effective stress of the membrane under the influence of the oxidative stress, or may be explained by the occurrence of obstruction in the gallbladder ducts \(^{(26)}\), there was a significant decrease (\(P<0.05\)) in Bilirubin levels for the treated rats, (G5 and G6) had a normal liver enzymes and Bilirubin levels.

**Histological Examination**

Histological section in (Fig. 3a) shows pancreatic lobules containing acini (*), islets of Langerhans (**) and unstained connective tissue septa (***) for control
group. Fig. (3b and c) showed damage and necrosis of endocrine cells of islet (*), Langerhans with shrinkage of islet (**), degenerated acini cells and islet of Langerhans (***) and presented different alterations of rat’s pancreas and many histological changes were detected in pancreas section of (G2) that administrated by dexamethasone, such as necrosis and damage of endocrine cells of islet Langerhans with shrinkage of islets, Dexamethasone induced necrosis is mediated by reactive oxygen species mediated lipid peroxidation which causes bursting of plasma membrane of the cell and disturbance of osmotic balance this osmotic alteration ultimately leads the cell towards necrosis.

Also the histological section show different change in pancreas of rats that administrated with dexamethasone and CeO$_2$ Nanoparticles (G3 and G4) these change increased by decreasing the dose of Nanoparticles in which cells look like normal histological structure of pancreas with dispersed necrosis cells, the healing effect of Nanoparticles from Fig. (4 a and b) photomicrograph it is observed that the empty spaces in islets created by dexamethasone induced necrosis is significantly decreased and the section showed dispersed necrosis of endocrine cells of inside the islet of Langerhans with certain reduction the contour of islets and ballooning degenerate of cytoplasm of cells; this indicates that Nanoparticles stimulates the regeneration of the damaged islets, from Fig. (4c and d) can see that Nanoparticles kept normal shape of pancreatic cells and they look like normal.

**Figure (3): Cross section of pancreas (a) Control group, (b and c) Group 2 X400 (H&E),**

**Figure (4): Cross section of pancreas (a) G3 (b) G4 (c) G5 (d) G6 X400 (H&E).**

**Conclusions**

Synthesis of biocompatible Nanoparticles was obtained by applying a novel and simple green technique using new extract of green tea (*Camellia Sinensis*) to prepare Cerium Oxide nanoparticles. It had investigated successful formation of nanoparticles by examine its structural and optical properties, nanoparticles administration provided a significant reduction against BGL after dexamethasone injection, using nanoparticles reduced serum blood glucose levels, improved Lever enzymes and Bilirubin to confirm this results the histological staining of pancreatic section have performed.

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The Study of the Effect of Cognitive-Behavioral Therapy (CBT) on Reducing Methadone Consumption and Increasing Self-Esteem in Drug Addicts

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Abstract

The purpose of this study is to determine the effectiveness of cognitive behavioral therapy (CBT) in reducing methadone consumption and increasing self-belief in addicted to substance people. This study is in terms of the objective of applied research and from the developmental branch and in terms of nature and method it is a quasi_experimental research. The study population of this study includes all clients of methadone clinic of razi hospital in qaeemshahr. The sample consisted of 30 subjects selected through targeted sampling available were divided into control and experimental groups, who referred to methadone clinic at razi hospital in Qaem-shahr during the study period. Data collection tool was a standard and researcher-made questionnaire. Franken’s Methadone Consumption Reduction Questionnaire (2002) and Self-confidence researcher-made questionnaire, which reliability was calculated to be 0.94 and 0.74 respectively, using Cronbach’s Alpha. Spss22 software was used to analysis the research hypotheses and data from the questionnaire. The data analysis method has been descriptive and inferential statistics (Kolmogorov–Smirnov, Levine, T2 and covariance test). The result of this study has been shown that cognitive behavioral therapy (CBT) is effective in reducing the consumption of methadone addicts. Cognitive behavioral therapy (CBT) increases self-belief in addicted people.

Keywords: Cognitive behavioral therapy, methadone, self-belief

Introduction

Addiction is a complex disease characterized by characteristics such as compulsive behaviors, irresistible temptations, drug-seeking behaviors, and persistent consumption even when there are many negative consequences for the individual. Continued use of substances over time and long-term toxic effects of its consumption on brain function have led to a wide range of behavioral, psychological, social and physiological abuses that prevent the behavior and normal behavior of addicts in the family, the workplace and at a wider level (1).

In society most addicts often leave the drug during adolescence, but after a while, they turn back to addiction. Addressing the psychological problems of addicts including self-esteem, self-esteem, accountability, and social and family problems is a direct link to the abandonment of addiction. Three-quarters of those who complete the course of relapse have relapsed within one year of completing the treatment. Therefore, it is essential to develop programs for treatment, reduction and prevention of it (1). It should be noted that the provision of non-medical treatments, as well as lifestyle modifications and social support, are essential for comprehensive treatment and with sustainable outcomes. Clinics working in methadone-based therapies should also provide such support. The success rate of methadone depends on the number of supplements used in various clinics and ranges from 45% to 90% (2).
The Cognitive Behavioral Therapy (CBT) approach has received high experimental support among various interventions for substance abuse treatment. The Psychiatric Association (1995) with the Public Assessment Organization (1996), although the risks of addiction to amphetamines have only been recognized in recent years.

Given the need for a thorough examination of the deficiencies of methadone treatment, which is the main treatment method currently in our country and most countries in the world, and the provision of non-pharmacological treatment is absolutely felt. These treatments and training can individually or collectively provide for the improvement of many of these problems, and, in parallel, holding private counseling sessions for the treatment and treatment of personal problems can help complete the treatment process and improve the references of the indicators. (3)

Therefore, its treatment has been very important. One of the most effective therapeutic treatments over the past years and now is the maintenance treatment program with methadone and buprenorphine. Opiate users, the dominant drug therapy, is the consumption of methadone which is currently in use in most countries of the world, and the results of studies have shown that this drug is a suitable alternative for opioids and has a beneficial effect on reducing drug intake, craving, Leak symptoms, psychiatric symptoms and dangerous behaviors of these patients during the course of treatment (4,5,6,7,8,9). The purpose of this study was to investigate the effect of cognitive-behavioral therapy (CBT) on reducing methadone consumption and increase self-esteem among addicted people.

Method

Study population, sample, measurement method

This is a descriptive study performed by collecting data on all of the clients of methadone clinic at Razi hospital in Qaem-Shahr. The sample consisted of 30 patients who referred to the methadone clinic of Razi Hospital in Qaem-Shahr during the study period. The subjects were selected through targeted sampling (15 subjects and 15 controls) and assigned to control and experimental groups.

(A) Franken’s Methadone Consumption Reduction Questionnaire (2002)

This questionnaire has 14 questions and Likert 5 options. Also, this questionnaire was designed to reduce the consumption of methadone as a motivational state by Franken (2002) and measuring the reduction of methadone consumption at the moment.

That includes 3 factors (desire and intention to use drugs, desire for consumption and negative reinforcement, pleasure and severity of lack of control). The original form of the questionnaire was designed by Lav in 1998 to measure decrease alcohol consumption, and in 2002, Francken, by adopting the alcohol consumption reduction questionnaire, has written the heroin intake reduction questionnaire. In the Iranian society this questionnaire has been used about reducing the consumption of Methamphetamine. Reliability of the components of this questionnaire in Makry, Ekhthari, Hasani Abharian and Ganjgahi (2010) survey in Consumer of the various types of opioids, including crack and heroin, are 0.89, 0.79, 0.4 respectively, and in metamphetamines Consumers was 0.25, 0.65, and 0.81 respectively.

B) Self-esteem increase questionnaire

Self-esteem increase questionnaire consists of 50 items that are used to measure self-esteem. The grading for the questionnaire is 4-point Likert spectrum, which is considered for “never,” “rarely,” “sometimes,” and “almost always,” points 1, 2, 3, and 4, respectively. The content validity of the questionnaire was confirmed in the research by Farzianpour et al. The reliability of the questionnaire with Cronbach’s alpha was more than 85%. Due to the standardization of the research questionnaires, their reliability in previous research has been proven repeatedly, but in order to ensure their reliability, 30 questionnaires before the final implementation were randomly assigned and implemented. The reliability of the questionnaires using the Cronbach’s alpha coefficient to reduce methadone consumption was 94 / 0 and for self-confidence increase was calculated 0.74.

In order to ensure the reliability of the content and formality of validity, the questionnaire was examined by several experts and supervisors and counselors and They all emphasized the validity of the questionnaires for the research.

The summary of the content of the therapeutic sessions:
First Session: introducing, Group Principles and Structure of Meetings, Motives, Objectives, and Amount of Obligations

Second session: The course of recovery and treatment, non-medical education, the problem of distrust

Third Session: Costs and benefits of consumption and Discontinue Usage, complete purity

Fourth Session: Reducing Energy, Temptation, and Starters

Fifth Session: Situations and external starters, internal factors and triggers

Sixth Session: Understanding auto Thought, Generating Negative Emotions (Depression, Anxiety, Aggression, Paranoid Thoughts, Phobia), shallow Beliefs of Negative Errors

Seventh Session: Control of Auto Thoughts, Technique A-B-C

Eighth Session: Logical Cognitive Errors, Appropriate Alternative Logical Thoughts

Ninth session: ways to change shallow thinking, to oppose with changing logic, to oppose with stimulus sentences

Tenth Session: Temptations and related beliefs, wrong ways of dealing with temptation, dealing with temptation.

Eleventh Session: Thoughts, emotions, and underlying behaviors associated with consumption, boredom, shame and sin, staying or job.

Twelfth Session: Relapse-prone activities, preventative relapses, prevention

Data analysis

Data analysis was done by descriptive and inferential methods through SPSS software. In this research, descriptive statistics have been used to calculate the mean and standard deviation of the research variables and to show the frequency and its related graphs. Inferential statistics are used to examine the research hypotheses. Before testing the hypotheses, the assumption of the normal distribution of data was done by Kolmogorov-Smirnov test. Then the homogeneity assumption of variances was assessed through Levin’s test in all variables of the two groups. To report the results, the significance of the statistical tests and the size of the independent variable was used. To analyze the inferential statistics, covariance analysis was used.

Result

According to demographic findings, 50% of the subjects formed the experimental group and 50% of the control group. Out of all patients, 63% of them were married. using the Kolmogorov-Smirnov test, we measured the normal variables.

According to the normal test table information (Table 1), the value of $p$-value in any of the variables is not significant. This indicates the normality of the research variables, which is one of the preconditions for using the covariance analysis test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$z$</th>
<th>fault</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pretest stage methadone consumption reduction</td>
<td>598/0</td>
<td>0.05</td>
<td>867/0</td>
</tr>
<tr>
<td>post-test methadone consumption reduction</td>
<td>764/0</td>
<td>0.05</td>
<td>604/0</td>
</tr>
<tr>
<td>pretest stage self-esteem increase</td>
<td>523/0</td>
<td>0.05</td>
<td>947/0</td>
</tr>
<tr>
<td>post-test self-esteem increase</td>
<td>637/0</td>
<td>0.05</td>
<td>811/0</td>
</tr>
</tbody>
</table>

Considering that in the Levine test, the $p$-value is greater than 0.05. We can conclude that, the assumption of the equality of the variances of the two groups is confirmed, which is another test precondition for the analysis of covariance. The Levine test table is also shown in table 2.
However, in the post-test, the difference between the two groups is significant and, in fact, according to the mean value, the self-confidence of the experimental group has increased. Evaluation of self-esteem score in pre-test and post-test, has been shown in figure 2.

According to the level of \( F = 39 \), and considering that \( p \)-value is less than 0.05, with a confidence level of 95%, the hypothesis of the study is confirmed and it is concluded that the treatment of cognitive-behavioral therapy (CBT) in reducing consumption in methadone addicts is effective. Regarding the average score of drug use in the post-test (Figure 1), we find that this is a positive effect, with the intensity of its effect being very high at 0.847. In fact, cognitive-behavioral therapy (CBT) is effective in reducing the consumption of methadone addicts.

Table 2. Levine test to verify the equality of variances

<table>
<thead>
<tr>
<th>Variable</th>
<th>f</th>
<th>fault</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>methadone consumption reduction</td>
<td>786/0</td>
<td>0.05</td>
<td>383/0</td>
</tr>
<tr>
<td>self-esteem</td>
<td>005/0</td>
<td>0.05</td>
<td>942/0</td>
</tr>
</tbody>
</table>

In the pretest stage, there is no significant difference between the mean score of substance use in both control and experimental groups (The value of \( p \)-value is greater than 0.05). We conclude that the subjects were divided into two groups of control and experiment equally. However, in the post-test, the difference between the two groups was significant and the test was significant, and in fact, according to the mean value, thoughts of substance use in the experimental group decreased. Evaluation on reducing drug intake in the pre-test and post-test phases, has been shown in figure 1.

Figure 1. Evaluation on reducing drug intake in the pre-test and post-test phases

In the pretest stage, there was no significant difference between the mean self-esteem score in the control and experimental groups (\( p \)-value greater than 0.05). We conclude that the subjects were divided into two groups of control and experiment equally.

Table 3. One-way covariance analysis of hypothesis 1

<table>
<thead>
<tr>
<th>Change</th>
<th>Squared</th>
<th>degree of freedom</th>
<th>mean squared</th>
<th>Fisher-modified</th>
<th>p-value</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed coefficient</td>
<td>731/0</td>
<td>2</td>
<td>366/0</td>
<td>81</td>
<td>000/0</td>
<td></td>
</tr>
<tr>
<td>Group effect</td>
<td>004/0</td>
<td>1</td>
<td>004/0</td>
<td>881/0</td>
<td>356/0</td>
<td></td>
</tr>
<tr>
<td>Pre-test score effect</td>
<td>580/0</td>
<td>1</td>
<td>580/0</td>
<td>128</td>
<td>000/0</td>
<td></td>
</tr>
<tr>
<td>Interaction of the pre-test score and the group</td>
<td>176/0</td>
<td>1</td>
<td>176/0</td>
<td>02/39</td>
<td>000/0</td>
<td>847/0</td>
</tr>
<tr>
<td>Error</td>
<td>122/0</td>
<td>27</td>
<td>005/0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>382/0</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Considering the level of $F = 111$ and considering that the $p$-value is less than 0.05, with a confidence level of 95%, the hypothesis of the study is confirmed and it is concluded that training anger management skills on reducing the amount of aggression Prisoners are effective. Considering the average amount of aggression of the subjects in the post-test stage (figure 2), we find that this is a positive effect, with the intensity of its effect being very high with respect to the 924/0. In fact, cognitive-behavioral therapy (CBT) is effective in increasing self-esteem among addicts.

### Table 4. One-way covariance analysis of hypothesis 2

<table>
<thead>
<tr>
<th>Change</th>
<th>Squared</th>
<th>degree of freedom</th>
<th>mean squared</th>
<th>Fisher-modified</th>
<th>p-value</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed coefficient</td>
<td>856/0</td>
<td>2</td>
<td>428/0</td>
<td>69/62</td>
<td>000/0</td>
<td></td>
</tr>
<tr>
<td>Group effect</td>
<td>139/0</td>
<td>1</td>
<td>139/0</td>
<td>40/20</td>
<td>000/0</td>
<td></td>
</tr>
<tr>
<td>Pre-test score effect</td>
<td>558/0</td>
<td>1</td>
<td>558/0</td>
<td>71/81</td>
<td>000/0</td>
<td></td>
</tr>
<tr>
<td>Interaction of the pre-test score and the group</td>
<td>347/0</td>
<td>1</td>
<td>347/0</td>
<td>88/50</td>
<td>000/0</td>
<td>810/0</td>
</tr>
<tr>
<td>Error</td>
<td>184/0</td>
<td>27</td>
<td>007/0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>163</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Hypothesis 1: Cognitive Behavioral Therapy (CBT) is effective in reducing the consumption of methadone addicts.

The results of the first hypothesis show that cognitive behavioral therapy (CBT) is effective in reducing the consumption of methadone addicts. This result is consistent with the results of research by Yaghoobi Nasrabadi (3), Keramaty (5).

In explaining this hypothesis, it can be said that in cognitive-behavioral therapy, the therapist and the patient provide a functional analysis for each case of drug intake. It identifies the thoughts, feelings and conditions of the patient before and after drug intake.

In the early stages of treatment, functional analysis helps the patient to identify determinants or risk situations that increase the likelihood of drug intake and reasons of drug intake.

This method of treatment will reduce the intake of drug in addicts, the desire to methadone and the pleasure of using methadone\(^{(10,11,12)}\).

**Hypothesis 2: Cognitive Behavioral Therapy (CBT) is effective in increasing self-esteem in addicts.**

The results of the second hypothesis show that cognitive behavioral therapy (CBT) increases self-confidence in addicted people. This result is consistent with the results of research by Jacob Nasrabadi (3), Keramati (5). In explaining this hypothesis, it can be said that cognitive-behavioral therapy is a kind of individual education program that helps abusers to abandon the old habits associated with drug abuse and to learn skills to develop healthier habits.

When the level of drug intake reaches a degree of severity that the person has to be treated. Probably the person used drugs as a way to confront a wide range of interpersonal and individual problems. Addicts usually have little self-confidence. The training of coping skills is the main core of cognitive-behavioral therapy. The
purpose of this training is helping patients to identify the most dangerous situations like high risk of drug intake and to obtain effective coping strategies to deal with those situations. The behavioral therapy helps addicted people not to be afraid of expressing thoughts and opinions and reaching their goals (13,14,15).

**Research constraints**

Obviously, the removal of research constraints will be the basis of further research and this will improve the science and research. The present research showed

A) Restrictions under the control of the researcher

1- The scope of research implementation was Qaemshahr.

2- This research was conducted in 95-94

3- This research was conducted among all patients referring to methadone clinic of Razi Hospital in Qaemshahr.

4- The tool used in this research was a questionnaire.

5- The subject of this research is the effect of cognitive-behavioral therapy (CBT) on reducing methadone consumption and self-esteem in drug addicts.

B) Restrictions beyond the control of the researcher

1. Unwillingness of some persons to answer related questions.

2. A small number of subjects

3. Lack of research done similar to the current research.

4. having problems in coordinating with addicts.

5. Subjects’ perceptual difference from the questions of questionnaire.

Future researchers are encouraged to do this research more widely in other cities. It is suggested to other researchers to investigate the role of cognitive-behavioral therapy in self-confidence and self-esteem of addicts in future research.

**Ethical Clearance:** Taken from ethics committee of Mazandaran University of Medical Sciences, Sari, Iran

**Source of Funding:** Mazandaran University of Medical Sciences, Sari, Iran

**Conflict of Interest:** None

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Study Effect of Clarithromycin drug on Cryptosporidium Parvum and Efficiency of ELISA Technique in Diagnosis Comparison with Some Traditional Methods \textit{in vitro}

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Abstract

This research aimed to study effect of Clarithromycin drug on \textit{Cryptosporidium Parvum} and efficiency of ELISA technique in diagnosis comparison with some traditional methods \textit{in vitro}. Samples collected from four different sources water were 400 liters: 100 liters tap water provider by Al-Kufa District River, 100 liters house tank water, 100 liters from sea of Al-Najaf and 100 liters stagnant water during period from October 2018 to April 2019. Examined samples by three laboratory methods: Modified Ziehl-Neelson stain, Flotation by saturated sugar solution method and ELISA to detecting from \textit{Cryptosporidium} oocysts in the water as used Clarithromycin drug effective against \textit{C.parvum} \textit{in vitro}. Capability test was implement impregnation isolated and determination cells of parasite counted when at 37\(^\circ\)C/ 48h incubated. Differ concentrations from Clarithromycin were 4, 8, 16 and 32 mg/L respectively compared with control group.

Results of the current study showed that oocysts were found in 182 from 400 liters 45.5\% as showed that height oocysts in stagnant water then followed Sea of Al-Najaf were 55\% and 45\% respectively, height percentage of oocysts were in April and March were 69.09\% and 66\% while decrease percentage of infection in October was 25\%. As showed study results presence significant differences in efficiency of ELISA technique was 83.33\% at detection from oocysts compared with Flotation by saturated sugar solution and Modified Ziehl-Neelson stain methods were 66.66\% and 61.11\% respectively. Shown results this study that Clarithromycin with 16 and 32 mg/L lead to reduction in number oocysts was 17.3±3.5 and 15.4±3.9, sporozoites counts was 17.1±3.4 and 14.9±4.0 compared with control group was 20.5±2.8. As observed significance differences \textit{P}<0.05 in the mean numbers the oocysts and sporozoites in three replicate plates after 48 h. These results proved that Clarithromycin have effectiveness in inhibition \textit{C.parvum} \textit{in vitro}.

Keywords: Cryptosporidiosis, Water, Oocysts, Sporozoites, Clarithromycin, \textit{in vitro}.

Introduction

Cryptosporidiosis is protozoan disease occurred by \textit{Cryptosporidium} \textit{spp} from phylum Apicomplexa is unicellular organisms include number of pathogenic infect human and mammals by food and contaminated water \textsuperscript{(1)}. Oocysts transmitted in arduous environment where not killed by conventional disinfectants and chlorination which causes diarrhea, massive dehydration, malnutrition and weight loss lead to abomasa cryptosporidiosis \textsuperscript{(2)}. Oocysts resisted tough environmental condition for six months where can stay alive for twelve weeks at 10\(^\circ\)C \textsuperscript{(3)}. Transmission riot by the fecal oral and involve drinking water, recreational water, person to person, animals to person and assist of sexual pursuit may locomotion through man with HIV \textsuperscript{(4)}.

\textit{C.parvum} infections diagnosed through knowledge number the infestation and non-inverting methods. The non-inverting methods, microscopic test more common \textsuperscript{(5)} or identification of these parasite is based on morphologic examination by using modified acid-fast staining method \textsuperscript{(6)}, important alternate the expansion of antigen the parasite (ELISA) showed these assay sensitive comparative with microscope examine, this lead to not request the observed of hale parasite \textsuperscript{(7)}.

Recently, observed there increase in number of infection with \textit{C.parvum} correlating with increase in number of immune-compromised patients and resistance against drugs, this disease currently endemic in 88 countries world and showed diffusion increase in immunosuppressive conditions such as HIV/AIDS \textsuperscript{(8)}. Clarithromycin known as
being semisynthetic antibiotic from macrolide derivative family (9). Also, good distribution perfect action against of parasites e.g.: *Toxoplasma gondii* (10) *Leishmania donovani* (11) and *Cryptosporidium parvum* (12) *invitro* as featuring effective, less side impacts, cheap and easy management. Clarithromycin, Azithromycin and Roxithromycin are active in inhibition *C.parvum* evolution in concentrations near those are achievable (13). The aim this research is study effect of Clarithromycin drug on oocysts and sporozoites *in vitro* and efficiency of ELISA technique in diagnosis comparison with some traditional methods.

**Materials and Method**

Water samples are collected from October 2018 to April 2019 period of Al-Najaf governate. A total amount was 400 liters: 100 liters of tap water supplies by Al-Kufa District River, 100 liters of house tank water, 100 liters sea of Al-Najaf and 100 liters of stagnant water, put in sterile bottles which delivered to the laboratory of pharmacognosy and medicinal plants in pharmacy college/ Kufa university.

**Diagnosis parasite:** 5 liters from water samples distributed in sterile test tubes placed in the centrifuged 1000g/10-15 min to get on sediment, taken 5 ml of sediment and added 15 ml of distilled water then aspirate during six layers of gauze for get rid of the suspended waste then centrifugation procedure was performed again 1000g/10 min. 10 ml add of saturated sugars solution to the sediment well mix (14). Taken drop from the upper layer of the tube by Pasteur pipette placed on glass clean slide left to dry in the air for 10 min, smear fixed by absolute methyl alcohol for 5 min and left to dry, added modified ziehl-neelson stain to the fixed smear and heating for 5 min by Bunsen burner until vapor appears and washed with tap water, the slide is immersed in 10% HCL for 10-15 sec and washed again with water then dye was stained with methylene blue for 2 min, wash the well slide with running water then air-dried, examined by microscope 40X and 100X magnification to investigate oocysts by several standard e.g form, size, color stain and surface merit (15).

**ELISA technique:** Used in these technique particular oocysts antibodies where placed these antibodies in the pits for the plate ELISA method and these antibodies have ability to interact with oocysts find in samples then plate incubated for 1 h at 21°C, wash the plate by washing solution and add the conjugation solution containing antibodies to the parasite associated with the peroxidase enzyme then incubate and wash the plate again. Add chromogen tetramethylbenzidine is solution of the reactive substance which contained bottle inside size 25 ml with ELISA diagnostic components, if the water container on oocysts, conjugation solution remains linked with the pit and the enzyme converts chromogen from colorless to blue color composite where strength this color is proportional with number of oocysts in the examined sample, stopped the enzyme reaction after addition of phosphoric acid solution then measured optical density at the wavelength of 450 nm using optical spectrum (16).

**Experiment study *invitro***: Oocysts isolated from the water by ELISA method was used throughout this study. Clarithromycin (Abbott, Ital.) dissolves in 50% Acetone and 50% Methanol to obtain 1 mg/ml of stock solution for perpetrated different concentrations where stored at −80°C in the dark. In these experiment used the methanol and acetone in alleviation of the drug not discouraged of *C. parvum* grows.

**Oocysts Preparation:** Occur by suspending portion of stock solution contain 9 portions sterile water and 1 portion 0.55% sodium hypochlorite then put in centrifuged and re-suspended during Dulbecco’s Modified Eagles Medium (DMEM) (Bio-Whittaker) then brood in Phosphate Buffered Saline (PBS) (Bio-Whittaker) contain 2000 U/mL of Penicillin G, 10 mg/L Amphotericin-B and 2000 mg/L Streptomycin busted when 37°C. Sporozoite excyst happened through oocyst incubated in buffer contain 0.75% Sodium Hypochlorite 0.25% Trypsin (Sigma-Aldrich.) at 37°C/60 min. Sporozoite liberated filtering by centrifuge 500g/10 min, re-suspended in (DMEM), counted in haemocytometer for culture (17).

**Cell Culture:** Isolated 500 cells from human stomach carcinoma preserve 25cm² tissue culture flacon. Put (DMEM) to medium with 10% Fetal bovine serum (Bio-Whittaker) in 4% L-Glutamine (Bio-Whittaker.), 1% non-essential amino acid, 100 mg/l Streptomycin and 0.5 mg/l Amphotericin-B. Lift cells from surface of the flacon by used 0.53 mM (EDTA) and 0.25% Trypsin in the (PBS). Coated 500 cells onto tissue culture glazy have 35mm in diameter of 10⁵ concentration grow able cells 5 ml overall size (18).

**Cells damage with *C. parvum***: Started by add 10⁵ sporozoite with 0.2 ml of the medium to happen connect and breakthrough of sporozoites incubate at 37°C/4 h with 5% CO2. Expel no inverting sporozoites by monolayer wash in (DMEM) also to oocysts less and epithelial cells no clinging. Cells infected are reservation in 5% CO2 during this of study (19).

**Antibiotics agent:** Clarithromycin concentrations prepared were 4, 8, 16 and 32 mg/L respectively, in
Triplicate of monolayers incubate at 37°C /48 h with 5% CO2 then washed four times in phosphate buffered saline to edit oocysts and prevent epithelial cells adhesion, well use 75% methanol for installation and Giemsa stained to estimate existence of sporozoites inwards cells to vision both intra and extracellular oocysts. Calculated the of parasite number by mean ± standard deviation are number mean of oocysts and sporozoites perceive in monolayers three for every to isolates ten to same concentration roofless of treatment, 40 field examine by 1000X magnification (20).

**Statistical analysis:** In these study association between presences of oocysts with various source of water depending to months this study and efficiency of the diagnostic methods used during the experiment by (P<0.05) (21).

**Results**

Table (1): Percentage of Infection with *Cryptosporidium* oocysts to different water sources in the Study.

<table>
<thead>
<tr>
<th>Source of water</th>
<th>No. of samples examined (liter)</th>
<th>NO. of Samples (+ve)</th>
<th>Percentage of Infection (+ve) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water provided by Al-Kufa river</td>
<td>100</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Tank / houses</td>
<td>100</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Sea of Al-Najaf</td>
<td>100</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Stagnant water</td>
<td>100</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>182</td>
<td>45.5</td>
</tr>
</tbody>
</table>

Table (2): Percentage of Infection with *Cryptosporidium* oocysts according to months the Study from October 2018 to April 2019

<table>
<thead>
<tr>
<th>Months</th>
<th>No. of samples examined</th>
<th>NO. of Samples (+ve)</th>
<th>Percentage of Infection (+ve) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>60</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>November</td>
<td>50</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>December</td>
<td>60</td>
<td>25</td>
<td>41.66</td>
</tr>
<tr>
<td>January</td>
<td>65</td>
<td>30</td>
<td>46.15</td>
</tr>
<tr>
<td>February</td>
<td>60</td>
<td>22</td>
<td>36.66</td>
</tr>
<tr>
<td>March</td>
<td>50</td>
<td>33</td>
<td>66</td>
</tr>
<tr>
<td>April</td>
<td>55</td>
<td>38</td>
<td>69.09</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>182</td>
<td>45.5</td>
</tr>
</tbody>
</table>

Table (3): Comparison between efficiency of ELISA technique in diagnosis with traditional methods

<table>
<thead>
<tr>
<th>Diagnosis methods</th>
<th>No. of samples examined</th>
<th>NO. of Samples (+ve)</th>
<th>Percentage (+ve) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flotation by Saturated sugar solution</td>
<td>90</td>
<td>55</td>
<td>61.11</td>
</tr>
<tr>
<td>Modified Ziehl-Neelson stain</td>
<td>90</td>
<td>60</td>
<td>66.66</td>
</tr>
<tr>
<td>ELISA</td>
<td>90</td>
<td>75</td>
<td>83.33</td>
</tr>
</tbody>
</table>
Table (4): Effect of clarithromycin drug on number of *Cryptosporidium* oocysts and sporozoites invitro.

<table>
<thead>
<tr>
<th>Mean number of C. parvum per 40 microscope fields with Clarithromycin</th>
<th>Concentration (mg/ml)</th>
<th>Samples examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>20.5±2.8</td>
<td>20.5±2.8</td>
</tr>
<tr>
<td>Oocysts</td>
<td>19.8±3.1</td>
<td>19.5±3.3</td>
</tr>
<tr>
<td>Sporozoites</td>
<td>20.4±2.8</td>
<td>19.7±3.1</td>
</tr>
</tbody>
</table>

**Discussion**

*Cryptosporidiosis* is great diarrheal disease happens by protozoan with health problem and poorly understood and many livestock farming industries as water consider of sources for zoonotic infections.

In the present study found oocysts 182 of 400 L from total samples 45.5% are rate varies depending to water exporter significant variation. Oocysts found about 40% in tap water provides by Al-Kufa river, as found 42% in tank houses water while oocysts appear 45%, 55% in sea of Al-Najaf and stagnant water samples respectively, this study agrees with (22) showed infection rate in the children at Al-Najaf city was 13.6% causes contamination drinking water contained on oocysts (23). As in Table1

As showed in the relation between oocysts presences and season appeared significant differences was recorded upper infection in April and March were 69.09% and 66% while decrease percentage of infection in October was 25%, this study agrees with (24) where recorded highest percentage in the spring and lowest winter, in Iraq which attributed to appropriate climatic conditions help on survival of oocysts in the environment and increased consumption of water contaminated. As in Table2

Results of the study current existence significant differences in efficiency of ELISA technique was 83.33% at detection from *Cryptosporidium* oocysts compared with Flotation by saturated sugar solution and Modified Ziehl-Neelson stain methods were 66.66% and 61.11% respectively, which may lead to examination of large number of samples, accuracy in reading results and shortening the duration of the diagnosis, this study agree with (25). As in Table3

Clarithromycin showed potent anthelmintic activity in vitro, mechanical through penetrate to larger number of the metacestode tissues in vitro, via metacestode fragment into small portions (20). In current study showed that clarithromycin when 16 and 32 mg/L lead to number decrease of oocysts was 17.3±3.5 and 15.4±3.9, sporozoites counts was 17.1±3.4 and 14.9±4.0 compared with positive control group was 20.5±2.8 respectively, lead to significance variation to mean of number the sporozoites and oocysts in three replicate plates after 48 h. As in Table4

Clarithromycin mechanism act number decline of parasite attributed to ability protein synthesis frustration by binding with transpeptidation sites for ribosomal subunits is target as *C.parvum* affect by protein synthesis frustration (26).

There are research showed that this drug is effective against protozoa such as *T. gondii*, *Cryptosporodium spp* (27) and *L. Major* in vitro action promastigotes mechanism effects through adverse binding to 50S ribosomal subunits and frustration the protein synthesis (28).

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Study of Tuberculosis Related Topics in Baghdad, Iraq 2012-2016

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Abstract

Objective(s): The present study designed to explore topics related to Tuberculosis, such as new smear positive cases, causes, treatment and new pulmonary positive cases in Baghdad City, Iraq for 2012-2016.

Method: A descriptive “retrospective” design was performed throughout the present study from the period of November 12th 2017 to February 13th 2018. All registered tuberculosis patients in Baghdad, Iraq for the period of 2012-2016. An instrument was constructed for the purpose of the study. Data were collected from the health records at the National Tuberculosis Center, State TB center, and district TB center for the period of 2012 to 2016 with the use of the study instrument. Data were analyzed through the application of descriptive statistical data analysis approach of frequency, percent, incidence rate and ratio.

Results: The study findings depicted that most of the new smear positive cases are accounted for the age group of (15-24) year old during the years of 2012-2016. Causes of Tuberculosis in the rural area were accounted for poverty (39.95%), smoking (22.39%), untreated cases (12.4%), alcoholism (11.32%), malnutrition (10.30%) and overcrowded (3.57%) respectively, and accounted for alcoholism (27.77%), overcrowded (27.16%), malnutrition (24.69%), smoking (11.93%), poverty (7.62%) and untreated cases of Tuberculosis (0.80%) respectively in the urban area.

Conclusion: The study concluded that Most of the new smear positive cases are accounted for the age group of (15-24) year old during the years of 2012-2016. Patients, in 2014, had benefited out of the Tuberculosis Program more than others based on the success rate.

Key Word: Study, Topics, Tuberculosis, Baghdad City, Iraq

Introduction

Roughly one-third of the world’s population has been infected with M. tuberculosis, and new infections occur at a rate of one per second (¹). However, not all infections with M. tuberculosis cause tuberculosis disease and many infections are asymptomatic (²). In 2007 there were an estimated 13.7 million chronic active cases, and in 2010 there were 8.8 million new cases, and 1.45 million deaths, mostly in developing countries (³,⁴). 0.35 million of these deaths occur in those co-infected with HIV. In 2015, across the world 1.8 million out of 10.4 million people affected by the disease died (⁵,⁶).

Any person who coughs and who was in contact with smear positive index case (smear positive pulmonary TB patient) should have three sputum examinations. Children aged less than 5 years: any contact aged less than 5 years who has a positive tuberculin that not previously vaccinated with BCG with signs or symptoms of TB should be treated as suffering from active TB. Those without signs or symptoms of disease should be given preventive chemotherapy (INH for 6 months) Children under one year of age with mothers who are being treated for smear positive pulmonary TB should be given Isoniazid if the tuberculin test is negative at the end of three months, INH may be stopped and BCG may be given (⁷,⁸,⁹).

Drug Resistance Tuberculosis is a man -made disease (due to non - compliance, improper drug regimen, etc.). Primary resistance is prevented by giving
the patient combination of drugs. Secondary (acquired) TB resistance is expected to be developed in (10-12):

1. A large bacillary population such as patient with cavitations.

2. Inadequate drug regimens (inappropriate drugs, insufficient dosage), drug side effects and complications.

3. Treatment of DR TB should be done by or in close consultation with an expert in the management of these cases and on hospitalization bases.

4. A single new drug should never be added to a failing regimen.

5. Treatment duration for DR TB patient may last 18 -24 months by using 4 -6 drugs (capriomycin, cyclocerin, ethionamide, levofoxacine, and PAS).

6. Second line regimens often represent the patient’s last hope for being cured inappropriate management can thus have life threatening sequences.

Based on the early stated facts, the present study ought to carry out a retrospective study to investigate related issues to detected cases of tuberculosis in Baghdad, Iraq for 2012-2016.

Method

A descriptive “retrospective” design was employed throughout the present study from the period of November 12th 2017 to February 13th 2018 in order to investigate related topics to Tuberculosis, such as new smear positive cases, causes, treatment and new pulmonary positive cases in Baghdad City, Iraq for 2012-2016. A convenient sample of (11680) registered patients with tuberculosis in Baghdad, Iraq for the period of 2012-2016. These patients were males and females and they were one year to over than 65 year of age.

An instrument was constructed for the purpose of the study. It was comprised of items that focused on patients’ characteristics of age, gender and type of Tuberculosis. A pilot study was conducted for the determination of the study instrument’s content validity, internal consistency reliability and adequacy. The study was carried out for the period of December 10th -20th 2017. Content validity of the instrument was determined through panel of (15) experts. These experts were (5) faculty members at the College of Nursing University of Baghdad, (5) Faculty members at the College of Medicine University of Baghdad and (5) epidemiologists at the Ministry of Health and Environment. They were presented with copy of the study instrument and asked to review it and provide comments for its modification to be more appropriate measure of the study. They had reviewed the instrument and presented their comments with an agreement that the instrument is content valid. Internal consistency reliability was determined for the study instrument through the use of split-half technique and measurement of Cronbach alpha correlation coefficient. The results indicated that Cronbach alpha correlation coefficient was r=0.85 which adequately reliable measure for the problem underlying the present study.

Data were collected from the health records at the National Tuberculosis Center, State TB center, and district TB center for the period of 2012 to 2016 with the use of the study instrument. Data were analyzed through the application of descriptive statistical data analysis approach of frequency, percent, incidence rate and ratio.

Results

Results out of table (1) indicate that most of the new smear positive cases are accounted for the age group of (15-24) year old during the years of 2012-2016.

Table (2) depicts that causes of Tuberculosis in the rural area are accounted for poverty (39.95%), smoking (22.39%), untreated cases (12.4%), alcoholism (11.32%), malnutrition (10.30%) and overcrowded (3.57%) respectively, and accounted for alcoholism (27.77%), overcrowded (27.16%), malnutrition (24.69%), smoking (11.93%), poverty (7.62%) and untreated cases of Tuberculosis (0.80%) respectively in the urban area. So, poverty and smoking are considered the most effective causes for patients in the rural area. In contrast, alcoholism, overcrowded and malnutrition are considered as the most effective causes for patients in the urban area.

Results of treatment out of table (3) reveal that success rate are (69%) in 2014, (61%) in 2013 and (51%) in 2012 respectively. Such rate can present evidence that patients in 2014 have benefited out of the program more than others.

Table (4) shows that age specific incidence numbers, percentage and incidence rate by gender. The percentage and the numbers in age group 15-24 years old was the highest and declined thereafter while age was increasing
for both male and female. But the incidence cases in age group 0-14 in both male and female were almost the same.

Though the incidence rate of both male and female cases the same in the age group 0-14 years old and difference was much higher as age groups increased from 15-24, 25-34, 35-44, and the highest was found among the age group 55-64 years old while the rapid decreasing was found in the age group 65+ years old.

**Conclusion**

The study concluded that Most of the new smear positive cases are accounted for the age group of (15-24) year old during the years of 2012-2016. The urban zone’s incidence rate was greater than that of the rural zone. Poverty and smoking were considered the most effective causes for patients in the rural area. In contrast, alcoholism, overcrowded and malnutrition were considered as the most effective causes for patients in the urban area. Patients in 2014 had benefited out of the Tuberculosis Program more than others based on the success rate. The incidence rate of both male and female cases the same in the age group of (0-14) years old and difference was much higher as age groups increased from (15-24), (25-34), (35-44), and the highest was found among the age group (55-64) years old while the rapid decreasing was found in the age group 65+ years old.

**Table (1). Percentage of New Smear Positive Cases in Different Age Groups (2012-2016)**

<table>
<thead>
<tr>
<th>Age Group (years)</th>
<th>Number of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>102</td>
<td>2.5</td>
</tr>
<tr>
<td>15-24</td>
<td>1025</td>
<td>24.9</td>
</tr>
<tr>
<td>25-34</td>
<td>815</td>
<td>19.8</td>
</tr>
<tr>
<td>35-44</td>
<td>676</td>
<td>16.5</td>
</tr>
<tr>
<td>45-54</td>
<td>642</td>
<td>15.6</td>
</tr>
<tr>
<td>55-64</td>
<td>479</td>
<td>11.6</td>
</tr>
<tr>
<td>65+</td>
<td>366</td>
<td>8.9</td>
</tr>
</tbody>
</table>

**Table (2). Causes of Tuberculosis in Urban and Rural Areas**

<table>
<thead>
<tr>
<th>Causes</th>
<th>Rural</th>
<th>Urban</th>
<th>Percentage</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poverty</td>
<td>380</td>
<td>818</td>
<td>39.95%</td>
<td>7.62%</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>108</td>
<td>2980</td>
<td>11.35%</td>
<td>27.77%</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>98</td>
<td>2650</td>
<td>10.30%</td>
<td>24.69%</td>
</tr>
<tr>
<td>Smoking</td>
<td>213</td>
<td>1280</td>
<td>22.39%</td>
<td>11.93%</td>
</tr>
<tr>
<td>Overcrowded</td>
<td>34</td>
<td>2915</td>
<td>3.57%</td>
<td>27.16%</td>
</tr>
<tr>
<td>Untreated cases of Tuberculosis</td>
<td>118</td>
<td>86</td>
<td>12.40%</td>
<td>0.80%</td>
</tr>
<tr>
<td>Total</td>
<td>951</td>
<td>10729</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (3). Results of Treatment of Drug Resistance Cases Detected among Tuberculosis Patients during (2012-2016)

<table>
<thead>
<tr>
<th>Years</th>
<th>Total Enrolled</th>
<th>Cure</th>
<th>Completed Treatment</th>
<th>Defaulted</th>
<th>Died</th>
<th>Failure</th>
<th>Treatment Extended</th>
<th>NA</th>
<th>Success Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>114</td>
<td>47</td>
<td>11</td>
<td>26</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>51%</td>
</tr>
<tr>
<td>2013</td>
<td>79</td>
<td>38</td>
<td>10</td>
<td>21</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>61%</td>
</tr>
<tr>
<td>2014</td>
<td>55</td>
<td>12</td>
<td>3</td>
<td>16</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>69%</td>
</tr>
<tr>
<td>2015</td>
<td>58</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>47</td>
<td>…….</td>
</tr>
<tr>
<td>2016</td>
<td>63</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>56</td>
<td>…….</td>
</tr>
</tbody>
</table>

Still Positive after 24 months of treatment (and extended is extended) Not applicable, Result should appear after following two years

Table (4). New Pulmonary Positive Cases by Age Groups and Gender in Baghdad during (2012-2016)

<table>
<thead>
<tr>
<th>Age groups (Year)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>29</td>
<td>73</td>
<td>102</td>
</tr>
<tr>
<td>15-24</td>
<td>392</td>
<td>633</td>
<td>1025</td>
</tr>
<tr>
<td>25-34</td>
<td>478</td>
<td>336</td>
<td>814</td>
</tr>
<tr>
<td>35-44</td>
<td>479</td>
<td>197</td>
<td>676</td>
</tr>
<tr>
<td>45-54</td>
<td>385</td>
<td>257</td>
<td>642</td>
</tr>
<tr>
<td>55-64</td>
<td>278</td>
<td>201</td>
<td>479</td>
</tr>
<tr>
<td>65+</td>
<td>190</td>
<td>176</td>
<td>366</td>
</tr>
<tr>
<td>Total</td>
<td>2231</td>
<td>1873</td>
<td>4104</td>
</tr>
</tbody>
</table>

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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Forensic Examinations and Therapeutic Agreement in Indonesia

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Abstract
This research describes the type of legal protection for doctors and patients in forensic examinations. The therapeutic agreement is an agreement between doctors and patients which gives doctors the power to give forensic examination services. In the legal aspect of the therapeutic agreement, there appears the rights and responsibilities of each party. This means that both patients and practitioners have their own rights and responsibilities. The method used in this research is the doctrinal method with deductive and inductive approaches. Meanwhile, before the forensic examinations, the doctor needs therapeutic agreements to fulfill rights and responsibilities. Without this agreement, the doctor’s work may not reach a maximum positive effect. Thus, there must be a balance. The doctors need the therapeutic agreement to fulfill their responsibilities in the forensic examinations.

Keywords: Quality, Legal Protection, Doctors, Patients, Therapeutic, Agreement

Introduction
The Constitution No. 36 year 2009 is an implementing regulation of health services. This regulation is a means to create harmony between the doctors’ rights and responsibilities and the patients’ interests to achieve success in forensic examinations. The law of health is an implementation of the health law and state’s governance apparatus. The society views doctors as legal subjects which are interrelated, which creates both medical and legal relations.1

A problem happens when someone or some institutions need forensic examinations. Anyhow, in undergoing forensic examinations, the doctors are required to have an agreement from the patient or the family, or an order from authorized institutions such as the police department or the court. This is what is called as the therapeutic agreement or therapeutic order.2

Without having the therapeutic agreement or therapeutic order, the doctors do not have the right to undergo any forensic examinations. Problems arise as the patients have limited knowledge on the forensic examination techniques and also because the information given by the doctors are not satisfying enough for the patients and the family.3 This is the main reason why the doctors need it. It is called an agreement, as the relation between the patients or family and the doctors have reached the elements of a valid agreement to undergo forensic examination as written in Article 132.4

The problem of forensic examinations has long happened. Patients (those who will be forensically examined or who will undergo visum et repertum) or the patient’s family (if the forensic examinations will be done to a body) do not understand the process. They might also feel that such procedures are against their religious beliefs. In the forensic examination of bodies, there is sometimes the need to carry out surgery or the process of cutting open some body parts. This may happen in post mortem examination, for example. According to some of the Indonesian people’s religious beliefs however, this is against the belief of honoring the dead body. The second problem is that the doctors must convince the family to understand that this is an important process, that the forensic examination is needed to find out some things such as why the victim was hurt or dead, the types of wounds or disease and other things which can only be found through the forensic examination.5 The problem happens also when the patient’s family actively inquires

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or objects the authority’s decision to undergo forensic examination. The doctors may be trapped between disputes. They may also be targeted by the family in the lobby. They may even be hurt.

Forensic test efforts which are beforehand aimed to know whether the patient or their body were affected by something. The doctors need legal protection which is integrated and sustainable. Therapeutic agreement with the right mechanism is the legal agreement for the doctor rights and responsibilities in forensic test or forensic agreement.

Research Method

The method used is the doctrinal method, with interacting deductive and inductive approaches. The problem above is analyzed based on the data which become the target of a deductive-method research. Then, based on the real condition, conclusions are made regarding the legal protection of the doctors’ and patients’ rights and responsibilities in the perspective of therapeutical agreement.

Findings and Discussion

The existence of forensic examinations always involves the interests of all members of the society. The society may experience some events and become a victim. They may also be suspicious that someone has become a victim. Sometimes the people are suspicious that the one who reject is the perpetrator. The arrangement of legal protection for doctors to undergo forensic examination is said to be real when a legal subject is given rights and responsibilities. Legal protection is defined as giving guarantee or protection, provisions, licences and so that they cannot be persecuted if there are mistakes in the diagnosis of the examination results which are unintended from the authority those protected from all dangers or risks which may arise.

The therapeutic agreements or the therapeutic orders for doctors are based on the problem of legal protection for the doctors regarding forensic test service and the treatments given by doctors. Informed consent is an agreement between doctors and patients or the family to undergo forensic examination and it is known as the therapeutic agreement. From the authorized institution in the case of criminality, it is known as the therapeutic orders. In the medical world, it is called the therapeutic agreement or informed consent, which is an agreement for doctors to undergo examination or therapeutic effort a patient after the patient or family or the authority has obtained information.

Therapeutic agreement in Indonesia is a crucial thing in the forensic examination. The therapeutical agreement contains two essential elements, which are the information given by the doctor and the agreement given by the patient or the authority. The therapeutical agreement will create interacting rights and responsibilities for doctors. The therapeutic agreement gives an excellent quality of legal protection for doctor in the case of forensic examination.

The therapeutic agreement gives a trust to the doctors in giving the forensic test or forensic examinations. It can be used as a legal protection if the any wrong results were given by the doctors. It is because in the therapeutic there are provisions. It is said that if the doctors fail to perform their obligations in the forensic examinations, the patients or their family or the authority will not sue that mistake.

Even so, the patients juridically have the right to sue them if the health workers did not undergo their professional standards well. The two essential elements in the basis of the therapeutic are the information from the doctors and the approval of the patient or their families or authority. The Republic of Indonesia’s Ministry of Health Decree Article 1 No. 290/MENKES/PER/III/2008 regarding Agreement of the Medical Treatment. Regarding the forensic examinations, the doctors have the obligation to obtain the correct, clear, and honest information from their examination results.

Approval will be given for the doctor. That agreement is in the form of clear approval, which is done in a written form by patient, families and the authority. The doctors will give an explanation and medical records. The patients must obtain clear information regarding the forensic examinations which will be given by the doctor. Obtaining information is an obligation for doctors.

The Constitution of Health Article 8 states that each person has the right to obtain information regarding one’s data of health, including the treatments and the medications which have or which will be obtained from the doctors. There are the rights of the doctors as a form of legal protection which must be taken consideration of, which are the rights to obtain forensic examination service, the rightsto give information, and the rights to be protected.
The therapeutic agreement between the doctors and the patients are based on Article 1320 B.W (Burgerlijk Wetbook/Indonesians Law Codifications). It does not only create rights of patients, yet there are also consequences if the doctors purposely fake the examination results. They may be imprisoned or their licenses may be annulled.

Regarding the points above, the rights of the doctors include working according to the standard. They may refuse to carry out procedures which cannot be professionally taken responsibility of. They may also refuse undergoing forensic examinations, except through the right process.¹²

These responsibilities are binding. If the doctors do not undergo their responsibility to carry out forensic test or if they undergo maltreatments which are not according to the procedures, thus there are other responsibilities which must be taken by the doctor. The said ‘other responsibility’ is in the perspective of legal protection, where there are two kinds: legal responsibility in the aspect of civil law and that of criminal law. If the doctors do not undergo their responsibilities, thus, the authority may sue them to pay for compensation or they may be punished as written in the Civil Law (KUH Perdata) Article 1365. It is said that if there is any action which violates the law, which brings loss to others, thus the perpetrator must pay compensation or they may be jailed if they undergo crime in the forensic examination process.

Meanwhile, the elements which must be fulfilled in Article 1365 of the Civil Law (KUHPerdata) is that there are actions which violate the law. There must be wrongdoingness by perpetrators, there are losses experienced by the victim, and there is a casualty effect, which is the relation between the perpetrator’s (the doctor’s) wrongdoingness and the losses experienced by the victim.¹³

Thus, in this relation, there exists rights and responsibilities which are interrelated.¹⁴ In practicing their responsibilities in the forensic examinations, doctors may have mistakes and errors which may bring a negative impact. Such cases may grow into various things, such as: how a doctor is considered to be doing malpractice or how they fake reports, or how a doctor underwent a default.¹⁵ There are three elements of a doctor’s error which may result to imprisonment in undergoing forensic examinations, which are: (1) the doctor underwent a medical profession error, (2) such actions are done carelessly, (3) they undergo fatal actions such as faking reports, purposely undergoing errors for certain motives. Thus, the doctors who underwent professional errors may be imprisoned, and his/her practice license may be revoked by the Ministry of Health.

Thus, to protect the doctors, they need to obtain the therapeutic agreement before forensic examination. it is better if therapeutic is stated in a written form, so as to become an authentic proof if things were to happen. This may avoid the accusation of undergoing wrongdoingsness if the doctors’ forensic examination are based on the procedures, and have been explained correctly.

## Conclusion

Doctors cannot undergo forensic examination without orders from the authority in the case of forensic examination to open up criminal cases. The doctors may also undergo forensic examination based on the agreement of the patients or the family in necessary conditions, for example if someone feels that he/she is a victim and therefore needs that process. The therapeutic agreement is a mechanism which ensures that the doctors will receive legal protection after undergoing forensic examination.

### Source of Funding
- author

### Conflict of Interest
- no

### Ethical Clearance
- yes

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The Effect of Ciprofloxacin Exposure on Ctx-M Gene Pattern of Escherichia Coli

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Abstract

Background: An exposure of ciprofloxacin on Escherichia coli (E.coli) may cause cross-resistance to beta-lactam groups. The researchers obtained that ciprofloxacin, aminoglycoside, cotrimoxazole and second generation of cephalosporins were the risk factors for infection caused by Extended-Spectrum β-Lactamases (ESBL)-producing bacteria. Exposure of ciprofloxacin to E.coli causes resistance of cefotaxime and produces ESBL which is genotypically evidenced by the presence of CTX-M.

Objective: To know the effect of ciprofloxacin exposure on CTX-M gene pattern of E. coli.

Method: A total of 30 E.coli isolates were exposed to ciprofloxacin for 14 days with the Kirby-Bauer antibiotic disc diffusion method. ESBL confirmatory test and Modified Double Disk Susceptibility Test (MDDST) method was used the antibiotic disc and continued electrophoresis using Polymerase Chain Reaction (PCR). The data analysis used the chi-square statistic test with α = 0.05.

Results: In the PCR examination, 10.00% of ESBL isolates were obtained to have CTX-M-15 gene, and 33.00% of non-ESBL E.coli isolates having CTX-M-15 gene. While 10.00% of E.coli ESBL isolates and 53.30% non-ESBL did not have CTX-M-15 genes. There was no significant difference between ciprofloxacin exposure and the presence of CTX-M-15 gene (p = 0.290).

Conclusion: Most of the E.coli isolates had CTX-M-15 gene after the exposure. There was no difference in ciprofloxacin exposure to the presence of CTX-M-15 genes of E. coli.

Keywords: Ciprofloxacin, Escherichia coli, ESBL, CTX-M genes

Introduction

Ciprofloxacin is a member of the fluoroquinolone antibiotic class. The mechanism of action of fluoroquinolone class antibiotics is to interfere with the enzymes Gyrase A, B, and Topoisomerase IV, thus inhibiting deoxyribonucleic acid (DNA) replication, and further disrupt protein synthesis and bacterial cell death. Escherichia coli (E.coli) is a species of the genus Escherichia which is a member of the Enterobacteriaceae family. After the discovery and use of the fluoroquinolone, group began to emerge the fluoroquinolone-resistant Escherichia coli strain by expressing a protein that protects the active site of the gryrase enzyme targeted by fluoroquinolone antibiotics. This leads to fewer antibiotic therapy options, prolonged treatment length, high morbidity and mortality, and increased maintenance costs.

The use of ciprofloxacin as part of fluoroquinolone is alleged to be a risk factor for urinary tract infection (UTI) caused by E. coli, resulting in the enzyme extended strain beta-lactamase (ESBL). In a study conducted on E. coli isolated from cats, it was found that the CTX-M
ESBL encoding genes were also present in the same plasmid as the Plasmid-Mediated Quinolone-Resistance (qnr) gene. Research conducted in Spain shows that the ESBL enzyme in E.coli was TEM (60.00%), CTX-M (24.00%), and SHV (16.00%). ESBL CTX-M-15 enzyme obtained at E.coli at Dr. Soetomo Teaching Hospital Surabaya for 94.50% in all specimens and 84.00% in pediatric patient feces.

The abundance of ciprofloxacin and cefotaxime as an empirical therapy option in UTI should be recognized as potentially causing resistant strains to many antibiotics. This is due to cross-resistance between one antibiotic and another, in this case, ciprofloxacin and cefotaxime because of the similarity in which the gene encodes resistance to both. An exposure of ciprofloxacin to E.coli may cause resistance to cefotaxime and produce ESBL which is genotypically attested by the presence of the CTX-M gene. We aimed to know the effect of ciprofloxacin exposure on the pattern of CTX-M gene in E. coli.

**Method**

The population was a clinical isolate of inpatients Dr. Soetomo Teaching Hospital Surabaya who grew E.coli at Clinical Microbiology Installation by using the automatic diagnostic method. The sample size used the Central Limit Theorem, i.e., 30 isolates of E.coli sensitive non-ESBL ciprofloxacin from clinical specimens of inpatients in Dr. Soetomo Teaching Hospital. The inclusion criteria of E. coli were from clinical specimens of hospitalized patients, calculated colonies of E. coli ≥105 CFU/mL if derived from middle transition urine and ≥102 CFU/mL if derived from catheter urine and any amount derived from suprapubic urine, and E.coli was sensitive to ciprofloxacin based on the results of the susceptibility test of the method for diffusion. The exclusion criteria included cefotaxime, ceftazidime, aztreonam, amoxicillin, and bacterial isolates by using the Phoenix-BDCLSI M100S 2016 automatic technique, then exposure to ciprofloxacin discs for 22 hours at 37°C. The second-day isolate was suspended in a 0.9% NaCl solution to a turbidity of 0.5 McFarland. On the second day was also prepared exposure discs ciprofloxacin and cefotaxime with isolate sensitivity to cefotaxime as a screening test. If cefotaxime resistant, then it was proceeding with the confirmatory test. If the modified double disk susceptibility test (MDDST) confirmatory test results, it will be stored at room temperature for PCR test. The next step was to read the sensitivity of antibiotics using sensitivity tests. Its classified to be sensitive on ciprofloxacin if the inhibitory zone showed diameter ≥21 mm and resistant if ≤15 mm. Whereas, cefotaxime classified to be sensitive if inhibit zone was ≥26 mm and resistant if the inhibition zone was ≥22 mm. The sensitivity test for ceftazidime said to be sensitive if inhibition zone was ≥21 mm and resistant if the inhibitory zone ≤17 mm. ESBL classified to be positive if resistant to cefotaxime. ESBL confirmation test was positive if found widening of inhibition zone on the side of the disc that faces amoxicillin clavulanate. Then, prepared PCR examination with plasmid extraction using commercial kit by PureYieldTM, performed PCR examination by mixing the target DNA of extraction into a 50 μL ependorf tube inserted into a PCR machine then electrophoresis.

**Results**

Isolate Resistance to CIP and CTX by Kirby-Bauer method

The subjects obtained 5 (13.50%) E. coli isolates were resistant to cefotaxime and 3 (8.10%) isolates were resistant to ciprofloxacin sequentially after confirmation of the test by the Kirby-Bauer disc diffusion method.
(Table 1). In the post-CIP recurrence of the eighth day, 5 (16.70%) isolates were resistant to cefotaxime. Then, day 12 obtained 4 (13.30%) isolates that became resistant cefotaxime and 1 isolate become sensitive again. On day 14, 4 (13.30%) of cefotaxime resistant isolates were detected and to date, no isolates were resistant to ciprofloxacin (Table 2).

**ESBL Confirmation Test from CTX resistant E.coli**

On the day 4, there was no isolate changed to ESBL. While on day 8, was found 10.00% of the isolates into ESBL. Additional of new three ESBL isolates were obtained on day 6, 7, and 8. On day 12 the ESBL isolate did not increase with total 4 (13.30%) isolates until day 14. There were 5 isolates of E.coli exposed to CIP (1357PS, 1564US, 1590SS, 2015SS, and 2707PS) resistant to cefotaxime but after confirmation testing, there were only 4 isolates (1357PS, 1564US, 2015SS, and 2707PS) confirmed ESBL. Isolate 1590SS was not confirmed as ESBL but the next day becomes sensitive to cefotaxime. In the E.coli isolate (2056US) of the cefotaxime-exposed group that was resistant to cefotaxime but after a confirmatory test, it was not proven to be ESBL. Its becomes sensitive to cefotaxime by the next day. However, in both groups, no isolates were obtained to be resistant to ciprofloxacin (Table 3).

**PCX gene CTX-M-15 examination results in E.coli exposed to ciprofloxacin**

A total of 30 isolates of E.coli exposed to ciprofloxacin, there were 13 (43.30%) had CTX-M-15, 1 (3.30%) ESXL isolates which did not have the CTX-M-15 and 10 (33.30%) genes isolated with the gene CTX-M-15 but phenotypically were not ESBL. All of 30 clinical isolates of E.coli non-ESBL and ciprofloxacin-sensitive exposed to ciprofloxacin for 14 days, was found 4 isolates became ESBL (Table 4). All of these isolates were then examined using PCR. Statistical results showed there is no significant differences the presence of CTX-M-15 genes after exposure to ciprofloxacin (p = 0.290).

**Table 1. Resistance of isolates to CIP and CTX by Kirby-Bauer method**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (%)</td>
<td>R (%)</td>
<td>S (%)</td>
<td>R (%)</td>
</tr>
<tr>
<td>CTX</td>
<td>100.00</td>
<td>0.00</td>
<td>83.30</td>
<td>16.70</td>
</tr>
<tr>
<td>CIP</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00s</td>
</tr>
</tbody>
</table>

CIP: Ciprofloxacin  CTX: Cefotaxime

**Table 3. ESBL confirmation test results from CTX resistant E.coli**

<table>
<thead>
<tr>
<th>Exposure to Antibiotics</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non ESBL (%)</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>ESBL (%)</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

CIP: Ciprofloxacin  CTX: Cefotaxime  S: Sensitivity  R: Resistance

**Table 2. Escherichia coli resistance to CIP and CTX post recurrent exposure of CIP method Kirby Bauer**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (%)</td>
<td>R (%)</td>
<td>S (%)</td>
<td>R (%)</td>
</tr>
<tr>
<td>CTX</td>
<td>100.00</td>
<td>0.00</td>
<td>83.30</td>
<td>16.70</td>
</tr>
<tr>
<td>CIP</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00s</td>
</tr>
</tbody>
</table>

CIP: Ciprofloxacin  CTX: Cefotaxime  S: Sensitivity  R: Resistance
Table 4. Polymerase Chain Reaction (PCR) results of CTX-M-15 gene on E.coli exposed to ciprofloxacin

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype CTX-M-15</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive n = 13</td>
<td>Negative n = 17</td>
</tr>
<tr>
<td>ESBL</td>
<td>10.00</td>
<td>3.30</td>
</tr>
<tr>
<td>Non ESBL</td>
<td>33.33</td>
<td>53.30</td>
</tr>
</tbody>
</table>

Discussion

There was no significant difference between ciprofloxacin exposure and the presence of CTX-M-15 genes. It was possible that there were other genes that encode ceftazidime (CAZ) resistance. This statement can be denied considering that in isolation E.coli was sensitive to CAZ or ESBL screening and confirmation methods were less accurate. The sensitivity of ESBL confirmation test with modified DDST method according to Clinical and Laboratory Standards Institute (CLSI) was 65.4% (21). This was because some ESBLs could not reach the concentrations that detected by the antibiotic disc diffusion test, thereby thwarting the therapy. The presence of porcine was quite instrumental in making antibiotics capable to killing bacteria. In small amounts of porins, beta-lactam antibiotics could not be able to enter the cells and phenotypically become a resistant bacteria. However, if the number of porins is large, there is an antifungal that not hydrolyzed (beta-lactamase) which is able to kill bacteria. In this condition, bacteria was sensitive to beta-lactam. This causes E. coli strains that genetically have CTX-M-15 genes but were phenotypically not ESBL. However, referring to several bands in the electrophoresis results, this was because the primers used were less specific for the CTX-M-15 gene, so bands from other genes become emerging. Each class of antibiotics has its own mechanism in inhibiting and killing bacterial cells. Such mechanisms of damaging antibiotic molecules, altering antibiotic action targets, and decreasing intracellular antibiotic concentrations. In these three mechanisms, repeated exposure to antibiotics will make bacteria remains alive. This was due to the mechanism of cross-resistance among antibiotic classes. In this study, the bacteria did not die or were outside the inhibition zone due to antibiotic concentrations.

The main mechanism of fluoroquinolone class antibiotic resistance through GyrA and GyrB gyrase encoding mutations was contained in chromosomes. In addition to the main mechanism, there was an additional mechanism, namely Plasmid-Mediated Quinolone Resistance (PMQR), in which all the genes were located in the plasmid. In certain plasmids (Inc-H, IncF, IncP) it containing the gene encoding of PMQR, there was also a CTX-M-15 gene encoding one of the ESBL enzymes. Repeated exposure of ciprofloxacin to E. coli may make it an effort to adapt by transferring plasmids Inc-H, Inc-F, and Inc-P which makes E. coli increase with ESBL-encoding genes to become dominant. The result showed that 4 isolates of E. coli exposed to ciprofloxacin were changed to ESBL while at exposure cefotaxime none became ESBL. All of E. coli ESBL was still sensitive to ciprofloxacin. PMQR was not the primary mechanism of bacterial resistance to quinolones, but the presence of an improved MIC-enhanced PMQR gene that does not always reach the limits of the resistance criterion (biological resistance). E.coli strain with PMQR were more sensitive to ciprofloxacin but not enough to kill the bacteria, or there was a non-dominant resistance strain. The number of resistant strains between 1-1.000 for each 109 bacteria.

The result of this research showed that the isolate of non-ESBL phenotype but on PCR examination was obtained CTX-M-15. There were 23.66% isolates become sensitive to ceftazidime and non-ESBL and 2.17% of K. pneumonia with CTX-M-Group IV genes are both sensitive ceftazidime of 93 E.coli isolates. CTX-M group IV refers to CTX-M-9, 13, 14, 16, 17, 18, 19, 21, and 27 as well as Toho-2. Another study stated that 38 isolates of E.coli non-ESBL (52.60%) had ESBL-encoding genes.

Conclusion

Most of the E.coli isolates (43.00%) had CTX-M-15 gene after exposure to ciprofloxacin. There was no difference between the presence of CTX-M-15 gene after ciprofloxacin exposure that caused by screening method, ESBL confirmation was less accurate, or the presence of porins. The recommendation may be that the exposure
time of ciprofloxacin is extended until proven for certain
time required by the bacteria to be resistant to cefotaxime
and ciprofloxacin and further studies are conducted to
determine the pattern of gene encoding resistance to
other antibiotics (aminoglycosides, tetracyclines, and
heavy metals) in isolates exposed to it.

**Ethical Clearance:** This research is in accordance
with ethical clearance, has not been published before
and is not being considered for publication elsewhere.

**Conflict of Interest:** There is no conflict of interest
in this study.

**Source of Funding:** This research was carried out
by a team and funded independently.

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The Visum Et Repertum Forensic Examination as Evidence of a Rape Crime in the Trial Process of Kupang District Court

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Abstract

This research aims to prove visum et repertum as an evidence of a rape crime in the trial process at Kupang District Court (PN Kupang), so as to know how the judge assembly accommodate all evidences in the court as a basis to make a verdict, regading the weight of the punishment which will be given the rape crime perpetrator. This is a juridical- empirical descriptive research, as it uses primary and secondary data in the form of the judges’ verdict at the rape crime trial which is absolute and also interviews conducted to subjects who are involved in the research. The health aspect in proving the rape case is evidenced in the visum et repertum papers and expert information from the doctors who examined the victim.

Keywords: Forensic examination, Visum et repertum, Rape crime, Judge’s verdict

Introduction

The Post-Reformation era in Indonesia is marked by the transparency of information and news. Reports show devastating information on rape crime. The data displayed in the Trauma, Violence, & Abuse journal suggest that rape victims are often children. In is said that between 8% until 13% of girls had experienced sexual violence. Meanwhile, the percentage showed 3% until 13% of boys are victims of sexual abuse. 1 The Annual National Commission of Women 2018 report said that there were 619 cases of rape in private space (families and relatives). Meanwhile, in the public/communal space, there were 669 cases. Such cases in public spaces usually happen at school, in public transportations, or even during deviating religious or spiritual rituals 2.

Anastasi Reni Widyastuti said that, “There are various possibilities which affect the high rate of reports on violence towards women. This may be because of the women’s awareness regarding their rights, their higher understanding, the law enforcement for women, or that the law is not effective enough in giving protection for women who are victims” 3. According to Reni, the various regulations which exist today only reflect the power of the patriarchic values. There are still discrimination and exploitation of justice-seekers and the common society in the enforcement of public regulations.

Results of the crime court system has brought disappointing empirical data. Harian Kompas newspaper survey showed that 72,7% of the people has not received just treatments. 45,2% of the public suggest that the judges’ verdicts are based on the consideration of money, and 30,5% of the respondents suggest that the verdicts are based on political considerations. Only 9,3% of the respondents believe that the Indonesian court verdicts are based on the consideration of law. 4

Thus, there needs to be a reformation of law and policies, especially in the law enforcement system which is just to all genders. 4 Usually, in the solving of crime cases, the law seems to emphasize the rights of the perpetrators, meanwhile the rights of the victims are ignored. 5
The judge poses a core role in the case-solving process, especially in rape crime cases. Regarding whether or not the defendant really did the action accused is the most important part in the criminal procedure law. Yet, what is not less important is the evidence given by the victim regarding the accused’s actions. There aspects of evidences: Evidences in the aspect of forensic based on Article 184 of the KUHP regulations which includes visum et repertum, expert forensic information, and DNA test for victims.

Meanwhile, the writer will use objects as an empirical study material to test the assumption regarding the verdict on the rape crime case at PN Kupang, as there is a high rate of sexual violence towards women. There are 153 cases between 2013 until 2017 which were inspected and decided upon by the PN Kupang. There are 2,573 gender-based cases which happen in East Nusa Tenggara. These numbers are accompaniment data from Kupang Women’s House or RPK from year 2000 until 2014 within the city/regency area. Meanwhile, there has been 144 cases between 2015 and 2017.

Physically, the victims experience fear, anger, disappointment, disgust to herself, and they blame themselves regarding the sexual violence they experienced. They will cry easily. Other consequences include insomnia, stress, aggressivity, social avoidance towards the family and the environment. According to Khudzifah Dimyati, there are various impacts which happens to rape victims, “Rape victim suffer losses, mental breakdown and/or suffering financially, physically, psychologically and socially, among many losses psychologically or mental, although rape victim suffers financially, physically, psychologically and socially, their position in the criminal justice system has been neglected.”

Method
This was non doctrinal research. Data were taken from State Court of Kupang, East Nusa Tenggara Indonesia, from 2013 until 2017, then analyzed descriptively. The analysis results were presented in the form of tables and narrations.

Findings and Discussion
Case Verdict of Rape Crime in PN Kupang

There are 153 numbers of cases investigated and decided upon by the PN Kupang from 2013 until 2017. If viewed from the groupings of the crimes’ maximum punishment, the punishments are very light; light (1-2 years); medium (2-4 years); severe (4-7 years); very severe (over 7 years), with further explanation as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Case</th>
<th>Verdict Category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rape</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>Inter-course</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molest-ation</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>2014</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>2015</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>2017</td>
<td>4</td>
<td>23</td>
</tr>
</tbody>
</table>

From the table above, we can see that the Defendants’ punishments, or the judges’ verdict at the PN Kupang are mostly in the categories of severe or very severe. It can also be seen that out of 55% of the sexual violence cases taken to the court, the punishment for the perpetrators usually falls under 6 years of imprisonment.
In the victims’ and the victims’ families’ point of views according Watty Bangang in a conversation with Watty Bangang as a Coordinator of the Accompaniment and Victim Division of RPK, East Nusa Tenggara, “The punishment given to the perpetrators are not equal with what they must go through after the sexual violence. The sexual violence experienced is not fully healed in a wider sense, and the healing process is not provided by the government.”

The Process of Proving Rape Crime by the PN Kupang

Munir Fuady defined the proving of law as a process in a civil court procedure, a criminal court procedure, or other courtly procedures. It uses the judge’s power procedure to consider facts and statements disputed in court to prove its truth. Then, according to M. Yahya Harahap, evidencing is also a procedure to manage evidences which are valid according to the constitution, which may be used by the judges to prove the accused’s guiltiness.

Proving is based on positive law in considering the evidences’ strength of value is based on the constitution. In this case, if a criminal action is proven based on the evidences required in the Constitutional regulation (article 184 of KUHAP), which are the forensic proof as visum et repertum or others include the expert information, the defendant’s letters, evidences, and information.”

The change of the judges’ views on rape crime is crucial. They must not perceive rape as a normal crime, but they should view it as a violation of human rights. This is parallel with Moerti Hadi Soeroso’s opinion, who said that, “The forms of rape towards women, including rape within marriage, may be categorized as gender-based violation.” Violence crime is categorized as an action which violates the women’s rights as human beings.

Other evidences, such as those from the forensic aspect, like in the form of visum et repertum, may be difficult to be proven as victims seldom report the case right after having experienced rape. This is usually because the rape victims felt scared and ashamed. They will usually clean themselves or take a bath as a manifestation of disgust towards the perpetrators’ actions and disgust towards herself. Thus, bodily evidences such as bruises, and fragments of the perpetrators’ bodies such as hair, sperm, or skin which stick to the victim’s body may not be obtained during the forensic examination. Meanwhile, if the victim is pregnant because of the perpetrator, the only way to determine the father of the fetus is through a DNA test. Yet, the DNA test is rather expensive, thus it may be hard for the victim, the family, to access.

Forensic doctors’ help to obtain legal evidences of rape cases are crucial. This is regulated in Article 133 paragraph (1) of KUHAP, which states that investigation for the sake of trial in treating victims of wounds, poisoning, or death, which are allegedly caused by criminal actions are allowed to be done based on expert information by medical experts or doctors from other expertises.

In a conversation with a pseudonym, Judge of PN Kupang on May 28th, 2018, the Judge said “The term rape is sometimes misused by the society to report the perpetrator. In this case, the society (or the victim’s parents) use the constitution to report the perpetrator, because usually the punishments of enprisonment or fine is rather high. Thus, they use this gap to obtain profit. So the visum et repertum as forensic proof was urgent” Based on the judge’s testimony above, thus the writer concludes that this visum et repertum may influence the judge’s consideration towards the victims, thus the verdict given to the perpetrator may be light, medium or severly based on forensic process.

Thus, the proposal of a visum et repertum evidence is crucial to be present in a criminal trial as it becomes the judge’s consideration in determining the severity of the criminal act’s sanctions to the rape perpetrators.

In the case No. 33 / Pid.B / 2017 / PN.KPG, the judges give consideration that the charge for the defendant is the first alternative charge, which is the violation of Article 285 KUHP, in which its principalities contain the following elements:

1. Whoever; fulfilled
2. With violence or threat of violence forces a woman who is not his wife to undergo sexual intercourse, strengthened with the evidence of Visum Et Repertum No. B/389/IX/2016/Kompartemen Dokpol Rumkit issued by dr. Chindy Tefà on the date of September 3rd, 2016 by the name of Adriana Paa, it is concluded that from the examination of the victim’s genitals, it is found that there are new tears in the hymen until the basis, with the locations in the direction of one o’clock, three
o’clock, five o’clock, seven o’clock, eleven o’clock, and reddish bruises on the labia minora in the direction of nine o’clock and three o’clock, caused by contact of a blunt object.

Judging:

States that the defendant has validly according to the visum et repertum and convincingly guilty of the crime of “rape”; and giving the verdict to the Defendant of enprisonment for 1 (one) year and 8 (eight) months;

Analysis

Regarding the case above, it seems that as always, The judges making a verdict base on the forensic process (via visum et repertum) even the judges not give severe verdict. Physical harm doesn’t become a point which makes the judge consider a severe punishment for the defendant, even though evidences has been shown through the visum et repertum report which stated that the victim experienced new wounds on the hymen on the directions of one o’clock, three o’clock, five o’clock, seven o’clock, eleven o’clock, and reddish bruises on the labia minora in the direction of nine o’clock and three o’clock, caused by contact of a blunt object. These evidences are in the form of valid and responsible medical information regarding the victim’s condition, especially that whih regards proofs of violence.

According to Susetiawan, rape in the macro vision is a form of ripping away human rights. Yet, rape in the sexual coercion vision may also be defined as ripping away rights, like the rights to be virgin, the rights to be free from suffering, the rights to be free from fear, and the rights to be free from inhumane actions. Because of the rape, the victim experienced impacts of the violation of human rights. The proving process of that case include the visum et repertum, should already be enough for the judge to give an equal punishment to the perpetrator, as it has been proven by the visum et repertum. Yet this is not accomodated.

According to the writer, in the case above, the judge assembly was not thoughtful enough in giving legal consideration as basis of the verdict, as there are some points ignored, such as: the victim’s visum et repertum examination which is written in the visum et repertum letter by the doctors, which concluded that there has been new wounds in the victim’s genitals. The forensic process via visum et repertum became evidence for the judges to made the verdict given to the Defendant by giving him a sentence in jail.

This case has fulfilled the requirements of two evidences, which are a forensic statement in the form of visum et repertum and the perpetrator’s admittance, thus these evidences may become the basis in dropping a verdict to the perpetrator which is equal to his actions, which is 12 years of enprisonment as written in Article 285 of KUHP. Based on the Police Investigation Report, in this case of rape, the crime of rape is categorized as Victim Precipita Rape which places the victim as the originator(15). This is done with the motive of coercion and threat.

Khudzaifah Dimyati says “that the judge, as an enforcer of law, must be able to discover, to read, to interpret, and to implement the legal codes rightly and correctly as part of an effort to encontent the world into what is legal and ilegal”.16 In this context, the judge must look through all evidences in the court to achieve a wholistic consideration as the basis of his/her verdict, to determine the severeness of the punishments given to the perpetrator of the rape crime, and to achieve justice, certainty, and benefit.

Conclusion

The judge assembly of a rape crime in PN Kupang, case No. 33 / Pid.B / 2017 / PN.KPG has accept the evidences in the aspect of the victim’s visum et repertum examination which is written in the visum et repertum letter by the doctors, which concluded that there has been new wounds in the victim’s genitals. The forensic process via visum et repertum became evidence for the judges to made the verdict given to the Defendant by giving him a sentence in jail.

Ethical Clearence: Yes
Conflict of Interest: No
Source of Funding: Author

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Inhalant Abuses in India: A Review

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Abstract

This review article explores the studies of inhalants and inhalant misuse in India. Since much research is focused on illegal drugs not much work had concentrated on the commonly available inhalants. The abuse of inhalants is a common phenomenon in the world. In this review, we will explain why these inhalants addiction is an inalienable thing which depressed youths can’t avoid. Then we will look into various inhalants commonly used and also into various case studies available in the literature hence knowing the abuse of inhalants among youths and techniques employed by them to get a kick.

Keywords: Inhalants, abuse, volatile solvents, youth.

Introduction

People inhale vapors of toxic substances to attain an elated experience as told by Scott Silvia and L Cruz.¹ By reaching this euphoria state makes them forget their past, worries. Therefore, it was necessity to look into inhalants used by youths in India. Inhalant mishandling has been present in India since 1970s, studies done notifies us the abuse has increased. It has been on the rise within students who are among low strata of the society and those who have broken families where students are forced to live on the street, rag pickers, who are unemployed, who gets into groups of gangs. Commonly used inhalants in capital of India are the Eraz-Ex diluter and whitener, made by Kores, commonly seen all through Delhi reported in his article by Gigengack.² A typical substance abuser can be either a person with academic failure or a dropout with socio-economic factors stated by Kumar, Suresh et al ³. Leading News outlets have reported about the addiction to whitener, The Hindu⁴, The Times of India ⁵, The New Indian Express.⁶

According to report of an activist of child rights Raj Mangal believed “that closely 50,000 children of streets with no support and the lakhs of others who have some care available, a vast majority are into substance abuse”. This shows the staggering rise of the children which can be affected with inhalants. The method the youth follow is mainly by sniffing or by pouring the whitener fluid on a cloth furthering using it as an inhaler, giving them elevation of rejuvenating experience for some hours as reported by The Hindu.⁴

Inhalants

Inhalant is a substance which is intentionally inhaled and prominently volatile for the purpose of triumphing an ecstatic state which has been reported worldwide highlighted by Ballard ⁷. From past 2 decades, inhalant abuse is an alarm raising problem among children and teenagers mostly seen who live in streets (gullies) and backward villages across the world as pointed out by Praharaj, Verma, P, & Arora, M.⁸ Coping with NIDA publications dated Feb 2017 has highlighted that these substances are chemical substances which have the capability to affect central nervous system and can delay brain activity. The main reason behind inhalant abuse addiction is availability of these dangerous chemicals in numerous common products such as petrol, glue, nail-polish remover, whitener (writer correction fluid), paint strippers, dry cleaning fluids, spray paints, deodorant sprays and electronic contact cleaners and so on, as pointed out by Weir based on addiction in Canada.⁹-¹⁰ The reason behind this epidemiology of this inhalant abuse was because of their low cost, ease of availability and lack of legislative control over control.
and acquisition pointed out by Dindwiddie.\textsuperscript{11}

When it comes to the chemical nature of this inhalants, hexane which affects peripheral nervous system, toluene and nitrous oxide which cause both central and peripheral nervous system purported by Charles W. Sharp and Fred Beauvais.\textsuperscript{12( pp1-8)} Symptoms of inhalants at acute level resembles to alcohol intoxication which include excitation, agitation, drowsiness. Increasing intake of inhalants further leads to dizziness, ataxia and disorientation. At extreme level of abuse leads to hallucinations, muscle weakness, insomnia, nystagmus (eyes make repetitive movements). Charles W. Sharp and Neil L. Rosenberg have highlighted that at chronic level abuse it leads to serious complications like loss in weight, disorientation, and coordination impairment \textsuperscript{13(pp 117 -120)}.

**Modes of inhalation**

Separate studies by Weir, McGarvey, Ford found that these inhalants are taken by self-administration where chemical like fuels, solvents and adhesives like glue, acetone and butane are inhaled by nose referred as sniffing, breathing fumes from an inhalant-soaked cloth mainly nitrate canisters or gas dusters bloated in the mouth termed as huffing, breathing fumes from inhalant like paints and adhesives are placed in a plastic bag seized and forcefully inhaled by mouth termed as bagging these studies had pointed out that most of the cases were seen in other countries like Mexico, Canada, USA, South Africa, Australia, United Kingdom, Croatia and Latvia.\textsuperscript{10,14- 15}

**Typewriter correction fluid (TCF)**

As there was no significant statistical data to reveal the usage of typewriter correction fluid in India but there are some cases which has been published in recent years. Because of its low cost and ease availability without any further restriction from drug and department stores use of this type of inhalant was increasing enormously. Symptoms of using TCF depend upon the mode of exposure for example if skin is exposed then symptoms like appearance of first and second degree burns, severe rashes and erythema. If intestine is exposed then related symptoms like Nausea, Vomiting, diarrhea, agitation, euphoria, headaches, dizziness and muscle weakness. If respiratory tract is exposed signs are similar to intestinal affects and sometimes leads to coma and sudden death as explained by Pointer.\textsuperscript{16} This inhalant consist of trichloroethane, trichloroethylene at a blend of 50-60% and toluene. These chemicals at extreme levels may leads to sudden death stated by Done.\textsuperscript{17} A study examined 10 children aged 11 to 12 years and are drop out of school and working as labor. Waraich et al had told that the children were inhaling not only TCF but also petrol, nail polish remover, tobacco and alcohol\textsuperscript{18}. A study evaluated 45 homeless children aged 6 to 14 years old in Delhi who were the victims of misusing TCF covering high toluene content told by Seth et al.\textsuperscript{19} Gupta et al had pointed that two children misusing TCF and an adhesive which is used to fix punctured tires.\textsuperscript{20} A national study on 100 children living in footpaths, platforms and streets, from that 83% of children were commonly misusing products containing toluene which was TCF by Ray. Further he reported another clinical data in 2011 that more than 33% of adolescence who were school children and school dropouts are using TCF as a primary inhalant.\textsuperscript{21,22} A study conducted to examine the inhalant abuse among street children in Delhi revealed that about 96% of them were regularly inhaling volatile substances; from those volatile substances 83% of them were using TCF with toluene content by Dhawan et al.\textsuperscript{23} Another case reported that 9 children aged 10 - 17 who were school dropouts have been huffing TCF regularly and they found more than 45 children were using TCF and paint thinners as inhalants in New Delhi by Praharaj.\textsuperscript{8}

**Glue/adhesive Sniffing**

Adhesives mainly glues have been misused by children in India and many other countries. It was found that these adhesives have been misused by sniffing so the term Glue sniffing has been appeared in many blogs and previous papers. Chemicals like toluene, acetone, n-hexane, trichloroethane, trichloroethylene are existing in these adhesives. Bass and Mondal proved in separate studies that sudden sniffing death syndrome is the outcome of many chronic glue sniffing cases.\textsuperscript{24, 25} A case by Sood, where a 16-year-old boy was admitted in emergency ward, symptoms he was experiencing were severe abdominal pain, vomiting and showing irregular respiration, hypertension. Hypertension in intracranial pressure is due to possibility of toluene. Later the boy died on 7\textsuperscript{th} day of admission because of intoxication. Other was 17-year-old male admitted to hospital with severe abdominal pain and renal failure and later he confessed that he had been regularly sniffing glue for kicks.\textsuperscript{26} Another meta-analysis study by Mondal states that streets boys living in railway platforms and footpaths of West Bengal, Bhutan, Bangladesh were addicted to
glue tubes, cans and rubber cement (type of adhesive) are favorite particulars of children. They also demonstrated that sniffing or huffing of glue/adhesive enhance the genotoxic affect in epithelial cells of mucosa of these children which suggest that there might be genetic alternations that may cause mutagenic events and cause cancers. Another case illustrates a 22-year-old man covered his face with plastic bag having large amount of glue in it. This person died while he was carried to the hospital, the reason of his death was sudden sniffing death which occurred by cardiac arrhythmia (mainly ventricular arrhythmia) and there was a presence of toluene ‘was’ demonstrated in blood and liver told by Jayanth et al in his study.

**Gasoline**

Thousands of children and teenagers were inhaling a vast varieties of organic solvents in order of achieving euphoric state. Those organic solvents include highly pressurized liquids which are aerosols like dry cleaning, lighter fluids and gasoline (petrol, diesel and kerosene). Symptoms and signs include euphoria, sleeplessness, changes in psychological activity because of toxic manifestations in central nervous system, multiple manifestations and sometimes leads to encephalopathy. A case assessed and reported that 15 teenagers aged 17 were sniffing cloth soaked in petrol and these had been identified by one of the boy showed psychotic behavior because of chronic inhalation and later hallucinations, illusions, amnesia and delusions have been observed in other students stated by Mahal. Another case reported that a 13-year-old girl was addicted to fumes of petrol, later she started sniffing cloth soaked in petrol and kerosene for one year and signs of becoming violent, pleasant feeling, loss of appetite concluded that she was experiencing inhalant abuse and was later confirmed by interviewing by Pahwa. Another case by Joshi and Vankar was about a 10-year-old boy huffing and sniffing petrol from vehicles, his parents observed over activeness, hangover like behavior, dizziness, restlessness and inattention. In other study, reported by Gautam a 22-year-old boy with a history of sniffing kerosene, petrol and diesel for past eight years on daily basis and on examination revealed poor grooming, delayed speech, insomnia and increased psychotic activity. Later the boy was treated boy with Clonazepam 0.5mg.

**Conclusion**

Inhalants abuse, one may overlook due to less awareness around this topic should not be rejected as normal issues faced by the young but should be given utmost care when dealing with them.

Researchers focus highly on the illegal drugs which has greater effects, they often overlook on inhalants since it is not used as much as other prohibited drugs but these inhalants overuse can turn deadly for anyone who does not think so, studies by Charles Wm. Sharp and his colleague have proven this. Hence our focus is to get back to basic items which are available in any stationery stores e.g. whitener, glue, nail polishers etc. which have been manipulated by deceived youths who think this as a source of excitement. Also we need to look into cases of acute users and chronic users of inhalants since we have to see the results of how exposure to these volatile solvents on long term affects young people, therefore a distinction has to be made on long and short term effects of these solvents. This has been a question long before, Charles W. Sharp and his colleague had discussed about these in their early studies. But the awareness about inhalants has not reached layman due to generation gaps, therefore government agencies, NGOs and other health care groups should educate parents, teachers and adolescent ones about the growing menace of this abuse. Also in this review we had discussed about the symptoms which can be identified if a person is addicted, hence on knowledge of these symptoms any one close to the student can counsel or report to elders or authorities.

Further bills should be passed in the parliament regarding ban of certain inhalants which can be toxic when overused. Since these inhalants are freely available hence the misuse is vast. Bio-friendly chemical components should be used as the composition for these volatile solvents. Finally, extensive research has to be carried out in these fields to get the accurate data about users of these inhalants, Mahal and Pahwa in separate earlier papers had highlighted this need for data. Reasons attributing to the lack of data could be less awareness concerning volatile solvent misuse, among healthcare professionals and parents. Clinical based studies regarding addiction of these inhalants among street children, abandoned kids should be taken into consideration. If any of the youths had been found using these substances of abuse they should be placed in de-addiction centers where they should be monitored, their
progress recorded.

Conflict of Interest: Nil.

Funding: No funding received.

Ethical Clearance: No ethical clearance needed

References


Evaluation of Antioxidant (GSH, Vitamin A, E, C) and MDA in Iraqi Women with Toxoplasmosis

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Abstract

Background: Pregnancy loss, also referred to as a miscarriage or spontaneous abortion, is generally defined as a nonviable intrauterine pregnancy up to 20 weeks gestation. Early pregnancy loss, which occurs in the first trimester, is the most common type.

Objective: The aims of the study were to assess the role of (MDA, GSH, and levels of antioxidant vitamins, C, E and IgG, IgM of Toxoplasma gondii) causes of Abortions.

Materials and Method: For this study, 45 aborted women have been selected in first – trimester, and 25 well-matched women as control group their ages range from ( 20 – 33 years).

Results: The study shows is an increasing level of Malondialdehyde (MDA) in women with toxoplasmosis. Furthermore, there is a decreasing level of antioxidant vitamins (A, C, and E), and Glutathione (GSH).

Keywords: vitamin A, E, and C, Toxoplasma gondii, GSH, MDA, Abortion women.

Introduction

Abortion is the term refers to the ending of pregnancy by loss of a fetus before it can be outside the uterus after 9 months. An abortion which occurs spontaneously is called miscarriage or may be caused purposely and is called induced abortion (¹). Infectious toxoplasmosis is a specific type of toxoplasmosis in which a fetus is infected by the placenta is associated with fetal death and abortion, and in an infant, it is associated with a defect in neurons, neurocognitive deficits, and chorioretinitis. Early pregnancy loss, which occurs in the first trimester, is the most common type. The nonspecific symptoms of vaginal bleeding and uterine cramping associated with pregnancy loss can occur in normal, ectopic, and molar pregnancies, which can be a source of frustration for patients and clinical confusion for care providers (²,³).

Toxoplasma gondii belongs to coccidia, habit the domestic cat and other fields as its definitive host and wide range of birds and mammals as intermediate hosts (⁴,⁵). T. gondii specific IgM antibody production, and low numbers of concanavalin A binding sites have been located on the surface of tachyzoites. Glycoproteins that bind specific IgG and IgM have been identified on the surface of T. gondii, and it appears that T. gondii is capable of both N. and O. glycosylation (⁵).

Glutathione is a sulfhydryl-containing compound making out of three amino acids: L-cysteine, glycine, and glutamate (⁶). play the main role in many metabolic processes polyunsaturated fatty acids (PUFAs) oxidizes by ROS in the cell membrane. This reaction will results in lipid peroxidation, that yields free radicals (⁷).

Malondialdehyde (MDA) in cells of the human body and tissue is an indicator of the oxidative stress arising from the lipid peroxidation (⁸). The Lipid peroxide is the derivative enzyme of feeble unsaturated fatty acid produced from the result of deposition of a set of complex component (⁹).

The antioxidant vitamin C, A, and E play a significant role in keeping cells and neutralizing free radicals. Cancer prevention agents are found in many plants based foods inclusive leafy vegetables. Normal doses are safe during pregnancy. Vitamin C is a fundamental supplement involved in the repair of tissue (¹⁰). Vitamin E is a fat-soluble antioxidant, it interrupts...
the propagation of reactive oxygen species (11).

**Materials and Method**

For this study, 45 aborted women have been selected in first – trimester, and 25 healthy normal group their ages range from (20 – 33 years). from the in-patients and out-patients of Kamal Al- Samarrai Hospital from the first of March 2019 till the end of May 2019. Temnlof blood was taken under aseptic technique and sent for virological and parasitological investigations including IgM and IgG antibodies detection by using enzyme-linked immunosorbent assay (ELISA) kits by abnova. com< 0.9 negative 0.9-1.1 Equivocal >1.1 positives. Glutathione (GSH) mg/dl is measured glutathione by the method (12). Malondialdehyde [MDA] has been measured level (umol/l) (13). Vitamin C,( mg/dl), vitamin E (mg / dl ), vitamin A (mg / dl ) are measured by (14-16) respectively.

**Statistical Analysis**

The analysis is carried out data utilizing the SPSS – 18 (statistical packages for social sciences – version 18 PASW statistics).

**Results**

The result shows significantly change (p<0.05) levels of Glutathione (GSH), and Malondialdehyde MDA when comparing between aborted women and the control groups, as seen in Table (1), and figure (1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients group n= 45</th>
<th>Control group n= 25</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (mg / dl)</td>
<td>40.78±3.5</td>
<td>46.60 ± 3.63</td>
<td>0.05</td>
</tr>
<tr>
<td>MDA (µ mol / L)</td>
<td>5.06± 9.4</td>
<td>3.73±7.70</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 2, and figure (2) shows levels of vitamin E, C, A for the patients and control group which shows a decreased level of these vitamins in patients compared with the control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients group(n= 45)</th>
<th>Control group ( n = 25)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E (mg / dl )</td>
<td>0.43 ± 0.582</td>
<td>0.57 ± 0.74</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin C (mg / dl )</td>
<td>1.20 ± 0.235</td>
<td>1.46 ± 0.163</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin A (mg / dl )</td>
<td>2.39 ± 0.547</td>
<td>2.82 ± 0.455</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Discussion**

Repetitive pregnancy forfeit is usually characterized as at least 3 or more miscarriages roughly 5% of couples endeavoring pregnancy have recurrent pregnancy loss. Newly, the advance has been made in understanding recurrent pregnancy loss approximately 10-15% of all first-time pregnancies bring about an unsuccessful miscarriage. In many instances, a similar miscarriage rate in subsequent pregnancies is expected (7). Jaslow (17) and Rai (18). Found there is a significant relationship between the history of miscarriage in the family and the studied group this is may be estimated to be due to the lifestyle in the family. It is well known that the most important anti-parasite influential mechanisms is produced of NO and the most important products of lipid peroxidation which is that MDA arises from fatty acids peroxidation. The products of MDA can reason...
cross-linkage of membrane elements by influencing the ion exchange from the cell membrane \(^{(19, 20)}\).

In the present study, reported the increase in MDA in toxoplasma women. It is reported the increase of MDA action in lymphocyte and erythrocyte in the dust mite positive or skin positive group which show the oxidative stress in patients with dust mite \(^{(20)}\). Another study shows an increase of MDA which refers to the increment of lipid peroxidation, due to the decreasing activity of the defense system protecting tissues from the free radical \(^{(21)}\). The Free radicals have been implicated in the pathogenesis of a variety of diseases. Thus, the large amount of lipid accumulates produced in the placenta in many diseases, involving toxoplasma infestation and must be prevented through the production of antioxidants \(^{(21)}\).

In Iraq, there is a study shows the increased level of MDA in toxoplasma seropositive patients who agree with the present study \(^{(22)}\).

The alteration of serum glutathione in patients with toxoplasmosis which shows a significant increase in serum glutathione concentration in pregnant women infected by toxoplasmosis \(^{(23)}\). This increasing of glutathione in pregnant women do not enough to decrease the detoxication capacity of the pregnant uterus. The increasing of glutathione simultaneous in response to the increase of radical and electrophilic compounds this disagreement with our study another study which shows a decrease of serum glutathione this agreement with our study \(^{(22)}\).

Antioxidant vitamins play a major role in the health of human rather than intoxication the decrease in the levels of vitamins A, E and E (nonenzymatic antioxidant) in women with toxoplasmosis may be turnover to increase for preventing oxidative damage \(^{(23, 24)}\). In a study has reported an increased vitamin E resulted in trends toward increased tissue cyst number, tissue pathology and weight loss during infection \(^{(25)}\). In Iraq, there is the first study that shows decreased the levels of antioxidant vitamins A, E and C during infection with toxoplasmosis in pregnant women which agrees with our result \(^{(26)}\).

**Conclusion**

In conclusion, there is an increasing level of Malondialdehyde and decreased levels of antioxidant vitamins (A, C, E), and Glutathione in patients with toxoplasmosis.

**Conflict of Interest:** There is no conflict of interest among the authors.

**Funding:** Self

**Ethical Clearance:** This study is ethically approved by the Institutional ethical Committee.

**References**

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Prevalence of Celiac Disease among Cases of Irritable bowel Syndrome in Baghdad- Iraq

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Abstract

Celiac disease is a common illness need not to be mistaken as IBS or GI motility disorders and careful evaluation of IBS patients especially those with diarrhea predominant type may need to be considered.

This study disclose that about more than 12% of patients who had been already diagnosed as IBS is discovered to have positive serology of gluten sensitivity and the diagnosis of Celiac disease had been confirmed by histopathology study.

Though and careful evaluation of presumed cases of IBS especially those with poor response to conventional therapy or those who with atypical presentations is essential in order to reach to an alternative diagnoses.

Keywords: Celiac disease, Gluten sensitivity, Irritable bowel syndrome, Anti tissue transglutaminase, Gluten free diet

Introduction

Celiac disease is a common cause of malabsorption of one or more nutrients. Recent observations have established that it is a common illness with protean manifestations, a worldwide distribution is approximately 1%.

Its incidence has been raised over the past five decades. Celiac disease has several other names, as nontropical sprue, celiac sprue, and gluten-sensitive enteropathy, the etiology of celiac disease is not completely understood, but immunologic; environmental; and genetic factors imply the major role in pathogenesis.

Celiac disease is considered an “iceberg” disease. A few number of patients have classic symptoms and manifestations linked to micronutrient malabsorption along with a varied natural history; the onset of symptoms can occur at all points of life, though the disease has two peaks of ages: the first is early in life, at approximately 2 years of age (after gluten containing diets has been introduced), or later in the second to fourth decades of life. It may first manifest after an attack of prolonged diarrhea following gastroenteritis or even after abdominal surgery.

A larger number of patients have “atypical celiac disease,” with presentations that are not obviously linked to small intestine malabsorption (e.g., anemia, infertility, osteopenia, and neurologic and psychological manifestations). Even larger figure of patients have “silent celiac disease”; they are essentially asymptomatic despite abnormal small-intestinal histopathology and positive gluten sensitivity serology.

Other symptoms of patients with celiac disease may range from significant malabsorption of multiple nutrients, with diarrhea; weight loss; steatorrhea; and the consequences of nutrient depletion (i.e., metabolic bone disease and anemia), to the total absence of gastrointestinal symptoms despite evidence of the depletion of a single nutrient (e.g., iron or folate deficiency; edema; osteomalacia from protein loss).

Both IBS and gluten sensitivity are common in the general population and both can coexist with each other independently without necessarily sharing a common pathophysiological basis.

Not all patient with IBS or IBS predominant diarrhea is candidate for screening for gluten sensitivity but testing should be considered in the following situations:

1. Patients with GI symptoms including recurrent or chronic diarrhea; weight loss; malabsorption and abdominal bloating or distension and severe lactose intolerance.
2-Patients with no alternative explanations for extraintestinal manifestations of combined nutritional deficiencies and/or anemia, persistent transaminitis, delayed puberty, short stature, females with recurrent abortions, hypofertility, recurrent aphthous ulcers, dental enamel hypoplasia, idiopathic peripheral neuropathy or cerebellar ataxia, or recurrent atypical migraine.

3-Patients with type 1 diabetes mellitus if they present with clinical manifestations of presumed celiac disease.

4-Asymptomatic first-degree relatives of patients with an established celiac disease.

These recommendations are consistent with the American College of Gastroenterology guidelines. The Rome III criteria can establish the diagnosis of IBS without further extensive testing and seeking for alternative diagnoses may be considered in cases of nocturnal diarrhea, symptoms unrelated to food or defecation. Alarm features that raise the concern of other diagnosis in presumed IBS cases are weight loss, anemia, bloody stool, positive family history of inflammatory bowel diseases, colonic cancer or celiac disease.

It is very important to remember that patients with celiac disease is at high risk of several malignancies like esophageal and intestinal adenocarcinoma, B-cell MALT lymphoma, hence increased mortality, so strict gluten free diet is essential.

Figure 1 Testing for celiac disease in cases with diagnosed IBS depending on Rome criteria, especially in cases of diarrhea predominant IBD or there is possible alternative diagnosis other than IBS.
**Diagnosis**

**SERUM ANTIBODY ASSAYS**

1. IgA endomysial antibody (IgA EMA)

2. Immunoglobulins tissue transglutaminase antibody (IgA tTGA and IgG tTGA).

3. Immunoglobulins deamidated gliadin peptide (IgA DGP and IgG DGP)

All patients with Celiac disease express the HLA-DQ2 or HLA-DQ8 allele, although only a minority of people expressing DQ2/DQ8 have celiac disease. Absence of DQ2/DQ8 excludes the diagnosis of celiac disease.

Endoscopic small bowel mucosal biopsy is the gold standard. Endoscopic biopsy is mandatory in suspected cases even if mucosa looks normal. As the histological changes can be patchy, multiple biopsies – usually, more than four biopsies from the second part of the duodenum in addition to one from the duodenal bulb – should be obtained.(7)

Histopathological feature mainly seen are villous atrophy or completely absent with a reduced villous-to-crypt ratio and crypts looks hyperplastic. There is increase in cellularity of the lamina propria with a mainly plasma cells and lymphocytes. The number of intraepithelial lymphocytes per unit length of absorptive epithelium is usually increased.(8)

---

**Modified Marsh Classification of histologic findings in celiac disease (Oberhuber)**

<table>
<thead>
<tr>
<th>Marsh Type</th>
<th>IEL / 100 enterocytes – jejenum</th>
<th>IEL / 100 enterocytes – duodenum</th>
<th>Crypt hyperplasia</th>
<th>Villi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;40</td>
<td>&lt;30</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>&gt;40</td>
<td>&gt;30</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>&gt;40</td>
<td>&gt;30</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>3a</td>
<td>&gt;40</td>
<td>&gt;30</td>
<td>Increased</td>
<td>Mild atrophy</td>
</tr>
<tr>
<td>3b</td>
<td>&gt;40</td>
<td>&gt;30</td>
<td>Increased</td>
<td>Marked atrophy</td>
</tr>
<tr>
<td>3c</td>
<td>&gt;40</td>
<td>&gt;30</td>
<td>Increased</td>
<td>Complete atrophy</td>
</tr>
</tbody>
</table>

- IEL/100 enterocytes, intraepithelial lymphocytes per 100 enterocytes
- Type 0: Normal; celiac disease highly unlikely.
- Type 1: Seen in patients on gluten free diet (suggesting minimal amounts of gluten or gliadin are being ingested); patients with dermatitis herpetiformis; family members of celiac disease patients, not specific, may be seen in infections.
- Type 2: Very rare, seen occasionally in dermatitis herpetiformis.
- Type 3: Spectrum of changes seen in symptomatic celiac disease.

---

**Patients and methods**

A cross sectional study conducted at Alyarmouk teaching hospital internal medicine outpatient clinic from the period of march 2018 to march 2019 involving 140 patients who had been labeled as IBS or presented with recurrent symptoms highly suggestive of IBS.

Inclusion criteria are patients had been previously diagnosed by general practitioner, physician, or gastroenterologistas having irritable bowel syndrome based on symptoms such as recurrent abdominal discomfort, colicky abdominal pain, altered bowel habits, and bloating at time of presentation.
Exclusion criteria are patients with recent infectious diarrhea, history of inflammatory bowel disease, peptic ulcer disease, gastrointestinal malignancies, previous gastrointestinal surgeries, alcoholism and patients with advanced chronic illnesses (chronic renal failure, long term diabetes mellitus, and congestive heart failure).

The patients had been re-evaluated regarding the diagnosis either due non convincing response to IBS therapy, insufficient initial work up, or new symptoms had been developed.

Screening for gluten sensitivity had been done by using anti tissue transglutaminase (tTGA) assay then the patient with positive results undergone upper endoscopy and histopathological analysis of multiple biopsy specimens from second part of duodenum to confirm the diagnosis.

All the patients who had been diagnosed as Celiac disease undergone complete evaluation for anemia and micronutrient deficiency with CBC, serum ferritin, B12, and vitamin D level.

The patient who had been confirmed to have celiac disease established on gluten free diet and set for follow up.

**Statistical Analysis**

Analysis of data was carried out by available statistical package (version 25). Statistical significance was considered at (P value ≤ 0.05).

**Findings**

This study which in cover 140 IBS cases, in which each case had been assessed for possible gluten sensitivity and the results were 17 patients of 140 (12.1%) have positive serology for gluten sensitivity, all the cases with positive serology undergone upper endoscopy which confirm the diagnosis of celiac disease.

Fourteen of 17 patient (82.4%) who were positive were females and 3 (17.6%) were males in which it carry no statistical significance. P =0.585.

Most of these cases were diarrhea predominant IBS 10 of 17 (58.8%) where as 7 (42.2%) were constipation predominant type. Table 1

### Table 1 Classification of patients according to age, gender and type variant of IBS in relation with positivity of tTGA

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Anti tissue transglutaminase</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Positive</td>
</tr>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>&lt;20y</td>
<td>9</td>
<td>6.4</td>
</tr>
<tr>
<td>20---29</td>
<td>22</td>
<td>15.7</td>
</tr>
<tr>
<td>30---39</td>
<td>35</td>
<td>25.0</td>
</tr>
<tr>
<td>40---49</td>
<td>41</td>
<td>29.3</td>
</tr>
<tr>
<td>50---59</td>
<td>28</td>
<td>20.0</td>
</tr>
<tr>
<td>=&gt;60y</td>
<td>5</td>
<td>3.6</td>
</tr>
<tr>
<td>Mean±SD (Range)</td>
<td>39.2±12.2 (15-79)</td>
<td>33.0±8.9 (19-50)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.585</td>
</tr>
<tr>
<td>Variant of irritable bowel</td>
<td></td>
<td>IBS-C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IBS-D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.006*</td>
</tr>
</tbody>
</table>

*Significant difference between proportions using Pearson Chi-square test at 0.05 level.

IBS-C irritable bowel syndrome – constipation, IBS-D irritable bowel syndrome - diarrhea
All the 17 cases with positive serology were undergone upper endoscopy and biopsy specimen had been obtained from second part of duodenum and sent for histopathological analysis.

The results of histopathological finding were positive for features of Celiac disease and were ranging from (2-3b) modified Marsh Classification of histologic finding in celiac disease (Oberhuber); 8 Patients type 2, 7 Patients type3a, and 2 patients type 3b. Figure 2

![HISTOPATHOLOGIC MARSH TYPE](image)

**Figure 2** Histopathological finding in patient with positive anti tissue transglutaminase.

Among the total 17 patients, 10 has anemia (7 cases hypochromic microcytic anemia and the rest 3 has normochromic normocytic anemia and no cases of megaloblastic anemia seen despite that there were 3 cases discovered to have B 12 deficiency in which they were had normal hemoglobin and MCV level)Figure 3. All the 7 cases of hypochromic microcytic anemia is confirmed to be iron deficiency anemia as well as 2 cases of normochromic anemia and 3 non anemic patients were had below normal ferritin level; so the total number was 12 of 17 hashad iron deficiency state. Lastly all patients found to have vitamin D level below the reference range. Table 2

All the cases who had been confirmed to be Celiac disease had been advised for gluten free diet and follow up.

![Screening for Hemoglobin State in Diagnosed Cases of Celiac disease](image)

**Figure 3** Hemoglobin state in cases of Celiac disease
Table 2 Micronutrient State in cases with Celiac disease

<table>
<thead>
<tr>
<th>Screening for micronutrient deficiency in Diagnosed cases of Celiac Disease</th>
<th>Vitamin D3</th>
<th>Vitamin B12</th>
<th>Iron State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Deficient</td>
<td>Normal</td>
<td>Deficient</td>
</tr>
<tr>
<td>0 (0%)</td>
<td>17 (100%)</td>
<td>14 (82%)</td>
<td>3 (17.6%)</td>
</tr>
</tbody>
</table>

Discussion

Celiac disease is one of the most readily missed illnesses in practice due to the complexity of gastrointestinal symptoms associated with this disease, such as flatulence, diarrhea or rarely constipation, abdominal cramps and fullness which may overlap with other various gastrointestinal illnesses, such as inflammatory bowel diseases, chronic infections and helminth infestation, other food allergies, lactose intolerance, motility disorders and irritable bowel syndrome.

The lack of history or features of micronutrient deficiency, mineral bone diseases and other cutaneous and neurological manifestations of celiac disease does not exclude the presence of this syndrome. So thorough evaluation for gluten sensitivity is essential in presumed cases of IBS which not respond to conventional dietary and medical approaches and carry the same importance of excluding inflammatory bowel disease or other pathologies that may simulate IBS.

Although it has to be pointed that some IBS cases with no evidence of gluten sensitivity may surprisingly get significant benefit in improvement of their symptoms after exclusion of gluten containing food from their diet.

According to this study there is significant number of those patients who had been diagnosed and managed as IBS are actually have Celiac disease, especially those with diarrhea predominant subtype.

The usual work up of patients who present with symptoms suggestive of IBS does not involve regularly screening for gluten sensitivity or any form of food allergies unless the patient has features of malabsorption of micronutrients, diarrhea, steatorrhea, weight loss or coexistence of dermatitis herpetiformis.

Still early diagnosis of Celiac disease and establishment of gluten free diet is essential to prevent long term serious complications other than vitamin and minerals malabsorption such as ulcerative jejuno-ileitis, small intestinal lymphoma and small bowel adenocarcinoma.

As compared with the study *The prevalence of celiac disease in patients with irritable bowel syndrome and its subtypes* by Danuta Domżał-Magrowska who had been published in Przegląd Gastroenterologiczny 2016 in which concomitant positive result of genetic testing and any elevated serum antibodies specific to celiac disease was found in 12.5% of IBS patients.

So this study carry a highly comparable results, Screening for anemia and micronutrient deficiency in the cases who had been confirmed to have Celiac disease reveals that anemia is present in 10 of 17 cases (58.8%) that was mainly of iron deficiency which is the most common type of nutritional anemia in Celiac disease, there is also significant number of cases with iron deficiency state to be added to cases of IDA to be a total of 12 of 17 (70.1%).

Mainly because of small size of sample there is no case with megaloblastic anemia detected but still there are 3 cases with low serum B12 level. It is also important to be referred that there is no data available about folate statues in our study because lack of availability of reliable test for red blood cell folate in our facility at time of the study and the level of serum folate does not reflect the actual folate level state.

Vitamin D deficiency is present in all cases who had been diagnosed as Celiac disease but it may reflect the high prevalence of vitamin D deficiency among Iraqi patients in general as well as the effect of Celiac disease on lipid soluble vitamins absorption.

Finally it is important to follow up all the patients who had been diagnosed as celiac disease regarding improvement in their GI symptoms following exclusion.
of gluten from their diet, if not, the coexistence of IBS or other GI pathologies still possible and careful clinical re-evaluation is warranted.

Conclusions

There is significant number of celiac disease patient had been diagnosed and managed as IBS cases (mainly diarrhea predominant type) and there may be coexistence of both illnesses in the same patient.

Ethical Clearance: No

Source of Fundings: Self

Conflict of interest: Nil

References


Psychological Empowerment and Organizational Commitment among Nurses

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Abstract

**Background** - Psychological empowerment plays an important role in behavioral, emotional and cognitive constructs, such as Job Satisfaction and organizational citizenship behavior. **Aim** - The study aimed to identify the relationships between Psychological Empowerment and Organizational Commitment among nurses. **Methods and Materials** - A cross-sectional, descriptive and correlational study design carried out in a period from 5th of September/2017 till 30th of May/2018 at five Public Hospitals in Mosul/Iraq. Non-probability sampling technique and accidental sampling method depended to select the sample of the study, which accounted for (317) nurse. Two instruments were used to collect data via an interview method with each subject “Psychological Empowerment Questionnaire and Organizational Commitment Questionnaire”. The findings of the study presented by mean, standard deviation, while the relationships among variables presented through stepwise regression. **Results** - Total PE and its domains had significant relations to total OC and its Affective and Normative domains.

**Keywords:** Psychological Empowerment, Organizational Commitment, Nurse.

Introduction

Psychological Empowerment is a factor affecting any work, whereas, managers need a deep understanding in order to help employees handling it, motivating, and eliminating disturbing factors appropriately\(^{(1)}\). The repository of knowledge, skills, and abilities are the employees who cannot be imitated by the competitors\(^{(2)}\). Among them are nurses who constitute the largest group of professionals, and are the cornerstone in determining and providing the quality and cost of the healthcare\(^{(3)}\). There are many factors and situations that put nurses under considerable stress, as high organizational demands, the climate of job uncertainty\(^{(4)}\), in addition to the psychological empowerment which is, in turn, having a great impact on the organizational success\(^{(5)}\).

Psychosocial empowerment can be defined as an active motivational approach to the role of the individual at work, in addition to the individual’s sense of control over work\(^{(6,7,8,9,10)}\). It is not the conditions of the work’s context, but the reactions of the employee to these conditions, that in turn, influence their organizational behavior\(^{(11)}\). It plays an important role in behavioral, emotional and cognitive constructs, such as Job Satisfaction and organizational citizenship behavior\(^{(9,12)}\). It is manifested as four-dimensional constructs of employees’ perceptions; Meaning, Competence, Self-determination, and Impact\(^{(13,14,15)}\).

In the healthcare environment, maintaining a committed workforce is a strong advantage. Committed nurses provide asset value of stable, training and developing costs, lower employee recruiting, dedicated workforce, retention of nurses with the knowledge, skills, and abilities that are critical to organizational success, improved organizational image within the community, in addition, a committed workforce influence customer loyalty\(^{(1)}\). Organizational Commitment is defined as an individual’s identification and involvement with a specific organization\(^{(16)}\). It is a reflection of individual’s psychological state, which defines the relationship between the employee and the organization, for which they work and that has deductions for their decision to
continue with the organization\textsuperscript{[17,18]}, identification with the organization and involvement in the organization\textsuperscript{[19]}, Normative commitment that means an attachment-based on perceived obligation and a sense of loyalty or duty\textsuperscript{[20]} and Continuance commitment that is an attachment-based on perceived cost and refers to an employee’s intention to leave or wish to remain a part of the organization and depends on their recognition of the availability of alternatives if they want to leave the organization\textsuperscript{[21,22]}.

The present study endeavored to identify the relationships between Psychological Empowerment and Organizational Commitment among nurses, for, they are many facets each employee can confront them in the work.

**Material and Method**

Cross-sectional, descriptive and correlational study design carried out for a period from 5\textsuperscript{th} of September/2017 till 30\textsuperscript{th} of May/2018 at five Public Hospitals in Mosul/ Iraq mentioned as A, B, C, D and E. Non-probability sampling technique and accidental sampling method depended to select the sample of the study, which accounted for (317) nurse. The inclusion criteria depended for the selection the sample were; providing bedside nursing care, having tenure in the current place ≥ 5 years, presenting at work during gathering data and didn’t engage in management or leadership position at the study period. Data gathered via an interview with each subject that lasted 20-30 minutes. Two instruments used to collect data; Psychological Empowerment questionnaire “PE” which is used to measure the level of four dimensions; (Meaning= 3 items, Competence=3 items, Self-determination=3 items and Impact=3 items), all items were of five values distributed as strongly disagree=1, disagree=2, no opinion=3, agree=4, and strongly agree=5\textsuperscript{[23]}, and Organizational Commitment questionnaire “OC” which is used to measure the level of four dimensions: (Affective Commitment “AC” =6 items, Normative Commitment”NC” =6 items and Continuance Commitment “CO” =6 items), four items of the questionnaire are reverse-keyed (labeled by “R” against each item), all items were of five-point scale ranging from strongly disagree=1, disagree=2, no opinion=3, agree=4, and strongly agree=5\textsuperscript{[24]}.

For each scale, all scores were summed and divided by the number of items to attain the means of scales. To determine the validity of the scales, content validity was measured by exposing the scales to five experts in nursing field to ascertain its consensual validity, they presented a few essential corrections that were undertaken in the final drafts, while the reliability analysis for the questionnaires yielded a Cronbach’s alpha of 0.79 for Psychological Empowerment scale and 0.87 for Organizational Commitment scale. Descriptive statistics (Mean and Standard deviation) used to review the levels of measured variables (PE, and OC), while stepwise regression analysis used to evaluate the impact of criteria variable on multiple prediction variables.

According to the administrative rules, informal permission from the participants obtained prior to starting data collection.

**Findings**

Table (1): Descriptive statistics of PE, and OC and their domains:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean of Score</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE (Total)</td>
<td>36</td>
<td>32</td>
<td>60</td>
<td>47.59</td>
<td>6.58</td>
</tr>
<tr>
<td>Meaning</td>
<td>9</td>
<td>8</td>
<td>15</td>
<td>12.47</td>
<td>1.87</td>
</tr>
<tr>
<td>Competence</td>
<td>9</td>
<td>6</td>
<td>15</td>
<td>12.68</td>
<td>2.07</td>
</tr>
<tr>
<td>Self-Determination</td>
<td>9</td>
<td>4</td>
<td>15</td>
<td>10.97</td>
<td>2.92</td>
</tr>
<tr>
<td>Impact</td>
<td>9</td>
<td>4</td>
<td>15</td>
<td>11.47</td>
<td>2.41</td>
</tr>
<tr>
<td>OC (Total)</td>
<td>54</td>
<td>33</td>
<td>66</td>
<td>49.98</td>
<td>7.98</td>
</tr>
<tr>
<td>Affective</td>
<td>18</td>
<td>9</td>
<td>23</td>
<td>16.98</td>
<td>3.56</td>
</tr>
<tr>
<td>Normative</td>
<td>18</td>
<td>8</td>
<td>26</td>
<td>16.55</td>
<td>3.78</td>
</tr>
<tr>
<td>Continuous</td>
<td>18</td>
<td>10</td>
<td>25</td>
<td>16.45</td>
<td>3.09</td>
</tr>
</tbody>
</table>
Table (1) demonstrates that the means of Total Psychological empowerment and its dimensions were above the mean of scores, whereas, the means of Total Organizational commitment and its dimensions were below the mean of scores.

### Table (2): Regression Findings for Organizational Commitment (OC) with respect to Psychological Empowerment (PE)

<table>
<thead>
<tr>
<th>Model</th>
<th>Adj. R2</th>
<th>B</th>
<th>t. Value</th>
<th>F</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.021</td>
<td>.297</td>
<td>2.805</td>
<td>7.865*</td>
<td>.156</td>
</tr>
<tr>
<td>Meaning</td>
<td>.034</td>
<td>.331</td>
<td>3.486</td>
<td>12.151**</td>
<td>.095</td>
</tr>
<tr>
<td>Competence</td>
<td>.167</td>
<td>.503</td>
<td>8.010</td>
<td>64.166**</td>
<td>.411</td>
</tr>
<tr>
<td>Self-Determination</td>
<td>.029</td>
<td>.266</td>
<td>3.246</td>
<td>10.535**</td>
<td>.180</td>
</tr>
<tr>
<td>Impact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) Predictors (Constant)=PE Domains DV=AOC *P. ≤ 0.01 **P. ≤ 0.001

<table>
<thead>
<tr>
<th>Model</th>
<th>Adj. R2</th>
<th>B</th>
<th>t. Value</th>
<th>F</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>.062</td>
<td>.515</td>
<td>4.682</td>
<td>21.922**</td>
<td>.110</td>
</tr>
<tr>
<td>Meaning</td>
<td>.051</td>
<td>.232</td>
<td>4.234</td>
<td>17.926**</td>
<td>.232</td>
</tr>
<tr>
<td>Competence</td>
<td>.143</td>
<td>.495</td>
<td>7.331</td>
<td>53.737**</td>
<td>.382</td>
</tr>
<tr>
<td>Self-Determination</td>
<td>.061</td>
<td>.396</td>
<td>4.640</td>
<td>21.531**</td>
<td>.253</td>
</tr>
<tr>
<td>Impact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(2) Predictors (Constant)=PE Domains DV=NOC **P. ≤ 0.001

<table>
<thead>
<tr>
<th>Model</th>
<th>Adj. R2</th>
<th>B</th>
<th>t. Value</th>
<th>F</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>.002</td>
<td>-.115</td>
<td>-1.236</td>
<td>1.527</td>
<td>-.069</td>
</tr>
<tr>
<td>Meaning</td>
<td>-.002</td>
<td>-.049</td>
<td>-.583</td>
<td>.339</td>
<td>-.033</td>
</tr>
<tr>
<td>Competence</td>
<td>-.003</td>
<td>-.011</td>
<td>-.183</td>
<td>.034</td>
<td>-.01</td>
</tr>
<tr>
<td>Self-Determination</td>
<td>.014</td>
<td>.169</td>
<td>2.364</td>
<td>5.587</td>
<td>.132</td>
</tr>
<tr>
<td>Impact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(3) Predictors (Constant)= PE Domains DV=COC

<table>
<thead>
<tr>
<th>Model</th>
<th>Adj. R2</th>
<th>B</th>
<th>t. Value</th>
<th>F</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>.024</td>
<td>.697</td>
<td>2.947</td>
<td>8.683*</td>
<td>.164</td>
</tr>
<tr>
<td>Meaning</td>
<td>.031</td>
<td>.705</td>
<td>3.312</td>
<td>10.97**</td>
<td>.183</td>
</tr>
<tr>
<td>Competence</td>
<td>.127</td>
<td>.987</td>
<td>6.868</td>
<td>47.168**</td>
<td>.361</td>
</tr>
<tr>
<td>Self Determination</td>
<td>.06</td>
<td>.831</td>
<td>4.613</td>
<td>21.283**</td>
<td>.252</td>
</tr>
<tr>
<td>Impact</td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

(4) Predictors (Constant)=PEDomains DV=TOC*P. ≤ 0.01 **P. ≤ 0.001

<table>
<thead>
<tr>
<th>Model</th>
<th>Adj. R2</th>
<th>B</th>
<th>t. Value</th>
<th>F</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>.124</td>
<td>.432</td>
<td>6.772</td>
<td>45.896**</td>
<td>.357</td>
</tr>
<tr>
<td>Total Psychological Empowerment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(5) Predictor (Constant)=TPE DV=TOC**P. ≤ 0.001

<table>
<thead>
<tr>
<th>Model</th>
<th>Adj. R2</th>
<th>B</th>
<th>t. Value</th>
<th>F</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>.122</td>
<td>.191</td>
<td>6.703</td>
<td>44.926**</td>
<td>.353</td>
</tr>
<tr>
<td>Total Psychological Empowerment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(6) Predictor (Constant)=TPE DV=AOC **P. ≤ 0.001

<table>
<thead>
<tr>
<th>Model</th>
<th>Adj. R2</th>
<th>B</th>
<th>t. Value</th>
<th>F</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>.163</td>
<td>.234</td>
<td>7.921</td>
<td>62.635**</td>
<td>.408</td>
</tr>
<tr>
<td>Total Psychological Empowerment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(7) Predictor (Constant)=TPE DV=NOC**P. ≤ 0.001

<table>
<thead>
<tr>
<th>Model</th>
<th>Adj. R2</th>
<th>B</th>
<th>t. Value</th>
<th>F</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>-.003</td>
<td>-.006</td>
<td>.244</td>
<td>.06</td>
<td>-.014</td>
</tr>
<tr>
<td>Total Psychological Empowerment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(8) Predictor (Constant)=TPE DV=CO

PE=Psychological Empowerment DV=Dependent Variable AOC=Affective Organizational Commitment NOC=Normative Organizational Commitment COC=Continuous Organizational Commitment TPE=Total Psychological Empowerment TOC=Total Organizational Commitment
Table (2) demonstrates that Psychological Empowerment as a total and its dimensions are significantly correlated with Organizational Commitment as a total and its dimensions at different levels except with Continuous Organizational Commitment.

**Discussion**

Empowerment gives a power of decision-making to employees, making employees own their work and thus increase commitment. When employees have high levels of perceived Psychological Empowerment, view their work as meaningful, acquire the skills and competencies needed, have the impact on actions in their work environment, provide a high level of autonomy, feel to influence the work outcomes, they perceive themselves as effective, see their work as important, and be more attached to their organizations(25,26). In line with many previous studies(9,27,28,29,30) and inconsistent with others(31), nurses’ Psychological Empowerment means as a total and as all dimensions in the present study are, they are higher than means of score of the scale, which indicates that nurses’ values and behaviors were, to some extent, in an accordance with the work goals and nurses’ capabilities and requirements of work, therefore, it must lead to increasing Organizational Commitment.

Organizational Commitment means in the present study were below the means of score of the scale, that means nurses were less loyal to their hospitals, also it reflects that they can leave their hospitals even they find a good offer elsewhere. A previous study found in their study that the means of Organizational commitment were less than that of the present study(32).

The results of the present study show a contradiction referring to the assumption that high Psychological empowerment must lead to high Organizational commitment for Psychological Empowerment means are higher than that the means of score of scale, whereas, the means of Organizational Commitment are below than that the means of score of scale*, in Iraqi health care agencies, may be due to the presence of hidden or unobvious factors so that the nurse possesses or enjoys the psychological empowerment without good affiliation to the healthcare agencies. These may be due to the inability of the health care agencies to meet the needs of the nurse while he/she is committed to staying in the work because of salary and wedges obtained, poor professional relationships between the nurse and other medical and paramedical colleagues, the legislation that limit the nursing role in carrying out the physicians’ orders without any nursing notes (in most settings) and any meetings corresponding the patient’s status, supremacy of physician on the health care team, cynicism from other medical and paramedical staff and verbal and physical violence and so on.

Total Psychological Empowerment indicated significant positive relationships with TOC, AOC, and NOC as 0.357, 0.353 and 0.408 respectively. The coefficient of determination ($R^2$) presents that PE predicted 12.4%, 12.2% and 16.3% of the variation in TOC, AOC, and NOC respectively. All dimensions of PE indicated significant positive relationships with TOC, AOC, and NOC at different levels, while the best was for self-determination. In line with these results were the findings of many previous studies(6,13,33,34,35).

As long as Meaning is a sense of the meaningfulness of work among employees, it must provide a suitable fit between the requirements and purposes of one’s organizational work roles and one’s personal value system, and this must lead to the creation of attachment with the work. In the present study, it is significant and positively related to AOC, NOC, and TOC as 0.156, 0.11 and 0.164 respectively, while it hadn’t any significant relation with COC. The coefficient of determination ($R^2$) presents that Meaning predicted the variation of AOC, NOC, and TOC as 2.1%, 6.2% and 2.4% respectively (Table- 2). These results are in line with the result of many previous studies(9,30, 35) for they indicated that Meaning dimension was the best significant correlation with OC. Meanwhile, another study demonstrated that Meaning was significantly influenced the AOC, and COC dimensions(13).

**Conclusions**

* Nurses possessed good Psychological empowerment.

* Nurses possessed weak organizational commitment.

* There were significant relations among all Psychological empowerment domains and two dimensions of Organizational commitment (Affective and Normative).

**Conflict of Interest:** Non

**Source of Fundings:** Non
Ethical Clearance: This research was carried out with the patient’s verbal and analytical approval before the sample was taken.

References
19. Choi S, Tran T, and Park B; Inclusive Leadership and Work Engagement: Mediating Roles of Affective Organizational Commitment and Creativity, Social Behavior and Personality: An International Journal,


The Position Medical Forensics and Visum Et Repertum in Adultery (Overspel) Cases in the Jenang Kutei Traditional Trial in Bengkulu, Indonesia

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¹Muhammadiyah University of Bengkulu, ²Muhammadiyah University of Surakarta, ³Muhammadiyah University of Ponorogo

Abstract

Proving is the action of presenting legally valid evidences by the Rajang Lebong customary judge to inspect cases of adultery (overspel), to achieve the truth according to the forensic science. This research uses a sosiological method with a descriptive-analysis approach regarding the forensics’ expert information and the position of visum et repertum in customary trials in the case of adultery. The aim of this research is to understand the real position of forensic experts and visum et repertum in the Rejang Lebong traditional trials. This research shows that the position of the forensics’ expert information of evidences and visum et repertum are accepted as proof in overspel cases.

Keywords: medical forensics, visum et repertum, adultery (overspel) cases

Introduction

Recognition towards customary law has just been explicitly expressed in the second amendment of the 1945 Constitution article 18B paragraph (2).¹ According to Soerojo Wignjodipoero, the sustainability of customary law is maintained by the decisions of the indigenous people, particularly the ones from the authoratives who are in charge of the implementation of legal actions.²

Ethnic customary law is an indigenous law which is applied to certain communities or some particular ethnic communities. While in Islamic law, it is acceptable for customary law to be applied based on the belief of the people. Oji continued that the implementation of the legal dualism was basically influenced by the British colonial law.³ So, it other words, the image of Indonesians was formed and built from the environmental image of inigenous people.⁴

In the Rejang Lebong community, customary issues are usually resolve through a traditional institution known as the Jenang Kutei Customary Institution. Aside from issuing regulations concerning Jenang Kutei court, the Rejang Lebong district government also issued regulations regarding law enforcement from Jenang Kutei, which are the village judges and other customary officials.

This can be referred into Regent Decree No. 152 of 2009 concerning the Appointment of the Village Consultative Body/Badan Musyawarat Adat (BMA) in Rejang Lebong Regency during 2009-2012 term. Aside from appointing Indigenous People Members, the Rejang Lebong Regency Government appoints a customary Judge through the Regent Decree No. 153 of 2009 concerning the Appointment of Jenang Kutei (Village Judges) in Rejang Lebong Regency in the 2009-2012 Service Period.

Problems solved in Jenang Kutei are ranged from mild to moderate criminal cases, such as theft, fights, to adultery and cases involving other civil matters. Regarding the adultery or overspel cases, according to the custom, in its settlement at the Jenang Kutei institution, the perpetrators will usually be chastised with the purgatorial ritual.

Rape is a case which is rather confounding to prove despite the whole amount of evidences which have been
examined and collected. In order to gain legal proof that a rape has indeed been committed, Forensic Medicine holds a major role in conducting examination for the medical explanation regarding the said occurrence. This study aims to determine the management of Forensic Medicine in proving rape cases in Jenang Kutei customary trial in Rejang Lebong, Bengkulu. Based on these issues, the writer would like to examine the role of Forensic Medicine and forensic expert explanation in proving cases of sexual violence crimes in Jenang Kutei customary trial in Rejang Lebong, Bengkulu, Indonesia.

**Methods**

This research is based on legal research which is conducted with a qualitative non-doctrinal approach. The research typology which is recently known as socio-legal research. The aim of using this approach for this research is to see the relationship between legal factors and extra-legal factors related to the object under study.

**Findings and Discussion**

Orientation towards Harmony Values and processes which are abundant of Discussion Values and Kinship Values are the real representation of the local community. Karolus mentioned that for the indigenous people, they instead looked at the issue of peace as an inseparable part of the dispute resolution process (the judicial process).

The existence of Jenang Kutei as a customary institution and Kelpeak Ukum Adat Ngen Riyan Ca’o Kutei Jang as a guideline for the Rejang tribe community, repeating what Von Savigny as Volkgeist or a nationalist. Law is a reflection of Volkgeist which is a human struggle with space and time. Volkgeist is an immanent, contextual, and local concept.

As for those who are authorized to impose sanctions are Jenang Kutei (Peace Institution), which is a customary institution with special authority by indigenous people to resolve any issue of customary rules and/or customary law violation and is decided by a customary judge.

In Kelpeak Ukum Adat Ngen Riyan Ca’o Kutei, there are several “cepalo” (actions and words of someone who violates customary rules) related to overspel cases, as well as the provisions which, if violated, may be imposed for sanctions, which includes:

- **Menebo** (a guy lures a girl on a runaway without clear motive and nowhere to go, under presumption they have committed something which are prohibited by their religion and custom).

Regarding legal culture, Lawrence M. Friedman interpreted it as values, ideas, attitudes, and behaviours related to law. Because of the complexity of the matters regarding judges and the court, using merely legal sociology and legal anthropology is not sufficient for the theoretical framework, hence the forensic science involvement.

Forensic in Indonesia is related to the court and in law may be interpreted as the result of the examination required in proceedings in court. Forensic science is a science related to crime or in other words, it has roles in solving criminal cases. The sciences related to forensic include forensic medicine, forensic chemistry, forensic physics, criminology, forensic psychology, and neurology. Forensic roots from the word forum which means comprehending the main problem of the object of study and forum itself is a meeting area in the era of ancient Rome for a trial.

In the Rejang Lebong community, the solving violations of Adultery Law has relied on witnesses, particularly those who bust and capture overspel perpetrators. As the time goes by, the customary judge of Jenang Kutei has conditioned in receiving forensic elements which is a multidisciplinary science to apply biology, chemistry, psychology, medicine and criminology to verify the overspel case by examining physical evidences in overspel cases.

After being proven guilty in customary law for overspel case (NGA’EM), the convicted person will be sentenced as follows: If the victim is pregnant, the sentence will be conducted after the child is born (approximately after 40 (forty days) then KUTEI will be sanctioned). But if the overspel case is known by the community immediately, KUTEI will be instantly sanctioned upon them.

Former Chair of the RL District Customary Council/Badan Musyawarah Adat (BMA), A Rauf said, Rejang custom is a spiritual treasure which has to be preserved and developed or better known by the term “Adat Nak Beak Nioa Pinang”. Related to the technological developments, the evidence via forensic medicine, expert statements and visum et repertum are accepted in Jenang Kutei customary trial for overspel cases, rape cases, etc.
Rauf also explained that through the said evidences, if the perpetrators are proven guilty in committing serious cepalo such as an overspel, they are required to sacrifice a goat. The said punishment is known as a purgatorial ritual called Empat Petulai in order to purify the village which commences by sacrificing a goat.\(^{(13)}\)

He admitted that the forensic medicine and visum et repertum procedure fields are used as legal basis in resolving cases which occur within society is because customary law does not stand against the development of technology or the modernity. The relationship between the existence of customary law alongside with its effectiveness will make customary law acknowledge verification methods in accordance to science and technology. In other words, the effectiveness of customary law may allow the law to achieve its goal, which includes for it to be applied and functioned through the help of forensic science and expert statements\(^{(14)}\).

Regarding overspel and rape cases, judge conviction in adjudicating in customary law is based on evidences at the trial. And via visum and forensic science, evidences related to human physical conditions are also acknowledged to be present at the trial. Customary judges admit that customary law requires assistance from other scientific disciplines, especially related to injury, health and lives of people in overspel cases. And forensic doctors may prove those conditions more precisely as the evidence.

The verification with the help of forensic doctor as witness at the trial in the form of visum et repertum written statement which explains the bodily condition of the overspel (adultery) defendant, concerning semen, bodily injuries, or sexual activity traces, and based on these then the Judge of Jenang Kutei remained guided by Kelpeak Ukum Adat Ngen Riyan Cao Kutei Jang passes his sentence. If there is a man who committed adultery (overspel perpetrators), the man is subject to customary punishment/customary fines namely:

1. Mencuci Desa (cleansing the village)/menepung matahari (pulverizing the sun). This should not be carried out at night\(^{(15)}\). Menepung matahari means paying the greatest village fines which is the same as murder and adding 2. Fines from the woman according to her demands which the man affords to pay. 3. The perpetrators are flogged with sticks as many as 100 strands and flogged 10 times\(^{(16)}\).

In this research, the author took an example of the medical forensic implementation towards the case of adultery which caused extramarital pregnancy but the man didn’t take the responsibility, namely Warni binti (the daughter of) Ujang Damori from Karang Jaya village, Bermani Ulu sub-district, in the case of overspel. The examination result from the forensic expert who was asked for the statement and the examination, finally proved the pregnancy even though she wasn’t legally married so that the customary judge of Jenang Kutei sentenced her guilty for committing overspel. There was the verification of injury on the genital caused by blunt object and the semen was identical with that of the male defendant. Hence, the judge dropped the sanction in the form of a customary ceremony with the decision that cleansing-the-village ceremony must be carried out and it was required to slaughter a goat. But according to the provisions, when the goat-slaughtering ceremony was carried out, the goat’s blood must be sprinkled to 4 (four) corners of the village and the two perpetrators would be hit by sticks with as many as 100 strands.

Additional punishment imposed on overspel perpetrators who are proved committing adultery, is parading them around the village as an example. It is an extra penalty for those overspel perpetrators. The offender usually obeys the punishment imposed because the evidence is strong and social sanctions await if the decision is not obeyed. The punishment for non-compliant families in carrying out the customary sanction is that the family will be given an excommunication punishment, in which according to Kadirman, their interactions will be socially restricted.\(^{(17)}\)

Although the customary law and sanctions must still be carried out with the customary philosophy, but in this case it is known that in handling the verification it has also been received and acknowledged by other sciences, such as medical forensic. The perpetrators of adultery who have been through the procession of customary trial so that it comes to the punishment execution turn out that they also went through the forensic stages, through visum et repertum, sperm and seminal examination added with the testimony of forensic doctor. The sentencing, of course, cannot be ruled out because the evidence is strong enough and the customary trial has clear legitimacy in the community. The overspel perpetrators, of course, are underestimated and even laughed at by the people for the deeds they have done, and this will usually continue to be practiced to the newborn child later on because the child will usually necessarily be called “kampang”
The punishment given is still a punishment with a customary philosophy even though the customary law has received forensic verification. The philosophy is the belief that punishment is given so that the local residents are saved from disaster. Such disasters are for instance, they will have difficulty in earning a living, there will be a prolonged famine, the atmosphere of the community will continue to heat because many residents quarrel with each other, floods and even other unexpected disasters as a form of anger from the God Almighty.\(^{18}\)

The customary trial realizes that the customary trial procession is not merely handled by traditional aspects, but it is also handled with the new elements in proving the criminal overspel cases to make it clearer\(^{19}\). The trial process through the assistance is able to handle the verification to the end of the case by the perpetrators based on bodily conditions examined by a forensic doctor. According to General Chief of Rejang Lebong BMA, Herman Firnadi, it is undeniable, so that the perpetrators will want to admit their crime, receive their punishment and are deterrent after serving the punishments so as to set an example for others not to repeat the same thing.\(^{20}\)

After going through the process of forensic verification and sentencing, they hold the village cleansing ceremony for the overspel defendant which was carried out in the traditional institution attended by many people. The offender certainly cannot avoid admitting his actions, considering that the evidence is very strong and cannot be refuted despite the embarrassment as conveyed by Susi Damayanti:\(^{21}\)

\[\text{“We who only saw are impressed with the trial process that has become more modern so that the defendant is not possible to evade with the existence of forensic evidences and the judge pass the sentence seeing the evidences.”}\]

**Conclusion**

Medical forensic can be accepted as an evidence in handling overspel cases at the Jenang Kutei customary trial in Rejang Lebong in the form of visum et repertum examination and forensic expert’s statement as in the general trial. The perpetrators who are proved guilty with the forensic verification cannot evade because the proof is modern, logical and strong as well as acknowledged by the parties in the form of forensic expert’s statement and the result of visum et repertum examination. The trial passes the verdict based on these proofs and then sentence them in accordance with the applicable customary law.

**Conflict of Interest-** No

**Source of Funding-** Authors

**Ethical Clearance-** Yes

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15. In a conversation with Herman Firnadi, General Chairperson of BMA Rejang Lebong Regency (23 November 2017, 11.20 a.m.)

16. In a conversation with Kadirman, Former Leader of BMA (June 2017, 01:40 p.m.).

17. According to Ahmad Faizir, the term “Adultery” is usually called as cleaning the village, as for the term “Adultery” get called as tempung mata bilai is the term usually used by Kadirman. In a conversation with Ahmad Faizir, Board Supervisor of BMA (1 May 2018).

18. In a conversation with Kadirman, Former Leader of BMA (20 January 2017, 03:25 p.m.).


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Evaluation Efficacy of Clarithromycin and Levofloxacin in the Eradication of Helicobacter Pylori (H.P) Infected Iraqi Patients at Al-Yarmouk Teaching Hospital

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Abstract

Helicobacter pylori (H.P) infection is an increasing concern in modern medicine due to its effect on the pathogenesis and management of peptic ulcer disease and gastritis and its possible link to gastric adeno carcinoma cases. The eradication of HP is a mandatory approach in documented cases of HP infection mainly gastric and duodenal ulcers. The treatment options is variable globally according to microbial susceptibility test and local guidelines. This study focused on two main options in the eradication of HP and found that levofloxacin based therapy is superior to clarithromycin based approach in the rate of eradication , with comparable rate of adverse events.

Keywords: Helicobacter pylori (H.P), Gastritis, Peptic ulcer, Urea breath test, Stool antigen, Clarithromycin, Levofloxacin

Introduction

In world about 50% of population , who have Helicobacter pylori (HP) that colonizing in , probably for life unless eradicated by anti-HP treatment. Colonization of HP concert main risk factor of peptic ulcer disease, also gastric mucosa associated lymphoid tissue (MALT) lymphoma gastric adenocarcinoma . Eradication of HP has revolutionized the treatment of peptic ulcer disease, and Treatment is permanent in most cases afterwards. Anti-HP therapy also represents first-line management for low-grade gastric MALT lymphoma cases. Treatment of HP is not recommended as a line of treatment of gastric adenocarcinoma, but preventing HP colonization or eradication may prevent gastric malignancy. In same time , increasing evidence suggests that lifetime HP colonization may give some protective effect against esophageal adenocarcinoma.

In most parts of the United States , prevalence of HP among adults is less than 30% , whilst in developed countries more than 80% (1).Humans are the only host of HP. Children may acquire the HP from their parents or their primary caregiver or other children essentially . Transport usually occurs either through the oral - stool or the oral-oral pathway , but HP is easily cultured from vomit us and gastroesophageal reflux fluid and to a lesser extent from stool (2,3)

Most of colonized people are healthy and asymptomatic, and only a fraction develop HP related illnesses. About 90% of patients with duodenal ulcers and 70% of gastric ulcer are infected with HP. The distribution and severity of HP–induced gastritis determine the future outcome. In most colonized patients, HP causes a mild pangastritis with little effect on stomach acidity environment and the majority develop no significant clinical outcomes.

In a minority , the infection causes an atrnal-predominant gastritis characterized by hypergastrinaemia and overt acid production by parietal cells and may end with duodenal ulceration. In a much smaller number of infected people(4), HP causes a corpus-predominant gastritis resulting in atrophy of the gastric mucosa and hypochlorhydria. The hypochlorhydria allows other bacteria to proliferate in the stomach; these other bacteria will result in chronic inflammation and produce mutagenic nitrites from dietary nitrates, predisposing to the development of gastric malignancies. The ulcer probably result as an end point of impaired mucusal defense resulting from a combination of HP infection;
smoking and NSAIDs, rather than highly acidic environment (4).

Patient and methods

This study was a single center open label randomized clinical trial to compare the efficacy of levofloxacin versus clarithromycin in the eradication of HP infection.

This study was a randomized, open-label randomized clinical trial to compare the efficacy of both clarithromycin and levofloxacin in the elimination of HP infection.

The study conducted in the outpatient internal medicine clinic at Al-Yarmouk teaching hospital during period (January 2018- January 2019) involving 130 patients. In clarithromycin group two patients was lost to follow-up and excluded and in the levofloxacin group, three patients were lost to follow-up and thus, were excluded, so the study actually covered 125 patient (52 female and 73 male), age patients from (19 to 59) years old.

Consent had been explained and obtained from the patients under supervision of ethical committee at Department of medicine at Al-Yarmouk teaching hospital.

Inclusion criteria for this study were as follows: diagnose patients have HP infection depending on history and clinical examination then confirmed by either stool antigen detection of HP or urea breath test or endoscopy and histopathology analysis.

Exclusion criteria for this study were as follows: pregnancy, previous use of clarithromycin and levofloxacin in the preceding month, consumption of alcohol or substancemisuse; advanced chronic gastrointestinal disease cirrhosis, gastric cancer, inflammatory bowel disease, cerebrovascular accidents, advance renal insufficiency, advance heart failure and severe debilitating illnesses.

Then the patient had been distributed randomly to be treated with either Clarithromycin based therapy (clarithromycin 500mg twice daily, metronidazole (500mg)twice daily and lansoprazol (30mg)twice ) or Levofloxacin based therapy (levofloxacin (500mg) twice daily, metronidazole (500mg)twice daily, and lansoprazole (30mg)twice daily) for a period between 10-14 days.

In this study we decided to replace amoxicillin; which is usually given with levofloxacin, with metronidazole in levofloxacin group for both reasons:

1- High rate of allergy to penicillins in Iraqi society.

2- Combination of metronidazole with levofloxacin is a new strategy to be evaluated for the future newer combinations in eradication of Hp.

At the end point (2) groups were compared with respect to proportion of HP eradication with either stool antigen or urea breathe test 45 days or more thereafter, then the results had been documented.

Any significant side effect of therapy had been documented and the patient followed up for any serious adverse events, discontinuation, or drug non-compliance.

Diagnosis

The current options available for diagnosis of HP infection are listed below:

- Invasive:
  1- Biopsy urease test
  2- histopathology
  3- Microbial culture

- Non Invasive:
  1- Serology
  2- Urea breath test
  3- Stool antigen testing

Breath tests or faecal antigen tests are best because of simplicity; accuracy and non-invasion (4).

Statistical Analysis

Analysis of data was carried out using the available statistical package of SPSS-25. Significance of difference of different percentages (qualitative data) were tested using Chi-square test ($\chi^2$-test) with application of Yate’s correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.
Finding

Current regimens consist of a Proton pump inhibitors (PPI) and (2 or 3) antimicrobial agents were given for (10-14) days(Table-1). The optimal regimens vary geographically, depending on the known rates of primary antibiotic resistance in most H. pylori strains locally, therefore, guidelines on optimal regimens for HP eradication in individual countries are evolving, and physicians should select the most up-to-date local guideline.

| Table (1): Commonly Recommendation Treatment systems for H.Pylori |
|--------------------------|--------------------------|--------------------------|--------------------------|
| **Regimen (Duration in days )** | **DRUG 1** | **DRUG2** | **DRUG3** | **DRUG4** |
| Regimen 1 (10-14) | Lansoprazole (30) mg bid | Clarithromycin (500) mg bid | Metronidazole (500) mg bid | - |
| Regimen 2 (10-14) | Lansoprazole (30) mg bid | Clarithromycin (500) mg bid | Amoxicillin (1g) bid | - |
| Regimen 3 (10-14) | Lansoprazole (30) mg bid | Bismuth subsalicylate (2) tabs qid | TertracyclineHCl (500) mg qid | Metronidazole (500) mg tid |
| Regimen 4 : concomitant 14 days | Lansoprazole (30) mg bid | Amoxicillin (1)g. bid | Clarithromycin (500)mg bid | Tinidazole (500)mg bid |
| Regimen 5 :10 days | Lansoprazole (30) mg bid | Amoxicillin (1) g bid | Levofloxacin (500) mg bid | - |

Two elements as most important in successful HP eradication is the patient’s commitment to the system and the use of drugs that the patient’s HP strain has not acquired.

Increasing levels of primary HP resistance to clarithromycin, levofloxacin are of growing concern(5). In most parts of the world (the main exception being northwestern Europe), the rate of primary clarithromycin resistance is high enough that regimens containing only clarithromycin plus one antibiotic often fail; regimens with clarithromycin and two other antibiotics remain superior as the other two antibiotics are likely to eradicate HP even if the strain is resistant to clarithromycin. When a patient is known to have been exposed—even distantly—to clarithromycin or levofloxacin, these two drugs should be avoided. Resistance to amoxicillin or tetracycline is unusual, even if these antibiotics have been given previously, and metronidazole resistance is only partial; thus there is no need to avoid using these antibiotics even if they have been previously used. Assessment of antibiotic susceptibilities before treatment is not usually done because endoscopy and mucus biopsy are necessary to obtain HP culture and because most microbiological laboratories lack experience in HP culture. If initial anti HP treatment fails, the usual approach is re-treatment with another antibiotic regimen(table-1).

The third-line ideally should include endoscopy; biopsy; culture as well as treatment based on antibiotic sensitivity. However, empirical third-line therapies can be applied(6).

From the total 69 patients who had been treated with Clarithromycin based therapy only 34 patient (49%) respond and failure rate is approximately (51%) 35 patients. While those who had been treated Levofloxacin based therapy 56 patients; 40 patients (71%) had respond, while failure rate is about (29%) 16 patients (table-2).
Table (2): Compare regarding success rate of therapy

<table>
<thead>
<tr>
<th></th>
<th>Clarithromycin based: 69(55%)</th>
<th>Levofoxacin based: 56(45%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respond</td>
<td>34 (49%)</td>
<td>40 (71%)</td>
</tr>
<tr>
<td>Failure</td>
<td>35 (51%)</td>
<td>16 (29%)</td>
</tr>
</tbody>
</table>

Total patient number : 125

For Response; P=0.012* Significant difference in proportions using Pearson Chi-square test at 0.05 level

![Figure (1): Incidence of anti HP therapy adverse effects.](image)

Incidence of minor adverse effect in both therapies is also reported; 19 cases (27.5%) in clarithromycin group and 14 cases (25%) in levofloxacine group. Diarrhea is the most documented side effect of therapy in both groups which happens in 8 cases of clarithromycin based therapy and 5 cases of levofloxacine lines, metallic taste also reported frequently but it may be due to metronidazole mainly other adverse effects had been reported in lower incidence rate but none of them resulted in discontinuation of therapy (table- 3).

Table (3): Adverse events of anti HP treatment in both study groups

<table>
<thead>
<tr>
<th>Study group</th>
<th>Clarithromycin based group</th>
<th>Levofoxacin based group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>8 (11.6%)</td>
<td>5 (8.9%)</td>
</tr>
<tr>
<td>Metallic taste</td>
<td>7 (10%)</td>
<td>6 (10.7%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (4.3%)</td>
<td>3 (5.3%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5 (7.2%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>2 (2.8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Headache</td>
<td>3 (4.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>2 (2.8%)</td>
<td>4 (7.1%)</td>
</tr>
<tr>
<td>Skin rash</td>
<td>1 (1.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19 (27.5%)*</td>
<td>14 (25%)*</td>
</tr>
</tbody>
</table>

*some cases develop more than one adverse events in the same time so the total is not necessarily equal the sum of total number of patients who develop adverse events in both groups.
Discussion

There were several findings in this study starting with comparing the efficacy of both line of management, there is significant difference in efficacy favoring levofloxacin based therapy as compared with clarithromycin which carry high failure rate exceeding 50% based line (P value 0.012). Also current study disclose that the rate of incidence of significant therapy adverse effect is almost equal in both lines; 25% for levofloxacin based therapy versus 27.5% for clarithromycin based therapy (P value 0.90).

If we compare that with the previous Iranian study regarding Comparison efficacy of both clarithromycin and levofloxacin in the eradication of Hp infection, levofloxacin-based therapy is preferable to clarithromycin-based regimen in HP eradication which achieved higher optimal rate of eradication less than 80% success rate (7), so the result of this study is almost comparable in which 71% was the success rate of levofloxacin group and 49% success rate in clarithromycin group compared with 80.4 and 57.4% for levofloxacin and clarithromycin group respectively (7).

This result may reflect the higher incidence of clarithromycin resistant strains in the population and that finding should be supported in the future by more extensive workup involving upper endoscopy and sampling for the microbiological evaluation (culture and antimicrobial sensitivity testing) or molecular based diagnostic assay for resistance detection. The incidence of adverse events with treatment had been reported in 19 cases with clarithromycin group and 14 in levofloxacin group but none of the resulted in termination of therapy but may result in discomfort and anxiety to the patient because most of these adverse events involve gastrointestinal system which is the host of HP.

We have to point that this study is a clinical trial depend mainly on the clinical response to therapy and the confirmation of eradication of HP with breath test or stool antigen, so it didn’t include microbiological antimicrobial sensitivity test so it didn’t necessarily suggest the real spectrum of bacterial resistance. Also the study is a single center study and more wide multicenter trial is needed for more clarification of the real incidence of HP clarithromycin resistance in the population.

Conclusions: Levofloxacian based therapy is superior to clarithromycin based therapy in eradication of HP infection. Both lines have comparable incidence of adverse events.

Recommendations: We recommend to depend on levofloxacian based triple therapy at least first alternative option if first line fails in eradication of HP cases in Iraq as it is effective, with accepted adverse events profile. Also we recommend against the use of clarithromycin based therapy in the management in new cases of HP infection due to high failure rate. Thorough antimicrobial sensitivity evaluation of emerging HP strains should be considered for determining local anti HP treatment guidelines.

Conflict of Interest: None

Source of Findings: Self

Ethical Clearance: None

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Job Satisfaction and Relationship with Organizational Commitment among Nurses

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Abstract

Background- Nurses’ job satisfaction is one of the most important factors affecting organizational commitment. Aim- The study aimed to identify the relationship between job satisfaction and Organizational Commitment among nurses. Methods and Materials- A descriptive, correlational and cross-sectional study design carried out between (5th/September/2017 till 30th of May/2018) at five hospitals in Mosul/Iraq. Depending on the convenience sampling method and Non-probability sampling technique, a sample of (317) nurses were selected. Two instruments were used to collect data via an interview method with each subject (Minnesota Job Satisfaction Questionnaire “JS” and Organizational Commitment Questionnaire “OC”). To measure the content validity of the questionnaires, they exposed to five experts in the nursing field, whereas, the reliability of them was checked by Cronbach’s alpha. The results of the study were demonstrated by descriptive statistic through (mean standard and deviation), and by inferential statistic through (stepwise regression). Findings- Total JS and its domains had significant relationships with total OC and its Affective and Normative domains, while they hadn’t any significant relationships with total OC.

Keywords: Job Satisfaction, Organizational Commitment, Nurse.

Introduction

There are many factors affecting any work, one of them is Job Satisfaction, which required deep understanding in order to handle appropriately by employees, motivating and eliminating any disturbing factor(1). Great competition and scarcer resources are confronting hospitals nowadays than ever before, in addition to the external and internal environment that are severely challenging to achieve its goals effectively and efficiently(2).

The employees consider the repository of knowledge, abilities, and skills that competitors find extreme difficulty for imitating(3). Nurses, as professionals, constitute a large group of them, who are responsible for determining and providing health care in good quality and less cost(4). They have the diverse tasks to perform, many roles to carry out, and profound experience needed to deal with many duties in many places in hospitals, so their role is very important and vital in hospitals(5). Many situations and factors make nurses stressful, as a climate of job uncertainty along with high organizational demands, in addition to the factors mentioned above which in turn have a significant impact on the organizational success(6).

Job satisfaction has a long-standing tradition in organizational research(7). It is defined as an individual’s affective reaction or cognitive attitude toward job; it is an extent to which individuals like or dislike their job(4,8,9). It can result from organizational climate together with individual’s perception and evaluation of a job, which is influenced by the individual’s unique circumstances, such as needs, values, and expectations(10,11). In addition to workload, incentives, job security and relationship with superiors, peer and organizational structure(6). Job Satisfaction is interrelated with emotions, and because of that, it affects organization as a whole productivity(12), efficiency(13), job performance, mental and physical health(14), fluctuation and absenteeism(15), also it affects the health of their personal relationships outside of work.
Later research has shown that Job Satisfaction can be arranged according to two dimensions: an Intrinsic versus an Extrinsic one. Extrinsic satisfaction refers to satisfaction with aspects that have little to do with the job tasks or content of the work itself, such as pay, working conditions, and co-workers. Intrinsic satisfaction refers to the job tasks themselves “e.g. variety, skill utilization, autonomy”(6).

Researchers have been investigating the relationship between employees and their employing organization for decades since they are aware of the importance of employees, who are the driving force of every organization(18). In the healthcare environment where nurses’ shortage is expected to increase beyond its current levels, a committed workforce must be maintained. Committed nurses provide a meaningful value of stable, professional workforce; lower employee recruiting; development and training costs; retention of nurses with knowledge; abilities and skills that are crucial to organizational success; improved organizational image within the community; in addition to that, a committed workforce and their loyalty to the organization(1).

Organizational commitment is the frame of employees’ mind to commit to facilitating the achievement of the objectives of the organization and includes levels of staff identification, participation, and loyalty. It is an emotional response driven by employee attitudes, belief, and behavior. There are three types of organizational commitment: Affective (Emotional), Continuance, and Normative. Emotional commitment (or moral obligation) occurs when employees cling to the organizational values and goals, be emotionally attached to their organization; and begin to feel responsible for the success of their organization(19). Continuance commitment occurs when the basis for staff participation with the organization is their related revenues, efforts, and costs. It describes as a kind of emotional attachment to the organization, it refers to the judgment of employees about whether spending on leaving the organization is greater than spending on continuing work(20), while feelings of the employees’ toward the organization call normative commitment(20,21).

The present study aimed to identify the relationship between job Satisfaction, and Organizational Commitment among nurses.

Material and methods

Descriptive, correlational and cross-sectional study design carried out between (5th/ September 2017 throughout 30th/ May 2018). Five hospitals in Mosul / Iraq were the setting of the study, they were pointed as A, B, C, D, and D. Depending on convenience sampling method and non-probability sampling technique, sample of (317) nurses selected based on inclusion criteria as; working at patient’s bedside, having working work ≥ 5 years, and present in the ward in the study period. An interview was depended for gathering data- each of them lasted “30-40” minutes-by using two tools; Minnesota Job Satisfaction questionnaire “JS” of two aspects; Intrinsic Job Satisfaction “IJS” (12 items) and Extrinsic Job Satisfaction “EJS” (8 items)”(22), each item had five options rated from strongly dissatisfied=1 to strongly satisfied=5, the other tool was Organizational commitment “OC” of three aspects; Affective Organizational Commitment ‘AOC’ (six items), Normative Organizational Commitment ‘NOC’ (six items), and Continuance Organizational Commitment ‘COC’ (six items)(23), each item had five options rated from strongly disagree=1 to strongly agree=5, four items of the tool were reverse-keyed (labeled by “R” against each item). The means of the scales and their aspects obtained by summation of all scores and division of their numbers. Content validity of the scales measured by opinions and comments of five nursing experts, whereas the reliability checked through test-retest on fifteen nurses outside the final sample of the study, it resulted in a Cronbach’s alpha of 0.83 for Job satisfaction and 0.87 for Organizational Commitment. The findings of the study demonstrated through mean and standard deviation as a descriptive statistic for the measured variables (PS and OC), and through stepwise regression to evaluate the impact of the prediction variables on the outcome results. Informal approvals were obtained from the participants prior to starting of collection data.
Findings

Table (1): Descriptive Statistics of JS and OC and their Aspects:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean of Scores</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS (Total);</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrinsic</td>
<td>60</td>
<td>40</td>
<td>80</td>
<td>61.93</td>
<td>8.47</td>
</tr>
<tr>
<td>Extrinsic</td>
<td>36</td>
<td>26</td>
<td>50</td>
<td>38.14</td>
<td>5.31</td>
</tr>
<tr>
<td>OC (Total);</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affective</td>
<td>54</td>
<td>33</td>
<td>66</td>
<td>49.98</td>
<td>7.98</td>
</tr>
<tr>
<td>Normative</td>
<td>18</td>
<td>9</td>
<td>23</td>
<td>16.98</td>
<td>3.56</td>
</tr>
<tr>
<td>Continuous</td>
<td>18</td>
<td>8</td>
<td>26</td>
<td>16.55</td>
<td>3.78</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>10</td>
<td>25</td>
<td>16.45</td>
<td>3.09</td>
</tr>
</tbody>
</table>

Table (1) demonstrates that the total means of JS and their aspects were around the means of scores of the scale, while the total means of OC and its aspects were below the means of scores of the scale.

Table (2): Regression Findings for Organizational Commitment (OC) with respect to Job Satisfaction (JS)

<table>
<thead>
<tr>
<th>Model(1)</th>
<th>Adj. R²</th>
<th>B</th>
<th>t. Value</th>
<th>F.</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic</td>
<td>.149</td>
<td>.261</td>
<td>7.502</td>
<td>56.276**</td>
<td>.035</td>
</tr>
<tr>
<td>Extrinsic</td>
<td>.085</td>
<td>.260</td>
<td>5.514</td>
<td>30.408**</td>
<td>.297</td>
</tr>
</tbody>
</table>

(1) Predictors (Constant) = JS Domains DV = AOC ** P ≤ 0.001

<table>
<thead>
<tr>
<th>Model(2)</th>
<th>B</th>
<th>t. Value</th>
<th>F.</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic</td>
<td>.230</td>
<td>.343</td>
<td>9.760</td>
<td>95.248**</td>
</tr>
<tr>
<td>Extrinsic</td>
<td>.094</td>
<td>.290</td>
<td>5.811</td>
<td>33.773**</td>
</tr>
</tbody>
</table>

(2) Predictors (Constant) = JS Domains DV = NOC ** P ≤ 0.001 level.

<table>
<thead>
<tr>
<th>Model(3)</th>
<th>B</th>
<th>t. Value</th>
<th>F.</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic</td>
<td>.006</td>
<td>.057</td>
<td>1.740</td>
<td>3.027</td>
</tr>
<tr>
<td>Extrinsic</td>
<td>.000</td>
<td>.036</td>
<td>.835</td>
<td>.697</td>
</tr>
</tbody>
</table>

(3) Predictors (Constant) = JS Domains DV = COC

<table>
<thead>
<tr>
<th>Model(4)</th>
<th>B</th>
<th>t. Value</th>
<th>F.</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic</td>
<td>.191</td>
<td>.662</td>
<td>8.705</td>
<td>75.782**</td>
</tr>
<tr>
<td>Extrinsic</td>
<td>.086</td>
<td>.586</td>
<td>5.549</td>
<td>30.792**</td>
</tr>
</tbody>
</table>

(4) Predictors (Constant) = JS Domains DV = TOC ** P ≤ 0.001

<table>
<thead>
<tr>
<th>Model(5)</th>
<th>B</th>
<th>t. Value</th>
<th>F.</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Job Satisfaction</td>
<td>.173</td>
<td>.394</td>
<td>8.189</td>
<td>67.055**</td>
</tr>
</tbody>
</table>

(5) Predictor (Constant) = TJS DV = TOC ** P ≤ 0.001

<table>
<thead>
<tr>
<th>Model(6)</th>
<th>B</th>
<th>t. Value</th>
<th>F.</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Job Satisfaction</td>
<td>.149</td>
<td>.162</td>
<td>7.43</td>
<td>55.2**</td>
</tr>
</tbody>
</table>

(5) Predictor (Constant) = TJS DV = AOC ** P ≤ 0.001

Model(7)
Table (2) depicts that Job Satisfaction as a total and its aspects were significantly correlated with Organizational Commitment and its aspects at a high level, whereas they don’t indicate any level of significance with Continuous Organizational Commitment.

**Discussion**

When work roles don’t conflict with the individual’s values and beliefs, they tend to experience Job Satisfaction because the job itself generates an inner sense of motivation, also when the employees view their work as meaningful, acquiring skills and competencies, impacting the outcomes within their organization, they can derive satisfaction to work(15). Other previous studies noted that nurses’ job satisfaction is one of the most important factors affecting organizational commitment(24,25).

Job Satisfaction means in the present study has been around the means of scores of the scale, whereas Intrinsic Job Satisfaction is best than Extrinsic Job Satisfaction (Table-1). If nurses are satisfied with intrinsic factors more than extrinsic factors, they can satisfy themselves interiorly more than the satisfaction promote from their environment and supervisors. This finding is consistent with the view of many previous studies(1,6,). This is an important issue for any organization, for, individuals who have the high level of Job Satisfaction generally have the positive attitude towards their work and vice versa(4), in another word, if employees are dissatisfied and they see a chance to work in other organization, they will leave an organization without a sense of guilt(11). Organizational Commitment means in the present study were below the mean of scores of the scale (Table-1), this means that nurses were less loyalty to their hospitals, also it reflects that they can leave their hospitals even they find a good offer elsewhere. the ignore the nurse’s role and their participation in the patient care plan. Many previous studies found that the commitment of nurses was on high levels(26,27,28), other studies found that the commitment of nurses was at a moderate level(19,29,30,31), low level among midwives(32), NOC had the lowest level(26), and COC had the highest level, highest level, and AOC had the lowest level (27).

Total Job Satisfaction (TJS) is significant and positively correlated with Total Organizational Commitment (TOC) and its dimensions AOC, NOC as 0.419, 0.386 and 0.451 respectively, while it doesn’t correlate with COC at any level of significance. The coefficient of determination (R2) presents that TJS predicted the variation of TOC, AOC, and NOC as 17.3%, 14.9%, and 20.1% respectively. From another hand, all JS dimensions present significant and positively correlated with TOC and with its aspects except COC, while IJS was best than EJS in predicting the variation of AOC, NOC and TOC as 14.9%, 23%, and 19.1% respectively (Table-4). It was revealed that Job Satisfaction was a significant predictor of Organizational Commitment and explained 36% of the variation, also Job Satisfaction was highly positively correlated with Organizational Commitment “r = .85, p < .001”(33), another study referred that Organizational Commitment explained 41% of the variance in Job Satisfaction(6).

As a total and in line with the present result, many previous studies found that Job satisfaction had a significant positive influence on Organizational Commitment(25,28,34), also it was found a significant relationship between total job satisfaction and AOC, and between extrinsic job satisfaction with Organizational
Commitment(34,35), significant relationship between job satisfaction with NOC(35).

So previous study indicated that there was no statistically significant correlation between TJS and TOC, and there was no statistically significant association between IJS and TOC domains (AOC, NOC, and COC). Also, there was no statistically significant association between EJS and TOC domains (AOC, NOC, and COC) (36).

Conclusions

* The nurses had an acceptable level of Job satisfaction.
* The nurses had an unacceptable level of Organizational Commitment.
* There were positive significant relationships among TJS and its aspects with TOC and its aspects except for COC.

Conflict of Interest: Non

Source of Findings: Non

Ethical Clearance: This research was carried out with the patient’s verbal and analytical approval .

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