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تقيم كلية الطب / جامعة الموصل
تحت عنوان
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مؤتمر اليوبيسيل الذهبي
والدورة الثانية للاجابة العلمية
والدورة الستة الرابعة لأمراض القلب استضافت ابي سينا التعليمي
في فندق نينوى الدولي للفترة من 3-5 نيسان 2012
# Annals of the College of Medicine Mosul

## Volume 37  Number 1&2  December 2011

### Editorial Board

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Manuscripts, including tables, or illustrations are to be submitted in triplicate with a covering letter signed by all authors, to the Editorial Office, Annals of the College of Medicine Mosul, Iraq. All the submitted material should be type written on good quality paper with double spacing and adequate margins on the sides. Rigorous adherence to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" published by the International Committee of Medical Journals Editors in 1979 and revised in 1981 should be observed. Also studying the format of papers published in a previous issue of the Annals is strongly advised (Ann Coll Med Mosul 1988; 14:91-103).

Each part of the manuscript should begin in a new page, in the following order: title; abstract; actual text usually comprising a short relevant introduction, materials and methods or patients and methods, results, discussion; acknowledgement; references; tables; legends for illustrations. Number all pages consecutively on the top of right corner of each page, starting with title page as page 1. The title page should contain (1) the title of the paper; (2) first name, middle initial(s) and last name of each author; (3) name(s) and address(es) of institution(s) to which the work should be attributed. If one or more of the authors have changed their addresses, this should appear as foot notes with asterisks; (4) name and address of author to whom correspondence and reprint request should be addressed (if there are more than one author);(5) a short running head title of no more than 40 letters and spaces.

The second page should contain (1) title of the paper (but not the names and addresses of the authors); (2) a self contained and clear structured abstract representing all parts of the paper in no more than 200 words in Arabic and in English. The headings of the abstract include: objective, methods, results, and conclusion.

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Detection of vascular invasion in colorectal cancer by using Weigert's stain for elastic fibers

Shuaib H.S. Al-Talib, Wahda Mohammed Taib Al-Nuaimy, Rana A. Azooz
Department of Pathology, College of Medicine, University of Mosul.

Received: 6th May 2010; Accepted: 13th Dec 2010.

ABSTRACT

Objective: Venous invasion is stage independent prognostic risk factor for distant metastasis in colorectal cancer. Elastic stain is among the various ancillary techniques that were described to increase the sensitivity of detection of vascular invasion. This combined prospective and retrospective study is aimed to assess the sensitivity of elastic tissue stain to detect venous invasion in comparison to the routine H & E stained sections, and to correlate intramural and extramural venous invasion with the Dukes staging system.

Method: Serial sections from 42 cases of colorectal cancer diagnosed between the years 2007 and 2009 were examined by using H & E and Weigert's stain. A comparison was drawn between the two stains in regard to venous invasion; the results were correlated with Dukes stage for colorectal cancer.

Results: The mean age of sampled patients was 50 years. Venous invasion was detected in 16 (38%) cases by the use of H & E stain, which was increased to 35 (83%) cases by the use of Weigert's stain for elastic tissue with a clear statistical significance (P<0.001). Venous invasion was correlated with Dukes B & C cases with a P value of more than 0.05 & less than 0.01 respectively.

Conclusion: The use of elastic tissue stain on one tissue block increases significantly the frequency of detection of vascular invasion of colorectal cancer.

Keywords: Venous invasion, colorectal cancer, Weigert's stain, elastic fibers
The use of elastic tissue stains in microscopical assessment has been proposed to be a sensitive technique for increasing the rate of detection of venous invasion within the tumour in a considerable proportion of tissue samples that are negative or suspicious on H & E staining alone (1,6,8-10).

This combined prospective and retrospective study is aimed to assess the sensitivity of elastic tissue stain to detect venous invasion in comparison to the routine H & E stained sections, and to correlate intramural and extramural venous invasion with the Duke's staging system.

Material and Methods
Forty two of surgically resected colorectal cancer cases between the years 2007 and 2009 were collected from histopathological laboratories of AL-Jamhouri Teaching Hospital and private laboratories in Nineveh province. The demographic data related to age and sex of patients, site of primary tumour and Dukes stage were obtained from the request forms and reports of histopathology. The available blocks of tissue that had been embedded in paraffin were retrieved from the archive.

The work was conducted at the Department of Pathology, College of Medicine, University of Mosul. Two serial sections of 4-5µ thickness were cut from each block, one section is stained with routine H & E stain the other with Weigert's stain for elastic fibers and counterstained with H & E.

All sections were scrutinized for the presence of venous invasion, which was defined as the presence of tumour cells within an endothelial lined space surrounded by a rim of smooth muscle and/or containing red blood cells on the basis of H & E stained sections.

By Weigert's stain for elastic tissue, venous invasion is defined as the presence of tumour deposits within a space with elastic fibers in its wall (which appears deep purple or black), Assessment of the location of venous invasion, whether intramural or extramural, was also done. Intramural venous invasion is defined as venous invasion within tumour, submucosa and muscularis propria, while extramural
venous invasion is located within the serosa or pericolic fat. When venous invasion was in doubt, the case was considered as negative.

The statistical analysis to compare the two stains was done by the use of $\chi^2$ test. A $P$-value $< 0.05$ was considered to be statistically significant.

**Results**

The mean age of sampled patients was 50 years (range is 18–70 years). Of the 42 cases of colorectal cancer, 16 were positive for venous invasion by the use of H & E stain. Of the remaining negative group, twenty were positive after the incorporation of Weigert's stain for elastic tissue.

Overall, the total frequency of venous invasion increased from 16/42 (38%) as detected by H & E stain only to 35/42 (83%) after the use of Weigert's stain for elastic fibers with a ($P<0.001$) (table 1; fig 1).

Regarding intramural and extramural venous invasion, the frequency of detection after using Weigert's stain increased from 9/42 to 31/42 and 9/42 to 16/42 respectively (P<0.001 and P<0.05) (table 1; fig 2,3,4).

Of the 16 cases with Dukes B disease, review of H & E and elastic tissue stained slides revealed venous invasion in 7 & 13 cases respectively; however the results did not reach statistical significance ($P>0.05$, table 2). While in Dukes C cases, eight of 23 cases were deemed positive on H & E stained slides whereas 20 cases of this group were confirmed by Weigert's stain for elastic fibers ($P<0.01$, table 2).

The study confirmed that the use of elastic stain on at least one tissue block increases the overall yield of venous invasion by 40% (table 3).

Table (1): Detection of venous invasion by H & E stain vs. Weigert's stain for elastic fibers.

<table>
<thead>
<tr>
<th>No.</th>
<th>Intramural VI</th>
<th>Extramural VI</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H &amp; E (%)</td>
<td>Elastic stain (%)</td>
<td>H &amp; E (%)</td>
</tr>
<tr>
<td>42</td>
<td>9 (21)</td>
<td>31 (74)</td>
<td>9 (21)</td>
</tr>
</tbody>
</table>

Table (2): Detection of vascular invasion with H & E stain vs. Weigert's stain in relation to Dukes staging system.

<table>
<thead>
<tr>
<th>Stage</th>
<th>No.</th>
<th>Intramural</th>
<th>Extramural</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H &amp; E (%)</td>
<td>Elastic Stain (%)</td>
<td>H &amp; E (%)</td>
</tr>
<tr>
<td>Dukes A</td>
<td>3</td>
<td>1</td>
<td>--</td>
<td>2</td>
</tr>
<tr>
<td>Dukes B</td>
<td>16</td>
<td>5 (31)</td>
<td>13 (81)</td>
<td>3 (19)</td>
</tr>
<tr>
<td>Dukes C</td>
<td>23</td>
<td>3 (13)</td>
<td>16 (70)</td>
<td>6 (26)</td>
</tr>
</tbody>
</table>

Table (3): Relation between the detection of venous invasion with the use of elastic tissue stain and the number of the available tissue blocks.

<table>
<thead>
<tr>
<th>No. of blocks</th>
<th>No. of cases</th>
<th>Venous invasion by elastic stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>2-4</td>
<td>22</td>
<td>12 (54%)</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>20 (48%)</td>
</tr>
</tbody>
</table>
Figure (1) A: Adenocarcinoma of the colon with no evidence of venous invasion (H & E x 200).

Figure (1) B: Adenocarcinoma of the colon with malignant glands surrounded by concentric layers of elastic fibers (Weigert's stain x 200).

Figure (2) A: Intramural venous invasion which is difficult to be detected using H & E stain alone (H & E x200).

Figure (2) B: Intramural venous invasion is identified when elastic tissue stain is added (Weigert's stain x 200).

Figure (3) A: Intramural venous invasion which is difficult to be detected using H & E stain alone (H & E x800).

Figure (3) B: Intramural venous invasion is identified when elastic tissue stain is added (Weigert's stain x 800).
Current study supports the use of elastic tissue stain on at least one tissue section to improve the detection of venous invasion in colorectal cancer. Venous invasion was identified in 35 cases, twenty of which were initially labeled as negative on the basis of H & E stain only. This led to an increase of the overall frequency of venous invasion from 38% to 83%. These results are comparable to those found in a previous similar study done by Sternberg et al. in which the addition of Weigert’s stain had enabled the diagnosis of 15 (39%) of 39 specimens which were initially negative on H&E sections, raising the overall incidence of venous invasion to 70.4% \(^{6,11}\). Furthermore, in another comparable study done by Inoue et al. the frequency of venous invasion was 31% as detected by H & E stain and increased to 81% after the incorporation of a Verhoeff van Gieson which was correlated with subsequent development of haematogenous metastasis \(^9\).

However, it has been proposed that the difference in the definition of vascular invasion, the type of stain used and perhaps the metastatic potential of the cells once they have gained access to lymphovascular spaces may be responsible for the variation in the frequency of vascular invasion \(^{12}\).

It is firmly established that extramural venous invasion is an independent indicator of poor prognosis \(^2,11,12\). In this study, the detection rate increased from 21% to 38% after the addition of elastic tissue stain; the results were mostly comparable with those observed by Vass et al. who demonstrated an increase of frequency of extravascular invasion from 24% to 43% after adding elastic fiber stain on tissue sections \(^9\).

On the other hand; the prognostic significance of intramural vascular invasion is more controversial \(^9,10\). A number of studies had demonstrated this type of vascular invasion at a lower frequency than the extramural type. This controversy and variability in the frequency of identification of intramural vascular invasion is attributed in part to the destruction of some veins beyond recognition by the invading tumour cells \(^2,11\) and the location of these vessels at the midst

**Discussion**

The influence of adding different elastic tissue stains to increase the detection rate of vascular invasion in colorectal cancer had been demonstrated by a number of investigators \(^8-11\). In a most recent study done by Howlett et al. who used Movat pentachrome on 92 cases of colorectal cancer, venous invasion was identified in 22 out of 50 cases (44%) which were initially grouped as negative on the basis of H & E stain only with an overall increase in the detection rate from 18% to 62% when comparing H & E and elastic tissue stains respectively \(^10\). In another study done by Vass et al. the addition of H & E/elastic stain on 75 cases of colorectal cancer specimens increased the overall detection rate from 21 (27%) to 43 (57%) \(^9\).
of the tumour and away from its leading edge\(^9\). Some studies regard the presence of vascular invasion as an indicator for the risk of haematogenous metastasis irrespective of its site\(^2,11\). In this study, intramural vascular invasion was found in nine cases (21%) of the H & E stained slides, the figure has raised to 31 cases (74%) when sections were stained with Weigert’s stain for elastic tissue. A comparable result was observed by Vass et al, who reported increases in detection of intramural vascular invasion of 29% when elastic stain was utilized\(^9,10\).

Previous studies recognized vascular invasion by tumour to be as good as a prognostic factor as Dukes stage and its presence may influence the therapeutic decision particularly in Dukes stage B disease\(^1,9\). Accordingly; many physicians now accept the recommendation of adjuvant chemotherapy for patients with Dukes B tumour with synchronous vascular invasion\(^1,9,13\).

In this present study, there were 16 cases with Dukes B disease, vascular invasion was detected in seven cases on the basis of H & E stain which increased to 13 cases when elastic stain was added. These data are similar to those found by Vass et al who demonstrated vascular invasion in four & 14 cases out of 27 cases with Dukes B disease on review of H & E and elastic stain respectively\(^9\). Despite this similarity; this factor did not reach a statistical significance which may be the result of the small number of cases harboring Dukes B tumour in the current study. It is also obvious that this study elicited vascular invasion at comparable values for Dukes B & C with the use of elastic stain, the results were also comparable for intramural and extramural types of vascular invasion for the two stages as this pathological marker is a stage-independent marker.

In this setting the use of elastic stain on at least one tissue block enables the identification of vascular invasion in many cases that were initially negative on the H & E stained sections thereby improving the therapeutic decision and patient outcome.

References


Serum heavy metals in patients with fragments and shells of improvised explosive devices

Mahmood A. Aljumaily*, Nabeel H. Alfhady**, Mr. Mowafak K. Hassan***
* Department of Surgery, ** Department of Anatomy, Section of Biology, College of Medicine; *** Department of Biology, College of Sciences, University of Mosul.

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ABSTRACT

Background: Iraq is the most affected country by injuries of improvised explosive devices (IED) including bomb car, bomb born on person and roadside bombs. Embedded fragments and shells can release heavy metal. High level of heavy metal in serum carries dangerous long term risk for injured patients.

Objective: The aim of this study is to estimate the serum level of heavy metals (copper, lead, cadmium, nickel, and zinc) in patients with fragments and shells from IED including bomb car, bomb born on person and roadside bombs.

Material and methods: Serum concentrations of heavy metals (copper, lead, cadmium, nickel, and zinc) were assayed using atomic absorption spectrophotometry in 52 patients with fragments and shells from IED. Serum concentrations of heavy metals were measured in 52 healthy adults as control group.

Results: The mean serum level of copper, lead, cadmium, nickel, and zinc in patients were 1304 ± 258.1 µg/L, 36.5 ± 16.7 µg/L, 6.203 ± 2.372 µg/L, 0.387 ± 0.183 µg/L, and 1651 ± 532 µg/L respectively, while in control were 824.2 ± 203.6 µg/L, 31 ± 15.1 µg/L, 1.654 ± 0.4 µg/L, 0.239 ± 0.028 µg/L, 619 ± 234 µg/L respectively. The differences in serum copper, lead, cadmium, nickel, and zinc between patient and control is highly significant, (P value < 0.0001), while the differences in serum lead between patient and control is not significant (P value > 0.05).

Conclusion: The present study demonstrates that a highly significant increase in serum level of copper, cadmium, nickel, and zinc in patient with fragments and shells from IED in comparison with control group, while the difference in serum lead in patients and control was not significant.

Keywords: Improvised explosive devices, fragment, shell, metal, serum level.
The improvised explosive devices (IED) ranged from bombs born on persons and bomb cars to roadside bombs (1). The explosive weapons in populated areas consistently cause unacceptable level of harm to civilians, this pattern of harm is seen in individual incidents of violence as well as in major conflicts (1). Explosive weapons produce a common pattern of wounding with variations depending on the force of blast, size of fragments, location of the blast, and susceptibility of the individual victims (1). The mechanisms of injury from blast have been divided into primary (effect of blast wave on organs and tissues), secondary (injuries caused by propagated flying debris and bomb fragments projection into body), tertiary (effect of blast wind cause propulsion of body into other objects) and quaternary (all other injuries and diseases following blast trauma) (1,2).

Primary Fragmentation can be part of the bomb casing itself or objects intentionally imbedded into the IED designed to cause further wounding like nails, screws, nuts, bolts and many variable materials, and any local material that was made airborne by projectile (secondary fragmentation) (1,2).

IED cause complex patterns of wounding and numerous penetrating wounds containing embedded fragments that require greater medical resources than other trauma. For survivors, immediate injuries can result in a range of long term physical condition (1). Information accumulated in the last 6 years in Iraq shows an increase in oncological, chronic and reproductive diseases and malformations at birth, particularly high in the areas most severely attacked (3). It is well-known that excess of the heavy metals can disrupt body functions and have pathogenic effects on human respiratory organs, kidney, skin and affect sexual and neurological development and functions (3-6). Cadmium, and nickel are known to be human toxic, carcinogenic and teratogenic. Lead, copper and zinc are well-known human multi-systemic toxic and fetotoxic (3-6). Systemic lead toxicity following gunshot, has been reported to occur anywhere from 2 days to 40 years after intra-articular gunshot injury (7).

In our community there is an un-estimated large number of injured patients with fragments and shells after explosion. There are no available studies on the level of heavy metals after injuries with fragments and shells of IED. The aim of this study is to estimate the serum level of copper, lead, cadmium, nickel and zinc in patients with fragments and shells from IED including bomb car.

Material and methods
This study was approved by the scientific research committee at the College of Medicine, University of Mosul. A written informed consent was obtained from all patients and controls. The chemical analysis and metal serum level measurement were conducted at laboratories of College of Medicine and College of Sciences, University of Mosul, during the period from September 2009 to September 2010. Fifty two patients with symptoms related to fragments and shell of IED including bomb cars, person born bombs, and road side bombs included in this study. All patients collected from the department of orthopedic surgery, Aljumhori Teaching Hospital, they had either pain at site of foreign body, acute flare up of infection or...
chronic discharging sinus. Forty four patients were males and 8 were females. The mean age of patients was 33.9 years ± 10, ranged 4 to 68 years. Forty six patients were injured in bomb cars while 4 patients injured by road side bombs and 2 by person born bomb. Forty nine patient had multiple shells and only 3 patients had a single shell. The mean duration between injury and samples taking was 14.6 months, ranged between one month to 6 years. Fifty two healthy adults share as a control group, they donated 10 ml of blood for chemical analysis for heavy metals. The control group mean age was 33.1 years ± 13 ranged 18 to 62 years, 28 of them were males and 24 were females.

All patients and controls were clinically examined. The exclusion criteria for patients and control were: the presence or history of any orthopedic or dental implants, renal insufficiency or chronic renal disease, or chronic medical treatment for systemic or local diseases, to avoid any bias in the serum metal determination.

**Preparation of samples**

Ten milliliters of venous blood was drawn from each patient and control. Blood samples were taken by blood sampling kit dedicated to trace element determination to avoid metal contamination. Serum samples were lysed for analysis of heavy metals by adding 10ml HNO3 to one ml of serum and heated at 60-70 C° for 3-4 hours. Then 1 ml perchloric acid was added to the same sample. Digestion process continued until the solution was clear.

Atomic absorption spectrophotometry flameless method was used to determine serum lead, cadmium and nickel by using Shimadzu AA-6650G instrument with electronic double – beam Graphite Furnace Atomic Absorption (GFAA) spectrophotometer. The copper and zinc serum level were measured with the flame atomic absorption spectrophotometer.

**Statistical analysis**

Results were reported as mean ± standard deviation. The unpaired two tail student (t) test used to calculate the differences between two means. The P value was considered significant if it was less than 0.05.

**Results**

The mean age of patients was 33.9 years ± 10, the mean age of control group were 33.1 years ± 13. Serum copper mean in patients with fragments and shells of IED was 1304 ± 258.1 µg/L, while in control group was 824.2 ± 203.6. The difference between the two means was highly significant (P value < 0.0001), (table 1). The mean of blood lead in patients with shells and fragments of IED was 36.5 ± 16.7 µg/L, while in control group was 31 ± 15.1. The difference between the two means was not significant (P value was 0.067), (table 1). The mean of blood cadmium in patients with shells and fragments of IED was 6.203 ± 2.372 µg/L, while in control group was 1.654 ± 0.4. The difference between the two means was highly significant (P value <0.0001), (table 1).

<table>
<thead>
<tr>
<th>Serum metals</th>
<th>Serum level</th>
<th>p- value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>copper</td>
<td>Patients</td>
<td>1304.6 µg/L</td>
<td>258.1</td>
</tr>
<tr>
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<td>Control</td>
<td>824.2 µg/L</td>
<td>203.6</td>
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<tr>
<td>lead</td>
<td>Patients</td>
<td>36.5 µg/L</td>
<td>16.7</td>
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<td>Patients</td>
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<tr>
<td>nickel</td>
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<td>0.387 µg/L</td>
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<tr>
<td>zinc</td>
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<td>1651 µg/L</td>
<td>532</td>
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<tr>
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<td>Control</td>
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</tbody>
</table>
The mean of serum nickel in patients with shells and fragments of IED was 0.387 ± 0.183 µg/L, while in control group was 0.239 ± 0.028. The difference between the two means was highly significant (P value < 0.0001), (table 1). The mean of serum zinc in patients with shells and fragments of IED was 1651 ± 532 µg/L, while in control group was 619 ± 234. The difference between the two means was highly significant (P value < 0.0001), (table 1).

Discussion

In Iraq gunfire remains the most common cause of death, and deaths from car bombing have increased (8). The expected numbers of injured patients would be much higher (9). Iraq is the country most affected by injuries of IED weapons (1). Bomb blast injuries are no longer confine to battlefield (10).

Data from Iraq suggest that explosive weapons, by comparison with other weapons types, have higher proportion of child and female deaths and injuries (1). Explosive weapons injuries represent a demanding surgical emergency (11). Heavy metal high level in serum carries dangerous long term risk for injured patients of IED (1). It is recommended to evaluate serum heavy metals in all patients with retained bullet and fragments (12).

In our study, there was highly significant difference in the level of serum copper between patients and control, (table 1). This might be explained by the intended use of copper containing covers of bullet and missiles to increase the fragmentation in explosion. Copper is an essential nutrient that is incorporated into a number of metalloenzymes, exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anemia, immunotoxicity, developmental toxicity, and carcinogenic (13-15). Copper serum level varied between 782-1690 µg/ L (4, 13-16), the reference value of serum copper in normal healthy human is 830 – 1530 µg/ L (17).

There was no significant difference in the level of serum lead between patients with fragment and shells of IED and control, (table 1). Lead poisoning from retained bullet or missile is rare (12). Lead is a dangerous highly toxic substance, exposure to which can produce a wide range of adverse health effects, it is harmful even in small amount. Humans get exposed to lead through their environment and diet (4,18,19). Lead is a multitargeted toxicant, usually affect kidney and nervous system (4,18). Lead toxicity remains one of the most important problems in terms of prevalence of exposure and public health impact (20). Mean lead concentrations in blood, tibia, and patella were 3.5 µg/ dl, 18.9 µg/g and 6.8 µg/g respectively(21). There is variation in gender and race, normal level was < 5 µg/dl (20, 21).

The level of serum cadmium shows a highly significant difference between patients and control, (table 1). This might be explained by the cadmium containing alloys used in cars and batteries used in explosion. Cadmium, a heavy metal is well known to be highly toxic to both human and animals. Cadmium exposure leads to long-term effects on human health even at low concentrations and it affect many systems, because cadmium confounds many physiological processes (22). Cadmium serum level is variable in literatures (4, 18, 22). Cadmium is toxic, carcinogenic and fetotoxic (4, 18).

There was highly significant difference in serum nickel between patients and control, (table 1). This might be explained by the wide use of nickel in cars bodies and parts, which produce many fragments and shells that contain nickel. Nickel is one of the heavy metals widely used in modern industry has been recognized as highly toxic to skin, respiratory system and reproductive system, and carcinogens (23, 24, 25). Reference value for nickel in healthy adults is 0.2 µg/L in serum and 1-3 µg/L in urine, it reaches 2-6.5 µg/L in serum of nickel industry workers (24, 25). The essentiality of nickel in humans has not been established, the nickel dietary recommendations has not been established for human (24,25). Stainless steel endoprostheses may release nickel, also administration of nickel contaminated medications lead to significant exposure (25).

Serum zinc in our study shows a highly significant difference between patients and control, (table 1). This might be explained by the wide use of zinc containing metallic parts.
in cars and in bomb designs. Zinc is an essential nutrient that is incorporated into a number of metalloenzymes, chronic zinc toxicity can impair cellular immunity and can cause hypochromic anemia \(^{5, 18}\). The normal value of serum zinc varied in different studies, its ranged between 700- 1348 µg/L \(^{4, 5, 13-16, 26}\).

It is wise to follow up patients with shells and fragments of IED for metals toxicity. More studies should be done in this field since this is very common medical problem in our country which subjected to the most heroic explosions and violence. In conclusion, the present study demonstrates that embedded fragments and shells form IED including bomb car causes a highly significant elevation in metals (copper, cadmium, nickel, and zinc) concentration in serum, while serum lead shows no significant differences in patient in comparison with control.

References

17. Walker SW. Appendix. In: Boon NA, Colledge NR, Walker BR, Hunter JAA. Davidson's principle and practice of
Measurement of serum magnesium concentration in type 2 diabetic patients on glibenclamide and metformin therapy

Isam Hamo Mahmood*, Zaynab M. Ali Hassan**, Zeina Satam*
*Department of Pharmacology, ** Department of Biochemistry, College of Medicine, University of Mosul.

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ABSTRACT

Objectives: To measure serum magnesium concentrations in patients with type 2 diabetes on glibenclamide, metformin or a combination of both drugs therapy in Mosul city.

Patients and Methods: One hundred type 2 diabetic patients formed the patients group. Another group of 27 non diabetic healthy individuals involved in the study as a control group. The patients’ group was divided into 3 subgroups according to the type of the oral hypoglycemic agent used (metformin, glibenclamide, metformin plus glibenclamide). The study was conducted in Al Wafaa Diabetes Center in Mosul city, and departments of Pharmacology and Biochemistry, College of Medicine during the period from May 2009 to May 2010. Design of the study is case control. Quantitative analysis of magnesium and glucose in serum were done by using commercial kits.

Results: The results showed a significant lower concentration of magnesium of the patients as compared with the controls and a significant higher serum glucose concentrations of the patients as compared with the controls.

Conclusion: This study demonstrated that low magnesium status is common in type 2 diabetics who were on therapy with the hypoglycemic agents, metformin, glibenclamide or a combination of both drugs, in Mosul city.

Keywords: Magnesium, type 2 diabetes mellitus, glibenclamide, metformin.
Magneesium deficiency has been reported in type 2 diabetes mellitus. In the United States, 25–39% of diabetic outpatients have low concentrations of serum magnesium (1). Low serum magnesium concentrations in patients with type 2 diabetes have also been reported in several European countries, e.g., Austria, Germany, Italy, France, and Sweden (2–5).

Magnesium is the fourth most abundant cation in the human body and the second most abundant intracellular cation. It may exist as a protein bound, complexed, or free cation. It serves as a co-factor for all enzymatic reactions that require ATP and as a key component in various reactions that require kinases. It is also an essential enzyme activator for neuromuscular excitability and cell permeability, a regulator of ion channel and mitochondrial function, a critical element in cellular proliferation and apoptosis, and an important factor in both cellular and humoral immune reactions (6).

Magnesium depletion has a negative impact on glucose homeostasis and insulin sensitivity in patients with type 2 diabetes, as well as on the evolution of complications such as retinopathy, thrombosis and hypertension. Moreover, low serum magnesium is a strong independent predictor of the development of type 2 diabetes (7).

A large body of evidence that shows a link between hypomagnesemia and reduction of tyrosine kinase activity at the insulin receptor level, which may result in the impairment of insulin action and development of insulin resistance, has been progressively accumulated in previous years (8). Although evidence suggests that magnesium supplementation could be useful in the treatment of diabetes and to prevent the development of its chronic complications, the possible benefits of magnesium administration as an adjuvant factor for the treatment of type 2 diabetes, based in a randomized controlled trial, are scarce and controversial (6).

The aim of this study was to determine the serum magnesium concentrations of patients with type 2 diabetes on hypoglycemic therapy and healthy controls in Mosul city.

Patients and methods
One hundred type 2 diabetic patients and 27 non diabetic controls participated in the study. The patient’s group was divided into 3 groups according to the type of the oral hypoglycemic agent used (metformin, glibenclamide, metformin plus glibenclamide) (table 1). The diabetic patients were recruited from Al-Wafaa Center of Diabetes Mellitus in Mosul city. Twenty six of the patients were taking metformin, 32 were taking glibenclamide, and 42 were using both. The doses of the drugs ranged from 500 mg to 2000 mg daily in case of metformin and 5 to 10 mg in case of glibenclamide. Durations of treatment were 2.65±2.76 years in case of metformin, 4.01±4.52 years in case of glibenclamide and 3.62±3.13 years in case of met+glib therapy.

Because loop diuretics are associated with higher urinary magnesium excretion, patients on loop diuretics were excluded. None were taking magnesium supplements. The study protocol was approved by the Ethical Committee of the College of Medicine, University of Mosul.

Venous blood samples from the fasting control subjects and patients were drawn. Serum was separated from blood cells by centrifugation at 3000 rpm for 15 minutes and stored at -25°C until analysis.

Quantitative analysis of magnesium in serum was done by a photometric method (9) available as a commercial kit for measurement of magnesium (Biolabo, France). Serum glucose concentrations were estimated by glucose oxidase peroxidase colorimetric method (10) available as a kit provided by Randox Company, UK.
Table (1): Patient’s and control’s characteristics.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Metformin</th>
<th>Glibenclamide</th>
<th>Met+Glib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>52.7±9.39</td>
<td>52.04±9.46</td>
<td>51.03±7.61</td>
<td>57.79±9.86</td>
</tr>
<tr>
<td>Male/ Female</td>
<td>12/15</td>
<td>17/9</td>
<td>15/17</td>
<td>22/20</td>
</tr>
<tr>
<td>Duration of treatment</td>
<td>---------</td>
<td>2.65±2.76</td>
<td>4.01±4.52</td>
<td>3.62±3.13</td>
</tr>
<tr>
<td>No Patients</td>
<td>27</td>
<td>26</td>
<td>32</td>
<td>42</td>
</tr>
</tbody>
</table>

Statistical analysis: Unpaired t-test was used to compare serum magnesium or glucose concentrations of the controls and the patients. Linear regression analysis (Pearson Correlation Coefficient, $r$) was performed for finding the degree of association between serum glucose concentration and serum magnesium concentration. Level of significance at 0.05 or less.

Results

Mean serum magnesium concentrations of the diabetics and the controls appeared in (table 2). A significant lower level of magnesium as compared with those of the control individuals, were obtained from the patients (P=0.001 for metformin, 0.004 for glibenclamide, and 0.0001 for the drug’s combination).

Mean serum glucose concentrations of the diabetic group were significantly higher as compared with those of the control group, and were uncontrolled cases as evident by their high serum concentrations (table 3).

No correlation was found between serum glucose concentration and serum magnesium concentration of the different groups ($r$=- 0.035 for glibenclamide, $r$= 0.26 for metformin, $r$=0.21 for met+glib, $r$= 0.07 for met+glib, P= 0.66).

Table (2): Serum magnesium concentrations in the control and patient’s groups (mg/ dl) (Mean±sd).

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Magnesium Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.22±0.69</td>
</tr>
<tr>
<td>Metformin</td>
<td>1.59±0.63</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>1.68±0.70</td>
</tr>
<tr>
<td>Met+Glib</td>
<td>1.30±0.72</td>
</tr>
</tbody>
</table>

Control values significantly differs from patient’s values: P Values; 0.001 for metformin; 0.004 for glibenclamide and 0.0001 for met+glib combination.

Table (3): Serum glucose concentrations in the control and patient’s groups (mg/ dl) (Mean±sd).

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum glucose Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113.29±59.34</td>
</tr>
<tr>
<td>Metformin</td>
<td>189.21±79.79</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>222.97±85.35</td>
</tr>
<tr>
<td>Met+Glib</td>
<td>231.14±92.10</td>
</tr>
</tbody>
</table>

Control values significantly differs from patient’s values: P Value; < 0.001 for metformin; glibenclamide and met+glib combination.

Discussion

The results in the present study showed that diabetic patients had low level of serum magnesium as compared with the control healthy subjects in Mosul city.

Similar findings have been obtained by other researchers in other countries. Pham et al.(6) stated that hypomagnesemia has been reported to occur at an increased frequency among patients with type 2 diabetes compared with their counterparts without diabetes in USA. Al-Osali et al.(11) showed that low total serum levels of magnesium are frequently seen in type 2 diabetic Omani patients. Seyoum et al.(12) reported that Ethiopians patients with diabetes mellitus have lower levels of magnesium and therefore at increased risk of complications related to magnesium. Low levels of magnesium in type 2 diabetic patients have also been reported in other countries including Italy(13), India(14), and Bangladesh(15).

The reasons for the high prevalence of magnesium deficiency in diabetes are not clear, but may include increased urinary loss, lower dietary intake, or impaired absorption of magnesium compared to healthy individuals(7).

Several studies showed that intake of magnesium can correct the magnesium status...
of the diabetic patients. Rodriguez-Moran and Guerrero(8) reported that oral supplementation with magnesium chloride solution restores serum magnesium levels, improving insulin sensitivity and metabolic control in type 2 diabetic patients with decrease magnesium levels. De Lordes Lima(16) showed that magnesium depletion is common in poorly controlled patients with type 2 diabetes, especially in those with neuropathy or coronary disease. More prolonged use of magnesium in doses that are higher than usual is needed to establish its routine or selective administration in patients with type 2 diabetes to improve control or prevent chronic complications.

In the present study no correlation was found between serum glucose concentrations and serum magnesium levels. Several authors have described a correlation between HbA1c and plasma magnesium in type 1 diabetics(4,17). However, no such a correlation was found in type 2 diabetes(4, 18, 19), similar to our results.

Clinically, there are significant data linking hypomagnesemia to various diabetic micro and macrovascular complications. In a study that involved 19 normotensive individuals without diabetes, 17 hypertensive individuals without diabetes, and 6 hypertensive individuals with diabetes, Resnick et al. (20) documented the lowest mean intracellular magnesium concentration among the last group. Two studies showed that not only did patients with diabetes have lower serum magnesium levels compared with their counterparts without diabetes, but also the serum magnesium levels among the cohort with diabetes had an inverse correlation with the degree of retinopathy(21, 22). In a comparative study that involved 30 patients who had type 2 diabetes without microalbuminuria, 30 with microalbuminuria, and 30 with overt proteinuria, Corsonello et al. (23) observed a significant decrease in serum ionized magnesium in both the microalbuminuria and overt proteinuria groups compared with the nonmicroalbuminic group. There also are data to suggest the association between hypomagnesemia and other diabetic complications, including dyslipidemia and neurologic abnormalities(24).

Serum glucose concentrations, in the present study, were high (the patient's diabetic state is uncontrolled, in spite of using of hypoglycemic agents). Several studies have shown elevated urinary magnesium excretion in both type 1 and 2 diabetic patients, and elevated urinary magnesium excretion in diabetes is associated with elevated fasting blood glucose and HbA1c concentrations(7). The lower magnesium concentrations in the present study may be due to the elevated levels of glucose concentrations reported in the present study. Pham et al. (25) reported that patients who have serum magnesium levels between 2.0 and 2.5 mg/ dl had the least degree of renal function deterioration and best glycemic control.

In conclusion, we have demonstrated that low magnesium status is common in type 2 diabetics who were on therapy with the hypoglycemic agents, metformin or glibenclamide or a combination of both drugs, in Mosul city in Iraq.

References


Effects of vitamin D, calcium, fluoride and vitamin C as dietary supplementation on bone healing in rabbits

Mahmood A. Aljumaily
Department of Surgery, College of Medicine, University of Mosul.

ABSTRACT

Objective: The aim of this study was to evaluate the effect of the daily oral administration of vitamin D, calcium, fluoride and vitamin C as dietary supplementation on bone healing in experimental animals (rabbits).

Material and methods: Eight young male rabbits divided into two groups after induction of open ulnar osteotomy, the experimental group received daily dose of vitamin D, calcium, fluoride and vitamin C as dietary supplementation from the second post operative day for 28 days. The control group received ordinary diet without any food supplementation. At the end of the fifth week, the animals were sacrificed and the specimens taken for radiological and CT scan densimetry, and histological evaluation carried out for calluses at site of osteotomy.

Results: All ulnar bone osteotomies in both groups united at the end of the fifth week macroscopically and radiologically. There was no significant difference in serum calcium, phosphate, and alkaline phosphatase preoperatively, and at the end of the fifth week. The callus density was measured in site of osteotomy by CT scan densimetry and its mean in experimental group was 331.1 ± 81.3, and control group was 199.7± 32.1. The difference between the experimental and control group was highly significant, (P value is < 0.001). The histological examination of the bone at site of osteotomy showed healing with woven bone predominantly and some lamellar bone and cartilage.

Conclusion: The present study demonstrates that a daily oral administration of vitamin D, calcium, fluoride and vitamin C as dietary supplementation enhance bone healing and increase callus density.

Keywords: Vitamin D, calcium, fluoride, vitamin C, dietary supplementation, bone healing.
many systemic and local factors influencing fracture healing: nutritional state including vitamins, minerals and trace elements supplementation is one of these factors (1). Calcium and Vitamin D3 administration had positive influence on fracture healing (2,3). Vitamin D3 (cholecalciferol) had been shown to be essential hormone for the process of fracture healing (4). Vitamin C supplementation enhances fracture healing by improving the mechanical resistance of fracture callus and improving the bone mineralization (5,6). Fluoride dietary supplementation accelerates the fracture healing (7).

Assessment of fracture healing is a common problem in orthopedic practice and research (8). Fractures healing can be evaluated through clinical, radiological, mechanical, histological, chemical or biological study (1,2,9). Bone mineral density measurement by computerized tomography (CT) is noninvasive, and a reliable tool for quantification of the fracture repair process in experimental animals (10). The mineral density of callus correlated positively with callus strength and stiffness (11).

To our knowledge there was no study on effects of the combinations of the vitamin D, calcium, fluoride and vitamin C as dietary supplementation on bone healing. The aim of this study was to evaluate the effect of the daily oral administration of vitamin D, calcium, fluoride and vitamin C as dietary supplementation on bone healing in rabbits.

Material and methods
This study was approved by the research ethics committee at the College of Medicine, University of Mosul, and follows the ethical code for animal experimentation of the Council for International Organization of Medical Sciences. Eight young male aged 4 months locally breeded New Zealand rabbits from animal house, College of Medicine, University of Mosul, were used in this study, from the first of November to the 30th of December 2009. Their average weight was 1460 grams (ranged between 1250 grams and 1600 grams). The animals were kept in separate metallic cages for one week, for adaptation in animal's house. In each cage one animal feed with standard ration and water.

Experimental technique
Food was suspended eight to ten hours prior to administration of anesthesia. To decrease the vagal tonus, each animal received 0.2 mg/kg dose of atropine sulphate by intramuscular injection. Animals were anesthetized by intramuscular injection of ketamine (50 mg/kg of body weight) and intramuscular injection of diazepam (5.0 to 10.0 mg/kg of body weight). Preoperative antimicrobial prophylaxis consisting of 50 mg/kg of ceftriaxone were injected subcutaneously in proximal part of the same limb. Sample of venous blood aspirated to measure serum calcium, phosphate, and alkaline phosphatase.

The right forelimb was shaved and cleaned by betadine solution. Under an aseptic conditions technique, the right ulna of each animal was accessed by an anteromedial longitudinal skin incision of approximately 20 mm. After division of the skin and subcutaneous tissue, the fascia, the muscles and tendon were retracted and the peristeum was opened and dissected from the ulna. The ulnar shaft was exposed; osteotomy was performed on the exposed portion of the ulna by means of a one mm blade thickness sterile hand saw. The incision was closed by layers, using absorbable 5-0 polyvycril sutures for the fascia and 4-0 monofilament PDS sutures for...
the skin, local dressing applied locally using sterile gauze covered with adhesive plaster.

The animals were assigned to one of the following groups, the first group (4 animals) as experimental group received daily dose of vitamin D, calcium, fluoride and vitamin C as dietary supplementation, the second group (4 animal) as control group.

A total dose of 100 IU vitamin D, 100 mg calcium, 25 µg fluoride, and 25 mg vitamin C dissolved in 10 ml water administrated orally on the second post-operative day and continued for 28 consecutive days thereafter. In the control group, the same volume of bi-distilled water was administered under similar conditions.

After five weeks, samples of blood aspirated to measure serum calcium, phosphate, and alkaline phosphatase from animals of both groups. Animals of both groups were anesthetized again as described previously and killed with a 2 ml intracardiac injection of potassium chloride. The right ulna of each animal was removed, dissected from the surrounding soft tissue.

The samples examined radiologically by Siemens- Sirography fluoroscopy equipment 62 K.T.; the KV used in taking x-ray was 30 KV, 50mA, (fig 1). The computerized tomography (CT ) scan examination carried out to measure the density of callus at the site of osteotomy. The CT scan equipment is light speed, multidetector equipment, General Electric (GE), 32 Yokogawa Medical System, taken TA 0.6 mm slice thickness. The mean of five points taken at the site of osteotomy to measure the density of callus and five points at the normal bone proximal to osteotomy, the means and standard deviations of these values calculated (fig 2).

The sites of osteotomy were carefully exposed by removal of all the soft tissue. The ulnar bones were removed, and fixed with 10% formaldehyde solution. After fixation, they were decalcified in 10% formic acid. The decalcification process demineralized the bone, leaving only the soft tissues and bone matrix. This was done to ensure that thin sections could be examined histologically. Thin sections embedded in paraffin wax were cut and stained with haematoxylin and eosin.

Figure (1): Radiological examination (X-ray) of rabbit forearm shows healed ulnar osteotomy in stage of union.

Figure (2): The site of osteotomy identified by CT scan and density measured in five points in site of osteotomy, and its means calculated.

Statistical analysis
Results are reported as mean ± standard deviation. The unpaired student (t) test used to calculate the differences between two means. The p value was considered a significant if it is less than 0.05.

Results
All animals survived to the end the study. Neither wound infection nor wound dehiscence were observed in the animals of either group. All animal had normal serum calcium (3.2 ± 0.22 mmol/dl), serum phosphate (1.35± 0.18 mmol/dl), and serum alkaline phosphatase (11.6 ± 2.4 IU unit/ dl) at time of osteotomy.
Five weeks after osteotomy, there was no statistically significant difference ($p > 0.05$) in the means of serum calcium ($3.1 \pm 0.14$ mmol/dl), serum phosphate ($1.4 \pm 0.1$ mmol/dl), and serum alkaline phosphatase ($12.1 \pm 1.6$ IU unit/dl), (table 1).

Macroscopic evaluations demonstrate that all osteotomies were united by the end of the study. The mean of CT scan density of callus at the site of osteotomy in experimental group was $331.1$ with a standard deviation $81.3$. The mean of CT scan density in normal bone proximal to site of osteotomy in experimental group was $930.7$ with a standard deviation $188.3$, (table 2). The mean of CT scan density of callus at the site of osteotomy in control group was $199.7$ with a standard deviation $32.1$. The mean of CT scan density in normal bone proximal to site of osteotomy in control group was $919.3$ with a standard deviation $186.8$, (table 2). There were highly significant differences in density of callus between the experimental group and control group, ($P$ value is $< 0.001$). There were no significant differences in density of bone proximal to site of osteotomy between the experimental group and control group, ($P$ value is $0.84$, table 2).

The histopathological examinations of the osteotomy site showed healed bone with predominantly woven bone with some area of mature (lamellar) bone with some area of cartilage, there was no evidence of infection or foreign body reaction, (fig 3). All histological examination of specimens showed approximately same stages of healing in both groups of animals.

![Histological examination showing new bone formation at the site of osteotomy in different stages of healing (woven bone with areas of cartilage and some lamellar bone).](image)

Table 1: The serum calcium, phosphate, alkaline phosphatase of rabbits in preoperative and at the end of the fifth week.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Preoperative values</th>
<th>Values at end of fifth week</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Serum calcium</td>
<td>3.2</td>
<td>0.22</td>
<td>3.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum phosphate</td>
<td>1.35</td>
<td>0.18</td>
<td>1.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Serum alkaline phosphatase</td>
<td>11.6</td>
<td>2.4</td>
<td>12</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Table 2: CT scan densimetry in site of osteotomy and in normal bone proximal to site of osteotomy, five week after osteotomy of right ulna of rabbits in Experimental group and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Experimental group</th>
<th>control group</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>CT scan densimetry in site of osteotomy</td>
<td>331.1</td>
<td>81.3</td>
<td>199.7</td>
<td>32.1</td>
</tr>
<tr>
<td>CT scan densimetry in normal bone proximal to site of osteotomy</td>
<td>930.7</td>
<td>188.3</td>
<td>919</td>
<td>186.8</td>
</tr>
</tbody>
</table>
Discussion

The production of a better and stronger healing bone has attracted the interest of many investigators in the past. Numerous substances have been used to increase both the strength and rate of production of fracture callus \(^{(12)}\).

Calcium ion is an essential structural component of the skeleton and essential for the acceleration of healing of fractured bones \(^{(13)}\). Vitamin D is critically important for development, growth and maintenance of a healthy skeleton from birth until death \(^{(4)}\). Vitamin D and its active derivatives could promote fractures healing by improving the histomorphometric parameters, mechanical strength and tendency to increase transformation of woven bone into lamellar bone \(^{(14,15,16)}\). The amount of ossified tissue was found to be significantly higher in the fluoride treated callus, the bone mechanical properties of healed bones improved also in the fluoride treated callus\(^{(17)}\). Vitamin C supplementation improved the mechanical resistance of fracture callus and made bone healing faster \(^{(18,19,20)}\). The vitamin D, calcium, fluoride and vitamin C well known drugs, used widely in treatment of many orthopedic diseases, and safe drugs in therapeutic doses. Their combination in therapeutic doses can be used as dietary supplementation to support bone healing process in humans.

In this study, the combination of 100 IU vitamin D, 100 mg calcium, 25 µg fluoride and 25 mg vitamin C as a daily dietary supplementation to rabbits with fractured ulna, highly significantly increased the density of callus in CT densimetry measurement at the site of osteotomy in comparison with control group, (P value is < 0.001), (table 2). There was no significant difference in density of normal bone proximal to the site of osteotomy between experimental and control groups (P value is 0.84), (table 2). Macroscopically all osteotomies were united by the end of the study in both groups. Histopathological examination showed good union without complications in both groups. It is well known that the mineral density of callus correlated positively with callus mechanical properties\(^{(11)}\).

There was no significant difference in serum calcium, serum phosphate, and serum alkaline phosphatase preoperatively, and at the end of the fifth week. Our results preoperatively and at the end of the fifth week fall within the normal range. The normal serum calcium, serum phosphate, serum alkaline phosphatase in normal rabbits were 3.0- 4.2 mmol/l, 1.28-1.92 mmol/l, and 10-70 IU/L respectively \(^{(21)}\). These findings indicate that animals had normal serum calcium and phosphate through all time of study.

The rabbits were chosen as the animal model because they are widely used in studies of bone preparations, and its bone is similar to human bone. The ulna was selected because it is easy to access, had good size, its fixation not essential, and it is easy to harvest. The small number of animals used in this experiment is sufficient to get a conclusion and to stimulate more wide clinical studies when financial and technical support are available. This also fits with animal studies protocol which should be designed to minimize the number of animals used \(^{(22)}\).

In conclusion, our study demonstrates that combination of vitamin D, calcium, fluoride and vitamin C improve bone healing process in rabbits model osteotomy; this effect is characterized by increase callus density.

References

Systemic inflammatory response syndrome (SIRS) in Mosul: Clinical characteristics and predictors of poor outcome

Rami M. A. Khalil*, Sabah Shallal**

*Department of Medicine, College of Medicine, University of Mosul; ** Ibn-Sina Teaching Hospital.


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ABSTRACT

Objectives: Systemic inflammatory response syndrome is one of the most important causes of intensive care unit (ICU) morbidity and mortality worldwide. The aim of this study is to explore the spectrum of diseases responsible for SIRS admission in Mosul, and to identify the mortality rate and the factors associated with poor outcome.

Methods: Fifty patients with sepsis or non-infective SIRS were studied during the period from June 1st to November 30th 2009. Patients were collected from the medical ICU and the general medical wards in Ibn-Sina Teaching Hospital in Mosul,. Acute physiology and chronic health evaluation II (APACHE II) score was utilized to assess the severity of illness on admission. The patients included in the study received the standard medical care according to their condition, and were followed to delineate the cause of their illness, the percentage of microbiological confirmation, the duration of hospital stay, the mortality rate and the factors that influence their outcome.

Results: Sepsis represented 86% of cases of SIRS, of which 82% of them were caused by community acquired infections. Pneumonia was responsible for 48.8% of sepsis cases, followed by acute pyelonephritis and intra-abdominal infection. Sepsis was microbiologically confirmed in 44.2% of patients, and blood culture was positive in 18.6% of patients. Impaired consciousness, anaemia, hyperglycaemia and high blood urea were associated with excess mortality rate; while positive blood culture and hypoalbuminaemia correlated with high APACHE II score. The overall mortality rate was 44%. Patients with severe sepsis had a mortality rate of 55.2%.

Conclusion: SIRS is an important cause of hospital admission in Mosul, with associated high mortality rate. ICU admission should be seriously considered for patients with certain risk factors that predict poor outcome.
Localized inflammation is a physiological protective response which is generally tightly controlled at the site of the injury. Loss of this local control results in an exaggerated systemic response which is clinically identified as systemic inflammatory response syndrome (SIRS). SIRS may be initiated by infection or by non-infectious causes such as trauma, autoimmune reactions, malignancy, cirrhosis and pancreatitis (1). SIRS associated with suspected or proved infection is called sepsis. The aim of this study is to identify the spectrum of diseases which are responsible for SIRS admission in a medical ICU and the general medical ward in Mosul.

Patients and methods
Fifty patients were studied prospectively; they were collected from the medical ICU and the general medical wards in Ibn-Sina Teaching Hospital in Mosul during the period from 1st June to 30th November 2009.

Patients were included in the study if they met the diagnostic criteria of SIRS according to the definitions given by the ACCP/SCCM consensus conference. Accordingly, included patients should have two or more of the following criteria:
1- Temperature >38 °C or <36 °C.
2- Heart rate >90 beats/minute.
3- Respiratory rate >20 breaths/minute.
4- White blood cell count >12000 cell/µl or <4000 cell/µl or >10% immature bands.

The acute physiology and chronic health evaluation II (APACHE II) score was calculated for every patient. This is the most widely used scoring system to assess the severity of illness and the excepted mortality of...
critically ill patients. The score utilizes the worst values of 12 physiological variables during the first 24 hours following admission, along with an evaluation of the patient’s chronic health prior to admission (7).

The following laboratory investigations were done routinely (and repeated when necessary):

1. Complete blood picture including platelet count, blood film and erythrocyte sedimentation rate (ESR).
2. Prothrombin time (with the International Normalized Ratio (INR)) and activated partial thromboplastin time.
3. Serum sugar, urea and creatinine.
4. Serum sodium, potassium and calcium.
5. Liver function tests (serum bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and albumin).

Additional investigations were ordered according to the requirements for the individual cases; these included:

1. Other biochemical investigations like serum amylase.
2. Hepatitis viral serology.
4. Urine culture.
5. Pleural fluid analysis and culture.
6. Cerebrospinal fluid examination and culture.
7. Wound or ulcer swab and culture.

Chest x-ray was the only imaging which was done as a routine. Ultrasound examination of the abdomen was done for most patients. Few patients had CT-scan, and to a lesser extent, magnetic resonance imaging.

Patients were considered to have an infection if this was microbiologically documented or at least clinically suspected requiring evidence such as the presence of white blood cells in a normally sterile body fluid, acutely inflamed abdominal organ, chest x-ray consistent with pneumonia or a clinical syndrome associated with high probability of infection (6,8).

Septic shock was defined as acute circulatory failure characterized by persistent arterial hypotension unexplained by other causes. Hypotension was defined by a systolic blood pressure <90 mmHg; mean arterial pressure <60, or a reduction in systolic blood pressure of more than 40 mmHg from baseline, despite adequate volume resuscitation, in the absence of other causes of hypotension. MODS were considered to be a dysfunction of more than one of the above organs, requiring intervention to maintain homeostasis (6).

The source of sepsis and the cause of the non-infective SIRS were determined, and daily follow up was made to record the last stage of sepsis reached, the duration of hospital stay and the final outcome (survival or death).

All variables were expressed as numbers and percentages and were compared with unpaired T-test, ANOVA test, Fisher Freeman Hallon test, Fisher Exact test and Chi-square test. The analysis was conducted using the SPSS package 16, p-value <0.05 was considered statistically significant and p-value <0.001 was considered highly significant.

Results
Fifty consecutive patients with SIRS were included in the study. Their age ranged from 12 to 89 years, with mean of 41.52 ± 20.53 years. Twenty six of them were males (52%) and twenty four were females (48%). Their mean duration of illness prior to admission was 7.43 ± 6.68 days.

Sepsis represented the major cause of SIRS in our study (43 patients (86%)), while non-infective SIRS was found in 7 patients (14%). Pneumonia was the leading cause of sepsis in our series; responsible for 21 (48.8%) cases, four of them were nosocomial. Acute pyelonephritis, intra-abdominal, and central nervous system infections were responsible for four cases each (8%). Two patients (4%) were found to have infective endocarditis. Although sepsis was suspected, the source of infection was not established in 3 patients. Community acquired sepsis represented (82%) of cases, the remainder (18%) were hospital acquired.

There was no statistically significant difference regarding the severity of illness (assessed by APACHE II score) or mortality between
hospital and community acquired cases. Four of seven patients with non-infective SIRS were found to have disseminated malignant diseases (carcinoma of the breast, prostate, teratoma and acute leukemia). Two patients had acute pancreatitis and a woman was diagnosed with active systemic lupus erythematosus (Table 1).

Sepsis was bacteriologically confirmed in 19 patients (44.2%). Confirmation was based on a positive blood culture in 8 patients (18.6% of all sepsis cases), sputum culture in 6 patients, CSF, pleural fluid, ulcer swab, urine and stool culture in one patient each. In the remaining 24 patients (55.8%), sepsis was suspected clinically, supported by laboratory and imaging results. There was no statistically significant difference between patients with positive and negative blood culture results in relation to APACHE II score, days of stay in hospital or mortality. However, patients with positive blood culture reached higher stage of illness (septic shock or MODS) when compared with those of negative blood culture (p=0.217).

The diagnosis of SIRS was based on two diagnostic criteria in 19 patients (38%), three criteria in 18 patients (36%) and the whole four criteria in 13 patients (26%). The increasing number of diagnostic criteria on which the diagnosis of SIRS was made was strongly associated with more advanced stage of illness (p<0.001) and higher mortality (p=0.0097).

An even stronger association was found between the level of consciousness assessed by Glasgow Coma Scale (GCS) and the subsequent stage of SIRS reached and the mortality rate. Patients with an initially reduced consciousness (GCS of 14 or less) reached higher stage of illness (more commonly passed to septic shock and MODS), and had higher mortality (77.3% versus 22.7%) compared with those having normal GCS on admission (p<0.001) (Table 2).

Anaemia was present in 22 patients (44%). These patients had significantly higher mortality than non anaemic patients (68.2% versus 31.8%, p=0.001). On the other hand, elevated ESR had no significant effect on the APACHE II score (p=0.115) or mortality rate (p=0.243), even when reached a level exceeding 70 mm/hr.

Hyperglycemia, defined as fasting blood sugar ≥7.8 mmol/l, had developed in 17 patients (34%); of whom 10 (58.8%) were non diabetic before their current illness (stress hyperglycemia). Patients with hyperglycemia had significantly higher APACHE II score (p=0.006), longer stay in hospital (p=0.029) and more advanced stage of SIRS (p=0.0109) (Table3). The mortality rate of these patients (58.8%) was higher than those who remained normoglycemic (36.4%) (p<0.001). Elevated serum urea (>7 mmol/l), rather than creatinine was associated with excess mortality rate (77.3% in those having high blood urea on admission, compared with 22.7% in patients with normal levels) (p=0.014).

Serum albumin level correlated significantly with the APACHE II score; the highest scores were encountered in those with serum albumin below 30g/ml (p= 0.007), and these patients reached more advanced stages of septic shock and MODS compared with those having normal serum albumin levels (p<0.001). There was also a non significant association between hypoalbuminaemia and a higher mortality rate and a longer stay in hospital (p=0.101 and p=0.301 respectively) (Table 4).

Overall, the most common organ dysfunction noticed in our study was related to the central nervous system (36% of cases), followed by the cardiovascular system (30%), kidneys (28%), liver (28%), lung (22%) and blood (10%). Two of our patients were already on ventilator therapy for respiratory paralysis caused by Guillain Barre syndrome before the development of sepsis (ventilator associated pneumonia). Eight patients (16%) required ventilator therapy to treat ARDS, or to support comatose patients. APACHE II score showed a very significant association with the stage of SIRS reached and mortality rate (p<0.001 for each).

The in-hospital mortality rate of our group of patients was (44%). Patients with sepsis had a mortality rate of 39.5%, while patients with non-infective SIRS had 71.4% mortality. This difference did not reach statistical significance (p=0.122). Nine patients (18%) had sepsis,
which did not progress further; one of them only died (mortality rate of 11.1%). Twenty patients (40%) reached a stage of severe sepsis (without further progression); of whom four died (mortality rate of 20%). Septic shock and MODS complicated severe sepsis in 6(12%) and 15(30%) of patients; their mortality rate were 50% and 93.3% respectively. Overall, the mortality rate of all patients who reached severe sepsis was 51.2%.

Twenty four of our patients were in the ICU (48%), and 26 were in the general medical wards (52%). Despite a higher mean APACHE II score (22.8 versus 15.2) and a more advanced stage of SIRS among patients admitted to the ICU, there was no significant difference in the mortality rate between the two groups (p=0.802). All ventilated patients were in the ICU.

Table (1): Causes of sepsis and non-sepsis SIRS.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia/Total</td>
<td>21</td>
<td>Disseminated malignancy/Total</td>
<td>4</td>
</tr>
<tr>
<td>Nosocomial pneumonia</td>
<td>4</td>
<td>Carcinoma of breast (lymphangitis carcinomatosis)</td>
<td>1</td>
</tr>
<tr>
<td>Empyema</td>
<td>1</td>
<td>Leukemic meningitis</td>
<td>1</td>
</tr>
<tr>
<td>Complicating measles</td>
<td>1</td>
<td>Carcinoma of prostate (cerebral metastasis)</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>15</td>
<td>Teratoma</td>
<td>1</td>
</tr>
<tr>
<td>Intra-abdominal infection/Total</td>
<td>4</td>
<td>Acute pancreatitis</td>
<td>2</td>
</tr>
<tr>
<td>Acute cholecystitis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver abscess</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelvic abscess</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Perforated acute appendicitis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute pyelonephritis</td>
<td>4</td>
<td>Autoimmune disease (SLE with lupus nephritis and cerebritis)</td>
<td>1</td>
</tr>
<tr>
<td>CNS infections/ Total</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encephalitis</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyogenic meningitis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurobrucellosis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infective endocarditis</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin and soft tissue infection/Total</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected decubitus ulcer</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulitis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others/Total</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary staphylococcal septicemia</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigellosis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppurative lymphadenitis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown cause</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td></td>
<td>7</td>
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</table>

Table (2): The association of Glasgow Coma Scale with the severity of sepsis and outcome.

<table>
<thead>
<tr>
<th>Stage of illness</th>
<th>GCS</th>
<th>Normal</th>
<th>Low</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>SIRS/sepsis</td>
<td>9</td>
<td>100</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Severe SIRS/severe sepsis</td>
<td>12</td>
<td>60.0</td>
<td>8</td>
<td>40.0</td>
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<tr>
<td>Septic Shock</td>
<td>2</td>
<td>33.3</td>
<td>4</td>
<td>66.7</td>
</tr>
<tr>
<td>MODS</td>
<td>1</td>
<td>6.7</td>
<td>14</td>
<td>93.3</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>48.0</td>
<td>26</td>
<td>52.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GCS</th>
<th>Mortality</th>
<th>Normal</th>
<th>Low</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dead</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alive</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>5</td>
<td>22.7</td>
<td>19</td>
<td>67.9</td>
</tr>
<tr>
<td>Low</td>
<td>17</td>
<td>77.3</td>
<td>9</td>
<td>32.1</td>
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<tr>
<td>Total</td>
<td>22</td>
<td>100</td>
<td>28</td>
<td>100</td>
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</table>
Table (3): The association of blood glucose level with the severity of sepsis and outcome.

<table>
<thead>
<tr>
<th>Stage of Illness</th>
<th>Blood glucose</th>
<th>&lt;7.8</th>
<th>%</th>
<th>≥7.8</th>
<th>%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>SIRS/sepsis</td>
<td>7</td>
<td>21.2</td>
<td></td>
<td>2</td>
<td>11.8</td>
<td>0.0109</td>
</tr>
<tr>
<td>Severe SIRS/severe sepsis</td>
<td>13</td>
<td>39.4</td>
<td></td>
<td>7</td>
<td>41.2</td>
<td></td>
</tr>
<tr>
<td>Septic Shock</td>
<td>5</td>
<td>15.2</td>
<td></td>
<td>1</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>MODS</td>
<td>8</td>
<td>24.2</td>
<td></td>
<td>7</td>
<td>41.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>100</td>
<td></td>
<td>17</td>
<td>100</td>
<td></td>
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</table>

Table (4): The association of serum albumin with the severity of sepsis and outcome.

<table>
<thead>
<tr>
<th>Stage of Illness</th>
<th>Serum albumin (g/l)</th>
<th>Normal</th>
<th>&lt;36</th>
<th>&lt;30</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>SIRS/sepsis</td>
<td>6</td>
<td>33.4</td>
<td></td>
<td>2</td>
<td>10.5</td>
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<tr>
<td>Severe SIRS/severe sepsis</td>
<td>9</td>
<td>50.0</td>
<td>9</td>
<td>47.4</td>
<td>2</td>
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<tr>
<td>Septic Shock</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td>5</td>
<td>26.3</td>
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<tr>
<td>MODS</td>
<td>3</td>
<td>16.6</td>
<td></td>
<td>3</td>
<td>15.8</td>
</tr>
<tr>
<td>Total</td>
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<td>100</td>
<td>19</td>
<td>100</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Serum albumin (g/l)</th>
<th>Normal</th>
<th>&lt;36</th>
<th>&lt;30</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Dead</td>
<td>6</td>
<td>33.3</td>
<td></td>
<td>7</td>
<td>36.8</td>
</tr>
<tr>
<td>Alive</td>
<td>12</td>
<td>66.7</td>
<td>12</td>
<td>63.2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>100</td>
<td>19</td>
<td>100</td>
<td>13</td>
</tr>
</tbody>
</table>

Discussion

Sepsis represented the majority of cases of SIRS in this study (86% of cases). Hernández et al noticed a similar proportion. They diagnosed sepsis in 79% of their patients, with the remaining 23% had non-infective SIRS (9). Sepsis was hospital acquired in 18% of cases only. Such cases constituted a much higher percentage in a recent Spanish study (49.5%) (8). The lower impact of hospital acquired infections reflects the under use of instrumentation (including intravenous catheterization and mechanical ventilation) in our hospital.

Pneumonia was the commonest cause of sepsis in both community and hospital acquired cases in our study (48.8%). Almost all recent studies in the field found the lungs (pneumonia) the major source of sepsis (4,8,10-14), this ranged from a percentage of 40% in a large multicentre trial in USA (4) to 86% in a pan-European study published in 2006 (10). The only notable exception was a recent Mexican study, where abdominal infection predominated over pulmonary infection (15). Abdominal and urinary tract infections were the second and third causes of sepsis in our study, shared by most other similar studies (8,10,12-14).

Sepsis was more frequently suspected than microbiologically documented. Periera et al from Portugal had a similar percentage of culture proven cases (14)(39% compared with 44% in our study). However in three other larger studies, 60% – 64% of sepsis cases were microbiologically documented (8,10,16). It seems that over-reliance on empirical therapy in our centre has largely replaced a thorough and careful search for microbiological confirmation.
Blood culture was positive in 18.6% of cases; a percentage quite similar to two other studies conducted by Rangel-Frausto et al (17)(17%) and Pereira (14)(20%), and a little less than the results of Selberge et al (18) (30%) who tried their best to differentiate sepsis cases from non-infective SIRS in order to compare certain biochemical markers. The low percentage of positive blood culture in general reflect the fact that sepsis does not indicate the presence of viable bacteria in the bloodstream, but rather an uncontained inflammatory response to infection. Moreover, many patients had received frequent courses of antibiotics before being admitted as sepsis (which reduces the chance of positive blood culture) and infections caused by non bacterial pathogens are undetectable by standard cultures. Variation in the number of blood culture positive cases in different studies is also influenced by the location of infection. For example, peritoneal infection results in a more frequent release of bacteria to the circulation compared with pulmonary infection (18). Positive blood culture was associated with higher prevalence of septic shock and MODS. Rangel-Frausto et al found a stepwise increase in the percentage of positive blood culture with increasing stage of sepsis (17%, 25% and 69% for severe sepsis, septic shock and MODS, respectively) (17). Two multi-centre trials in Portugal (14) and France (16) found bacteraemia (manifested by positive blood culture) a risk factor for early mortality. Despite the higher mortality rate in blood culture positive patients in our study (75% Vs 38%), the small sample size did not mount a statistical significance.

The increasing number of diagnostic criteria on which the diagnosis of SIRS was made strongly correlates with more advanced stage of illness and higher mortality. Sprung et al found that fulfilling more than two criteria carries a higher risk of subsequent development of severe sepsis, septic shock and MODS (19). This finding was confirmed by Rangel-Frausto et al who stated that “SIRS with only two criteria – as initially proposed – is less helpful in defining a subset of ICU and ward patients who are at especially high risk of severe sepsis than SIRS with three or all four criteria” (17).

Our findings regarding anaemia in sepsis patients is consistent with the accumulating evidence that anaemia in critically ill patients is common and correlates with poor outcome (20,21). The mechanism of anaemia in these patients is similar to that of chronic disease anaemia, except that the onset is generally rapid (21). Despite the deleterious effect of anaemia of critical illness, aggressive treatment with blood products can be as detrimental as no treatment with associated increase in morbidity and mortality (21,22). The use of erythropoietin stimulating agents is rapidly gaining acceptance as a substitute to transfusion therapy (22).

High ESR had no relation with severity of illness assessed by APACHE II score or mortality. This could be due to the fact that ESR is a crude indirect measure of acute phase response. Even an ESR higher than 70 ml/hr was not found a poor outcome index in these patients.

Acute hyperglycaemia is frequently present in situations of stress in both diabetic and non-diabetic patients (23,24). The prevalence of hyperglycaemia in critically ill patients depends on the defining criteria. In one study conducted in a medical ICU, admission blood glucose above 11.1mmol/L was present in 23% of patients (25). In another study, conducted in a surgical ICU, admission glucose level was >6.1mmol/L in 86%, almost all of patients became hyperglycaemic during ICU stay (26). Applying our definition of 7.8 mmol/L, a prevalence of 34% in our study is almost similar.

The strong association between ICU hyperglycaemia and excess morbidity and mortality noticed in our study was also shown by similar studies. Van der Berghe et al reported dramatic (42%) relative reduction in mortality in a surgical ICU when blood glucose was normalized to 4.4 – 6.1 mmol/L by means of insulin infusion (compared with 10 – 11.1 mmol/L in the control group) (26). The benefit of glucose reduction in the medical ICU was less certain (27,28).
The adverse effect of hypoalbuminaemia in acute illness has been confirmed in a meta-analysis. Hypoalbuminaemia was found a potent and dose dependant predictor of mortality, independent of nutritional status or inflammation. Each 10 gm/L decline in serum albumin concentration significantly raises the odd ratio of mortality by 137%, morbidity by 84% and ICU stay by 28% (29). However, the use of albumin for volume resuscitation of critically ill patients with serum albumin concentration ≤ 25 gm/L was not associated with reduction of mortality, duration of ICU stay or mechanical ventilation (30,31). A potential beneficial role of albumin in patients with sepsis requires further study (31). The association of low serum albumin with disease severity was clearly shown in our study, but significant correlation with mortality rate and hospital stay has not been reached, perhaps because of small sample size.

The overall mortality rate of sepsis in our study was somewhat high (39.5%). In recent epidemiological studies, the mortality rate of sepsis has ranged from 9% (17) to 48.2% (13). In the above mentioned pan-European study (10), wide variation in mortality of severe sepsis has been noticed in different centres around Europe; being lowest in Switzerland (10%) and highest in Portugal (64%). In comparison, our result of 51.2% mortality rate of these patients seems acceptable. Despite the more advanced stage of SIRS reached, and the higher mean APACHE II score of our ICU patients compared with those in the general medical wards, there was no significant difference in mortality between these two groups. This result was in agreement with Guidet et al, who found a mortality rate of 49% in severe sepsis patients in the general medical wards and 42% in ICU patients (32). Blanco et al showed a mortality rate 55% in septic patients in the general wards and 48% in the ICU (8). The similar mortality rate (despite less severe illness of SIRS patients who remained on the general wards) calls for serious consideration of ICU admission for most cases of SIRS, especially for those who develop severe sepsis.

References


P53 in breast carcinoma: an immunohistochemical study

Zainab W. Aziz*, Shuaib H. Saleem**

* Department of Pathology, Nineveh College of Medicine, ** Department of Pathology, College of Medicine, University of Mosul.

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ABSTRACT

Objectives: The aims of the present study are; first, to find out the relative frequency of p53 over-expression in different types of breast cancer. Second, to correlate the p53 over-expression with different parameters, including the age and menopausal status of the patient, size, grade, stage, type of the tumor, and the status of axillary lymph nodes. Third, to compare our results with others

Methods: The study was both pro and retrospective and included 60 cases of breast carcinoma. Data were obtained from archives of the pathology department, at Al-jumhuri Teaching Hospital and collected in a period spanning from August 2008 to January 2009. P53 over-expression was assessed immunohistochemically.

Results: The patients ages ranged from 25 to 78 years (mean: 51.5 year); most of them were in the fourth decade (41.2%). There was a significant inverse relation between p53 over-expression and the age of the patients (p<0.001), in which the largest percentage of p53 positivity seen in the third decade. P53 over-expression was detected in 38.3% of the cases. P53 over-expression was found in (100%) of medullary carcinoma, 19/47 (40.4%) of invasive ductal carcinoma (NOS), 1/3 (33.3%) of ductal carcinoma in situ, and 1/6 (1.7%) of invasive lobular carcinoma. P53 over-expression was not detected in mucinous and papillary carcinomas.

There was a significant direct correlation between p53 over-expression and tumor size (p=0.0274), grade (p=0.032), and stage (p<0.001).

There were no statistically significant relations between p53 over-expression and the menopausal status (p=0.262) or axillary lymph node metastasis (p=0.471).

Conclusions: Immunopositivity for p53 tumor suppressor protein was detected in 38.3% of the cases in this study. P53 over-expression was significantly correlated with patient's age, tumor grade, stage, and size, but no correlation was found with menopausal status and axillary lymph node metastasis.

Keywords: Breast carcinoma, P53 over-expression
Breast cancer is the most common type of cancer among women in both high-resource and low-resource settings, and is responsible for over 1.3 million of the estimated 10 million neoplasms diagnosed worldwide each year in both sexes (1). Since the p53 tumor suppressor gene has been found to be mutated in more than 50% of human cancers, it has attracted the interest of numerous researchers (1). In breast cancer the reliability for detecting p53 mutations by IHC was found to be high enough with particular attention to the simplicity and cost-effectiveness when compared to other techniques.

Several studies believe that it is important to investigate the correlation between p53 immunostaining with traditional factors, to predict the prognosis, drug responsiveness, and survival rate among patients with breast cancer (2). In general p53 over-expression in breast cancer is in the range of 19%–40% (2). P53 is encoded by Tp53 gene, located at 17p13, this contains 11 exons spanning 20 kb (2, 3).

There are 3 functional distinct regions in p53:
1- Acidic n-terminal region (codons 1-101).
2- Central DNA binding core region (codons 102-292).
3- Basic c-terminal region (codons 293-393).

P53 acts as brakes to the cycle of cell growth, DNA replication and division into two new cells (3), and preventing inappropriate cell proliferation and maintaining genome integrity following genotoxic stress (4).

Our aims of the present study are: (1) finding out the relative frequency of P53 over-expression in different types of breast carcinoma. (2) correlating the P53 over-expression with different parameters including the age and the menopausal status of the patient, size, grade, stage, type of the tumor, and status of axillary lymph nodes. (3) comparing our results with others.

**Patients and Methods**

**Patients:** A pro and retrospective study was carried out in a series of sixty consecutive patients diagnosed with primary breast carcinoma. All patients had operable tumors with no evidences of distant dissemination at the time of diagnosis, and they underwent lumpectomy or modified radical mastectomy as well as axillary lymph node clearance. A representative tissue block of each case was chosen for IHC.

Clinicopathological data were obtained from archives of the department of pathology laboratory, at Aljumhuri Teaching Hospital, Mosul City and these included:
Age (divided into six groups 21-30, 31-40, 41-50, 51-60, 61-70, 71-80), menopausal status (divided into two groups ≤50 and >50 years), tumor size in centimeters (<2, 2-5, >5); histological type which was evaluated according to the WHO classification. Tumor grade was assessed by using Nottingham modification of the Bloom Richardson Grading system and tumor stage by adopting the TNM system.

**IHC staining:** Tissue paraffin blocks were cut into sections of 4µm and mounted on silanized slides. The slides were left in the oven at 65 °C overnight. Sections then deparaffinized and rehydrated by descending grades of ethanol and distilled water. The slides were immersed in preheated retrieval solution and the Coplin jars were put in water bath (95-99) °C for 30 minutes. Next, they were left to cool at room temperature for 20 minutes. Levamisole blocker was added, and slides were incubated for 20 minutes, and then rinsed with wash buffer. Slides were incubated with 100µl primary antibody for 30 minutes, followed by rinsing with buffer. Slides were incubated with secondary antibody for 30 minutes in warm area, and then rinsed with wash buffer bath for 5 minutes followed by preparing working solution of 1ml of permanent red substrate buffer and substrate buffer solution with +10µl of permanent red chromogen solution and lastly were put in dark area. Two hundred µl of this solution was added on each slide for 10-15 minutes and then the slides were incubated for 15 minutes. The slides were rinsed with distilled water and bathed for 5 minutes, which was followed by another wash. Positive and negative control slides were involved in each run. Known positive breast carcinoma samples were used as positive control. For negative control the same protocol was followed with omission of the primary antibody and incubation with Tris Buffered saline (TBS).

**IHC analysis:** H-score was used to indicate positivity of p53 nuclear staining. The intensity of immunostaining on each slide was rated on a four-point scale: 0 none; 1+ light; 2+ moderate; 3+ heavy; and 4+ intense. The percentage of immunopositive tumor cells was determined by counting a minimum of 200 cells from at least three representative high-power fields. H-scores were then calculated as the product of intensity (0 to 4) X distribution (0% to 100%), with H-score ranging from 0 to 400. A cut-off value for the H-score of ≥50 was used to indicate p53 positivity.

**Statistical analysis:** The relationship between p53 over-expression and various clinicopathologic parameters was analyzed by Fisher Freeman Holtons’ test. The results were considered statistically significant if the p value was ≤0.05.

**Results**

The patients’ age range was 25-78 years (mean 51.5 year), most of them were in the fourth decade (41.2%), however data concerning the age were missing in 9 patients. Forty four patients (73.3%) were premenopausal and sixteen (26.7%) were post menopausal.

The tumor size ranged from 1 to 10cm (mean 4.2cm) most of the tumors (61.6%) were T2. Histologically, there were 47 infiltrative ductal carcinoma (NOS) and 13 cases showing different patterns (6 invasive lobular, 3 DCIS, 2 medullary, 1 mucinous and 1 papillary).

Most of the cases of IDC were categorized as grade II (56.9%); grade III and grade I formed (37.2%) and (5.9%) respectively. Thirty three cases (55%) presented with axillary lymph node involvement by metastatic disease.

**Correlation between p53 over-expression and the studied clinicopathological parameters:** There was a significant correlation between p53 over-expression and the age of patients (p<0.001) with largest percentage of p53 positivity seen in patients in the third decade, (Table 1). An increased relation was found between p53 positivity and the age of the patients.

There was no statistically significant relation between p53 over-expression and the menopausal status, (p=0.262). (Table 2).

P53 over-expression statistically had significant correlation with tumor size (p=0.0274), as the largest proportion of p53 positivity occurred in T3 (53.3%), (Table 3).
Regarding tumor type (Table 4), P53 was positive in 100% of medullary carcinoma, in 40.4% of invasive ductal carcinoma (NOS), in 33.3% of ductal carcinoma in situ, in 1.7% of invasive lobular carcinoma and in 0% of mucinous and papillary carcinomas. (Fig 1-3).

Significant direct relation was found between p53 and tumor grade (p=0.032), p53 immunopositivity increased from 33.3% in grade I to 36.9% in grade III, (Table 5).

P53 over-expression failed to show a significant relation with axillary lymph node metastasis (p=0.471), although p53 was positive in 42.2% compared to 33.3% in tumors with no ALN metastasis, (Table 6).

There was a significant direct relationship between p53 over-expression and the tumor stage (p<0.001), (Table 7).

Table (1): P53 over-expression and patient's age.

<table>
<thead>
<tr>
<th>Age (year)</th>
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<th>-ve</th>
<th>p-value</th>
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<td>7</td>
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<td>2</td>
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<td>71-80</td>
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<table>
<thead>
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<td>&gt;5</td>
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<table>
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<td>ILC</td>
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<td>Medullary</td>
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<td>Papillary</td>
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Table (5): P53 over-expression and tumor grade.

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<td>%</td>
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<td>36.9</td>
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Table (6): P53 over-expression and ALN.

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<td>%</td>
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<td>9</td>
<td>33.3</td>
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<tr>
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<td>60</td>
<td>100</td>
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<td>38.3</td>
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Table (7): P53 over-expression and stage of tumor.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total</th>
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<th>P53-ve</th>
<th>p-value</th>
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</thead>
<tbody>
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<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
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<td>0.0</td>
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<td>60.0</td>
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<tr>
<td>Stage IIIA</td>
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<td>6</td>
<td>40.0</td>
</tr>
<tr>
<td>Stage IIIB</td>
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<td>15</td>
<td>4</td>
<td>44.4</td>
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<td>7</td>
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<td>4</td>
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<tr>
<td>Total</td>
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<td>100</td>
<td>23</td>
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Figure (1): IHC staining for p53 (control) (x100), left positive; right negative.

Figure (2): Infiltrative lobular carcinoma. Positive staining. Left, (x100); right, (x400), (H Score=270).
Discussion
Among the biological markers investigated, p53 gene has received a considerable attention as a promising prognostic and predictive marker in breast cancer. P53 overexpression is believed to be an early stage in tumorigenesis, thus immunostaining to detect mutant p53 provides sufficiently reliable information for clinical decision making. In the current study p53 immunostaining was positive in 38.3%. However others reported different results.

Our study showed a significant inverse correlation with increasing age (p=0.001), with higher expression being found in younger patients and lower expression in older age group. This is comparable to the findings of most of other studies, but different from Pich A and Levesque et al. There was a tendency for occurrence of p53 immunopositive tumors in the premenopausal women although this did not achieve statistical significance. Similar results were obtained by others. However, Uchikawa et al. revealed a weak correlation with postmenopausal status.

The higher level of positive p53 immunostaining was detected in larger tumor size. These results were comparable with the data in the literature.

In the current study, the strong positivity of p53 seen in medullary carcinomas and the low reading of p53 in lobular carcinoma are explained by the relation of p53 with the grade of the tumor. Medullary carcinoma is always of high grade so high correlation with p53 while invasive lobular carcinomas are of low grade so weakly correlated with p53. These findings are comparable with those found by Mhjoub et al. On the other hand Iwaya and Eteebary in their study failed to show a particular predilection of p53 immunostaining to certain types of breast cancer.

The frequency of p53 mutation is directly correlated with the grade of the breast cancers i.e. higher expression is detected in higher grade. This fact is also reflected in our study. Other people reported no significant correlation.

We couldn’t find a significant correlation between p53 and ALN metastasis and this is similar to most studies.

Most of our cases were in stages II and III, the same as in other studies which are rather advanced for this type of tumor. We found a significant direct relation between p53 over-expression and tumor stage (p<0.001), which is similar to those of Uchikawa and Mourao et al.

Conclusions
The 38.3% p53 positivity in breast carcinoma was within the general range observed by others. P53 over-expression showed a significant correlation with the patient age, and size, grade and stage of tumor. However there was no significant correlation with ALN metastasis and menopausal status. P53 over-expression was positive in the two cases of medullary carcinoma, showed lower positivity in lobular carcinoma and was negative in mucinous and papillary carcinomas. Finally

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validity and simplicity of application of IHC technique in determining the status of p53 protein, as it was clearly noticed encourage its use as a prognostic factor.

References
The effect of concentrated bone broth as a dietary supplementation on bone healing in rabbits

Mahmood A. Aljumaily
Department of Surgery, College of Medicine, University of Mosul.

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ABSTRACT

Background: A variety of substances, biological and physical interventions have been used to enhance bone fractures healing. There is growing evidences for the importance of nutrition in maintenance of bone health and support of fracture healing.

Objective: The aim of this study was to evaluate the effect of the daily oral dose of concentrated bone broth as dietary supplementation on bone healing in experimental animals (rabbits).

Material and methods: Twelve young male rabbits divided into two groups after induction of open ulnar osteotomy, the experimental group receive daily dose of concentrated bone broth as a dietary supplementation. The control group received ordinary diet. In the end of second weeks three animals from both group were killed, at the end of fifth week, the other animals were sacrificed and the specimens taken for radiological and computerized tomography (CT) scan densimetry and histomorphometric evaluation carried out for the callus at site of osteotomy.

Results: The ulnar bone osteotomies in both groups were united at the end of the fifth week macroscopically and radiologically. The callus density was measured in site of osteotomy by CT scan densimetry, at the end of 2nd week the difference between the experimental and control group was not significant, (P value > 0.05), and at the end of 5th week the difference between the experimental and control group was highly significant, (P value < 0.001). The histomorphometric evaluation of healing in site of osteotomy carried out, and at the end of 2nd week the difference between the experimental and control group was not significant, (P value > 0.05), and at the end of 5th week the difference between the experimental and control group was significant, (P value < 0.05).

Conclusion: The present study demonstrate that a daily oral administration of concentrated bone broth as dietary supplementation in rabbits enhance bone healing by increase callus density in CT, and improve histomorphometric evaluation of healing.

Keywords: Bone broth, fracture, healing, and dietary supplementation.
Traditional recipes for treatment of physical and mental illness exist in all major ancient civilizations of the world. All cultures around the globe have used bone broth as part of their traditional dishes for centuries and used it as healing tonics for many ills. The use of bone broth dietary supplementation by the common folk for promoting fracture healing process is an old practice in our community. Bone broth simply have met and stood the test of time within human cultures. Simmering bones in water extract many constituents contained in them like proteins, amino acids, glucosamine, hyaluronic acid, chondroitin sulphate, minerals, vitamins and other trace substances. Bone broth is excellent source of minerals, since these naturally derived minerals are extracted from bone, they are in an ideal balance and easily utilized by the body. By reviewing the available literatures, there were no registered studies on the effects of concentrated bone broth as dietary supplementation on bone healing.

Bone mineral density measurement by computerized tomography (CT) is noninvasive, and a reliable tool for quantification of the fracture repair process in experimental animals. The mineral density of callus correlated positively with callus strength and stiffness. Bone histomorphometry is a quantitative histological examination of decalcified and undecalcified bone biopsy performed to obtain quantitative information on bone remodeling and structure. Histomorphometric evaluation provides clear evidence of the healing process. CT evaluations of bones show highly significant correlation with those determined using histomorphometry.

The aim of this study was to evaluate the effect of the daily oral administration of concentrated bone broth as dietary supplementation on bone healing in experimental animals (rabbits).

Material and methods
This study was approved by the scientific research committee at the College of Medicine, University of Mosul, and follows the council for international organization of medical sciences ethical code for animal experimentation. Twelve young male aged 4 months locally breed New Zealand rabbits from animal house, College of Medicine, University of Mosul were used in this study at 1st of November 2009 to 30 of April 2010. Their average weight 1470 grams ranged between 1260 grams and 1520 grams. The animals were kept in separate metallic cages for one week for adaptation in animal's house. In each cage one animal feed with standard ration and water.

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Bones broth preparation
The bone extract done to some extent to that prepared by our population as bone soup given to patients. Fifty hundred grams of bone taken from bovine femoral head and neck, broken into pieces and cooked with 2 liters of distilled water in a pot for 4 hours, the bone removed and allowed to cool, and placed in refrigerator overnight for excess fat to congeal. The excess fat on surface of fluid removed, and the pot back on the stove and allowed to concentrate to 100 ml. The fluid stored in 4ºC, and three milliliters of concentrated broth mixed with animal daily diet of experimental group.

Experimental technique
Food was suspended eight to ten hours prior to administration of anesthesia. To decrease the vagal tonus, each animal received 0.2 mg/kg dose of atropine sulphate by intramuscular injection. Animals were anesthetized by intramuscular injection of ketamine (50 mg/kg of body weight) and intramuscular injection of diazepam (5.0 mg/kg of body weight). Preoperative antimicrobial prophylaxes consisting of 100 mg/kg of ceftriaxone were injected subcutaneously in proximal part of the same limb. Sample of venous blood aspirated to measure serum calcium, phosphate, and alkaline phosphatase.

The right forelimb was shaved and cleaned by betadine solution. Under aseptic techniques, the right ulna of each animal was accessed by an anterior longitudinal skin incision of approximately 20 mm. After division of the skin and subcutaneous tissue, the fascia, the muscles and tendons were retracted and the periosteum was opened and dissected from the ulna. The ulnar shaft was exposed; osteotomy was performed on the exposed portion of the ulna by means of a one millimeter blade thickness sterile hand saw. The incision was closed, using absorbable 5-0 polyvycril sutures for the fascia and 4-0 monofilament PDS sutures for the skin, local dressing applied locally using sterile gauze covered with adhesive plaster. No surgical fixation or external splintage were used in these osteotomies.

The animals were assigned to two groups; the first group (6 animals) as experimental group received a daily dose of 3 ml of concentrated bone broth as dietary supplementation in addition to their ordinary diet and continued for five weeks. The second group was the control group (6 animals) received ordinary diet. At the end of 2nd week, 3 animals from each group were sacrificed, other 3 animals from each group were sacrificed at the end of 5th week. Animals were anesthetized as described previously, a sample of blood aspirated to measure serum calcium, phosphate, and alkaline phosphatase, and killed with a 2 ml intracardiac injection of potassium chloride. The right ulna of each animal was removed, dissected from the surrounding soft tissue and prepared for radiological, CT, and histological examination.

The samples examined radiologically by Siemen- Sirography fluoroscopy equipment 62 K.T.; the KV used in taking x-ray was 30 KV, 50 mA. The CT scan examination carried out to measure the density of callus at the site of osteotomy. The CT scan equipment was light speed, multidetector equipment, General Electric (GE), 32 Yokogawa Medical System, taken TA 0.6 mm slice thickness. The mean of five points taken at the site of osteotomy to measure the density of callus, the means and standard deviations of these values calculated.

The sites of osteotomy were carefully exposed by removal of all the soft tissue. The ulnar bones were removed, and fixed with 10% formaldehyde solution. After fixation, they were decalcified in 10% foramic acid. The decalcification process demineralized the bone, leaving only the soft tissues and bone matrix. This was done to ensure that thin sections could be examined histologically. Thin sections embedded in paraffin wax were cut and stained with haematoxylin and eosin. The site of osteotomy examined histologically. The progression of fracture-healing in each specimen was quantified with the use of a scale that assigns a grade based on the relative percentages of fibrous tissue, cartilage, woven bone, and mature bone in the callus (histomorphometric evaluation) (8).
Grade 1 indicates fibrous tissue; grade 2, predominantly fibrous tissue with some cartilage; grade 3, equal amounts of fibrous tissue and cartilage; grade 4, all cartilage; grade 5, predominantly woven bone; grade 6, entirely woven bone; grade 7, woven bone and some mature bone; and grade 10, lamellar (mature) bone. Five views were examined at site of osteotomy, and the mean of fracture healing scores were calculated for each group. The histomorphometric examination carried out in Al-Jumhori hospital laboratory. The grading was done blindly without knowing which treatment had been given.

Statistical analysis
Results were reported as mean ± standard deviation. The unpaired student (t) test used to calculate the differences between two means. The P value was considered a significant if it was less than 0.05.

Results
All animals survived to the end of the study. Neither wound infection nor wound dehiscence were observed in the animals of either group. All animal at time of osteotomy had normal serum calcium (the mean was 3.4 ± 0.22 mmol/dl), serum phosphate (the mean was 1.45± 0.18 mmol/dl), and serum alkaline phosphatase (the mean was 11.6 ± 2.4 IU unit/ dl). At the time of scarifying animals, the serum calcium was normal (the mean was 3.4 ± 0.14 mmol/dl), the serum phosphate was normal (the mean was 1.42 ± 0.1 mmol/dl), and serum alkaline phosphatase was normal (the mean was 12. ± 1.6 IU unit/ dl). By using student (t) test to compare the differences between two mean, there was no statistically significant difference (p> 0.05) in the means of serum calcium, phosphate, alkaline phosphatase at starting experiment and at scarifying animals. Macroscopic evaluations demonstrate that all osteotomies were united completely by the end of 5th week of the study, and there is soft callus in the end of 2nd week in both groups. Radiological examination shows that osteotomies were at different stage of bone healing according to dating of scarification.

The callus density was measured in site of osteotomy by CT scan densimetry, its mean at the end of 2nd week in the experimental group was 110.73 ± 51.23 and in the control group was 98.7 ± 26.24, the difference between the experimental and control group was not significant, p value was 0.69, (P value > 0.05), at the end of 5th week in the experimental group was 510.47  ± 134. 84 and in the control group was 261.13 ± 70.03, with p value 0.0001, the difference between the experimental and control group was highly significant, (P value is < 0.05), (table 1).

Table (1): Histomorphometric evaluation of bone healing and callus density evaluation by CT scan in site of osteotomy in experimental group and control group at the end of 2nd and 5th week.

<table>
<thead>
<tr>
<th>Evaluation type</th>
<th>Time of end</th>
<th>Experimental group</th>
<th>Control group</th>
<th>P value</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Standard deviation</td>
<td>Standard deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histomorphometric evaluation of bone healing in site of osteotomy</td>
<td>At end of 2nd week</td>
<td>4.33</td>
<td>0.72</td>
<td>4.27</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>At end of 5th week</td>
<td>7.67</td>
<td>0.62</td>
<td>7.03</td>
<td>0.74</td>
</tr>
<tr>
<td>Callus density evaluation by CT scan in site of osteotomy</td>
<td>At end of 2nd week</td>
<td>116.4</td>
<td>46.8</td>
<td>95.8</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>At end of 5th week</td>
<td>510.47</td>
<td>134.84</td>
<td>261.13</td>
<td>70.03</td>
</tr>
</tbody>
</table>
The mean of histomorphometric evaluation of healing in site of osteotomy at the end of 2nd week were 4.33 ± 0.72 in experimental group, while in control group were 4.27 ± 0.70, the p value was 0.8, the difference between the experimental and control group was not significant, (P value > 0.05), and at the end of 5th week were 7.67 ± 0.62 in experimental group while in control group were 7.13 ± 0.74, the p value was 0.041, the difference between the experimental and control group was significant, (P value < 0.05), (table 1). The Histopathological examinations of the osteotomy site shows healing bone at different stages according to time of bone harvesting, there is no evidence of infection or foreign body reaction in site of osteotomy.

Discussion
In this study, the concentrated bone broth shows highly significant increased the density of callus in CT densimetry measurement in site of osteotomy at the end of 5th week in comparison with control group. The histomorphometric evaluation show significant improvement in healing process in site of osteotomy at the end of 5th week in comparison with control group. At the end of 2nd week the difference in CT callus density and histomorphometric evaluation was not significant between experimental and control group. Histopathological examination shows good union without complications (infection or giant cell reaction) in both groups. The findings in this study support the common folk idea that this dietary supplementation enhances bone healing when given in course of fracture healing for sufficient time. Bone broth, made from the bones of animals, has been consumed as a source of nourishment for humankind throughout the ages (2). It is a traditional remedy across cultures for the sick and weak (2).

There is growing evidences for the importance of nutrition in maintenance of bone and joint health (9). Bone is complex tissue that requires many nutrients (10). The recommended therapeutic supplementation includes about 20 bone building nutrients for acceleration of bone healing and to reduce complications(10). Multi-nutrient supplementation includes proteins and amino acids, carbohydrates, fatty acids, antioxidants, calcium, phosphorus, vitamin D, fluoride, vitamin C, zinc, copper, magnesium, silicon, and trace of other substances, help in the acceleration of bone healing(8,11-16). Strength of callus in the process of fracture healing depends on an adequate intake of dietary protein as well as an adequate supply of minerals (17).

American Academy of Orthopedic Surgeons statement on animal use in experiments insists on: that the protocol should be designed to minimize the number of animals used (18). The number of animals used in this experiment is sufficient to get a conclusion and to stimulate more wide clinical studies. The serum calcium, serum phosphate, serum alkaline phosphatase were normal in rabbits. This finding indicates that animals are healthy and had no systemic bone disease through all the time of study.

To compare the findings of our study with other studies, we reviewed the available scientific literatures. There were no registered studies on the effects of concentrated bone broth as dietary supplementation on bone healing, whether in experimental animals or in humans. Modern sciences are beginning to validate what tradition has valued for centuries. The bone broth is available, cheap, easy to be prepared, and can be very useful for patients in developing poor community with limited medical resources. Randomized prospective clinical studies indicated to support this finding in human.

In conclusion, this study demonstrates that concentrated bone broth as dietary supplementation improved significantly the healing process in rabbit's ulnar osteotomy, this effect characterized by increase callus density in CT, and improved results of histomorphometric evaluation in fracture healing.

References
C-reactive protein and lipid profile among depot-medroxyprogesterone acetate injections users

Wahda B. Al-Youzbaki
Department of Pharmacology, College of Medicine, University of Mosul.

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ABSTRACT

Objective: To study the effect of depot-medroxyprogesterone acetate (DMPA) injections on C-reactive protein (CRP) and lipid profile and to find the predictors (body weight, body mass index (BMI), blood pressure (BP) and lipid profile) that significantly predict the risk of cardiovascular disease (CVD) among DMPA injections users.

Method: A prospective cohort study was performed during the period from March 2009 to March 2010 included thirty apparently healthy married women, their age ranged between 20-35 years, who were attending Al-Batool and Al-Khansa Family Planning Centers in Mosul and started (for the first time) to use DMPA injections (150 mg medroxyprogesterone acetate), called "Depo-Provera" as contraceptive. These (DMPA users group) were compared to another 30 healthy married women who did not use any hormonal contraceptives (non users group). Both groups were followed for one year, during which blood samples were obtained from both groups, before starting to use DMPA, after 6 months and after 12 months. Sera were used for the estimation of the biochemical studied parameters using commercial kits except serum low density lipoprotein (LDL) and atherogenic index (AI) which were calculated by special equations.

Results: DMPA injections caused a non significant increase in body weight but a significant increase in BMI after 12 months. There was a significant increase in the mean diastolic blood pressure (DBP) of DMPA users according to the duration of use. The DMPA caused non significant changes in the CRP levels. There was a significant increase in serum triglycerides (TG) after 6 months of DMPA uses with respect to the duration of use. But there were non significant changes in mean serum total cholesterol (TC), high density lipoprotein (HDL), LDL and AI. Among all variables that were studied, only body weight and BMI showed a significant positive correlations with CRP. Using a stepwise multiple regression analysis, it was found that the predictors that significantly predict the risk of CVD among DMPA users were AI, DBP and TG.

Conclusion: This study found that there is a significant positive association between CRP and CVD risk factors in DMPA injections users as contraceptive. Furthermore AI, DBP and TG were found to be significant predictors for the risk of CVD among DMPA users. This study confirmed the safety of DMPA use as contraceptive medication, but that special care should be directed for patients with CVD and other patients who were more sensitive to the harmful effects of lipid in the blood.

Key words: Depot-medroxyprogesterone acetate, CRP, lipid profile.
Since atherosclerosis may in part be an inflammatory disease, CRP, a marker of low grade chronic inflammation, has been identified as biomarker for cardiovascular disease (CVD) in general population. CRP, a marker of acute phase reactant, has been shown to provide additional prognostic information to LDL cholesterol, TC and HDL cholesterol in women. Recent data also indicate that C-reactive protein is not simply a short term marker for risk, even if it is derived from acute phase reactants. Depo-Provera, a single intramuscular injection of Medroxyprogesterone acetate (DMPA), is a highly effective, convenient non-daily hormonal contraceptive option that has been available worldwide for many years. It is approved by the US Food and Drug Administration (FDA) since 1992 and used worldwide by more than 90 million women. Long term use of DMPA injections may cause a reduction in menstrual blood loss, decreasing the risk of endometrial cancer and suppression of endogenous estrogen secretion which leads to reversible reduction in bone density and changes in plasma lipids associated with increased risk of atherosclerosis.

Since atherosclerosis may in part be an inflammatory disease, circulating factors related to inflammation may be predictors of CVD in general population. CRP, a marker of low grade chronic inflammation, has been identified for both men and women as an independent predictor for CVD and has recently been shown to provide additional prognostic information to LDL, TC and HDL in women. Recent data also indicate that level of CRP adds to the predictive value of lipid parameters in determining risk of a first myocardial infarction and screening based on lipid levels may provide an improved method of identifying women at risk of CVD.

C-reactive protein is not simply a short term marker for risk, as it has previously been demonstrated in patients with unstable angina, but a long term marker for risk, even...
for events occurring six or more years later\(^5\). The relationship between inflammatory factors and coronary heart disease (CHD) suggests that subclinical chronic inflammation may have a major role in the development of atherosclerosis\(^11\). It is now well established that atherosclerosis originates in early life, and that its risk factors track to adulthood\(^4\).

The relative CVD risk associated with elevated TG levels is greater in women than in men\(^12\) and the threshold for increased risk from low HDL is higher\(^13\). HDL is atheroprotective as evidenced by a strong inverse association between HDL levels and coronary heart disease (CHD) risk\(^12\). Beside that, HDL levels greater than 60 mg/dl (1.55 mmol/l) which are more commonly found in women than in men, are so protective as to essentially negate the effect of one of other CHD risk factors\(^14\).

Inflammatory processes, along with plasma lipids and lifestyle behaviors, play a pivotal role in the pathogenesis of cardiovascular diseases\(^7,15\). In both men and women, several epidemiological studies now indicate that the relationship between the inflammatory biomarker of high-sensitivity C-reactive protein (hsCRP) and future vascular events is strongly independent of other risk factors and the association of hsCRP with vascular events provided a strong argument for screening in the primary prevention population\(^7\).

The relationship between CRP and the risk of CHD has been shown in adults\(^4\). Despite its widespread use, the cardiovascular effects of DMPA in young women are unclear, so the current study was conducted to investigate the association of serum CRP with body weight, BMI, BP and lipid profile among young DMPA injections users as contraceptives and to find the significant predictors of the risk of CVD among these users.

**Subjects and methods**

The approval of the study protocol by an ethical committee was obtained from local health committee of Ministry of Health, and College of Medicine, University of Mosul. This study included 30 apparently healthy married, not pregnant, not lactating women, were fertile at the time of study, having regular menstrual cycle, who were attending Al-Batool and Al-Khansa Family Planning Centers in Mosul. A written consents were taken from the women after explanation.

The following inclusion criteria were put: age 20-35 year, BMI < 25, hemoglobin not less than 10.5 g/dl, and no hormonal contraceptives before, or any medications during the period of the study. No history of allergy or any disease that interferes with the immune system, non smokers, and not alcoholics. They were just started to receive (for the first time) 150 mg DMPA injection (called "Depo-Provera" of Pharmacia NV/SA Puurs-Belgium) every 3 months. These women were called DMPA users group. The non users group included another 30 apparently healthy volunteer women who have the same inclusion criteria as the DMPA users group except that they were not using any hormonal contraceptives, instead, they used either a barrier method or mechanical methods. Anthropometric measures (blood pressure (mmHg), body weight (Kg) and height (cm)) were taken. Ten ml venous blood were withdrawn into plain tube, using a disposable syringe at about 8.30-10.00 am (after 12 hours fasting) from the DMPA injections users group at the beginning before they start taking the injection, after 6 months, then after 12 months of use, and from the non contraceptive users group using the same schedule. The blood was allowed to clot, then serum was separated by centrifugation at 3000 rpm for 10 minutes and then kept frozen at – 20 °C to be analyzed:

1- Serum CRP was measured by slide agglutination using Biokit, Spain.
2- Measurement of serum TC and TG concentration was done by the enzymatic colorimetric method, using (BioMerieux kits, France) for each.
3- Serum HDL was measured by the precipitation method, using HDL Cholesterol/ Phospholipids kit (BioMerieux, France).
4- Serum LDL was calculated by using Friedewald equation\(^16\):
\[
\text{LDL (mmol/l)} = \text{TC-HDL-} \left( \frac{\text{TG}}{2.19} \right)
\]
5- Atherogenic index (AI) was calculated by the following equation: \(\text{AI} = \frac{\text{TC}}{\text{HDL}}\).\(^17\)
Standard statistical methods were used to determine the mean, standard deviation (SD) and the range. Paired t-test was used to compare the results of various biochemical parameters among the two groups. Linear regression analysis (Pearson correlation coefficient $\rho$) was performed for finding the degree of association between different parameters. ANOVA test (analysis of variance) was used to identify the variation in the different variables in relation to the duration of DMPA users group. Duncan’s test was used to identify groups responsible for statistical difference through comparison. Linear Stepwise Multiple Regression Technique was applied to detect the significant independent (predictors) variables that predict CVD risk among DMPA users. All values quoted as the mean ± SD and a P-value of $\leq 0.05$ was considered to be statistically significant.

**Results**

The obligatory use of DMPA injections every 3 months led the users women to visit the Family Planning Center regularly and eventually every 3 months to take the injection, so all women enrolled in this study can be followed up with less possibility of loss to follow up.

There was no significant difference between mean ± SD of age of the DMPA users (28.36 ± 4.14 years) and of the non users (27.40 ± 4.71 years). There was no significant difference between mean ± SD height of the DMPA users (157.57 ± 3.33 cm.), and that of the non users (159.30 ± 3.25 cm.).

This study indicates that DMPA caused a non significant increase in body weight among DMPA users in comparison with non users after 6 and 12 months. Although DMPA use caused an increase in BMI of the DMPA users in comparison with non users after 6 and 12 months, only the increase after 12 months was significant. However ANOVA analysis of the DMPA users group revealed a non significant ($F=1.67$, p=0.207) increase in the mean BMI of the DMPA users in relation to the duration of usage (table 1).

<table>
<thead>
<tr>
<th>Period of use (Months)</th>
<th>(Mean ± SD) BMI (kg/m²)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMPA Users (n=30)</td>
<td>Non Users (n=30)</td>
</tr>
<tr>
<td>0</td>
<td>22.186 ± 1.986 a</td>
<td>22.25 ± 1.94</td>
</tr>
<tr>
<td>6</td>
<td>23.24 ± 2.54 a</td>
<td>21.95 ± 2.11</td>
</tr>
<tr>
<td>12</td>
<td>24.36 ± 3.28 a</td>
<td>21.83 ± 2.33</td>
</tr>
</tbody>
</table>

- (a, b) different letters (vertically), means significant difference.

This study demonstrated that the use of DMPA injection causes a non significant increase in SBP and DBP among DMPA users in comparison with the non users after 6 and 12 months. ANOVA analysis among the DMPA users group indicated a non significant increase in the mean SBP of the DMPA users from non users, but a significant ($F=3.41$, p=0.048) increase in the mean DBP of DMPA users according to the duration of DMPA injections use.

There were no significant changes in the mean serum CRP among DMPA users in comparison to the non users after 6 and 12 months. ANOVA analysis of the DMPA users indicated that there were non significant changes in serum CRP among DMPA users in relation to the duration of DMPA injections usage.

Table (2) demonstrates that there was a significant increase in serum TG after 6 months in the DMPA users in comparison with the non users. There was no significant difference in the mean serum TG of the DMPA users and non users at the baseline time (0 month). ANOVA analysis among the DMPA users group indicated a significant ($F=5.27$, p=0.012) increase in the mean serum TG according to the duration of DMPA injections use.
Table (2): Comparison between mean serum TG of DMPA users and non users after 6 & 12 months.

<table>
<thead>
<tr>
<th>Period of use (Months)</th>
<th>(Mean ± SD) Serum TG (mmol/l)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMPA Users (n=30)</td>
<td>Non Users (n=30)</td>
</tr>
<tr>
<td>0</td>
<td>0.97 ± 0.402 a</td>
<td>1.324 ± 0.732</td>
</tr>
<tr>
<td>6</td>
<td>1.82 ± 0.79 a</td>
<td>1.025 ± 0.57</td>
</tr>
<tr>
<td>12</td>
<td>1.913 ± 0.66 b</td>
<td>1.66 ± 0.84</td>
</tr>
</tbody>
</table>

- (a, b) different letters (vertically), means significant difference.

There were non significant changes in the mean serum TC, HDL, LDL, and AI among DMPA users in comparison to the non users after 6 and 12 months. ANOVA analysis of the DMPA users group indicated that there were non significant changes in serum TC, HDL, LDL and AI among DMPA users in relation to the duration of DMPA injections usage.

This study demonstrated that among all variables that were studied, only body weight and BMI showed a significant positive correlations with CRP (r=0.733, P=0.016 ; r= 0.612, P=0.057 respectively). By using linear stepwise multiple regression to account for any co depended effects of different biochemical parameters (using CRP) and other biochemical parameters (variables), the predictors that increase the risk of CVD significantly among DMPA users are AI, DBP and TG serum levels. (table 3).

Table (3): Linear multiple stepwise regression model for predictor of CVD in the DMPA users after 12 months.

<table>
<thead>
<tr>
<th>Variable Xi (Predictors)</th>
<th>Regression Coefficient</th>
<th>Standard Error (SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI</td>
<td>-2.700</td>
<td>0.394</td>
<td>0.021</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.312</td>
<td>0.053</td>
<td>0.028</td>
</tr>
<tr>
<td>TG</td>
<td>-2.000</td>
<td>0.677</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Discussion

This study found that there is a significant positive association between CRP and CVD risk markers in DMPA injections users as contraceptive, also CRP can be used as independent risk factor to predict other risk factors by using linear stepwise multiple regression to account for any co depended effects of different biochemical parameters (variables). It is found that the predictors that increase significantly the risk of CVD among DMPA users are AI, DBP and TG serum levels. To the best of our knowledge no previous study done to investigate such theory in DMPA injections users as contraceptive.

This study found that DMPA caused a non significant increase in body weight among the DMPA users in comparison with the non users after 6 and 12 months but caused a significant increase in BMI after 12 months in comparison to non users. This is not of clinical importance since comparison of changes with their respective pretreatment values were not statistically significant. This is in agreement with some studies\(^{(15,16)}\), while other studies found that prolonged use of DMPA for 1-2 years in Navajo women\(^{(19)}\) and for 3-5 years\(^{(20)}\) caused a significant increase in body weight.

This study demonstrated that among all variables that were studied, only body weight and BMI showed significant positive correlations with CRP, but neither body weight nor BMI were significant predictor for the risk of CVD among DMPA users. Studies performed among different ethnic groups showed diverse results, but all these studies confirmed a relationship between serum CRP and both generalized and abdominal obesity\(^{(21)}\).

Among men and women with CHD, CRP was correlated with traditional risk factors and to a lesser degree to manifestation of CHD and BMI is the main contributor to CRP variability, explained by these factors among women\(^{(22)}\). Another study found a significant positive association between CRP and atherosclerotic risk factors in healthy young people, as well as an increase in these markers in the upper quartiles of waist circumference, but not BMI\(^{(23)}\).
In this study there was a non significant increase in SBP and DBP among DMPA users in comparison with the non users after 6 and 12 months. Non significant increase in the mean (SBP) but a significant increase in the mean (DBP) of DMPA users in comparison of changes with their respective pretreatment values was found. The study of Mia et al., (20) found that long term use of DMPA caused insignificant increase in SBP and DBP, but a significant increase in body weight, while the study of Al-Banna (24) found that the use of hormonal contraception cause a non significant changes in body weight, SBP and DBP.

This study revealed that DBP is one of the significant predictors for the risk of CVD among DMPA users by using CRP as the co depended variable. Hashimoto et al., (25) found that in hypertensive patients being managed by drug therapy or lifestyle modification, CRP is an equivalent or superior independent predictor of the progression of carotid atherosclerosis than the pulse pressure or systolic blood pressure.

This study found that there were non significant changes in mean serum CRP level among DMPA users in comparison to the non users after 6 and 12 months and in relation to the duration of use. This is in agreement with the study of Goldstein et al., (26) who found that the CRP was not significantly altered by the use of DMPA for 12 months.

This study also revealed that the use of DMPA injections as contraceptive in young women caused a significant increase in serum TG after 6 months in the DMPA users in comparison with the non users and according to the duration of use. However there were non significant changes in mean serum TC, HDL, LDL and AI among DMPA users in comparison to the non users after 6 and 12 months nor in relation to the duration of use. This is in agreement with the study of Garza-Flores et al., (27) who found that the use of DMPA for 5 years causes a moderate increase in the serum TG, but a moderate non significant decrease in TC and HDL, with unchanged LDL. Fahmy et al., (28) found that after 3 months use of DMPA, there were no significant changes in TC and TG, while there was a significant decrease in HDL, and a significant increase in LDL. After 15 months there was a significant increase in TC and LDL and a significant decrease in HDL. The study of Faddah et al., (29) although demonstrated that neither mean serum TC nor TG were affected by DMPA use, only AI was gradually but non significantly increased in comparison to control group as in this study.

Controversial results from different studies were found of the metabolic effects of long term DMPA use. Some studies found that there are non significant changes in lipid profile parameters (26,30,31) including AI (32) after one year use of DMPA, and concluded that DMPA may be considered as a safe contraceptive medication as the overall data indicate that acute and/or chronic DMPA use at the dose currently employed for contraception does not induce major abnormalities in serum lipoproteins. While other studies found that long term use of DMPA injections as contraception causes significant increase in the mean serum TC, TG and LDL levels, but a non significant decrease in HDL in comparison to that of the control group (33). Other studies found that DMPA use for 12 weeks (34) or for one year (35) caused significant decrease in HDL level, and suggested that DMPA should not be prescribed to women with abnormally high risk for atherosclerosis such as heavy smokers and women with adiposity and /or diabetes mellitus.

This study found that one of the predictors that significantly predicts the risk of CVD among DMPA users were AI and TG. Despite extensive research, it has not yet been determined whether TG represent an independent risk factor for CHD. The association has been obscured by imprecision in TG measurements, individual variability, and complex interactions between TG and other lipid-nonlipid parameters. Although current guidelines do not mandate screening for elevated TG levels in the general population, obtaining TG levels in those with known CHD or with other risk factors can provide valuable prognostic information and therefore be of aid
in therapeutic decisions (36). Models incorporating both hs-CRP and lipid parameters have significantly greater ability to model using lipid alone (37).

Conclusion
In the present study, the predictors that predict significantly the risk of CVD (represented by CRP) among DMPA users were AI, DBP and TG serum levels. This means that incorporating both CRP level, lipid parameters and blood pressure have a significantly greater ability to predict CVD risk among DMPA users than model using lipid profile alone.

References


35. Kremer J, de Bruijn HW, Hindriks FR. [Injectable contraceptive, DMPA, serum

Profile of Pseudomonas aeruginosa in burn infection and their antibiogram study

Haitham M. Al-Habib, Asmaa Z. Al-Gerir, Ansam M. Hamdoon
Department of Microbiology, College of Medicine, University of Mosul.

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ABSTRACT

Objectives: 1- To evaluate the incidence of Pseudomonas aeruginosa bacterial pathogens in burn patients. 2- To determine the antibiogram profile of Pseudomonas aeruginosa to selected antibacterial agents. 3- To assist their production of β-lactamases.

Patients and methods: This study enrolled 90 burned patients including 63 (70%) females and 27 (30%) males. Pus and wound swabs were collected aseptically from these patients and assessed microbiologically. The isolates of P. aeruginosa were tested for their susceptibility to 10 selected antimicrobial agents, and evaluated for β-lactamases using iodometric and double disk approximation methods.

Results: Out of the 90 studied patients with second and third degree burns, 60 (66.7%) yielded positive bacterial growth, while 30 (33.3%) were culture negative. From the total 105 bacterial isolates 88 (83.8%) were gram negative and the remaining 17 (16.2%) were gram positive. The predominant microorganism was P. aeruginosa (50%), whereas the least isolated one was Proteus (3.3%). The antibiogram study of P. aeruginosa showed that the least resistance was against piperacillin, while the highest resistance was noted in cases of carbencillin and cefoxitin. Multidrug resistance (MDR) P. aeruginosa formed 44.4% of the total isolates of P. aeruginosa and they had statistical association with ceftriaxone, meropenem, ceftazidime and amikacin consumption. Ninety percent of P. aeruginosa were β-lactamases producer and 10% of them produced the inducible β-lactamases.

Conclusion: The bacteria isolated from Burn Units are the best examples for the study of pathogenic bacterial species, specially Pseudomonas aeruginosa, other enteric bacilli and Staph. aureus which frequently responsible for human colonization. Also, Pseudomonas aeruginosa and other gram negative bacilli are frequently associated with nosocomial burn infection. Furthermore, most isolates of P. aeruginosa from Burn Units are β-lactamases producers and most of these isolates were MDR pseudomonas aeruginosa.

الخلاصة

الأهداف: تقييم دور الزوانف الزراقية والجراثيم الممرضة الأخرى في مرضى الحروق. وكذلك تعني حساسية جراثيم الزوانف الزراقية المعزولة لبعض المضادات الحيوية المختارة، بالإضافة إلى تحديد إنتاج العزلات المذكورة لإنتاج البيتا لاكتاينز.

المرضى والطريقة: هذه الدراسة شملت 90 من مرضى الحروق، كان عدد الإناث 63 (70%), بينما كان عدد الذكور 37 (30%). أخذت نماذج الحروق من الجروح وكذلك الفيتي، ودشست من الناحية الجرثومية، وأيضاً تم قياس مدى حساسية جراثيم الزوانف الزراقية المعزولة لـ 10 أنواع من المضادات الحيوية، وكذلك تحديد أنواع إنزيمات البيتا لاكتاينز المنتجة من قبلها باستخدام الطريقة اليودية وطريقة تقريب القرص الثنائي.

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The skin forms a protective barrier against invasion by bacteria, fungi and viruses. Any breach in this barrier provides easy access for microbial invasion. In spite of the enormous advances in medicine and specific treatment of burns, infection continues to pose the greatest danger to burn patients. Following the initial period of shock, sepsis is the major complication in burns, and it has been estimated that about 75% of the mortality associated with burn injuries is related to sepsis specifically in developing countries. In addition, overcrowding in burn units is an important cause of cross-infection. Gram positive bacteria from hair follicles and sweat glands colonize the wound within 48 hours of injury.

Burn infections are caused by both gram positive and gram negative microorganisms, currently the common pathogens isolated from burn patients are Pseudomonas aeruginosa, Staphylococcus aureus, β-haemolytic streptococci, Escherichia coli, Klebsiella species and various coliform bacilli. Fungi like Candida albicans and Aspergillus species are also associated with burn infections. P. aeruginosa is a gram negative opportunistic pathogen found along with other Pseudomonas species as part of the normal flora of human skin. It rarely causes infection in healthy individuals, although it is responsible for serious infections in immunocompromised hosts, such as those with severe burn wounds, cystic fibrosis patients, cancer patients, and patients with HIV infection. Burn causes a breach in the protective skin barrier which suppresses the immune system, rendering the patient highly susceptible to infection by P. aeruginosa which then easily colonizes and infects the burn wound.

The burn wound is rarely an important health problem, but its infection results in severe complications in patients who sustained burns. Infection of burn wounds with P. aeruginosa which disseminates into distant organs via blood stream often leads to bacteremia, endotoxic shock and sepsis.

The mortality rate in burn patients who developed septicaemia was greater than 75%. Multidrug resistant bacteria (MDR) have been frequently reported as the cause of nosocomial outbreaks of infections in Burn Units or as wound colonizers in burn patients. Multidrug resistant strains of P. aeruginosa (resistant to at least three of the following antimicrobials cefotaxime, imipenem, gentamicin and ciprofloxacin) are often isolated among patients suffering from nosocomial infections. One of the greatest sources for this resistance is the production of β-lactamase. The chromosomally encoded β-lactamases of Pseudomonas spp. are serine based Ambler class C enzymes (AmpC).
whose expression is often strictly repressed. The enzyme production can be induced to a high level of expression yielding sufficient enzyme to confer resistance\(^{(17)}\). Therefore \(\beta\)-lactamase detection and identification is valuable\(^{(18)}\).

Oral antibiotics are generally ineffective against most serious skin and soft tissue \(P\). \(aeruginosa\) infections \(^{(14)}\). Treatment of such infections is confounded by the innate and acquired resistance of \(P\). \(aeruginosa\) to many antibacterial agents\(^{(19)}\). Hence the development of new therapeutic and prophylactic agents for the control of bacterial infection in patients with burn wounds is mandatory. An alternative to antibiotic therapy is phage therapy which involves the use of bacterial viruses to target bacterial infections \(^{(20)}\).

**Aims of the study**

The aims of the current study are to evaluate the incidence of \(P\). \(aeruginosa\) and bacterial pathogens isolated from burn patients at the Burn Unit in Al-Jumhori Teaching Hospital in Mosul City. Also, to determine the antibiogram profile of the isolated \(P\). \(aeruginosa\) to some selected antibacterial agents and to evaluate their production of \(\beta\)-lactamases. This will help to assess the burden of infections at Burn Unit and to formulate antibiotic policy for better management of burn patients.

**Patients and methods**

This study was approved by the Scientific Research Committee at the College of Medicine, University of Mosul. Formal consent taken from all patients after clear explanation.

**Patients**

This is a prospective study based in the Burn Unit in Al-Jumhori Teaching Hospital in Mosul-Iraq, and the Department of Microbiology, College of Medicine, University of Mosul. The study was conducted during the period from October 2009 to May 2010. This study enrolled 90 patients admitted to the Burn Unit of whom 27 (30%) were males and 63 (70%) were females (figure 1). The male to female ratio was (1:2.3). The age of the patients ranged from 1-52 (18 \(\pm\)2SD) years.

Regarding the degree of burn, 21 patients were with second degree burn and 69 patients were with third degree burn.

A history of age, sex, cause, duration at time of study, site, extent of the burn, and the use of antibiotics was taken. None of the patients included in this study had any signs and symptoms of urinary tract infection, blood stream infection, and wound infection based on NNIS system criteria within the first 48 hours after admission.

**Microbiological methods**

Clinical specimens used in the current study were pus and wound swabs which were collected aseptically from the patients after bathing on the third day after admission. The specimens were inoculated directly onto 5% sheep blood agar, nutrient agar and MacConkey’s agar which were incubated at 37°C for 24 hours, with further 48 hours incubation if there is no growth. Identification of the isolates was relied upon their colonial morphology, gram reaction and standard biochemical tests\(^{(21)}\). Further confirmative diagnostic tests for \(P\). \(aeruginosa\) were attempted including growth at 42°C in brain heart infusion, oxidase test, motility test, oxidative-fermentation test for carbohydrate and pigment production.

Pure cultures of the isolates were prepared for further identification and antibiogram study. The isolates were tested for their sensitivity to 10 selected antibiotics which were piperacillin 100 mcg, norfloxacin 10 mcg, ciprofloxacin 5mcg, gentamicin 10 mcg, tobramicin 10mcg, ceftriaxone 30 mcg, ticarcillin 75mcg, cefotaxime 10 mcg, cefoxitin 30 mcg, and carbencillin 100 mcg (Bioanalyse, UK) on Mueller-Hinton (Oxoid UK) using the standard disc diffusion method following NCCLS recommendations\(^{(22)}\).

\(P\). \(aeruginosa\) isolates were also tested for their ability to produce \(\beta\)-lactamases enzymes using the rapid iodometric method and they were tested for the production of Amp C inducible \(\beta\)-lactamase by double disc approximation test. In this test, plates were inoculated and cefotaxime 30 mcg and cefoxitin 30 mcg discs were placed 20 mm apart. \(\beta\)-lactamase inducibility is recognized by
blunting of the cefotaxime zone adjacent to the cefoxitin disc(17).

The iodometric test: using benzylpenicillin in phosphate buffer and bacterial growth from agar is suspended heavily in them. After the addition of starch and iodine, β-lactamase activity is demonstrated by decolorization of iodine within 5 minutes(17).

**Statistical analysis**

Z two proportional test was used to evaluate the relationship between the administration of different antibiotics and the isolation of MDR Pseudomonas aeruginosa.

**Results**

Among the examined 90 patients with 2nd and 3rd degree burns, 60 (66.7%) of them yielded a positive bacterial growth while 30 (33.3%) showed a negative growth. Out of the 60 positive cases, pure culture isolation was recovered in 34 (56.7%), while mixed growth was seen in 26 (43.3%). A total of 105 bacterial isolates were detected, most of them 88 (83.8%) were gram negative and the remaining 17 (16.2%) were gram positive (figure 2). The predominant microorganism was P. aeruginosa (50%), followed by Klebsiella pneumoniae (26.7%), Staph. aureus (11.1%). E. coli (10%), while Acinetobacter and Staph. epidermidis were recovered in similar rate (7.8%). The least isolated microorganism was Proteus (3.3%), as shown in table 1.

Concerning the relation between the depth of burn and the development of sepsis, from the 21 patients with second degree burns 57.1% were infected. Whereas from the 60 patient with third degree burns, the incidence increased to 69.6%. Such result reflects the importance of burn depth in development of infection.

The antibiogram profile of the isolated P. aeruginosa was determined against a panel of antimicrobial agents. Piperacillin showed the lowest resistance rate (60%), while the highest resistance was detected against carbencillin and cefoxitin and reached to 96% of the total isolates (table 2).

In the current study, distribution of β-lactamases in the isolates of P. aeruginosa was 90%. The Amp C inducible β-lactamase was further investigated and it was found in 10% of the total isolated P. aeruginosa. All P. aeruginosa isolates that produce inducible β-lactamase were MDR Pseudomonas. MDR Pseudomonas in this study formed 20/45 (44.4%) of the isolated P. aeruginosa strains. Using two proportional Z test the relation between MDR P. aeruginosa and antibiotic administration was also analyzed. The statistical analysis revealed that there was a significant statistical association between MDR P. aeruginosa and the consumption of ceftriaxone (P≤0.006), meropenem (P≤0.042), ceftazidime (P≤0.001) and amikacin (P<0.014), while the intake of other antimicrobial agents was statistically not significant.

![Figure (1): Sex distribution of cases.](image1)

![Figure (2): Percentage of gram negative and gram positive isolates from burned patients.](image2)
Table (1): Isolated microorganisms in burn infection.

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>No. of isolates</th>
<th>% from total patients</th>
<th>% from culture positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>45</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>24</td>
<td>26.7</td>
<td>40</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>10</td>
<td>11.1</td>
<td>16.7</td>
</tr>
<tr>
<td>E. coli</td>
<td>9</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Staph. epidermidis</td>
<td>7</td>
<td>7.8</td>
<td>11.7</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>7</td>
<td>7.8</td>
<td>11.7</td>
</tr>
<tr>
<td>Proteus</td>
<td>3</td>
<td>3.3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Resistance of Pseudomonas aeruginosa isolates to antibiotics.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Conc.</th>
<th>% of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin</td>
<td>100 mcg</td>
<td>60</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10 mcg</td>
<td>73.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 mcg</td>
<td>80</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 mcg</td>
<td>86.7</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>10 mcg</td>
<td>86.7</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30 mcg</td>
<td>91.1</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>75 mcg</td>
<td>93.4</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>10 mcg</td>
<td>93.4</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>30 mcg</td>
<td>96</td>
</tr>
<tr>
<td>Carbencillin</td>
<td>100 mcg</td>
<td>96</td>
</tr>
</tbody>
</table>

Table (3): Multidrug resistant Pseudomonas and antibiotic intake.

<table>
<thead>
<tr>
<th>Type of antibiotic</th>
<th>No. of patients on antibiotics</th>
<th>No. of patients showed pseudomonas growth(%)</th>
<th>No. of MDR Pseudomonas (%)</th>
<th>% of MDR pseudomonas in patients using these antibiotics</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin + cloxacillin</td>
<td>38</td>
<td>16(42.1)</td>
<td>13(34.2)</td>
<td>81.25</td>
<td>0.477</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>21</td>
<td>12(57.1)</td>
<td>4(19.04)</td>
<td>33.3</td>
<td>0.006</td>
</tr>
<tr>
<td>Meropenem</td>
<td>6</td>
<td>4(66.7)</td>
<td>1(16.7)</td>
<td>25</td>
<td>0.042</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6</td>
<td>2(33.3)</td>
<td>0(0)</td>
<td>0</td>
<td>0.083</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>6</td>
<td>4(66.7)</td>
<td>2(33.3)</td>
<td>50</td>
<td>0.221</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>4</td>
<td>3(75)</td>
<td>0(0)</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>Amikacin</td>
<td>3</td>
<td>2(66.7)</td>
<td>0(0)</td>
<td>0</td>
<td>0.014</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>5</td>
<td>2(40)</td>
<td>0(0)</td>
<td>0</td>
<td>0.016</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>45(50)</td>
<td>20(22.2)</td>
<td>44.4%</td>
<td></td>
</tr>
</tbody>
</table>

*P value: Z two proportion test.

Discussion

Despite significant improvement in the survival of burn patients, infection complications continue to be the major cause of morbidity and mortality (21). Though control of invasive bacterial burn wound infection, strict isolation techniques and infection control policies have enormously minimized the occurrence of burn wound infection (22). The current study showed a high prevalence of bacterial infections among burned patients which was in agreement with the result of other investigators (23), but in contrast to another study (24). In the present work burn wound swab and pus yielded positive bacterial growth in 66.7% of examined cases which was similar to the observation of other workers (25,26,27). Solitary isolates were found in 56.7% of the studied cases which was in accordance with the result reported by Daher et al., who obtained pure culture isolation in 58.7% of their patients (25), while a higher isolation rate (89.3%) was reported by Demarco and Santo (27).

Different types of gram positive and gram negative microorganisms were detected in the current study of which gram negative bacteria...
constituted (83.8%), and gram positive ones were (16.2%). This finding goes with that of kehinde et al., who mentioned that gram negative bacteria constituted (72%) of their isolates (26). However, other investigators reported lower isolation rates which ranged between 33-51.1% (25,27,28). Furthermore, the frequency of hospital infection by gram negative enteric bacilli specially P. aeruginosa has been increased during the last decade. Other studies described P. aeruginosa as the common cause of nosocomial burn infection (29). In the current study P. aeruginosa was proved to be the major cause in burn patients which constituted 50% of the total isolates and found in 75% of culture positive cases. This result was in concinnity with the findings of Song et al., who reported a percentage of (50%) (28). However, other studies reported a lower prevalence of P. aeruginosa in burn infections (25,29,30), while Mansour and Enayat reported a higher isolation rate (68.3%) (31). The second most frequent organism recovered in this work was Klebsiella pneumoniae (26.7%), which agrees with the result obtained by Kehinde et al., (34,3%), and in contrast with that reported by others (32) who mentioned that Proteus was ranked in the second place. One study (32) reported that Staph. auerus was the commonest microorganism associated with burn injuries which was in contrast to the present result where Staph. auerus came in the third place (11.1%) after P. aeruginosa and Klebsiella. In addition E. coli recovered from 10 %of the total cases and this rate was similar to that detected by Daher et al., (28). The bacterium Acinetobacter was isolated from 7.8% of the examined patients which was a lower percentage compared to that obtained by others (13.4%) (28).

The difference in prevalence of bacterial isolates may be attributed to the environmental condition of a specific area and contamination of the burn units. Due to the increased resistance to various antibiotics and cross infection in the hospital environment there is clear change in the bacterial spectrum. Before few decades the predominant bacteria was Streptococcus which then followed by Staph. aureus, but with the frequent use of topical antibiotics, fungi and viruses become more prevalent. Also, due to the introduction of a wide range of antibiotics, resistant gram negative bacteria become more prevalent. This increased resistance to various antibiotics poses a challenge to Burn Care Units because it reduces the effectiveness of treatment and may increases morbidity and mortality.

In the present work antibiogram study of the isolates revealed that most P. aeruginosa isolates were resistant to the antibiotics in common use such as gentamicin, ciprofloxacin and ceftiraxan which are being indiscriminately prescribed as empirical treatment for long time. This high resistance to the above mentioned antibiotics was also noticed by other investigators (16,25). Also, resistance to tobramycin was high (86.7%) and this findings goes with that of Strateva et al. (16).

In this study the most effective agent against P. aeruginosa was piperacillin which yielded the least resistance percentage (60%). This could be explained on the basis that piperacillin is not commonly prescribed against Pseudomonas infection in this locality. However, higher resistance to this drug (86.2%) was reported by other investigators (16) where the use of this antibiotic is more frequent in their locality. Actually the MDR P. aeruginosa is a major problem at the mean time. In the current work, it constituted 44.4% out of the total recovered P. aeruginosa, and this finding was in agreement with Strateva et al., (49.8%) (16) although it was higher than that reported by other workers (33). Also, the association between MDR P. aeruginosaa and antimicrobial consumption was analyzed in this study. The statistically significant association was found to be with ceftiraxone, meropenem, ceftazidime and amikacin administration which was in contrast to the study performed by Messadi et al., who found the significant association was with the use of ciprofloxacin (34). This discrepancy in the results may be due to the difference of antibiotics use in different localities.
Production of the enzymes β-lactamases is the mechanism by which Pseudomonas resists antibiotics. In the current study, P. aeruginosa isolates were tested for β-lactamases production using the iodometric method and it was found that 90% of them showed a positive result, which was in concinnity with that reported by another study (30). Moreover, AmpC inducible β-lactamase production was detected using the disk approximation test and it was found in only 10% of the total isolated P. aeruginosa. This rate was lower than that determined by other researchers (16), but closely similar to that found by Supriya et al., (7%) (35). Furthermore, inducible β-lactamase producers were MDR, this result reflects the role of inducible β-lactamase in antibiotic resistance.

This increasing rate of MDR may be attributed to the subinhibitory concentration of antibiotics in vivo due to the administration of an inappropriate dosage of β lactam antibiotics, or the regular administration of aminoglycosides in combination with β lactam drugs which provide optimal conditions for persistence of MDR P. aeruginosa strains. These findings highlighted the need for further attention to disinfect inanimate hospital environment and to control contact between staff and patients in order to limit transfer of P. aeruginosa in Burn Units. Moreover, the use of some antimicrobial agents must be restricted due to the existence of high resistance. Also, the use of combined effective antibiotics is recommend.

In conclusion, bacteria isolated from Burn Unit's Patients are the best examples for the study of pathogenic bacterial species, specially P. aeruginosa, other enteric bacilli and Staph. aureus which are frequently responsible for human colonization. Also, P. aeruginosa and other gram negative bacilli are frequently associated with nosocomial burn infection. Furthermore, most isolates of P. aeruginosa from burn units are β- lactamases producers and most of these isolates were MDR Pseudomonas aeruginosa.

References


Measurement of total serum IgE antibody in patients with atopic dermatitis

Amir A. Al-Hafedh*, Haitham B. Fathi**

* Department of Medicine, College of Medicine, ** Department of Medicine, Nineveh College of Medicine, University of Mosul.

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ABSTRACT

Objectives: To estimate the difference in total serum IgE level between patients with atopic dermatitis (AD) and healthy control subjects and to correlate age and gender of patients, and severity of AD with total serum IgE level.

Patients and methods: This is a case-series study on 52 patients with AD, recruited from dermatology clinic at Al-Jumhoori teaching hospital in Mosul, during the first half of 2010. Twenty five healthy non-atopic subjects were recruited from same clinic as a control group.

Results: The total serum IgE level in patients with AD was 286.0 (SD 83.1) IU/ml; corresponding value in control group was 47.8 (SD 38.1) IU/ml. Thus, total serum IgE level was significantly higher in patients with AD than in control group (p = 0.007). The highest concentration of total serum IgE level was found among females (p = 0.04), age group 11-15 year (p = 0.09), and severe cases of AD (p = 0.007).

Conclusion: Majority of patients with AD have a raised total serum IgE level, which in turn correlates well with female gender, age group 11-15 year, and severity of disease.
A topic dermatitis (AD) is a chronic, remitting relapsing immune-mediated inflammatory skin condition. The main immunological abnormality is excessive formation of total IgE\(^1\). Despite the extensive studies, picture of AD is still fragmented and controversies remain unsolved. Some of these controversies are the relationship of gender, age, and severity of AD with total serum IgE level. Gender of patient was the interest of many investigators but their conclusions were controversial\(^2,3\). From a clinical perspective, supported by epidemiological investigations, there is a decline with age in both incidence and severity of atopic diseases. This is in association with general humoral alteration manifested by decline in IgE level with age\(^4\). The severity of AD also has some positive correlation with serum IgE level, but this is not a consistent observation\(^5\). Hence, the current study was performed to elucidate the correlation between age, gender and the severity of AD with serum total IgE level. To the best of our knowledge, this is the first study of its kind from our locality.

**Patients and methods**

The study was conducted as a case-series study. Fifty two consecutive patients with atopic dermatitis were recruited from dermatology clinic at Al-Jumhoori teaching hospital in Mosul, from January to June 2010. The inclusion criteria adopted were clinical manifestations of AD when enrolled in the study and patients agreement. The following patients were excluded: patients with other allergic diseases, disease inducing higher serum IgE level, or patients using topical/ or systemic immune-modulator treatments.

The diagnosis of AD was based on fulfilling Hanifin and Rajka’s major and minor criteria\(^6\). The severity of atopic dermatitis was based on the use of Six Areas, Six Signs Atopic Dermatitis (SASSAD) severity score\(^7\). The clinical severity was graded as mild (localized chronic forms with <10% of the body surface area involved), moderate (disseminated lesions over trunk and extremities), and severe forms (e.g., more generalized eczema).

Twenty five relatives of other patients, who attended the clinic and who have no history of atopic diseases were asked to participate in this study, as a control group. They were matching patients in age and gender.

Five ml of blood was taken from both patients and control group for estimating total serum IgE antibody. The blood was left to clot at room temperature and then centrifuged at 3000 rpm. The total serum IgE antibody was measured by Enzyme Linked immune-Fluorescent Assay technique (ELFA) performed by mini VIDAS. The kit was provided by bio Mérieux, France. The kit provided a quantative in vitro assay for human total IgE antibody.

**Statistical methods**

Different descriptive statistical methods were used to summarize the data in this study. Independent two sample student T-test was used to compare the mean differences in total serum IgE level between patients and control group, also between male and female patients with AD. One way ANOVA test with post hoc dunnette’s test were used to assess mean difference in total serum IgE level among different categories of severity of AD. Person linear regression test was used to evaluate age-associated changes in total serum IgE level. A p-value of <0.05 was considered significant. Statistical processing were conducted by the use of statistical package SPSS ver. 10 (SPSS inc, Chicago, Ill).

**Results**

The age of patients with AD ranged from 4 to 25 year with mean and standard deviation (SD) of 14.5 and 6.1 year respectively. There were 20 (38.5%) females and 32 (61.5%) males. The age of control group ranged 6 to 24 year with mean (SD) of 13.1 (5.5) year, and included 10 (40%) females and 15 (60%) males.

The mean total serum IgE level in patients with AD was 286.0 IU/ml (SD 83.1) and the corresponding value 47.8 IU/ml (SD 38.1) for control group. The difference was statistically highly significant P = 0.007 (fig 1).

The mean total serum IgE level among females 356.2 IU/ml (SD 301.4) was higher than that in males 217.4 IU/ml (SD 266.9) and
The difference was statistically significant $P = 0.04$ (fig 2).

The scattered plot (fig 3) showed poor linear relation of age of patient with total serum IgE level ($r = 0.1$). After breaking down age into 5 years age groups, highest mean total serum IgE level 427.7 IU/ml (SD 412.3) was found among those (11-15) year age group, and the lowest 81.7 IU/ml (SD 44.4) was among those of (21-25) year age group (fig 4).

A statistically significant rise in the mean total serum IgE level was found with increasing severity of AD. Figure (5) showed two and half time rise in mean serum IgE level in severe cases of AD 432.3 IU/ml (SD 326.2) in comparison to mild cases 173.3 IU/ml (SD 219.8) and the difference was statistically significant ($p = 0.007$).
Table (1): Correlation of total serum IgE (IU/ml) with different demographic and clinical variables in patients with atopic dermatitis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean (SD)</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>217.4 (266.9)</td>
<td>121.2-313.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Female</td>
<td>356.2 (301.4)</td>
<td>215.1-497.3</td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5 years</td>
<td>144.9 (10.4)</td>
<td>128.3-161.5</td>
<td></td>
</tr>
<tr>
<td>6-10 years</td>
<td>293.5 (262.3)</td>
<td>105.9-481.9</td>
<td></td>
</tr>
<tr>
<td>11-15 years</td>
<td>427.7 (412.3)</td>
<td>189.7-665.8*</td>
<td>0.09</td>
</tr>
<tr>
<td>16-20 years</td>
<td>268.6 (206.2)</td>
<td>149.6-387.7</td>
<td></td>
</tr>
<tr>
<td>21-25 years</td>
<td>81.7 (44.4)</td>
<td>49.9-113.5</td>
<td></td>
</tr>
<tr>
<td>Severity of atopic dermatitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>173.3 (219.8)</td>
<td>75.8-270.7</td>
<td>0.007</td>
</tr>
<tr>
<td>Moderate</td>
<td>207.2 (237.5)</td>
<td>56.3-358.1</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>432.3 (326.2)</td>
<td>270.1-594.6*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference at p-value 0.05 (Dunnett's post hoc test).

Discussion

The evidence for the central role of IgE in etiopathogenesis of AD was proved by several studies\(^{1-5}\) including the current one. The results showed that mean total serum IgE level was six times higher among AD patients in comparison to control non-atopic subjects. According to manufacturer's reference figure, 100 IU/ml is considered as a cutoff value. In this study, total serum IgE level exceeded 100 IU/ml in 60% of patients. Raised IgE level was consistent with the findings of some studies and was slightly lower than that reported by others. Reported figures in the literature varied from 70% \(^{8}\) to 88% \(^{9}\) of atopic patients. These differences may be explained by the different chosen cutoff value, methods of estimating IgE, age of participants, and severity of disease. In the above mentioned studies\(^{8,9}\), they used 75 IU/ml as a cutoff value, patients were younger and the proportion of severe cases was larger. Our findings revealed that normal serum IgE level do not rule out the presence of AD and that serum IgE may not be the only stimulus for signs and symptoms of AD, but definitely play an important role in their exacerbation.

Marked controversy was noted in the literature regarding the relation between gender and total serum IgE level. While Ahmed and Nasreen concluded absence of an association\(^{8}\), Johanson et al, concluded a statistically significant association\(^{10}\). Moreover, controversy can be noticed in the direction of association; while Siroux et al concluded male preponderance\(^{3}\) Johanson et al and the current study showed a female preponderance\(^{10}\). The dilemma of gender-IgE association requires a large population-based study.

The study revealed a gradual rise in total serum IgE level starting from the first year of age to reach its peak at age 11-15 years then, starts to decline. This pattern explains the failure of demonstrating linear relationship between age and IgE. This pattern is almost similar to the age-pattern reported by other investigators\(^{11}\). The age at IgE decline lags behind that at clinical improvement\(^{12}\). In another word, many of the patients with AD showed clinical improvement (limited lesions, less severity, infrequent relapses, and long remission) at age 5-10 year, while serum IgE needs further five years to match the clinical improvement. This time gap, is probably due to tolerance and to the fact that cascade of immunological events becomes less easily provoked by exogenous and endogenous triggering factors.

The two and half folds rise in IgE among patients with severe AD in comparison to mild cases and the p-value of 0.007 support the hypothesis of association of severity of AD and total serum IgE level. Corresponding to the influence of severity of AD on serum IgE data in the literature are contradictory either showing no influence of severity\(^{5}\) or high level of correlation\(^{13}\). The negative association probably is attributed to: first, small sample
size that fails to illustrate statistical significance
of difference; second, sampling error which
does not cover the full spectrum of severity of
the disease; and third, absence of objective
measure of severity of AD as the available
criteria are subjective with low inter-observer
agreement. Probably, future studies showing
the changes in total IgE after successful
treatment will provide new insight to this
association.

In conclusion, high level of total serum IgE
was found in patients with AD, especially
among females, in 11-15 year age group, and
in severe cases of AD. Further studies to
assess the total serum IgE level in follow up of
patients with atopic dermatitis is highly
recommended.

References
1. Frue M. Atopic dermatitis: immunological
abnormalities and its background. J
Dermatol Scien 1994;7:159-68.
2. Yang KD, Liu CA, Chang JC. Polymorphism of the immune-braking
gene CTLA-4 (+49) involved in gender
discrepancy of serum total IgE levels and
allergic diseases. Clin Exp Allerg
3. Siroux V, Curt V, Maccario M, Kauffmann F. Role of gender and hormone-related
events on IgE, atopy, and eosinophils in the Epidemiological Study on the Genetics and Environment of Asthma, bronchial
hyperresponsiveness and atopy. J Aller
4. LeMaoult J, Szabo P, Weksler ME: Effect
of age on humoral immunity, selection of
the B-cell repertoire and B-cell
development. Immunol Rev 1997;160:115-
126.
5. Laske N, Niggermann B. Does the severity
of atopic dermatitis correlate with serum
IgE levels? Pediatric Allergy Immunol.
6. Hanifin JM, Rajka G. Diagnostic features
of atopic dermatitis. Acta Dermatol
Venereol (Stockh) 1980;92:44-9.
7. Berth-Jones J. Six area, six sign atopic
dermatitis (SASSAD) severity score: a
simple system for monitoring disease
activity in atopic dermatitis. Br J Dermatol
8. Ahmed I, Nasreen S. Frequency of raised
IgE level in childhood atopic dermatitis. J
9. Somani VK. A study of allergen-specific
antibodies in Indian patients of atopic
dermatitis. Indian J Dermatol Venereol
Leprol 2008;74:100-104.
10. Johanson CC, Peterson EL, Ownby DR. Gender Differences in Total and Allergen-
specific Immunoglobulin E (IgE)
Concentrations in a Population-based
Cohort from Birth to Age Four Years. J Am
Epidemol 1998;147:1145-52.
CW. Total serum IgE level in each age
group of patients with atopic dermatitis.
12. Linno O. Ten year prognosis for
generalized infantile eczema. Arch Paed
13. Dhar S, Malakar R, Chattopadhyay S,
Banerjee R. Correlation of the severity of
atopic dermatitis with absolute eosinophil
counts in peripheral blood and serum IgE
Assessment of nutritional status indicators in children under five at Al-Hamdaniya District, North of Iraq

Anwar K. Matee*, Asma A. Al-Jawadi**

* M.sc. Candidate, **Department of Community Medicine, College of Medicine, University of Mosul.

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ABSTRACT

Objectives: To provide a data base for nutritional assessment indicators among children under five in Al-Hamdaniya district, North of Iraq.

Materials and Methods:
Study design: A cross sectional study.
Study setting: The present study was carried out in 3 primary health care centers. These are: Al-Hamdaniya primary health care center which has a population size in the catchment area of 38310, Bartella primary health care center (41029) and Al-Namrood primary health care center (25466). These centers are located in Al-Hamdaniya district in Nineveh Governorate, North of Iraq which has an overall population of 180980.
Study participants: Under five children who were brought to the chosen primary health care centers for vaccination purposes.
Outcome measures: Anthropometric measurements (weight and height/length) and WHO growth standard charts were used to compute the nutritional assessment indicators. Age and sex of the examined children were tested as a demographic attributes affect the study indicators.

Results: A total of 775 under five children were included in this study; of this sample, 41.4% were less than 6 months old, 53.3% were males. The prevalence of stunting, under weight, wasting, risk of over weight, over weight and obesity were 7.9%, 3.7%, 2.5%, 12.9%, 3.0% and 0.5%, respectively. Stunting is significantly prevalent at age group 24-<60 months with no sex difference. Wasting showed the same association with age group 0-<6 months. Risk of over weight, over weight and obesity were significantly present among children 24-<60 months old and among males.

Conclusion: The present study provides a base line data for nutritional assessment indicators among under fives in a local community in Iraq.

Keywords: Under nutrition, under fives, stunting, under weight, wasting, obesity.
the World Health Organization (WHO) defines malnutrition as "the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific function". Contrary to the common use, the term malnutrition refers not only to deficiency or excess or imbalance in the intake of calories, proteins and/or other nutrients. Developing nations are not exempted from the upward secular trend in the pandemic of obesity. Obesity is now considered by WHO as the biggest unrecognized public health problem. The prevalence of obesity in some developing countries has reached even higher levels than in many industrialized nations.

Stunting can coexist with underweight or with overweight/obesity. In the line of co-existence of stunting and overweight in children, there are risk factors for chronic diseases in adulthood. Management of many chronic diseases that may develop due to the increased incidence of obesity would be beyond the capacity of many nations. It is equally important to identify the coexistence of both undernutrition and overnutrition, as an intervention that is designed to prevent only one problem could exacerbate the other.

Growth indices in the form of length/height for age, weight for age, weight for height, and body mass index (BMI) for age are important tools for the assessment of nutritional status of children. The prevalence of nutritional indicators in the form of stunting, underweight, wasting, risk of overweight, overweight, and obesity in children under 5 years of age is one of the ways of assessment of nutritional status of the population and used as a nutritional surveillance indicators among this age group.

Concerning length/height for age; it can help identify children who are stunted (short) or severely stunted due to prolonged undernutrition or repeated illness (chronic malnutrition). While weight for age; it is used to assess whether a child is underweight or severely underweight. On the other hand weight for length/height is especially useful in situations where children's ages are unknown (e.g. refugee situations). It helps identify children with low weight for height who may be wasted or severely wasted (acute malnutrition). Beside that, BMI for age is an indicator that is especially useful for screening for risk of overweight, overweight and obesity.

Surveillance of these indicators is commonly practiced regularly in many countries as a measure of public health status. The concept of nutritional surveillance is derived from disease surveillance which means to watch over the nutritional status of the population and used as a nutritional indicators in the form of stunting, underweight, wasting, risk of overweight, overweight, and obesity to assess whether a child is underweight or severely underweight.
population in order to make decision that lead to improvement of their health \(^{(12)}\). Concerning this issue Mason and Mitchell \(^{(12)}\) have defined three objectives, primarily in relation to problems of malnutrition in developing countries to aid long-term planning in health, to provide input for program management, and to give timely warning of the need for intervention to prevent critical deterioration in food consumption.

Accordingly, the aim of the present study is to provide a data base for some nutritional status assessment indicators which are considered as a backbone for nutritional surveillance of children less than 5 years in Al-Hamdaniya District, which is a part of Nineveh governorate in Iraq.

**Materials and methods**

Prior to data collection, an official permission was obtained from Nineveh Health Directorate (NHD) to facilitate data collection from the Primary Health Care Centers (PHCCs) that were involved in this study. In addition informed oral consent was obtained from the concerned PHCCs managers, workers, and attendants.

**Study setting, design and sampling method**

This study was conducted in Al-Hamdaniya district, which is one of the largest districts in Nineveh Governorate, on the south east of Mosul city. This district has a total population of 180980, with an estimated number of 30767 children less than five years age \(^{(13)}\).

In the present study the target population was children less than five years of age attending the chosen PHCCs with their mothers or care givers for immunization. To achieve the aim of the present study, a cross-sectional clinic based design was adopted. Multistage cluster sampling technique was carried out, in which Al-Hamdaniya district was divided into three main sub-districts upper, middle and lower \(^{(13)}\). From each sub-district one main PHCC which has the largest catchment's area was selected. Therefore, the selected centers were: Al-Hamdaniya PHCC which has a population size in the catchment area of 38310, Bartella PHCC (41029) and Al-Namrood PHCC (25466) \(^{(13)}\). In this study minimum sample size approximation was done according to the method used by Gorstein et al., of calculation of sample size in cross-sectional studies \(^{(14)}\).

The ultimate sample size calculated was 744 children. In this survey the total number collected was 775. Such figure was distributed among the chosen PHCCs taking the proportion of children under five years in each catchment's area into consideration. In each PHCC, selection of children was carried out by circular sampling in which the start was made randomly and every third thereafter was selected in a circular manner until the total sample size was reached from the target PHCC \(^{(15)}\).

Data collection was carried out by interviewing mothers of the target children using a standard questionnaire form. In order to assess the reliability of the information derived from the mothers, a pilot sample consisted of 50 mothers who were interviewed by using a test and re-test approach where two different interviewers were used. The reliability index was calculated \(^{(16)}\) which is the percentage of agreement number of mothers during test and re-test occasions. The calculated reliability index was 85.0%.

To assess the validity of the questionnaire form a modified Angoff method was used \(^{(17)}\) in which the questionnaire form was reviewed by a committee consisted of seven experts in the field of community medicine, family medicine, and medical statistics from the Department of Community Medicine - College of Medicine/University of Mosul. Each one of the experts gave his standpoints about the Coverage, Clarity and Reality of the contents of the form. The overall percent agreement among the experts was 88.3% which indicates that the form is valid.

The included items were chosen from multiple modules \(^{(11, 14, 18)}\). However, some modification was done according to the feedback from the pilot study and stand points of the scientific panel in the Department of Community Medicine. From the related modules, questions about age and sex of the child, and father's job were used in order to determine social class of the target child \(^{(18)}\).
Also there were questions about residence whether the family resides in the center of the district i.e. the city of Karakush, which is considered an urban region while the outside vicinity is rural. Migration status whether migrant (those who are reported in the Migration Office) or non migrant.

**Outcome measures and statistical analysis**

Weight in Kg, length/height in cm, age and sex data were used to calculate z-scores i.e. standard deviation score (SDS) of the different nutritional indicators. Age was determined by months (exact age). Baby was weighted with minimum amount of clothing and the result was rounded to the nearest 50 grams. Measurements were carried out using WHO/Seca scale for infants and children. The scales were checked for zero error daily.

Length/ Height was taken without shoes using wooden board for height measurements and plastic board for length measurement, both of them are of WHO/Seca, and the figure was rounded to the nearest centimeter.

Identification of underweight, stunting, wasting, risk of over weight, overweight, and obesity among study sample was calculated using the published WHO child growth standards (19). After plotting results of anthropometric measurements to the reference population in WHO growth standard (11), each of the four anthropometric nutritional status indicators were expressed in standard deviation score from the median of this reference population, which is called (z-score). The child was categorized either as stunted, underweight, wasted or has risk of overweight, overweight or obesity The prevalence of moderate and severe underweight was defined as the proportion of children whose weight for age was below -2 and -3 (SDS), respectively. Similarly, the prevalence of moderate and severe wasting and stunting was defined as the proportion of children with weight for height (wasting) or height for age (stunting) who were below -2 and -3 (SDS). Finally, the prevalence of risk of overweight, overweight and obesity was defined as the proportion of children whose BMI for age was more than +1, +2, and +3 (SDS), respectively (11).

Data collection was started on 1st of November, 2010 and ended on the 31st January, 2011.

Data entry and analysis were done using MINITAB version 13. SPSS package version 12 was also used for statistical analyses. Chi squared test was used to determine the presence of association between variables. Odd ratio and its 95% confidence limit interval (95% CI) were also computed.

**Results**

A total of 775 children aged 0-<60 months were enrolled in this study; of the total sample 53.3% were males and 46.7% were females. Almost two fifth (41.4%) fell in the age group 0-6 months and 16% were 24-<60 months old. The sample was predominantly rural (70.0%).

Regarding migration status, 20.8% of study children were of migrant families. Concerning social class, two thirds of study sample fell in social class III (33.4%), and 36.5% in social class IV.

Table 1 reveals that 7.9%, 3.7%, 2.5% of examined children were stunted, underweight, and wasted respectively. While, 12.9%, 3.0% and 0.5% of the total children included in the survey were presented with risk of overweight, overweight and obesity, in that order. Four children only showed combination of stunting and obesity.

Table 2 demonstrates distribution of study subjects according to the nutritional assessment indicators by age and sex during study period. In regard to male sex the fraction of stunting is minimum at the age group 6-<12 month (8.6%), in female the lowest proportion is at the age group 12-<24 months (11.5%).

Underweight is highly prevalent among males and females whose age was 0-<6 month (50.0% and 57.8%) respectively. Similar trend is shown in wasting. At the same time the present survey found that almost half (46.8%) of males with risk of over weight, overweight and obesity were at the age group of0-<6 month old. While one third (31.3%) of females were at the same age group.
Table (1): Database of nutritional status assessment indicators among study population.

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Malnutrition type</th>
<th>Malnourished (No.)</th>
<th>Normal (No.)</th>
<th>Total (No.)</th>
<th>Overall prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>height/age</td>
<td>Stunting</td>
<td>61</td>
<td>714</td>
<td>775</td>
<td>7.9</td>
</tr>
<tr>
<td>weight/age</td>
<td>Underweight</td>
<td>29</td>
<td>746</td>
<td>775</td>
<td>3.7</td>
</tr>
<tr>
<td>weight/height</td>
<td>Wasting</td>
<td>19</td>
<td>756</td>
<td>775</td>
<td>2.5</td>
</tr>
<tr>
<td>BMI/age</td>
<td>Risk of overweight</td>
<td>100</td>
<td>675</td>
<td>775</td>
<td>12.9</td>
</tr>
<tr>
<td>BMI/age</td>
<td>Overweight</td>
<td>23</td>
<td>752</td>
<td>775</td>
<td>3.0</td>
</tr>
<tr>
<td>BMI/age</td>
<td>Obese</td>
<td>4</td>
<td>771</td>
<td>775</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table (2): Distribution of study sample according to age, sex and nutritional status assessment indicators.

<table>
<thead>
<tr>
<th>Age</th>
<th>stunting</th>
<th>underweight</th>
<th>wasting</th>
<th>Risk of overweight, overweight and obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>0-&lt;6</td>
<td>11(31.4)</td>
<td>10(38.5)</td>
<td>5(50.0)</td>
<td>11(57.8)</td>
</tr>
<tr>
<td>6-&lt;12</td>
<td>3(8.6)</td>
<td>4(15.4)</td>
<td>2(20.0)</td>
<td>4(21.1)</td>
</tr>
<tr>
<td>12-&lt;24</td>
<td>11(31.4)</td>
<td>3(11.5)</td>
<td>3(30.0)</td>
<td>0(00.0)</td>
</tr>
<tr>
<td>24-&lt;60</td>
<td>10(28.6)</td>
<td>9(34.6)</td>
<td>0(00.0)</td>
<td>4(21.1)</td>
</tr>
<tr>
<td>Total</td>
<td>35(100)</td>
<td>26(100)</td>
<td>10(100)</td>
<td>19(100)</td>
</tr>
</tbody>
</table>

The significance of certain age and sex categories which affect study indicators are shown in Table 3. Statistical analysis of the results demonstrated that children with 24-<60 months carried a significant risk of stunting when compared with the reference age group (age group with lowest prevalence), (OR: 4.11, 95% C.I for OR: 1.669 - 10.119, p=<0.001).

Table (3): Association of age and sex with nutritional status assessment indicators.

<table>
<thead>
<tr>
<th>Category</th>
<th>Significance</th>
<th>stunting</th>
<th>underweight</th>
<th>wasting</th>
<th>Risk of over weight, over weight and obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-&lt;6</td>
<td>OR</td>
<td>1.59</td>
<td>2.76</td>
<td>3.58</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>95% CI OR</td>
<td>0.66-3.82</td>
<td>0.79-9.66</td>
<td>1.01-12.85</td>
<td>0.83-2.50</td>
</tr>
<tr>
<td></td>
<td>p-value*</td>
<td>=&lt;0.296</td>
<td>=&lt;0.097</td>
<td>=&lt;0.037</td>
<td>=&lt;0.199</td>
</tr>
<tr>
<td>6-&lt;12</td>
<td>OR</td>
<td>-----</td>
<td>1.99</td>
<td>2.16</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>95% CI OR</td>
<td>----</td>
<td>0.49-8.09</td>
<td>0.48-9.79</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>p-value*</td>
<td>----</td>
<td>=&lt;0.330</td>
<td>=&lt;0.307</td>
<td>----</td>
</tr>
<tr>
<td>12-&lt;24</td>
<td>OR</td>
<td>2.12</td>
<td>----</td>
<td>----</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>95% CI OR</td>
<td>0.83-5.39</td>
<td>----</td>
<td>----</td>
<td>0.67-2.35</td>
</tr>
<tr>
<td></td>
<td>p-value*</td>
<td>=&lt;0.108</td>
<td>----</td>
<td>----</td>
<td>=&lt;0.476</td>
</tr>
<tr>
<td>24-&lt;60</td>
<td>OR</td>
<td>4.11</td>
<td>1.69</td>
<td>----</td>
<td>2.48</td>
</tr>
<tr>
<td></td>
<td>95% CI OR</td>
<td>1.67-10.12</td>
<td>0.37-7.71</td>
<td>----</td>
<td>1.32-4.66</td>
</tr>
<tr>
<td></td>
<td>p-value*</td>
<td>=&lt;0.001</td>
<td>=&lt;0.49</td>
<td>----</td>
<td>=&lt;0.004</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>OR</td>
<td>1.20</td>
<td>0.47</td>
<td>1.63</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>95% CI OR</td>
<td>0.71-2.03</td>
<td>0.21-1.02</td>
<td>0.64-4.21</td>
<td>1.06-2.32</td>
</tr>
<tr>
<td></td>
<td>p-value*</td>
<td>=&lt;0.505</td>
<td>=&lt;0.053</td>
<td>=&lt;0.304</td>
<td>=&lt;0.023</td>
</tr>
<tr>
<td>Female</td>
<td>OR</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>95% CI OR</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>p-value*</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

*Chi-square test was used.
The blank boxes referred to reference group (age group with lowest prevalence) used in the calculation of odds ratio.

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The lower prevalence of underweight among males (34.0%) demonstrates that they are evidently protected from risk of underweight. On the other hand children 0-<6 month old showed significant risk for the development of wasting (OR=3.58, 95% C.I=1.01-12.845, p=<0.037).

What is more, the result of analysis showed that children who were 24-<60 months old were at a significant risk of over weight, over weight and obesity (OR=2.481, 95% C.I=1.320-4.661, p=< 0.004). Males sex presents the same picture (OR= 1.57, 95% C.I= 1.061-2.321, p=< 0.023).

Discussion
Knowing the prevalence of stunting, underweight, wasting, and over weight is important for determining the overall health status of the community (20).

In developing countries and marginalized groups in affluent societies, under nutrition is increasing as a result of the debt crisis and consequent economic adjustment policies. On the other hand, the rapid growth of some economies and changes in lifestyles, including diet and physical activity patterns, contribute to other malnutrition problems at the other end of the spectrum (5).

Al-Hamdaniya district is one of the largest districts of Nineveh Governorate in the North of Iraq. It showed many changes after possession in 2003. Before that it was a small town, but the last events that occurred in Iraq especially forced migration lead to an increase in its population size with its adverse effect on the health of the people particularly children under five years of age. Ever since, such population group is the most vulnerable subset that may be affected by abnormal conditions. Fairly, Al-Hamdaniya district did not suffer from the events that affect the rest of the country after possession. Consequently, this district was chosen as a study setting for this work.

The design of this study is a cross-sectional. This has many advantages as it is easy to conduct, needs less time and measure the prevalence of an event which highlights the extent of the problem in the community. Along side, the main characteristics of the affected person are detected, and could be related to disease occurrence (16).

The present study found that 7.9% of children were stunted, 3.7% were under weight, 2.5% were wasted, and 16.4% was with risk of over weight, over weight and obesity. Corresponding figures reported by the rapid assessment carried out by the UNICEF (21) in Baghdad during 2003 were 15.9% for stunting, 13.2% under weight and 7.7% of examined children were wasted. If results of the present study compared with that of WHO in 2010 (22): a different figures were reported which were 27.5% for stunting, underweight 7.1% and 15% for over weight. This is probably due to differences in sample size and different population distribution. Fifteen years ago a study was conducted by Al Jawadi (23) at Mosul city showed that 37.9% of children were stunted and the prevalence of wasting was 19.7%, these higher figures were most probably caused by the effect of economic sanction and material blockade applied by U.N on Iraqi people at that time. Three years later and at the same city a similar study was carried out showed a lower figures than that used by Al-Jawadi (23) (10% and 18.9%) respectively (24).

Regarding age and sex of the examined children, they are important demographic variables and are the primary basis of demographic classification in the survey. The findings of the present work showed that children in the age group 24-<60 months were significantly at higher risk of stunting compared with children in the younger age category (6-<24 months); similar results was shown a decade ago by Al-Jawadi (23) in Mosul city. In addition to it is consistent with other study carried out by Yimer (25) in Ethiopia, and other fare away developing countries such as India (26). The second year of life is a critical period for under nutrition, because the child is dependent on someone for nutritional intake, complimentary foods are introduced, and the child is exposed to food-born pathogens (27). The result of this survey highlights the first two years of life as the most critical period for intervention suggesting an urgent need to institute programs which improve the
nutritional status of most vulnerable children in the study area. Such programs are probably most effective if they are instituted among children in the first three years of life.

Cross-sectional study conducted by Rao et al. (28) in North-East India in 2004, has been shown that female children were at higher risk of stunting than male children. Other studies showed boys were more malnourished than girls such as that carried out by Malla and Shrestha (29) in Nepal, and by El Mouzan et al. (30) in Saudi Arabia. The present study revealed no significant gender difference in the prevalence of stunting. The same result has been reported by Schoenbaum et al., (31) in Gaza Strip in 1995. While under weight so called general malnutrition seems to be evidently more prevalent in females, with males being evidently protected yet this relationship is of no significant value, this result unlike the results in the study of El Mouzan and his co-workers (30) in Saudi Arabia; who reported an increasing fractions of under weight with increasing age this paradoxical findings may be attributed to different study locality, design and sample size.

Regarding wasting, it reflects the proportion of achieved body mass to chronological age and is particularly sensitive to acute growth disturbance. This measurement is used in less developed areas where age assessment is difficult. The result of this study showed that wasting was significantly more prevalent among children < than 6 months old (OR=3.58, 95% C.I=1.01-12.845, p=<0.037), probably due to the repeated attacks of common infection during this period. A similar finding was reported by Anderson et al., (32) in Ghana.

On the other hand the results of BMI for age measurement showed that 16.4% of children suffered from risk of over weight, over weight or obesity. This figure is higher than that reported by the study of Tinh and Nhan (33) in Viet Nam, who examined a smaller number of children (n=270) with quite varied nutritional environment. In the present study over weight and obesity estimates were 3.5% unlike that of WHO (15.0%) which covered a ten years period (1999-2009) (22). This dissimilarity is probably due to change in the life style, dietary habits and increased time of watching television and computer games among older age groups.

The high prevalence of obesity problem with statistical significance at age group 24-60 months needs further attention as obese child will become obese adult, Pediatric obesity has been documented in low and middle income communities (LMICs) and is the driving force behind pediatric metabolic syndrome risk that has become a growing public health concern in such settings (34). It is associated with health problems for the child/adolescent including discriminating risk of psychosocial morbidity, cardiovascular complications, and type 1 and type 2 diabetes (35).

Conclusion and recommendation
This study has helped in determining the nutritional status indicators of under five children in Al-Hamdaniya district. These data can be used as a baseline for the development of local and national nutritional surveillance system.

References
24. Al-Rashedi MW. Assessment of malnutrition of children under five years of age in Al-Thawra District/Mosul. DPH Dissertation, Community Medicine Department, College of Medicine, Mosul University, Iraq; 1998: 24-26.
30. El Mouzan MI, Foster PJ, Al Herbish AS, Al Salloum AA, Al Omar AA, Qurachi MM.


Alternative diagnosis for pain in patients who underwent appendectomies for normal appendices and the incidence of negative appendectomies

Ali M. Muhammed*, Hiwa O. Ahmed**
* General Surgeon, Sulaimaniyah Teaching Hospital;
** College of Medicine, University of Al-Sulaimaneyah.

ABSTRACT

Background and objectives: Some gastrointestinal and genitourinary tract pathologies can simulate clinical features of acute appendicitis; we aim to determine the alternative diagnosis for the pain in which appendicitis was considered, and to find the incidence of negative appendectomies in our practice.

Methods: An observational study, including 558 patients, who underwent an appendectomy at Al Sulaimaniyah Teaching Hospital (STH) from the 2nd of January to the 30th of June 2009. Only patients who underwent urgent appendectomy and the specimen subjected to tissue examination were included. Appendices were labeled acutely inflamed when, macroscopically there were injections of mucosa, fibrinous or purulent film, edematous or necrotic changes of the wall and blood or pus on opening the appendix.

Results: Most of the patients were young between 20-40 years age with median age of 22 ±7.7 years. Other pathologies presented in patients with macroscopically normal appendices, included 35 (6.27%) patients had purulent peritoneal fluids occurred in young female with tubo-ovarian infections, 12 (2.15%) patients had mesenteric lymphadenitis and 37 (6.63%) patients had rupture Graafian follicles. Histologically normal appendix was present in 178 (31.89 %) patients, 61 (10.93%) of them were males and 117 (20.96%) were females.

Conclusion: Normal appendectomies were found in 32 % of the patients, more frequently in young female patients, undergoing early (within 6 hrs since the pain) appendectomy, with the most common alternative diagnosis of tubo-ovarian infections.

Keywords: Acute appendicitis, alternative diagnosis, normal appendix.
Acute appendicitis is the most common cause of urgent abdominal surgery (1), the accuracy of a clinical diagnosis of acute appendicitis based on patient's history and physical examination ranges from 70% to 84%, because other gastrointestinal and genitourinary tract abnormalities can present similarly. (2) A delay in diagnosis or an incorrect diagnosis causes serious complications. (3,4) Although history and physical examination results remain the cornerstone of the diagnosis of acute appendicitis, many additional adjuncts have been proposed to increase diagnostic accuracy (3). These include laboratory investigations like WBC count, C reactive protein, serum markers, scoring systems, ultrasonography, computed tomographic (CT) scanning, and laparoscopy (3,5), but no laboratory or radiological test is 100% accurate. (6)

Unfortunately, the clinical benefit of none of the scores has been tested in an adequate controlled study, (7, 8) negative appendectomy rate of 7% was found. (7)

Regarding laboratory investigations, normal appendices were found in 13.4% and 32.5% of the patients who had high and normal white blood cell counts, respectively. (9)

Both ultrasonography (US) and computed tomography (CT) help in diagnosis of acute appendicitis (10) Sonography is preferred more than CT; as the initial imaging study for young, female, and slender patients, (11) although CT is less operator dependent than sonography. (10,12) It may help in surgical planning, (10, 13) has sensitivity and specificity rates of 93% and 92%, respectively (12, 13), can safely exclude appendicitis (12) without CT rates of appendectomy with normal findings (negative appendectomies) was of 13.4 to 33% (4, 7, 10, 13-15).

On the other hand there are authors claiming that it has no significant contribution to the diagnosis of acute appendicitis, and that they in fact delay treatment and therefore result in increased perforation rates with negative appendectomy rates reaching up to 20% and the rates of negative appendectomy have remained unchanged (4,8).

We aim to determine the alternative diagnosis for the pain in which appendicitis was considered and to find the incidence of negative appendectomies in our practice.

**Patients and methods**

An observational study including 558 patients, who underwent appendectomy at Al Sulaimaniyah Teaching Hospital (STH) from the 2nd of January to the 30th of June 2009. Only patients who underwent urgent appendectomy and specimen subjected to tissue examination were included. All the patients were operated for appendicitis by open appendectomy on the basis of history, physical findings, relevant clinical data, investigations, plain abdominal radiography and ultrasound. The Ethics committee of Al Sulaimaniyah University – Medical College approved the research protocol, and written informed consent was obtained from all patients.

Demographic data regarding age, gender, occupation, duration, details of symptoms and clinical signs of acute appendicitis were recorded

Baseline laboratory assessment included leucocytes count and urine analysis, imaging including; plain radiography of abdomen was performed in 89 patients and ultrasonography was performed in 480 (84% of) patients and the remaining patients 78 (13.97%) underwent surgery without diagnostic ultrasonography.
because they were admitted during the night when the ultrasound is not available.

All the patients were told not to take anything orally for 4 hours. After the decision was made by the on call surgeon, and then the patients were seen by anesthetist doctors. During induction of anesthesia prophylactic antibiotics given as 1 g Ampicillin-Cloxacillin (or Ceftriaxone when the patient was allergic to penicillin), and 80 mg (or the dose was adjusted to the weight in children) of gentamicin intravenously.

Through right sided grid iron incision (3-7 cm), centered on the McBurney point, formal minilaparotomy was done; peritoneal cavity was inspected for any fluid, pus, blood, Meckel's diverticulum, ovarian pathology, etc. Caecum was identified, the appendix was found, retrograde appendectomy was done in most of the cases 549 (98.38%) and ante grade for the others.

Appendices were labeled acutely inflamed when, macroscopically there were intravascular injection of mucosa, fibrinous or purulent film, edematous or necrotic changes of the wall and blood or pus on opening the appendix. (15)

All excised appendices or any excised tissue were sent for histopathology. Patients were discharged on the base of day case surgery when they fulfilled discharging criteria as following: stable vital signs, apyretic: no wound or airway problems, tolerating diet, and established autonomy at discharge, possession of a telephone, suitable home accommodation and adequate home support upon discharge (16), to report on need, or after 7 days.

Histopathological criteria of acutely inflamed appendix were granulocytic invasion of the mucosa (erosive), deeper lesions to the submucosa (erosive or ulcerated), or into the muscular wall (ulcer or inflammation). Perforating appendicitis was diagnosed in cases of periappendiceal abscess, gangrene, or when lesions penetrated the wall, and were verified macroscopically. Scarring and specific lesions were not considered as acute appendicitis. (17)

The data were analyzed using SPSS version 16, A P-value of less than .05 was considered to indicate statistical significance.

Results

A total of 558 patients had appendectomy for clinically diagnosed acute appendicitis over 6 months at Al Sulaimaniyah Teaching Hospital. Most of the patients were young between 20-40 years age with median age of 22 ±7.7years. Three hundred and five patients (54.66%) from sum of 558 patients were females, two hundred fifty three (45.34%) patients were males and with female to male ratio 5/4.

More than three quarters of the patients presented within 6 hours from the onset of abdominal pain and three quarters underwent operations within 6-12 hours and the rest within first 24 hours after admission, (Table 1). Only 491 (87.99%) patients presented with right iliac fossa pain, while the rest had generalized or central abdominal pain, and 312 (55.91%) had tenderness in the right iliac fossa. More than three quarters of the patients 451 (80.82%) had nausea, while 257 (46.05%) of the patients had vomiting before the onset of the pain and 30 (5.37%) patients had vomiting after the onset of pain. Three hundred and sixty eight (65.94%) patients were anorexic, 62 (11.11%) had dysuria and only 89 (15.94%) patients had mild pyrexia up to 38.2 degree centigrade.

Histological normal appendix was present in 178 (31.89 %) patients, 61 (10.93%) of them were males and 117 (20.96%) were females (Table 1) (P-value=0.0259).

Among 558 patients who were suspected clinically of having acute appendicitis, intraoperative finding revealed that 206 (36.92%) patients had acutely inflamed appendix, 113 (20.25%) suppurative appendicitis, 32 (5.73%) gangrenous appendicitis, 12 (2.15%) had perforated appendix . One patient had Carcinoid tumor in the base of the appendix and in 7 (1.25%) cases there were Enterobeeous Vermicularis (E.V) found inside the lumen of the acutely inflamed appendices, (Table 2).

Fifty nine (10.03%) patients were diagnosed intraoperative by naked eyes as normal appendix, 18 of them showed pathological
changes on the histological examination. While 14 (2.51%) appendices labeled during the operations as appendicitis were histopathologically normal.

Other pathologies presented in patients with macroscopically normal appendices, included 35 (6.27%) patients had purulent peritoneal fluids occurring in young female with tubo-ovarian infections, 12 (2.15%) patients had mesenteric lymphadenitis and 37 (6.63%) of the patients had rupture graafian follicles (Table 3).

Table (1): Negative appendectomies and alternative diagnosis; difference in gender, time of appendectomy, in each age group with their complications.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Time from pain to operation (hours)</th>
<th>Age (years)</th>
<th>Co morbidity</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂</td>
<td>6-12</td>
<td>12-24</td>
<td>&gt;24 0-10</td>
<td></td>
</tr>
<tr>
<td>♀</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Table (2): Histopathological results of all the patients underwent open appendectomy.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Frequency no. and (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute appendicitis</td>
<td>206 (36.92%)</td>
</tr>
<tr>
<td>Normal appendix</td>
<td>178 (31.89%)</td>
</tr>
<tr>
<td>Suppurative appendicitis</td>
<td>113 (20.25%)</td>
</tr>
<tr>
<td>Gangrenous appendix</td>
<td>32 (5.73%)</td>
</tr>
<tr>
<td>Perforated appendicitis</td>
<td>12 (2.15%)</td>
</tr>
<tr>
<td>Acutely inflamed appendices</td>
<td>7 (1.25 %)</td>
</tr>
<tr>
<td>obstructed with E.V*</td>
<td></td>
</tr>
<tr>
<td>Carcinoid at the base of appendix</td>
<td>1 (0.179 %)</td>
</tr>
<tr>
<td>Total</td>
<td>558 (100%)</td>
</tr>
</tbody>
</table>

* Enterobeous Vermicularis.

Table (3): Alternative diagnosis were found in normal appendices.

<table>
<thead>
<tr>
<th>Findings in the patients with normal appendix</th>
<th>Frequency no. and (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rupture Graafian follicle</td>
<td>37 (6.63%)</td>
</tr>
<tr>
<td>Purulent peritoneal fluid (pelvic infection, tuboovarian infections)</td>
<td>35 (6.27%)</td>
</tr>
<tr>
<td>Mesenteric lymphadenitis</td>
<td>12 (2.15%)</td>
</tr>
</tbody>
</table>

**Discussion**

The present work showed that more than 60% who had normal appendix were females and their mean age was 18±5.6 years. The findings are in the line with the reported difficulties in female adolescence and young females. The accuracy of a clinical diagnosis of acute appendicitis based on patient’s history...
and physical examination ranges from 70% to 84%. In women of childbearing age, this figure decreases to 60–68% because of the overlap of symptoms from acute gynecologic abnormalities (2).

In the current study results showed that the incidence of negative appendectomy to be 178 (31.89%) which is comparable to recently published literatures, declaring that rates of appendectomy with normal findings (negative appendectomies) were in the range of 13.4 to 33% (4,7,10,13-15,21). Appendectomy for a normal appendix is associated with both morbidity and mortality. (21,22) Although the morbidity and mortality are of the same quality, but with higher frequencies i.e., up to 5% of patients will develop intestinal obstruction following surgery for a normal appendix. (23)

The diagnostic tools like white cell counts, urine analysis and ultrasonography have not been shown conclusively to improve the outcome in terms of negative finding on appendectomy. (3, 4, 5, 12, 13, 24, 25)

The surgeons who did the operations considered all the patients (n=558) to have acute appendicitis, while pathological results showed 178 excised appendices to be normal. One of the reasons for this finding may be that the majority of surgeons favor early operation. It was stated that negative appendectomy is higher among patients who received immediate surgery after admission to a hospital. (8,26) Furthermore a recent retrospective study found no significant differences in complications between early (less than 12 hours after presentation) and late (12-24 hours) appendectomy. (8)

In this work all (n=588) appendices were removed, even when they look macroscopically normal. The points of defense are, first endoluminal appendicitis occurs in 11-58% of apparently normal appendices which were removed. Secondly it is accepted generally to remove normal appendices during open appendectomies. (8, 27) The third point is that normal-looking appendices have a 22% chance of being inflamed on further sophisticated investigations. (18) It is also reported that children underwent appendectomy for either infected or normal appendix have reduced chance of developing ulcerative colitis. (27)

During appendectomy, 14 appendices were labeled by naked eyes to be acutely inflamed, but histopathology showed them to be normal appendices. Five of which were associated with tubo-ovarian infection, and three were associated with rupture graafian follicles, while the rest have no any alternative pathology. Jane E. et al, gave an explanation for that the appendix in some patients with colonic diverticulitis, colitis, or pelvic inflammatory disease had a secondary edema or serosal inflammation. (28)

There was one case of carcinoid tumor presented as acute appendicitis, it was evident as yellowish small oval mass (8mm x 4mm) in the base of the appendix, this presentation is not going in line with literature. Studies were reporting that carcinoid tumors were not evident macroscopically. The incidence (0.17%) out of 558 patients also higher than what found in the literature (0.1%) out of 1000 patients (29,30) P-value=0.001.

In the current work seven patients had Enteroboeous Vermicularis in appendicular lumen, when histological results showed inflamed appendix. This is comparable to literature “Enteroboeous infection is often associated with acute appendicitis and perforation of an inflamed retrocaecal appendix” (10, 18, 31).

Imaging will help in accuracy of diagnosis and decreasing negative appendectomy, it also may detect alternative diagnosis in patients with features of acute appendicitis. It may be necessary to use imaging to raise the accuracy of clinico-laboratory diagnosis of acute appendicitis, but not routinely, saving for special groups with difficulty in clinical diagnosis of acute appendicitis.

The limitations of the work
One of the limitations of the current work is that, we haven’t started diagnostic laparoscopy in our centre, which may significantly reduce the rate of removal of histo-pathologically normal appendices, (18,19) accordingly we are not able to discuss the effect of laparoscopy on reducing negative appendectomies. Another limitation is the CT
of the abdomen not ordered although it is available in restricted time in our Emergency Department (from 08.00 to 1400). This makes us have no wide concept of the effect of the CT scan on reducing negative appendectomy, although there are studies claiming that CT have been used to diagnose appendicitis with no additional diagnostic specificity. (2,12,13, 32,33) Also it needs ionizing radiation; it is not routinely available at all hours and has false negative results as high as 15%. (3,4,7,11-13,28) The effect of ionizing radiation may be a drawback especially the patients with uncertain clinical diagnosis, who need a CT usually are adolescence or young child bearing ladies. (2, 12, 13, 18,19,28).

Conclusion
Normal appendices were found in 32 % of the patients, more frequently in young female patients, undergoing early (within 6 hrs since the pain) appendectomy, with the most common alternative diagnosis of tubo-ovarian infections.

Acknowledgements
We are grateful to Dr. Adnan Hamawandi Professor of pediatrics, for his statistical guidance, and to all medical and paramedical staff at the Emergency Department of Al-Sulaimaniyah Teaching Hospital for their technical help.

References
Sciatic nerve injury following gluteal intramuscular injection

Mohammed M. Fathi Alsheikh
Department of Medicine, College of Medicine, University of Mosul.

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ABSTRACT

Objective: To study the type of injected agents, clinical features and outcome of conservative treatment of patients with sciatic nerve injury following gluteal intramuscular injection.

Methods: A prospective study of patients with sciatic nerve injury following gluteal intramuscular injection from private neurological clinic and neurophysiology unit in Ibn Sena Teaching Hospital in Mosul between January 2008 and July 2010.

Results: The total number of the patients was 36. Male patients were 19 and females were 17. There were 28 patients under age of 5 years. Thirty four patients sustained their nerve injury by nurses, and antibiotics were the offending agents in 91.33% of patients. Foot drop is the commonest presenting clinical feature. Complete recovery was reported in 29.6% of the patients, partial recovery in 44.5% and no measurable clinical improvement in 25.5% of them.

Conclusion: Children are at higher risk of injury. The injection should be prescribed only when mandatory and administered by a well qualified and competent personnel.

Keywords: Intramuscular injection, sciatic nerve, sciatic nerve injury.

Sciatic neuropathy is the second commonest mononeuropathy in the lower limbs after common peroneal nerve injury. The vulnerability of the nerve to damage is attributed to its long anatomic course from lumbosacral plexus, through the sciatic notch down to its bifurcation just above the popliteal fossa (1,2). Sciatic nerve injury following intragluteal injection is the second most common cause of sciatic nerve injury after hip arthroplasty (2). Apart from the risk of transmission of blood-borne diseases, improper injection technique can cause peripheral nerves damage; most commonly of

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the radial nerve in the upper limbs and sciatic nerve in the lower limbs especially in paediatric populations\(^\text{(3,4,5)}\).

Damage to the sciatic nerve by injection has been reported from several parts of the world; these injuries are related mainly to a faulty injection technique and lack of trained manpower capable of administering paranteral drugs in the developing countries\(^\text{(1,6,7)}\). Injection injury account for 50% of sciatic nerve injuries in one large series reported from the USA\(^\text{(4)}\).

The degree of nerve fiber injury depends on the site of injection and upon the specific agent injected. The pathological alteration in the nerve is evident as early as 30 minutes following injection injury in the form of widespread axonal and myelin degeneration\(^\text{(7,8)}\).

The mechanism of injury includes direct needle trauma, secondary constriction by scar and direct nerve fiber damage to both axon and Shwan cell, with breakdown in the blood-nerve barrier by neurotoxic chemicals in the injected agent\(^\text{(9)}\). A review of literature on relevant injection procedure found that injury to sciatic nerve is associated with the use of dorsogluteal site for injection because the sciatic nerve commonly courses this site\(^\text{(10)}\). The World Health Organization (WHO) estimates that of the 12 billion injections administered worldwide annually, 50% are unsafe and 75% are unnecessary\(^\text{(11)}\).

This study was conducted to analyze the type of injected agents, clinical features and outcome of a conservative treatment of patients with sciatic nerve injury following gluteal intramuscular injection.

Materials and methods
This was a prospective study for the period from January 2008 to July 2010. We were able to identify a total of 36 patients from private neurological clinic, and neurophysiology unit in Ibn Sena Teaching Hospital in Mosul that satisfied our inclusion criteria (i.e. neurologcal symptoms and / or signs in lower limb following an intragluteal injection).

All patients had history of recent gluteal intramuscular injection and they did not have any weakness in the limbs or associated sensory disturbances before the injection. They had received complete polio immunization together with stool examination to exclude patients with poliomyelitis.

The motor symptoms and signs were graded using the Medical Research Council (MRC) grading score (0-5) for knee flexion, foot dorsiflexion and planter flexion muscles\(^\text{(11,12)}\). But this is was not possible in three patients because they had only sensory symptoms and signs.

The relative involvement of the common peroneal and posterior tibial nerve were determined by comparing the power grade in the dorsiflexion and planter flexion of the foot at the time of the first presentation.

The nerve conduction study was done in the lower limb nerves: the common peroneal and posterior tibial. The recording was done over the extensor digitorum brevis and the abductor hallucis longus muscles, the sensory nerve conduction study involves the sural and superficial peroneal nerves.

A total of 36 patients were followed up every 2 months during the study with clinical evaluation to assess the degree of improvement by using MRC clinical grading of power and they were divided in 3 groups:
1- Complete recovery: when MRC clinical grade of power is 5 on the last follow up visit.
2- Partial recovery: when the power recovered by > 1 grade from the baseline on the last follow up visit.
3- No recovery when there was no measurable improvement from the baseline in the last follow up visit\(^\text{(12)}\).

Results
Of the 36 patients fulfilling the inclusion criteria, 19 patients (52.77%) were males and 17 patients (27.33%) females, male:female ratio 1.12 : 1.

There were 28 patients (77.77%) under the age of five years.\(^\text{(Fig.1)}\)

The majority of these patients 34 (94.45%) has sustained their nerve injury by nurses and 2 patients (5.55%) by doctors.
Unfortunately the exact site of the gluteal injection could not be identified in all the patients.

The indication for administration of the gluteal intramuscular injection is shown in Table (1); by far the most common condition for which intramuscular injection of the drug was given was upper respiratory tract infections 69.44%.

Antibiotics injections (cefotaxime, ampicillin, procaine penicillin) were the offending agent in 33 patients (91.33%), while 3 patients (8.33%) had diclofenac sodium and acetaminophen injections. The sex distribution according to the side affected was show in table (2).

Twenty three patients (63.88%) were affected on the left side while 13 patients (36.12%) had the injury on the right side and the left side was more commonly affected in both male and female patients.

Table (1): Indications for giving injections and the number and percentage of patients.

<table>
<thead>
<tr>
<th>Indication for injection</th>
<th>Number affected</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper respiratory tract infections</td>
<td>25</td>
<td>69.44</td>
</tr>
<tr>
<td>Diarrheal diseases</td>
<td>5</td>
<td>13.88</td>
</tr>
<tr>
<td>Back pain</td>
<td>3</td>
<td>8.33</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>2</td>
<td>5.55</td>
</tr>
<tr>
<td>Otitis media</td>
<td>1</td>
<td>2.77</td>
</tr>
</tbody>
</table>

The onset of the symptoms was immediate in 34 patients (94.44%) and delayed for few hours in 2 patients (5.55%).

Table (3) showed the chief complaint at presentation. There was post injection foot drop in 19 patients (52.77%), weakness of the leg and foot in 11 patients (30.55%), pain and numbness in 3 patients (8.33%), equinovarus deformity in 2 patients (5.55%) and fibrotic scar in gluteal region in 1 patient (2.77%).

All the patients had undergone an initial electrophysiological study at mean of 3 months (3 weeks to 15 months) from the onset of the symptoms. The results of the electrophysiological study revealed that 3 patients (8.33%) had decreased sensory nerve action potential (SNAP) in the sural nerve without any motor conduction abnormalities (these in patients presented with sensory symptoms and signs only). Twelve patients (33.33%) had isolated involvement of the common peroneal nerve, seven patients (19.44%) had involvement of the posterior tibial nerve, and 14 patients (38.88%) had involvement of both common peroneal and posterior tibial nerves equally.

Table (2): Sex distribution of the limbs affected.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of patient</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>Left</td>
<td>10</td>
<td>27.77</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>4</td>
<td>11.11</td>
</tr>
<tr>
<td>Left</td>
<td>13</td>
<td>36.11</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Symptoms at presentation.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Number of patients</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot drop</td>
<td>19</td>
<td>52.77</td>
</tr>
<tr>
<td>Leg and foot weakness</td>
<td>11</td>
<td>30.55</td>
</tr>
<tr>
<td>Pain and numbness</td>
<td>3</td>
<td>8.33</td>
</tr>
<tr>
<td>Equinovarus deformity</td>
<td>2</td>
<td>5.55</td>
</tr>
<tr>
<td>Fibrotic scar</td>
<td>1</td>
<td>2.77</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>
Thirty two patients (88.88%) were treated conservatively by vitamins, physiotherapy, ankle brace and pain medication (for the patients with pain and numbness).

Four patients (12.9%) were treated by tendon transfer because of their late consultation (more than 15 months) and yielded good results.

Five patients out of the 32 patients who were treated conservatively discontinued their follow up, the reminder 27 patients were followed up 8-18 months (mean followed up period 10 months) in the prospective phase of the study with clinical evaluation to assess the degree of improvement.

Eight patients (29.6%) completely recovered, 12 patients (44.5%) showed partial recovery and 7 patients (25.5%) had no clinically measurable improvement from the baseline.

Three patients of those with complete recovery had only sensory signs and symptoms at presentation.

During follow up, 3 patients (9.6%) developed atrophic changes in forms of wasting and ulceration in the leg and toes.

**Discussion**

Post injection sciatic nerve injury can occur in both adults and children. In this study the children are affected more than adult and 77.77% of the patients were under the age of 5 years. In other two studies 77.5% and 86.36% of the patients respectively were under this age\(^\text{13,14}\). Since children have various thickness of the subcutaneous tissue and depth of gluteus musculature, the chance of involvement of sciatic nerve is more than in adults\(^\text{14,15}\) and gluteal intramuscular administration of drugs is presently a common practice among the paediatric age groups. Some health workers have strong preferences for injection and infusions even when oral and other alternative routes are equally good and safe\(^\text{13}\).

Male to female ratio in this study is (1.12:1) and this is in agreement with a study done by Ezeukwu AO. where the ratio was (1.13:1)\(^\text{16}\) but it is lower than another study done by Odyedeji AO et al where the ratio was (1.7:1)\(^\text{13}\).

The majority of the patients 94.45% had sustained their nerve injury by nurses while in a similar study 51.1% of the patients had sustained their nerve injury by unqualified medical practitioners\(^\text{16}\). This is due to inadequate health care facilities and to security condition which prevent the patients in this study from reaching the health centers or hospitals specially during the night. The exact site of injection can not be identified in all patients this is in agreement with other study\(^\text{14}\).

Upper respiratory tract infections, common during cold winter season in our locality, were the main indication for intramuscular injections. Antibiotics were the common offending agents in this study, because of their wide use; while in a study done at Nigeria, malaria was the main indication which is endemic there\(^\text{13}\). Chloroquine, pyrazolone, and procaine penicillin were the main offending agents in study done by Fatunde OJ and Familusi JB\(^\text{17}\) and quinine dihydrochloride\(^\text{14}\).

The onset of the symptoms was immediate in 94.44% which is in agreement with another study done by Maqbool W, et al where the onset was immediate in 90.9% of the patients\(^\text{18}\).

All the cases presented with unilateral lower limb involvement, the left lower limb affected in 63.88%. This is in agreement with another study\(^\text{13}\), while Ezeukwu AO. found that the right lower limb was more affected (in 52.1% of the patients) \(^\text{16}\). The male had higher percentage for either side to be affected and similar to other studies\(^\text{13,16}\). Ndiaye, et al performed sciatic nerve gluteal dissection on 10 fresh adult African cadavers, on both sides. The nerve pathway was 19 times out of 20 in the subpiriformis canal. In all cases the pathway was identical with an oblique and vertical portion running down through the ischio-trochanteric channel. The cutaneous projection of the sciatic nerve was distant from the upper lateral quadrant of the buttock\(^\text{10}\), (Fig. 2).
Figure (2): Anatomy of the sciatic nerve in the gluteal region.

The chief complaint at presentation are foot drop in 52.33%, followed by leg and foot weakness in 30.55%, pain and numbness in 8.3% while in another study done in India the weakness of the foot and leg in 55.7%; foot drop in 33.9% and pain and numbness in 18.8% of the patients. Equinovarus deformity in 5.55% while in other study was 8.7% and fibrotic scar in gluteal region in 2.7% which is similar to that noticed by Singh Khaimar. The loss of sensory nerve action potential of sural nerve and a great vulnerability of the common peroneal nerve to injury by injection as well as by other mechanism has been noticed previously and the prognosis is good in patient with sensory nerve conduction abnormalities alone in the first electrophysiological study after the injection.

In this study 8.33% of the patients have decreased sensory nerve action potential of the sural nerve alone and they completely recovered. Twelve patients (33.33%) had isolated involvement of the common peroneal nerve, 7 patients (19.44%) had involvement of the posterior tibial nerve, and 14 patients (38.88%) had involvement of both common peroneal and posterior tibial nerves equally while Maqbool W, et al found that 1.9% of the patient had decrease amplitude of SNAP in sural nerve, 47.1% had isolated or predominant involvement of the common peroneal nerve, 17.9% had predominant involvement of the posterior tibial nerve and 26.4% of the patients had equal involvement of the common peroneal and posterior tibial nerves.

Treatment of sciatic nerve injury following intramuscular injection is not specific although it is important to maintain joint movement and avoid damage to anaesthetic skin, ankle brace with vitamins may be helpful and there is no real evidence that exploration of the sciatic nerve with or without excision of the damaged portion and grafting is of any use but tendon transfers should be considered when there is permanent residual paralysis 15 months after the onset of the injury.

The outcome of conservative treatment in this study was 29.6% of the patients completely recovered while 70.4% showed partial or no recovery this is in agreement with the results of three studies which showed partial or no recovery in 64%, 72% and 82% of the cases respectively.

During follow up 9.6% of the patients develop wasting of leg and ulceration in the toes, while in other two studies 14 and 32% of the patients respectively had wasting of the leg and ulceration of the toes this may be due to a good care and physiotherapy of the affected limb of patients in this study.

Conclusion
- Children are at higher risk of injury than adults.
- Sciatic nerve injury presents most commonly as foot drop.
- In order to reduce for frequency of this handicapping condition, injection should be prescribed only when mandatory and administered by well qualified and competent personnel.
- The outcome of conservative treatment was poor.

References
Cyclorefraction of healthy children in the first two years of life

Azzam A. Ahmed
Department of Surgery, College of Medicine, University of Mosul.

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ABSTRACT

Objectives: To provide a description of refractive errors in healthy, term-born children, aged 1 through 24 months, and to test the hypothesis that spherical equivalent becomes significantly less hyperopic and less variable with increasing age.

Methods: This is a prospective, cross-sectional design, cycloplegic retinoscopy was used to measure the refractive error in both eyes of 100 healthy, term-born children in four age groups. Spherical equivalent, cylindrical power and axis were analyzed as a function of age.

Results: Spherical equivalents of right and left eyes did not differ at any age. Hyperopia declined significantly with increasing age. The variability in spherical equivalent also decreased significantly with age. Cylindrical error of one diopter or more was found in 15% of children; the proportion with astigmatism was highest in infancy and then waned. Myopia and anisometropia were rare, occurring in 5% and 2% of the sample, respectively.

Conclusions: Significant declines in hyperopia and variability of spherical equivalent appear to be features of emmetropization. The normal prediction limits provide guidelines against which data from individual patients can be compared.
It has been long and widely recognized that infants, on average, are hyperopic, and that the hyperopia gradually decreases during infancy and early childhood. These changes in normal refractive error are presumed to reflect finely regulated eye growth, controlled at least in part by the retina. The involved processes, known collectively as emmetropization, are accompanied by a high prevalence of astigmatism in infants. Despite numerous studies of refractive development, the new millennium has been entered without sufficient specification of refractive development in healthy infants and young children to support quantitative comparison with populations with disease, or to diagnose abnormal refraction in an individual youngster. The aim of this study is to provide a review of cycloplegic retinoscopy in 100 normal children. This data permits specification of normal refractive characteristics, including the limits of normal spherical equivalent for each four age groups ranging from 1 through 24 months.

Methods
This is a prospective study, refractive errors were measured in 100 healthy children aged 1 to 24 months and were categorized into four age groups:
- Group 1: age from 1 month to 6 months.
- Group 2: age from 7 months to 12 months.
- Group 3: age from 13 months to 18 months.
- Group 4: age from 19 months to 24 months.

More than half of them aged 12 months or younger. All subjects were born at term (gestational age ≥ 37 weeks) with Apgar scores of at least 8, had an uncomplicated neonatal course, were free of medical problems, and, by parental report, were experiencing normal development. Of 120 children undergoing refraction, 20 were excluded because of ophthalmic abnormality (cataract [n= 3], disc anomaly [n=2] or incomplete cycloplegia [n=10] as judged by ophthalmologist at the time of measurement and five miscellaneous ophthalmic problems including esotropia). Each child underwent a complete eye examination including cycloplegic retinoscopy.

Retinoscopy was performed in a dim room by streak Combi Keeler retinoscope with same hand (to avoid interexaminer variability), at least 45 minutes after instillation of 0.5% of cyclopentolate for those children below 12 months of age and 1% of cyclopentolate for those children above 12 months of age. For each eye of the 100 children, spherical equivalent, power and axis of cylinder were recorded.

Astigmatism was defined as 1.0 diopter (D) or more of cylinder. Axis of cylinder was categorized as with-the-rule (minus cylinder axis at 180° ± 15°), Against –the-rule (minus cylinder axis at 90° ± 15°) or oblique (all else). Myopia was defined as spherical equivalent of at least 0.5 D. Anisometropia was defined as difference of at least 1.0 D between eyes in spherical equivalent or cylinder.

Results
Astigmatism was found in 25% (25 cases) of 100. High cylindrical errors were uncommon; only 3% (3 cases) had 2 diopters (D) or more of cylinder. Hyperopia represents 68% (68 cases), myopia represents 5% (5 cases) and anisometropia represents only 2% (2 cases) of the sample (table 2, figure 1).
rule astigmatism was more common (56%) than with-the-rule-astigmatism (29%) or oblique (15%) astigmatism. There was a significant decline in hyperopia with age ($P \leq 0.01$), being higher for children in group 2 (7-12 months) representing 40% (40 cases) then decline to 20% (20 cases) in group 3 (13-18 months) and 15% (15 cases) in group 4 (19-24 months), (figure 3). Distributions of spherical equivalent appeared broader at younger age groups (table 1).

Table (1): Spherical equivalent (diopters) and prediction limits*

<table>
<thead>
<tr>
<th>Age, Months</th>
<th>Spherical Equivalent, Mean (SD), D</th>
<th>95% Prediction Limits, D</th>
<th>99% Prediction Limits, D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>1</td>
<td>2.20(1.60)</td>
<td>5.51</td>
<td>-1.12</td>
</tr>
<tr>
<td>1.5</td>
<td>2.08(1.12)</td>
<td>4.36</td>
<td>-0.20</td>
</tr>
<tr>
<td>2.5</td>
<td>2.44(1.32)</td>
<td>5.13</td>
<td>-0.26</td>
</tr>
<tr>
<td>4</td>
<td>2.03(1.65)</td>
<td>5.21</td>
<td>-1.16</td>
</tr>
<tr>
<td>6</td>
<td>1.79(1.27)</td>
<td>4.39</td>
<td>-0.81</td>
</tr>
<tr>
<td>9</td>
<td>1.32(1.13)</td>
<td>3.63</td>
<td>-0.99</td>
</tr>
<tr>
<td>12</td>
<td>1.57(0.78)</td>
<td>3.16</td>
<td>-0.01</td>
</tr>
<tr>
<td>18</td>
<td>1.23(0.91)</td>
<td>3.09</td>
<td>-0.64</td>
</tr>
<tr>
<td>24</td>
<td>1.19(0.83)</td>
<td>2.89</td>
<td>-0.50</td>
</tr>
</tbody>
</table>

* D indicates diopters.

Table (2): Distribution of refractive errors among the age groups.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Group 1 (1-6) months</th>
<th>Group 2 (7-12) months</th>
<th>Group 3 (13-18) months</th>
<th>Group 4 (19-24) months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperopia</td>
<td>17</td>
<td>27</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Astigmatism</td>
<td>3</td>
<td>12</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Myopia</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anisometropia</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure (1): Percentage of refractive errors in 100 healthy full-term born children from 1-24 months.
Discussion
The mean spherical equivalents analyzed herein and those reported in other studies, there are no conspicuous discrepancies between the results for healthy children and those with presumptively normal eyes, compared with previous studies\(^6\)\(^-\)\(^10\). We sampled more ages during the first year, when the rate of change appears to be rapid \(^7\). Besides gradually decreasing hyperopia, we found another feature of normal development: the significant decrease in variability of the spherical equivalent. Furthermore, as development proceeds, there is significant variation in cylindrical power that in healthy infants is determined mainly at the cornea. The application of these data pertains to diagnosis in the individual child. Data from the 100 healthy children provide a definition of the limits of normal spherical equivalent in infants and young children. The results from an individual child can be specified as within, or outside, these limits (table 1). The broad prediction interval during infancy may bear consideration when planning screening programs that depend on refraction. Given the broad prediction intervals during infancy, screening refractions at 12 months of age or older may be more efficient. Nevertheless, detection of high cylindrical errors and
anisometropia can contribute to the diagnosis of amblyogenic factors in infancy (10).

**Conclusion**
This data further define the characteristics of refractive errors in the healthy, developing eye, and so specify limits of normal refractive error at 1 through 24 months of age.

**References**
The value of step-sectioning in the diagnosis of lymph node micrometastasis in breast cancer

Rana A. Azooz
Department of Pathology, College of Medicine, University of Mosul.

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ABSTRACT

Objectives: Routine practice is to examine one histological section for evaluating secondary tumor deposits in the axillary lymph nodes in patients with breast cancer. The aim of the present study is to evaluate whether multiple levels of histological section detect significantly more metastatic deposits in axillary lymph nodes from breast cancer resection specimen than the standard practice of examining one section.

Methods: A retrospective study of 30 patients with node negative breast cancer was performed whose specimens were received at AL-Jamhuri Teaching Hospital and private laboratories in Mosul city between the years 2008 and 2010. The original slides and the paraffin wax blocks containing the lymph nodes were retrieved from the archive, and 4 extra levels (separated by 30 µm) were cut from each block and were stained with H & E stain.

Results: Of the 30 cases with node negative breast carcinoma, 4 (13.3%) were found to contain extra tumor deposits at deeper levels that were not detected at the original sections.

Conclusion: Multiple levels of histological sections separated by relatively small intervals detect more tumor deposits in the axillary lymph nodes than the current practice of examining a single section.

The status of the axillary lymph nodes remains one of the most important prognostic factors in patients with invasive breast cancer (1-3). Survival rate, disease-free
interval, and treatment failure, are largely influenced by the pathological staging of these lymph nodes\(^2,4\).

Two pathological staging systems are commonly used, the TNM staging and the anatomical stage\(^6\). It is well known that lymph nodes can be classified as positive or negative on the basis of the presence or absence of metastatic carcinoma, which in most cases, is a straightforward diagnosis. However, in some cases there are only clusters of tumor cells and a diagnosis of metastasis is not easily established\(^1\).

It is also known that different pathologists in different laboratories follow different protocols for the processing and examination of lymph node specimens\(^6\). The recommendations published by the Association of Directors of Anatomic and Surgical Pathology for the processing and reporting of lymph node biopsies being evaluated for metastatic carcinoma include the submission of the entire node for microscopical examination, slicing it into 3-4 mm slices and microscopical examination of several levels of each slice, stained with hematoxylin and eosin (H & E) only\(^6,7\). However, for lymph node dissection specimens being studied for metastatic carcinoma, the Association recommended submission of every node for microscopical examination with one H & E slide per cassette\(^6,7\).

It has long been recognized that the microscopical examination of a single H & E stained section fails to identify all metastatic foci in a lymph node, and some investigators have proposed serial subgrossing and histologic step-sectioning of each node which has been shown to up stage nodal status in a significant number of patients\(^1\).

This has also been recommended by the updated protocol for the examination of specimens with invasive breast cancer\(^5\). Additional recent techniques such as immunohistochemistry and reverse transcriptase-polymerase chain reaction have been used to detect isolated tumor cells or micrometastases\(^1,5-7\). However, the prognostic importance of these metastases remains unknown\(^1,3,5\). In addition, such detailed histopathologic evaluation of the entire axilla is prohibitively expensive\(^1\).

The aim of this retrospective study was to evaluate whether multiple levels of histological sections detect significantly more metastatic deposits in axillary lymph nodes from breast cancer resection specimens than the standard practice of examining one section.

**Patients and methods**

In order to achieve the aim of the present study, a retrospective study design was adopted. Thirty women with clinical stage Tis – T\(_3\) No Mo breast cancer who underwent surgical resection with axillary clearance that were received at the histopathological laboratory of AL-Jamhuri Teaching Hospital in Mosul city, and from private laboratories of the same city, between the years 2008 and 2010 were included in the study.

After obtaining approval from Nineveh Health authorities, the original slides and paraffin wax blocks containing the lymph nodes were retrieved from the archive and reviewed at the Department of Pathology - College of Medicine, where an extra four levels (separated by 30 µm) were cut from each paraffin wax block containing lymph nodes and stained with H & E only. These multiple levels were labeled as A, B, C, and D, respectively. All those four levels were examined and carefully searched for the presence of tumor metastasis that was not detected in the original H & E sections. Any tumor deposit visible at any extra level was counted as metastasis. The number of examined lymph nodes and the histological pattern of the primary tumors were also recorded.

Basic statistical methods were used to calculate means, numbers and percentages.

**Results**

The mean age of the study sample was 51.4 year. Review of the slides from 30 females with breast carcinoma was done, according to pTNM staging; they were segregated into 4 groups: 3 were Tis N\(_0\) M\(_0\), 11 were T\(_1\) N\(_0\) M\(_0\), 13 were T\(_2\) N\(_0\) M\(_0\) and 3 were T\(_3\) N\(_0\) M\(_0\).

The total number of examined lymph nodes was 148 with a mean of 5 lymph nodes for each case. A total of 53 paraffin wax blocks...
containing lymph nodes were cut resulting in a total of 212 sections with a mean of 7 sections for each case and 1.4 sections for each lymph node.

Extra tumor deposits were detected in 4 (13.3%) of the 30 cases, 2 of them were detected at the 4th level (level D), and their corresponding initial pathological stage was T1N0M0 and T3N0M0. In the third case the tumor deposit was detected at the 2nd level (level B) (T2N0M0). In all these cases the primary tumor type was invasive ductal carcinoma (figure 1and 2). While in the last case, the tumor deposit was detected at the 3rd level (level C) (T1N0M0) and the tumor type was invasive lobular carcinoma.

**Discussion**

Detailed pathological examination of axillary lymph nodes with serial sectioning and H & E examination has resulted in an increased rate of detection of tumor deposits for breast cancer patients (1,5,9).

The present study included 30 patients with node negative breast cancer, as detected by current practice of using single histological section. Of these, four (13.3%) patients are found to have new tumor deposits in the lymph nodes after subjecting them to serial sectioning and H & E examination of multiple levels which correlates with previous published data that found unequivocal lymph node metastases in four (12%) of 33 patients with node negative invasive breast cancer on additional H & E sections (8). However in that study the addition of cytokeratin cocktail fails to show metastases over that detected by H&E apart from single strongly keratin positive sinus-based cell in one lymph node with unclear nuclear nature (benign vs malignant) (8).

Previous publications from groups such as The International Breast Cancer Study Group had reported that 9% of axillary lymph nodes which are judged to be negative on one routine histological section were subsequently found to contain metastases if they were subjected to levelling (9). Based on this publication, step-sectioning had been also applied on lymph nodes extracted from 100 colorectal cancer resection specimens in a previous study in whom 11 cases were found to have extra tumor deposits in level 2 and 3 that would have gone undetected when only one level had been examined. This resulted in upstaging of only one patient from N1 to N2 (pTNM). Despite this, the authors justified the small increase in the workload pressure at pathology laboratories to increase the accuracy in staging (10).

In spite of the excellent prognosis for patients with node negative breast cancer, recurrence or distant metastasis within 10 years was encountered in 25% to 30% (1,8). This was attributed in part to undetected occult metastases in the lymph nodes which are not observed during the initial routine.
histopathological evaluation but become apparent at deeper levels of routine histologic sections or on immunohistochemical analysis \(^{(1)}\).

The identification rate of micrometastases in sentinel lymph node biopsy depends on the number of sections evaluated from each paraffin block and the interval between each section\(^{(1)}\). There is no question that the examination of more levels at smaller intervals will increase the detection rate of micrometastases\(^{(1)}\). However, according to the recommendations of the Association of Directors of Anatomic and Surgical Pathology, it is not currently clear that how many sections (and from what levels of the block) are optimal \(^{(6,7)}\). It is also unclear whether immunohistochemical stains add clinically relevant information and are not routinely advocated in any consensus recommendations\(^{(1,6,7)}\). They are particularly helpful for detecting tumor cells which do not form cohesive clusters in lymph nodes such as metastatic invasive lobular carcinoma\(^{(1)}\) but the results should be interpreted in the context of H & E stain\(^{(1,6,7)}\).

A large retrospective study done by the International Breast Cancer Study Group that reassessed the axillary nodal status (negative for metastases by routine histology) of 921 patients with serial sectioning and H & E examination showed that the presence of micrometastases was associated with an increased chance of relapse when compared with patients without micrometastases on reassessment \(^{(1)}\). Thus far, processing of lymph nodes in serial sections and careful analysis of the H & E stained sections results in good accuracy and greater practicality in the identification of metastasis \(^{(4)}\).

The pathological stage of the axillary nodes remains a major prognostic determinant in patient with operable breast cancer \(^{(1-4,11,12)}\) and is crucial in the decision on the need and type of adjuvant therapy \(^{(2,11,12)}\). Guidelines and recommendations proposed at the 8th International Conference on Adjuvant Therapy of Primary Breast Cancer emphasized a substantial difference for treatment guidelines between patients with node negative breast cancer and patients with node positive disease \(^{(11,12)}\). In general, patients without nodal involvement or with minimal involvement received significantly less chemotherapy and less anthracycline-containing chemotherapy in particular, when compared with patients with node positive breast cancer \(^{(11,12)}\). Furthermore, patients with hormone responsive node negative disease were prescribed significantly less chemotherapy within the adjuvant program \(^{(11-13)}\).

In conclusion, multiple levels of histological sections separated by relatively small intervals detect hidden tumor deposits in the axillary nodes that were otherwise missed by the routine practice of evaluation of a single section and will increase the detection rate of lymph node micrometastases despite the slightly increased workload at pathology laboratories. This may influence the stage, choice of adjuvant therapy and prognosis of node negative operable breast cancer.

References


Evaluation of causes of acute hip pain in children at Erbil teaching hospital

Zohair M. Al-Saffar *, Sherwan J. Shareef **
* Department of Surgery, College of Medicine, Hawler Medical University; ** Department of Surgery, Erbil Teaching Hospital, Erbil.

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ABSTRACT

Objectives: Painful hip in children is a common pediatric problem and there are several causes for hip pain. In this study we will show the most common causes of hip pain in children and evaluate the procedures performed at our hospital, with a view of establishing which parameters most relevant to make a clinical decision.

Methods: This is a case review study which was carried out at Erbil Teaching Hospital between December 2006 and October 2007. Sixty two patients who had consultations and/or been admitted at our hospital complaining of hip pain were included in this study. We did investigations for them and we reviewed their clinical, laboratory and imaging documents.

Results: The differential diagnosis was transient synovitis (TS) 54.8%, Legg-Calves-Perthes disease 24.2%, septic arthritis (SA) 8.1%, traumatic synovitis 4.8%, avulsion fractures 3.2 %, brucellosis 1.6%, tuberculosis 1.6%, and non specific synovitis 1.6%. We found that three or more criteria, (the clinical and laboratory parameters), were present in all cases of septic arthritis (100% sensitivity), but also were present in 10% of non septic conditions (90% specificity). Radiographs showed abnormalities in 50% of the cases. Ultrasound showed joint effusion in 43 patients with 100% of sensitivity.

Conclusions: Transient synovitis is the most common cause of irritable hip. Application of our scheme might result in a reduction of the number of patients who need hospital admission and also reduction of invasive procedures and containment of the cost.

Keywords: Diagnosis of painful hip.
The causes of painful hip may include numerous disorders\(^{(1)}\). Some of these conditions are benign and self-limiting disorder, like transient synovitis\(^{(2,3)}\). On the other hand, septic arthritis is a medical emergency that needs rapid confirmation and treatment and usually hospital admission\(^{(3,4)}\). Because early treatment is the single most important prognostic factor for a favorable outcome; therefore, a fast and reliable investigation scheme for irritable hips is mandatory; which, for feasibility, should be based on simple, affordable, and non-invasive medical techniques.

Various strategies for the work-up of children with hip pain have been proposed, some of them were based on clinical signs and laboratory parameters \(^{(3,4)}\) while others relied more on medical imaging and aspiration of the hip joint \(^{(2-9)}\). Imaging was usually performed with ultrasound and radiography. Other modalities, such as bone scintigraphy, computed tomography, and magnetic resonance imaging, although were used by some authors \(^{(10)}\), were not routinely used for primary evaluation of painful hips \(^{(5,6,11)}\).

In this study we depended mainly on clinical signs and symptoms, simple laboratory tests, radiography and ultrasound, aiming to identify the most common causes of hip pain in patients who presented to our hospital; and to evaluate the procedures which are performed at our hospital with a view to establish which parameters are most relevant to make a clinical decision.

**Material and methods**

Sixty two patients were included in this study; their ages ranged 2-13 year. For each patient we recorded the duration of signs and symptoms, limping or inability to walk, hip or knee pain (referred pain) and temperature. All patients had a standard anterior-posterior radiograph of the pelvis, with an additional lateral or oblique view of the affected hip, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white cell count and hemoglobin concentration. Brucella agglutination test and latex slide agglutination test were done for some patients according to their presentation.

Ultrasound was performed for patients with negative, or positive but not conclusive, radiological findings regardless of the results of laboratory tests of the patient (i.e. ultrasonography was not done for patients with positive x-ray findings because of the conclusive diagnosis reached.

Patients in whom septic arthritis of the hip was suspected, based on their clinical presentation and laboratory findings, and who had effusion shown on ultrasound, were subjected to arthrotomy (surgical drainage) performed under general anesthesia. A sample of joint aspirate was taken for bacteriological study. The appearance of the aspirate and the results of the bacteriological tests were recorded.

After performing all investigations, radiographs and follow up, the diagnosis was not certain in two patients, so we proceeded for synovial biopsy. The histopathological results of the biopsies were recorded.

Diagnosis was made if the following criteria were met:

- Septic arthritis: presence of signs and symptoms (fever, joint swelling, tenderness on local examination and limited movement), positive laboratory tests (high erythrocyte sedimentation rate (E.S.R.), positive C-reactive protein (CRP), leucocytosis, and low hemoglobin concentration), joint effusion on ultrasound, and pus was drained from the
hip joint, a sample of joint aspiration was taken for bacteriological study. The appearance of aspirate and the result of the bacteriological tests were recorded.

- Perthes disease: the radiographs showing characteristic features and evolution of sclerosis, subchondral fracture, fragmentation, and flattening of the femoral head during the follow up.
- Transient synovitis is certain when joint effusion demonstrated by ultrasound with normal laboratory tests, no bony changes on x-ray and uneventful recovery during follow up.
- Trauma: is put as a diagnosis when the patient had a history of recent trauma and laboratory tests were normal with effusion which is shown by ultrasound or positive radiography.
- Other causes: this group included patients with data not fit with criteria of diseases mentioned above, so further blood tests and synovial biopsy were done.

The following conditions were excluded: Major trauma with fractures of the proximal femur and the hip, referred pain from the spine, femoral head and neck deformity, already diagnosed or old cases, and developmental dysplasia of the hip.

Fisher's exact test was used in statistical analysis. Fisher's exact test a statistical significance test used in the analysis of contingency tables where sample sizes are small. P value significant when p<0.001.

**Results**

The age of patients ranged from 2-13 year, with an average of 5.5 year. There was no patient with bilateral hip involvement.

The causes of hip pain are shown in (table 1) which shows high percentage of hip pain in children caused by transient synovitis in 34 patients (54.8%). Fever was present in 12 patients (table 1). The average temperature was (38.2 C). The degree of the fever was mild in cases of TS, tuberculosis and brucellosis with a range of (37.6 C) to (37.9 C), and it was moderate to high fever in cases of SA with a range of (38.6 C) to (39.3 C). Fever was present in all patients with septic arthritis (p<0.001 by Fisher's exact test). WBC count ≥ 10 x 10^9/L was found in 9 patients (14.5%), five patients had transient synovitis and four patients had septic arthritis. The average was 7.5 x 10^9/L. ESR ≥ 10 mm/hr found in 36 patients (table 1), the average was 20.2 mm/hr. ESR >10 mm/hr found in 54% of the cases so, it is not significant parameter (p=.068 by Fisher's exact test). CRP was positive in 11.3% of cases (table 2), it is an acute phase reactant protein, and it is a good tool to differentiate septic from non septic cases (P value is missed because n is small). Brucella agglutination test was positive in one case with a titer of 1/320. Anemia was found in three children only, one with tuberculosis and the other two with transient synovitis.

Table (1): Differential diagnosis of hip pain and prevalence of fever and ESR level more than 10 mm/hr.

<table>
<thead>
<tr>
<th>Diagnosis of hip pain</th>
<th>Prevalence of fever</th>
<th>E.S.R. Level more than10 mm/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Transient synovitis</td>
<td>34(54.8%)</td>
<td>29(85.3%)</td>
</tr>
<tr>
<td>Perthes disease</td>
<td>15(24.2%)</td>
<td>0(100%)</td>
</tr>
<tr>
<td>Septic arthritis</td>
<td>5(8.1%)</td>
<td>0(100%)</td>
</tr>
<tr>
<td>Traumatic synovitis</td>
<td>3(4.8%)</td>
<td>3(100%)</td>
</tr>
<tr>
<td>Avulsion injuries</td>
<td>2(3.2%)</td>
<td>2(100%)</td>
</tr>
<tr>
<td>brucellosis</td>
<td>1(1.6%)</td>
<td>0(100%)</td>
</tr>
<tr>
<td>tuberculosis</td>
<td>1(1.6%)</td>
<td>0(100%)</td>
</tr>
<tr>
<td>Non specific synovitis</td>
<td>1(1.6%)</td>
<td>1(100%)</td>
</tr>
<tr>
<td>Total</td>
<td>62(100%)</td>
<td>62(100%)</td>
</tr>
</tbody>
</table>
Table (2): Cases with positive C-reactive protein and Radiological findings.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Case with positive C-reactive protein</th>
<th>X-ray findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Transient synovitis</td>
<td>32(94.1%)</td>
<td>2(5.8%)</td>
</tr>
<tr>
<td>Perthes disease</td>
<td>15(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Septic arthritis</td>
<td>0</td>
<td>5(100%)</td>
</tr>
<tr>
<td>Traumatic synovitis</td>
<td>3(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Avulsion injuries</td>
<td>2(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>1(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Non specific synovitis</td>
<td>1(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>55(88.7%)</td>
<td>7(11.3%)</td>
</tr>
</tbody>
</table>

Radiographs were abnormal in 31 patients in the form of widening of the joint space (indirect evidence of the joint effusion), bony changes or both of them. The bony changes were one or more features of sclerosis, fragmentation, subchondral fractures and flattening of the femoral head, irregular bony contour with rarefaction and subchondral cystic changes. In 31 patients the radiographs were normal (table 2).

Total number of patients who had an ultrasound was 44. Joint effusion was found in all cases except in one patient. History of recent trauma was present in five cases (8.1%), while six more patients (9.6%) gave the history of trauma within the last few months before the time of the presentation. Arthrotomy was done in seven patients (11.3%). In two cases the synovial fluid was non purulent and synovial biopsy was performed. The histopathological study showed tuberculosis in one case and non specific synovitis in the other. Purulent Synovial fluid was found in 5 cases. The result of culture was positive in all except in one patient who received antibiotics preoperatively (table 3). We had fortunately no complications from this procedure.

Table (3): Synovial fluid culture.

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>H. influenza</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

In patients who had Legg-Calve-Perthes disease, female to male ratio was 1:4 and this ratio was near to other studies (12-14).

Most of the patients were suffering transient synovitis (54.8% of the cases), and this was in agreement with other studies (13-15). In our study (14.7%) of the cases had mild fever, elevated ESR (41.9%) and high WBC count; while widening of the joint space on radiographs was found in 6 patients (17.6%). These are in agreement with some studies (3,15-19), and disagreement with others (14).

Legg-Calve-Perthes disease was present in 24% of the cases; this is higher than in other studies which showed the prevalence of Perthes disease as 4% of hip pain with a range of 1.5–5/10 000 (1), and one in 10,000 (15). There was no patient with bilateral Perthes disease which might be seen in approximately 12% of cases (13). Probably because of short duration of our study since bilateral perthes disease is usually at different stage of the disease.
In septic arthritis, the average age of patients was 3.8 years like in other studies (19) (because children in this age are active and they frequently get trauma) which state that overall average age of patients with septic arthritis is 3-6 years. Elevated CRP and ESR were found in all cases (100%), other studies showed elevated CRP and ESR in at least 95% of cases (20). The anterior approach was applied for joint drainage which is the procedure of choice in children (12,21).

Traumatic synovitis was seen in three patients in whom the hip pain was related to a recent accident or trauma, and other clinical and laboratory parameters were normal, except hip effusion on U/S; as in other studies (22).

Avulsion fracture injuries of hip adductors were found in two cases that had history of trauma, one of them also had ecchymosis. X-ray findings were seen immediately in one but, after ten days in the other. like other studies (16).

Brucellosis was found in one patient who had hip pain with positive brucella agglutination test. Brucella agglutination test is significant when there is a titer of 1/320 or four fold rising of the titer (23).

Tuberculosis should be considered in the differential diagnosis of arthritis and chronic osteomyelitis specially in patients from endemic area (12). We found a case of tuberculosis and fortunately, we confirmed the diagnosis by synovial biopsy, which is helpful in establishing the diagnosis (12,22).

Non specific synovitis was found in one patient who had atypical clinical and laboratory characteristics and the result of the biopsy showed non specific inflammatory cells. WBC count was more than 10 x 10⁹/1 in five cases with transient synovitis and in four patients with septic arthritis (p=0.002). These results are in agreement with other studies (2, 8). We found that three or more criteria were present in all cases of septic arthritis (100% sensitivity), but these criteria (pain, fever, elevated W.B.C., elevated E.S.R., elevated CRP) were found also in 10% of non septic conditions (90% specificity). This agreed with other studies which identified four important, independent, diagnostic variables: fever, non-weight bearing on the affected limb, ESR 40mm/hr and WBC count of ≥12 x 10⁹/1, when present together, it is 99.6% sensitive for diagnosis of septic arthritis of the hip, and 93% for those with 3 factors (12,17). Also it agreed with investigation schemes based on clinical examination and laboratory tests (ESR and complete blood count) which identified patients with a septic arthritis of the hip with a sensitivity of up to 97% but had a relatively low specificity which resulted in a large number of hospital admissions (2,6,8). Anemia was present in three patients, one patient with tuberculosis and the other two patients had non specific or nutritional anemia.

According to our results, radiographs showed changes in 50% of the patients and these changes were not specific in all conditions; therefore, radiography was not a significant parameter. Our study agrees with other reportes (24). An exception to this, radiographs were highly significant in cases of Legg-Calve Perthes disease who had long-lasting symptoms (more than two to three weeks) and in cases of trauma. This agreed with other studies (24).

Ultrasound detected hip joint effusion in 43 cases with a sensitivity of 100%, like other authors who had 95% to 100% sensitivity (7). Other studies reported lower results (9,11). Ultrasound only detected the presence of effusion without prediction of the nature of the effusion whether it is blood or fluid, but it could show whether the fluid contains debris or not and also the presence of cartilage thickening but actually it needs experience. Ultrasound guided joint aspiration had been done in some studies (17,18), but, unfortunately, we could not perform this procedure in our study, because our patients do not accept to be operated twice (one operation for joint aspiration and a second operation for drainage), and because the ultrasound guided joint aspiration is invasive and can occasionally cause complications, so we do not support routine aspiration in patients with hip joint effusion as proposed by some authors (7,22).

Synovial fluid culture was done for five patients, in three cases (60%) the result of
culture and sensitivity was S. aureus, in one case was H. influenza and it was negative in one case who received preoperative antibiotics. This result is identical to other authors (14,20).

In conclusion, transient synovitis is the most common cause of painful hip, and the patient may have mild fever and elevation of WBC count and ESR. There was high incidence of Legg-Calve-Perthes disease. Hip ultra sound, temperature, E.S.R. & CRP were the most relevant parameters for diagnosing and differentiating possible septic and non-septic effusions of the hip. Avoid unnecessary and expensive investigations for large number of population with simple and benign disorder.

Finally, application of our scheme might result in a reduction of number of patients who need joint aspiration, hospital admission, and imaging, and also reduction of invasive procedures and containment of the cost.

References
Effects of quetiapine on body mass index and reproductive hormonal levels in male schizophrenic patients

Imad A. Thanoon, Shamil H. Othman, Omar M. Y. Shindalla
Department of Pharmacology, College of Medicine, University of Mosul.

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ABSTRACT

Objective: To assess the effects of quetiapine therapy on body mass index (BMI) and serum level of testosterone, prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) in newly diagnosed male schizophrenic patients in comparison to healthy controls.

Patients and methods: Thirty male patients with schizophrenia were included in this study. The diagnosis of schizophrenia was made according to DSM-IV criteria of the American Psychiatric Association (APA). Another thirty apparently healthy male individuals were included in the study as a control group. Blood samples were taken initially from patients and controls and assay of serum testosterone, prolactin, FSH, LH, and E2 were done using enzyme–linked fluorescent immunoassay (ELFA) technique by vidas instrument. Later, after 6 months of quetiapine therapy, other blood samples were taken and assay of the same parameters were done. Calculation of BMI was done for the patients and controls using special equation.

Results: There was an insignificant difference in the mean BMI, serum testosterone, prolactin, FSH, LH, and E2 levels between patients in the pre-therapy stage group and in both the post-therapy stage group and controls.

Conclusion: Chronic quetiapine therapy might be regarded as a safe drug with regard to effects on BMI and serum levels of reproductive hormones in newly diagnosed male schizophrenic patients.

Keywords: Schizophrenia, quetiapine, BMI, testosterone, prolactin, FSH, LH, E2.
Q uetiapine (atypical antipsychotic drug) is a novel dibenzo-thiazepine antipsychotic drug developed by Zeneca Pharmaceuticals in 1985. It is marketed under the trade name Seroquel (1). It was approved in September 1997 by the Food and Drug Administration (FDA) and has since been introduced in most western European countries as well as in 70 other countries worldwide for the treatment of schizophrenia and related psychotic illnesses (2). The use of atypical or second generation antipsychotic drugs is gaining popularity as a result of their tolerability and efficacy (3). However, the use of these drugs has been associated with metabolic and endocrinal adverse effects beside their effects on body weight (4,5).

According to our knowledge, no study was done previously evaluating the effect of quetiapine on BMI, testosterone, prolactin, FSH, LH, and E2, so the present study was conducted to assess the effect of quetiapine therapy on such parameters in newly diagnosed male schizophrenic patients.

Patients and methods

Out of the 37 selected newly diagnosed patients with schizophrenia, only 30 patients completed the study. Patients were referred from private psychiatric clinics. The criteria of selection included: newly diagnosed male patients, above the age of 20 years (mean ± SD 33.70±6.17 years, ranged between 21 and 41 years), cooperative relatives, no other diseases (cardiovascular, hepatic, renal or endocrinal). Diagnosis of schizophrenia was made according to DSM-IV criteria of American Psychiatric Association (APA). Another thirty healthy male individuals above the age of 20 years (mean ± SD 32.62± 5.06 years, ranged between 21 and 42 years) participated in the study as a control group.

Initially, blood samples were taken from patients and controls and assay of serum levels of testosterone, prolactin, FSH, LH, and E2 were done using enzyme-linked fluorescent assay (ELFA by VIDAS instrument using commercial kits for each hormone from bioMerieux - Lyon-France). Patients then put on quetiapine (Seroquel) in a daily dose ranged between 200 mg and 400 mg for 6 months. By the end of the suggested period of the study, another blood samples were taken and assay of the same parameters was done. BMI was calculated for both patients and controls using the following equation:

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (m)}^2}$$ (6)

Statistical methods

Standard statistical methods were used to determine the mean and standard deviation (SD). Unpaired t-test was used to compare the results of measured parameters of patients in the (pre and post-therapy stages) and controls. Paired t-test was used to compare the results of measured parameters in patients in the pre- and post- therapy stages. P- values equal or less than 0.05 were considered significant (7).

Results

There were insignificant differences in the mean values of BMI, serum levels of testosterone, prolactin, FSH, LH, and E2 in patients in the pre- and post- therapy stages and the controls (Table 1).

There were insignificant differences in the mean values of BMI, serum levels of testosterone, prolactin, FSH, LH, and E2 in patients in the pre- and post- therapy stage groups (Table 2).
Table (1): Comparison of BMI and serum sex hormones levels between patients and control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>Controls</th>
<th>Patients Pre-therapy</th>
<th>Patients post-therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>25.01 ± 2.28</td>
<td>23.91 ± 2.27**</td>
<td>24.37 ± 2.31**</td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>5.61 ± 1.25</td>
<td>5.72 ± 1.47**</td>
<td>6.05 ± 1.16**</td>
<td></td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>10.97 ± 2.57</td>
<td>11.89 ± 6.13**</td>
<td>12.31 ± 2.94**</td>
<td></td>
</tr>
<tr>
<td>FSH (m IU/ml)</td>
<td>3.69 ± 0.93</td>
<td>4.18 ± 2.54**</td>
<td>3.85 ± 1.50**</td>
<td></td>
</tr>
<tr>
<td>LH (m IU/ml)</td>
<td>3.52 ± 1.93</td>
<td>3.53 ± 1.65**</td>
<td>3.78 ± 1.70**</td>
<td></td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>27.30 ± 6.19</td>
<td>28.57 ± 6.86**</td>
<td>27.88 ± 6.50**</td>
<td></td>
</tr>
</tbody>
</table>

**Non significant difference from control at p<0.05.

Table (2): Comparison of BMI and serum sex hormones levels between patients before and after quetiapine therapy.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>23.91 ± 2.27</td>
<td>Patients pre-therapy</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>5.72 ± 1.47</td>
<td>6.05 ± 1.16</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>11.89 ± 6.13</td>
<td>12.31 ± 2.94</td>
</tr>
<tr>
<td>FSH (m IU/ml)</td>
<td>4.18 ± 2.54</td>
<td>3.85 ± 1.50</td>
</tr>
<tr>
<td>LH (m IU/ml)</td>
<td>3.53 ± 1.65</td>
<td>3.78 ± 1.70</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>28.57 ± 6.86</td>
<td>27.88 ± 6.50</td>
</tr>
</tbody>
</table>

* Non significant difference at p< 0.05.

**Discussion**

The present study demonstrated that schizophrenic patients have normal BMI and that the administration of quetiapine for 6 months to schizophrenic male patients resulted in a non significant effects on BMI and serum levels of testosterone, prolactin, FSH, LH, and E2.

Emsley et al \(^8\), in a randomized control trial, studied the effects of quetiapine and haloperidol on BMI and glycemic control. They reported that switching treatment from a conventional antipsychotic to quetiapine is not associated with weight gain or worsening of glycemic control even in the long term therapy which is also in agreement of this study. Furthermore, Gorobets \(^5\) studied the weight gain in patients with schizophrenia and schizoaffective disorders induced by the long term treatment with atypical antipsychotic drugs. He concluded that long term therapy with olanzepine, clozapine and risperidone exert a more marked influence on the body mass as compared to quetiapine and amisulpiride regardless of the patient sex, which is consistent with our findings.

Regarding serum levels of reproductive hormones and in agreement with our findings, Kaneda and Ghmoor \(^9\), evaluated the effect of quetiapine administration for a mean duration of 104.7 days on hypothalamic-pituitary-gonadal axis (HPG) hormones in patients with chronic schizophrenia and reported that quetiapine might not affect prolactin and HPG axis hormones at least in chronic schizophrenic patients with normal levels of HPG axis hormones.

On the same line but with other atypical antipsychotic drugs, Konarzewska et al \(^10\) studied the effects of risperidone and olanzapine on reproductive hormones, psychopathology, and sexual functioning in male patients with schizophrenia. They
reported that the mean serum prolactin level was markedly higher in patients taking risperidone, whereas FSH levels were lower than in patients receiving olanzapine, and the mean levels of LH, testosterone, and estradiol were within normal reference ranges.

**Conclusion**

Chronic quetiapine therapy might be regarded safe, with regard to effects on BMI and serum levels of reproductive hormones in newly diagnosed male patients with schizophrenia.

**Acknowledgment**

We wish to express our thanks to Dr. Mahfoudh S. Al-Noaimy (psychiatrist) for his help and support.

**References**

5. Gorobets LN. Weight gain in patients with schizophrenia and schizoaffective disorder induced by the long-term treatment with atypical antipsychotics. Zh Nevrol Psikhiatr Im SS Krasakova 2008; 108 (9): 52-56.
Body mass index and some biochemical parameters among valproate treated male epileptic patients

Othman A. Pachachi*, Imad A. Thanoon**

* Department of Clinical Pharmacy, College of Pharmacy, **Department of Pharmacology, College of Medicine, University of Mosul.

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ABSTRACT

Objective: To assess the effect of continuous valproate monotherapy (VPA) on body mass index (BMI), serum leptin, malondialdehyde (MDA) and lipid profile in male epileptic children and adult patients in comparison to healthy male controls.

Subjects, materials and methods: A case-control study design was adopted. Samples from 44 male patients (22 less than 18 years old, and 22 over than 18 years old), with primary generalized epilepsy, on continuous VPA monotherapy, for at least six months before participation in the study, were collected over the period from October 2009 to March 2010. Forty-four apparently healthy male volunteers (22 less than 18 years old, and 22 over than 18 years old) without previous history of epilepsy were recruited as controls. Serum levels of leptin, and lipid profile indices were estimated using commercially available kits and a manually prepared reagent for MDA assay. (BMI) was calculated as weight in kilograms divided by the squared height in meters.

Results: The results revealed that epileptic children and epileptic adults receiving continuous VPA monotherapy had a significantly higher BMI (p<0.001), serum leptin, serum MDA and atherogenic index (AI) and a significantly lower (p<0.001) serum high density lipoprotein cholesterol (HDL-c) as compared to their matched control subjects. The results also revealed insignificant difference in serum total cholesterol (TC), triglycerides (TGs) and low density lipoprotein cholesterol (LDL-c) between epileptic children and epileptic adults on VPA and their matched control subjects. Serum leptin was positively correlated with body mass index standard deviation score (BMI SDS) of epileptic children (r=0.542; P<0.001) and duration of using VPA (r=0.215; P<0.001) in epileptic children. The results showed insignificant difference in serum TC, TGs and LDL-c between epileptic adults on VPA and control subjects. This study also revealed that the increase in serum leptin was significantly higher (p<0.001) in epileptic adults receiving continuous VPA monotherapy than in epileptic children.

Conclusion: Continuous VPA monotherapy was associated with higher BMI and serum leptin and an increase in the oxidative stress marker MDA in both male epileptic children and adults. There was no effect of VPA use on lipid profile indices in both epileptic children and adults except significant decrease in serum HDL-c and significant increase in AI.

Keywords: Valproate, male epileptic children and adult, BMI, leptin, malondialdehyde, lipid profile

الخلاصة

أهداف الدراسة: تقدير تأثير الفالبروات كعلاج أحادي مستمر على دليل كتلة الجسم، اللينتين والمالوندايلديايد وصورة الدهون في مصل الدم عند مرضى الصرع من الأطفال والبالغين الذكور بالمقارنة مع مجموعة الضبط من الأصحاء.

© 2011 Mosul College of Medicine
المريض وطرق العمل: تم اعتماد تصميم دراسة العينة والشاهد. عينات من 44 مريضا من الذكور (22 أعمارهم دون 18 سنة 22 أعمارهم دون 18 سنة 18 سنة) مصابين بالصرع الأحادي لعقار الفالباروات على الأقل 6 أشهر قبل البدء بالدراسة تم تجميعها للفترة من تشيرين الأول 2009 إلى أيار 2010. أيضا تم تجميع 44 شخسا سبما (22 أعمارهم دون 18 سنة 22 أعمارهم دون 18 سنة 18 سنة) كمجموعة سلامة. تم سحب نماذج الدم في حالة الصياح ومصل الدم، وبعدها قياس لإجراء الفحوص الكيميائية. أما مستوى الفالباروات، فقد تم قياسه بطرقية الكشف المحضر بدويا ومستوى الكولسترول الواطي الكافحة معالفة فيرسول. وتم حساب دليل كتلة الجسم من خلال قسمة الوزن (كمف) على مربع الطول (متر).

النتائج: أظهرت نتائج الدراسة أن مرضى السرطان وارتفاع معيون في ذيل كتلة الجسم، الليبين والمالونةديهيدايديس في مصل الدم وجود مؤثر تصلب الشرايين. وكان لديهم انخفاض معيوني في الكولسترول العالي الكافحة، والكولسترول الكلي، الدهون الثلاثية، الكولسترول الواطي الكافحة بالمقارنة مع مجموعة السليمة.

أظهرت الدراسة أيضا أن مرضى السرطان المستenum على الفالباروات لديهم ارتفاع معيون في ذيل كتلة الجسم، مستوى الليبين والمالانونديهيدايديس وكذا مؤثر تصلب الشرايين وانخفاض معيوني في مستوى الكولسترول العالي الكافحة في مصل الدم. بينما لم يكن هناك فروقات معينة في مستويات الكولسترول الكلي، الدهون الثلاثية، الكولسترول الواطي الكافحة.

الاستنتاج: استنتجت هذه الدراسة أن الاستمرار بالعلاج الأحادي لعقار الفالباروات ترافقت مع زيادة في وزن الجسم والليبين في مصل الدم مع زيادة في مؤثر جيد الأوكسيديات (الفالونديهيدايديس) في مرضى السرطان. كان هناك انخفاض معوني في الكولسترول العالي الكافحة وزاد في مؤثر تصلب الشرايين مما يتوافق مع دراسة أوسوم من حيث عدد المرضى للتحقيق مع الأجر.

مفتاح الكلمات: الفالباروات، مرضى السرطان، والأوقاع الخفيفة، دلالة كتلة الجسم، الليبين، والمالانونديهيدايديس، دهون الدم.

Epilepsy and its medications are associated with weight changes in which weight gain is the most common and distressing particularly with Valproate (VPA) therapy. Weight gain is a difficult problem at any age, particularly in adolescence, a period of increased awareness to body weight and image. Epilepsy and its medications may alter weight homeostasis regulating process including the two main homeostatic hormones, leptin and insulin. Increased blood levels of leptin and insulin due to leptin and insulin resistances are observed in patients with epilepsy.

The most important effect of free radicals is lipid peroxidation which results in disruption of cell membranes, and this may explain the role of oxidative stress in the etiology of seizure-induced neuronal death. It has been reported that increased generation of free radicals or decreased activity of antioxidant defense systems can cause some forms of seizures and in addition can increase the risk of seizure recurrence. Many antiepileptic drugs (AEDs) are metabolized to generate reactive metabolites with the capability of covalent binding to macromolecules as proteins or other vital biomolecules and hence eliciting systemic toxicity. Lipid peroxidation caused by increased generation of free radicals or decreased activity of antioxidant defense systems have been suggested to be critically involved in seizure control.

Many studies, mainly comprising adult patients, have provided the evidence that there is a significant influence of long-term AED therapy on total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c). VPA is not a microsomal enzyme inducer, and lipid profile during VPA treatment is controversial. Decreased, no effect, and increased levels of serum lipids have been reported in epileptic patients.
The aim of this study was to assess the effect of continuous VPA monotherapy on BMI, serum leptin, MDA and lipid profile in epileptic children and adults in comparison with those of healthy controls.

**Subjects, materials and methods**

**Epileptic patients**

This study included 44 male patients (22 less than 18 years old with mean age ±SD of 11.26±4.41, and 22 more than 18 years old with mean age ±SD of 30.55±6.54), with primary generalized epilepsy, on continuous valproate [VPA, Depakine, Sanofi-Aventis, France] monotherapy in a mean ±(SD) dose of 386.36±99.02 mg/d, for at least six months before participation in the study. These patients were referred from a private clinic over the period from October 2009 to March 2010. Patients with the following criteria were excluded from this study:

1. Patients with secondary epilepsy.
2. Patients with other neurological, medical, or psychiatric disorders.
3. Patients with rapidly progressive disorders that could alter their weight.
4. Patients with family history of body weight disorders.

**Control subjects**

Forty four apparently healthy male volunteers (22 less than 18 years old with mean age ±SD of 10.95±4.10, and 22 more than 18 years old with mean age ±SD of 30.05±5.95) without previous history of epilepsy were recruited as controls (with age matching to the patients group) from employees at the College of Pharmacy and Mosul College of Medicine, and from relatives who fulfilled the criteria adopted for the study. The control group was judged free of any illness by history and clinical examination. They were included in the study to compare the normal values for serum leptin, MDA and lipid profile indices.

**Specimens collection and analysis**

For epileptic patients, about 5 mL of venous blood was drawn, using a disposable syringe between 8.00 to 10.00 a.m., and after an overnight fasting. The blood was allowed to clot in a plain tube at room temperature, and then the serum was separated by centrifugation at 3000 rpm for 10 min., and kept frozen at -20 °C to be analyzed later on. Samples from the control subjects were collected and processed in the same way. Serum leptin was measured by enzyme linked immunosorbtent assay (ELISA) technique, using the IBL leptin ELISA Kit (Germany), which is an immunoassay for the quantitative in vitro diagnostic measurement of leptin in serum and plasma. Serum MDA levels were estimated using TBA assay method (14). Determination of serum TC and TGs concentrations was done by the enzymatic colorimetric method (Allain et al., 1974) (15), using total cholesterol BIOLABO Kit (France). Determination of serum HDL-c concentration was done by the precipitation method (Lopez-Virella et al., 1977) (16), using HDL-c BIOLABO Kit (France). Serum LDL-c concentration was calculated using Friedewald equation (17):

$$LDL-c \ (mmol/L) = TC - HDL-c - TG/2.2.$$  
Atherogenic index (A.I) was calculated by the following equation: 

$$AI = TC / HDL-c$$  

BMI was calculated according to the equation:

$$BMI= Weight (Kg)/ Height (m^2)$$  

Age and sex specific standard deviation scores (SDS) for height, weight and BMI were calculated for children according to charts developed by the National Centre for Health Statistics in collaboration with the National Centre for Chronic Disease Prevention and Health Promotion (20).

**Statistical analysis**

The data were analyzed using Statistical Package for Social Sciences (SPSS) (version 11.5). Standard statistical methods were used to determine the mean and standard deviation. Unpaired t-test was used to compare the results of different biochemical parameters. Linear regression analysis [Pearson correlation coefficient (r)] was performed to identify the relationship between different biochemical parameters. p-value ≤ 0.05 was considered to be statistically significant (21).

**Results**

Tables 1 and 2 demonstrated the demographic characteristics of the epileptic patients and the control subjects respectively.
Table 3 demonstrated the comparison of BMI SDS, serum levels of leptin, MDA and lipid profile indices between epileptic children and epileptic adults receiving continuous VPA monotherapy and their matched control subjects.

The results of this study revealed that epileptic children receiving continuous VPA monotherapy had significantly higher values for BMI, serum leptin, MDA and AI and a significantly lower (p<0.001) serum HDL-c as compared to their matched controls, while non significant differences were demonstrated for serum TC, TGs and LDL-c . Serum leptin was positively correlated with BMI SDS of epileptic children (r=0.542; P<0.001) and duration of usage VPA (r=0.215; P<0.001) in epileptic children. The results of this study also revealed that epileptic adults receiving continuous VPA monotherapy had a significantly higher (p<0.001) BMI, serum leptin, MDA and AI and a significantly lower (p<0.001) serum HDL-c as compared to their matched controls. This study also revealed that the increase in serum leptin was significantly higher (p<0.001) in epileptic adults receiving continuous VPA monotherapy than that in epileptic children. On the other hand, there was insignificant difference in serum MDA, and lipid profile indices between epileptic children and epileptic adults receiving continuous VPA monotherapy.

Table (1): The characteristics of the epileptic patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean± SD</th>
<th>Children (≤ 18 years) (n=22)</th>
<th>Adults (&gt; 18 years) (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients age (years)</td>
<td></td>
<td>6-18 (11.26±4.41)</td>
<td>19-44 (30.55±6.54)</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m²)</td>
<td></td>
<td>27.75±4.41</td>
<td>26.86±3.31</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td></td>
<td>2.34±2.09</td>
<td>5.17±6.64</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td></td>
<td>9.38±4.42</td>
<td>25.39±9.38</td>
</tr>
<tr>
<td>Duration of using VPA (years)</td>
<td></td>
<td>2.07±2.18</td>
<td>2.86±2.98</td>
</tr>
<tr>
<td>Dose of VPA (mg/day)</td>
<td></td>
<td>386.36±99.02</td>
<td>595.45±247.80</td>
</tr>
</tbody>
</table>

Table (2): The characteristics of the controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean± SD</th>
<th>Children (≤ 18 years) (n=22)</th>
<th>Adults (&gt; 18 years) (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (years)</td>
<td></td>
<td>6-18 (10.95±4.10)</td>
<td>22-44 (30.05±5.95)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td>23.86±5.53</td>
<td>24.59±2.92</td>
</tr>
</tbody>
</table>

Table (3): Comparison of BMI, serum leptin, MDA and lipid profile indices levels between the epileptic patients and the controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean± SD</th>
<th>Children (≤ 18 yrs) (n=22)</th>
<th>Adults (&gt; 18 yrs) (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td>27.75±4.41**</td>
<td>23.86±3.53</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td></td>
<td>6.06±5.54 **</td>
<td>2.89±1.71</td>
</tr>
<tr>
<td>MDA (µMol/L)</td>
<td>2.03±0.44 **</td>
<td>1.26±0.48</td>
<td>2.06±0.51**</td>
</tr>
<tr>
<td>TC(mmol/L)</td>
<td>4.46±0.78</td>
<td>4.48±0.79</td>
<td>4.54±0.52</td>
</tr>
<tr>
<td>TGs (mmol/L)</td>
<td>1.44±0.45</td>
<td>1.36±0.48</td>
<td>1.34±0.44</td>
</tr>
<tr>
<td>LDL-c (mmol/L)</td>
<td>3.01±0.83</td>
<td>2.84±0.57</td>
<td>3.18±0.62</td>
</tr>
<tr>
<td>HDL-c (mmol/L)</td>
<td>0.79±0.13</td>
<td>1.02±0.27</td>
<td>0.75±0.11**</td>
</tr>
<tr>
<td>AI</td>
<td>5.64±1.50**</td>
<td>4.55±0.77</td>
<td>6.07±1.51**</td>
</tr>
</tbody>
</table>

**: significant differences p< 0.001.
**Discussion**

The current study demonstrated higher body weight and significant increase in BMI in both children and adult epileptics on VPA therapy. Clinically significant weight gain has been reported with several AEDs including the conventional agents VPA, carbamazepine (CBZ) and the newer medications gabapentin and vigabatrin and may result in lack of compliance with or discontinuation of therapy \(^{(22)}\). Among all of the AEDs, VPA a broad-spectrum antiepileptic drug commonly used to treat children with focal and generalized epilepsy, has been the best studied with respect to effects on body weight \(^{(23)}\). Its etiology is most likely multi-factorial and controversial. Pylvanen et al. \(^{(24)}\) reported that 52% of men treated with VPA had BMI scores within the obesity category. Numerous pooled and specifically assigned data from clinical trials plus retrospective and cross-sectional analysis also reported that treatment with VPA is associated with a significant weight gain in 3—71% of patients which ranged from 5 to 49 kg \(^{(25,26)}\). In agreement with our results the study of Rauchenzauner et al., \(^{(27)}\) who reported that long-term therapy with VPA in childhood was associated with significant increase in body weight, as well as Hamed et al., \(^{(28)}\) who also concluded that BMI was significantly elevated in VPA treated epileptic children compared with controls, untreated and those treated with other AEDs.

Several mechanisms have been suggested to explain VPA-related weight gain including:

1- The effect on the hypothalamus: this is supported by the observation that epileptic patients treated with VPA and reported weight gain also developed increased appetite thirst, and quenching with calorie-rich beverages \(^{(23)}\), all of which indicate hypothalamic stimulation \(^{(29)}\).

2- VPA-induced hyperinsulinemia and insulin resistance: this is supported by the observation that weight gain during VPA treatment is related to increase in insulin concurrent with decrease in glucose level which can stimulate appetite and may cause weight gain \(^{(23)}\).

3- VPA-induced hyperleptinemia and leptin resistance: this is supported by the observation that women who became obese after VPA therapy reported high leptin levels and insulin resistance while patients who remained lean did not show any changes \(^{(2)}\).

4- VPA-induced changes in circulating levels of leptin and its relation to ghrelin and adiponectin: this is supported by the observation that weight gain in VPA-treated patients was associated with increased concentrations of leptin and decreased concentrations of ghrelin and adiponectin. These changes were correlated with the extent of body weight, patients’ BMI, the amount of adipose tissue and fasting plasma levels of insulin and leptin \(^{(30)}\). The down-regulation of ghrelin is considered as a consequence of increased insulin or leptin.

5- Genetic susceptibility to develop hyperleptinemia: this is supported by the observation that VPA-treated patients may manifest high serum leptin levels without weight gain and leptin level is correlated to BMI \(^{(31)}\).

The results of the current study also revealed an elevated serum leptin in both epileptic adults and children on VPA monotherapy. This is consistent with the finding of Rauchenzauner et al., \(^{(27)}\) and Hamed et al., \(^{(28)}\), both reported an increase in serum leptin in epileptic patients on continuous VPA therapy and that hyperleptinemia was common among epileptic children who gained weight with VPA therapy suggesting a state of leptin resistance. The mechanism of hyperleptinemia and leptin resistance with VPA treatment is still controversial. The increase in serum leptin levels in VPA-induced weight gain may be a consequence of increase in adipose tissue \(^{(4)}\). It has been suggested that VPA causes direct secretion of leptin from adipocytes.

Lipid peroxidation is an indicator of free radical metabolism and oxidative stress in human beings and other organisms. Malondialdehyde (MDA), is an end product of lipid peroxidation that can react with thiobarbituric acid. The estimation of MDA is a
sensitive measure of lipid peroxidation (32). This study revealed a significantly higher serum MDA in both epileptic children and adults receiving VPA monotherapy. In agreement with our finding, is the study conducted by Yukset et al. (33). They concluded that epileptic patients on VPA had an increased lipid peroxidation levels. Consistent with our results, also the study conducted by Solowiej and Solaniec (34) who reported that the concentration of MDA was elevated in all epileptic patients significantly in both VPA monotherapy and polytherapy, while insignificant elevation was noticed in newly diagnosed epileptics and in CBZ monotherapy group. In their more recent research, Sobaniec et al., (35), and Yiş et al., (6) both reported insignificant elevation in MDA concentration in epileptics on VPA monotherapy. While in contrast to our results, Peker et al., (36) who investigated the effects of VPA on lipid peroxidation among other parameters, reported no significant differences in serum MDA in comparison to healthy controls. In explanation to our results, there were several studies suggesting that excessive generation of free radical intermediates are associated with VPA administration possibly as a consequence of VPA biotransformation (37), or alterations in glutathione homeostasis (38) and/or depletion of cofactors required for antioxidant defense (9).

The effect of VPA on lipid profile remains unclear. VPA has been reported to be associated with a decrease of serum LDL-c or TC (39,40) and increased HDL-c (8). No significant changes in serum lipid has been reported by others (13,41). Our study revealed a significantly lower HDL-c and a highly significant increase in A.I in epileptic children on VPA monotherapy in comparison to healthy controls. In agreement with our results, is the study conducted by Zeitlhofer et al., (42). They reported that VPA-treated epileptic patients showed decreased HDL-c levels. Eiris et al., (43) also reported that epileptic children receiving continuous VPA monotherapy had significantly lower HDL-c than their matched control subjects. While in contrast to our results, Heldemberg et al., (40), reported that epileptic children receiving continuous VPA monotherapy had increased serum HDL-c.

This study also revealed insignificant differences in TC, TG and LDL-c in epileptic children on continuous VPA monotherapy which is in concordance with the results of Luef et al., (44), who found insignificant differences in serum TC, TG and LDL-c between epileptic children receiving continuous VPA monotherapy and their matched controls. Furthermore, this study also demonstrated that epileptic adults receiving continuous VPA monotherapy had significantly lower HDL-c and significantly higher AI than their matched controls. This is consistent with the results of the study conducted by Hamed et al., (28). They reported that epileptic adults on continuous VPA monotherapy had significantly lower HDL-c than their matched controls.

In conclusion, VPA monotherapy in epileptic children and adults can cause a rise in BMI, serum leptin and MDA (as a reflection of oxidative stress) and also causes a significant reduction in HDL-c and a significant rise in AI which needs further evaluation with larger number of patients.

References
5. Aydin K, Serdaroglu A, Okuyaz C, Bidect S and Gucuyener K. Serum insulin, leptin, and neuropeptide Y levels in epileptic
26. Zimmermann U, Kraus T; Himmerich H, Schuld A and Pollmacher T. Epidemiology, implications and mechanisms underlying


Detection of extended spectrum B-lactamase in E. coli from clinical samples

Ansam M. Hamdoon
Department of Microbiology, College of Medicine, University of Mosul.

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ABSTRACT

Objectives: 1- To study the frequency of ESBL (extended spectrum beta lactamase) among E. coli clinical isolates. 2- To determine the antibiotic profile for the isolates. 3- To determine the difference between the antimicrobial susceptibility of the ESBL producing E. coli and non producers.

Methods: A 4-months review of patients from three different hospitals who were diagnosed to have genitourinary tract infections with E. coli. These isolates were identified and assessed for their production of B-lactamase, and their antibiotic susceptibility to 21 different antimicrobial agents was determined.

Results: Out of the total 136 E. coli isolates, 58.82% were found to be ESBL producers. The most effective antimicrobial agent against the isolates was amikacin (85%), followed by ciprofloxacin (67.6%), while all the isolates were fully resistant to penicillin, cephradine, cephalothin and carbencillin. Multi-drug resistance (MDR) were found to be more among the ESBL producers. There was a statistical association between the production of B-lactamase and the resistance to Amikacin, nitrofurantoin, levofloxacin, kanamicin, nalidixic acid, gentamicin, piperacillin, cefotaxime and cephalixin.

Conclusions: This study shows that E. coli recovered from clinical specimens produce B-lactamase in high percentage and are resistant to penicillins and most cephalosporins. In addition, the MDR was higher among the B-lactamase producers. Therefore, determination of B-lactamases production, antimicrobial sensitivity of the isolates and strict antibiotic policy should be adopted in hospitals to take steps for reducing the bacterial resistance.

الخلاصة

الأهداف: دراسة وجود أنزيم البيتا لاكتاماسي في العزلات السريرية للشرشليات القولونية وتحديد حساسيتها للمضادات الحيوية. بالإضافة إلى دراسة الفرق بين منتجات أنزيم البيتا لاكتاماسي وغير المنتجة للإنزيمي من ناحية حساسيتها للمضادات الحيوية.

طرق العمل: كانت مدة جمع العينات 4 أشهر من مرضى من ثلاث مستشفيات مختلفة. تم عزل الشرشليات القولونية عندهم من خمح الجهاز البولي والتناسلي وفحصت العزلات من ناحية إنتاجها لإنزيمي البيتا لاكتاماسي ومدى حساسيتها لـ 21 نوعا مختلفا من مضادات الحيوية.

النتائج: خلال فترة الدراسة وجد أن 84.7% من العزلات مكونة لإنزيمي البيتا لاكتاماسي وقد أظهر مضادات الامكانيك أعلى نسبة حساسية (85%) لليه السيروفوکساسين (7.17%). كانت كل العزلات مقاومة للبنسيلين والسيفرادين والسيفالوشين والكابريسين. كما وجد أن العزلات مقاومة لأكثر من نوع من المضادات الجرثومية كانت الأكثر ضمن منتجات إنزيمي البيتا لاكتاماسي. كانت هناك علاقة إحصائية إيجابية بين منتجات الإنزيمي ومضادات الامكانيك، نايفوروفرانتين، ليفوکساسين، كاتاماسي، ناليدكسين، سيفوناكم، تاباسيلين، سيفالوكسين، سيفالكسين.
The emergence of ESBL has increased the possibility that traditional empiric antimicrobial regimens may be ineffective, resulting in limitation of therapeutic options and making urinary tract infection (UTI), which remains the most common bacterial infection in human populations, and other infections difficult to treat.\(^1,11\)

In genital tract infections E. coli which normally inhabits the rectum can cause infection if spread to the vagina in which the normal balance of bacteria may be disrupted, resulting in the overgrowth of harmful bacteria at the expense of protective bacteria.

Furthermore, antibacterial agents such as trimethoprim-sulphamethoxazole, aminoglycosides, fluoroquinolones, tetracyclins and chloramphenicol are often co-transferred on a resistance plasmid resulting in multidrug resistance\(^7\). However, carbapenems are the treatment of choice for serious infections due to ESBL producing organisms\(^8\).

The National Committee for Clinical Laboratory Standards (NCCLS) recommends ESBL screening methods and confirmatory tests, because delay in the detection and reporting of ESBL production is associated with prolonged hospital stay, increased morbidity, mortality, and health care costs\(^5\).

The aims of this study are to:

1. Study the frequency of β-lactamase and ESBL among E. coli recovered from urinary tract and genital tract infections.
2. Study the antibiotic susceptibility pattern and multiple drug resistant E. coli in these patients.
3. Evaluate the difference between the antimicrobial susceptibility patterns of β-lactamase producing E. coli and non producers.
Materials and methods

This study was conducted in the Microbiology Laboratory, Department of Microbiology, College of Medicine, University of Mosul. A total of 136 E. coli isolates (112 UTI and 24 genital tract infections) were collected from patients attending Al-Khansa, Al-Batool and Ibn-sena Teaching Hospitals. The period of sample collection was between September 2010 and December 2010. The identification of the isolates was based on morphological features and standard biochemical tests(12).

All the isolates were tested for their susceptibility to 21 selected antibacterial agents using the standard disc diffusion method(12). A sterile cotton swab soaked in the bacterial suspension in Muller Hinton broth was used to inoculate the organisms onto the surface of Muller Hinton agar plates, then the antimicrobial discs were applied and the plates were incubated at 37°C for 24 hours. The resultant inhibition zone diameter for each disc was measured and compared with the control measure(13). The used antibacterial discs were: penicillin 10 U, levofloxacin 5 µg, nalidixic acid 30 µg, nitrofurantoin 100 µg, gentamicin 10 µg, ticarcillin 75 µg, cefixime 5 µg, ampicillin 10 µg, amikacin 30 µg, cephalexin 30 µg, cefoxitin 30 µg, cefotaxime 10 µg, kanamycin 30 µg, cloxacillin 10 µg, ciprofloxacin 5 µg, cephradine 30 µg, enrofloxacin 5 µg, cephalexin 30 µg, carbenicillin 25 µg, ceftriaxone 10 µg and piperacillin 30 µg (Bioanalyse.UK). The interpretation of the results was as recommended by NCCLS.

For the detection of β-lactamase enzyme production, both the rapid iodometric tube method and ESBL activity were tested. In the latter method (ESBL), the double disc synergy test was performed using ceftriaxone and a combination disc of amoxicillin 20 µg and clavulanic acid 10 µg(11).

Statistical analysis was performed using chi square test where appropriate, and P value < 0.05 was considered significant.

Results

In the current study the β-lactamase enzyme production was detected in the isolated E. coli from urinary tract and genital tract (Figure 1). Out of the total 136 tested E. coli, 80 (58.8%) were found to be ESβL producers (Figure 2).

There was no statistical association between the production of β-lactamase enzyme and the source of isolation (UTI and genital tract infection) (P>0.05). The antibiogram profile of the E. coli isolates was determined against a panel of 21 antimicrobial agents. The highest sensitivity percentage was noted in case of amikacin (85.3%) followed by ciprofloxacin, enrofloxacin, nitrofurantoin and levofloxacin (67.6%, 66.2%, 64.7% and 58.8% respectively). In addition, all the isolates were fully resistant to penicillin, cephalothin, cephradine and carbenicillin (Table 1).

The sensitivity to certain antibiotics was statistically decreased (P<0.05) with the production of β-lactamase enzyme particularly in case of amikacin, nitrofurantoin, levofloxacin, kanamicin, nalidixic acid, gentamicin, ticarcillin, piperacillin, cefotaxime and cephalexin (Table 2).

Broad spectrum resistance, which is defined as the resistance to ampicillin or cephalothin, was present in the current work for all the isolates, apart from two (98.5%) (5).

Extended spectrum beta lactam resistant E. coli, is defined as resistance of bacteria to ceftriaxone, which was observed in 114 isolates (83.8 %)(5).

The MDR ESBL was considered as resistance to 3 of the following 4 antibiotic groups: trimethoprim- sulphamethoxazole, aminoglycosides (amikacin or gentamicin), fluoroquinolones (ciprofloxacin, norfloxacin, or nalidixic acid), and nitrofurantoin. This MDR was detected in 34 isolates (25%) and were all β-lactamase producers, hence, a co-resistance to non β-lactam antibiotics was observed more with ESBL producing E. coli(5).
Table (1): The antimicrobial sensitivity of E. coli isolates from UTI and genital tract infections.

<table>
<thead>
<tr>
<th>Antimicrobial Agents</th>
<th>UTI No.(%)</th>
<th>GTI No.(%)</th>
<th>Total No.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>94(83.9)</td>
<td>22(91.7)</td>
<td>116(85.3)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>72(64.3)</td>
<td>20(83.3)</td>
<td>92(67.6)</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>68(60.7)</td>
<td>22(91.7)</td>
<td>90(66.2)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>76(67.8)</td>
<td>12(50)</td>
<td>88(64.7)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>66(58.9)</td>
<td>14(58.3)</td>
<td>80(58.8)</td>
</tr>
<tr>
<td>Kanamicin</td>
<td>46(40)</td>
<td>14(58.3)</td>
<td>60(44.1)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>40(35.7)</td>
<td>8(33.3)</td>
<td>48(35.3)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>36(32.1)</td>
<td>8(33.3)</td>
<td>44(32.4)</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>22(19.6)</td>
<td>0</td>
<td>22(16.2)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>16(14.3)</td>
<td>6(25)</td>
<td>22(16.2)</td>
</tr>
<tr>
<td>Cefixime</td>
<td>10(8.9)</td>
<td>6(25)</td>
<td>16(11.8)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>14(12.7)</td>
<td>0</td>
<td>14(10.3)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>8(7.1)</td>
<td>0</td>
<td>8(5.9)</td>
</tr>
<tr>
<td>Cephalixin</td>
<td>8(7.1)</td>
<td>0</td>
<td>8(5.9)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>4(3.6)</td>
<td>0</td>
<td>4(2.9)</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>2(1.8)</td>
<td>0</td>
<td>2(1.5)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2(1.8)</td>
<td>0</td>
<td>2(1.5)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cephradine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table (2): The antimicrobial susceptibility percentages of ESBL producing and non-producing E. coli.

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>B-lactamase producer</th>
<th>B-lactamase non-producer</th>
<th>Total sensitive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Amikacin</td>
<td>75</td>
<td>25</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>65</td>
<td>35</td>
<td>71.4</td>
<td>28.6</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>65</td>
<td>35</td>
<td>67.9</td>
<td>32.1</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>42.5</td>
<td>57.5</td>
<td>96.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>47.5</td>
<td>52.5</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>Kanamicin</td>
<td>15</td>
<td>85</td>
<td>85.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>17.5</td>
<td>82.5</td>
<td>60.7</td>
<td>39.3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>5</td>
<td>95</td>
<td>71.4</td>
<td>28.6</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>0</td>
<td>100</td>
<td>39.3</td>
<td>60.7</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>12.5</td>
<td>87.5</td>
<td>21.4</td>
<td>78.6</td>
</tr>
<tr>
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<td>92.5</td>
<td>17.9</td>
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<tr>
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<td>14.3</td>
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<td>85.7</td>
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<td>3.6</td>
<td>96.4</td>
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<td>100</td>
<td>3.6</td>
<td>96.4</td>
</tr>
<tr>
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<td>0</td>
<td>100</td>
</tr>
<tr>
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<td>100</td>
<td>0</td>
<td>100</td>
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<td>Cephalothin</td>
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<td>100</td>
<td>0</td>
<td>100</td>
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<td>Carbenicillin</td>
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<td>0</td>
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</tr>
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</table>
Discussion

ESBLs have been detected worldwide, and they are forming a major contributor of drug resistance in many Enterobacteriaceae\(^{(14)}\).

In the present work the ESBL E. coli was detected in 58.8% of our isolates which is in accordance with results of other studies that ranged between 41%-68% \(^{(3,10,15-18)}\). Another study done in a Turkish Hospital has reported a higher percentage (79.3%) \(^{(19)}\), while other researchers reported a lower percentages of such isolates ranging between (11.4-38.2%) \(^{(1,4,7,11,20)}\).

The prevalence of ESBLs among clinical isolates varies greatly in different geographical areas and are rapidly changing over time\(^{(14)}\). This variation may be attributed to the difference in the use of antibiotics between different localities particularly B-lactam antibiotics.

Broad spectrum resistance, in the current study, was detected in 98.5% of E. coli isolates, which was somewhat relative to the result of study\(^{(5)}\) done in Iran where 87.9% of the isolated E. coli were found to have a broad spectrum resistance.

Extended spectrum B-lactam resistant E. coli was recorded in 16.2% of the isolates. Such finding is in contrast with the results of another work\(^{(5)}\), where 45.2% of their isolates found to be ESBL resistant. However in Pakistan a lower result (8%) was reported\(^{(3)}\).

Multi drug resistance (MDR) is a major problem in the management of uropathogens. It has been noticed that the clinical isolates of E. coli that are ESBL producers are more likely to be resistant to other non β-lactam antimicrobial agents. This MDR may be due to plasmid carrying several genes coding multi-resistance which are transferred from one bacterium to another. The future treatment of MDR ESBL producing E. coli may become more complex because of further limitations of the available drugs.

In the present work, MDR ESBL formed 25% of the isolates, where all are β-lactamase producers. Aminzadeh, et al\(^{(5)}\) in Iran also reported 25% to be MDR E. coli\(^{(5)}\). Other studies reported much higher percentages of MDR ESBL-E. coli (69.6% and 90.5%) \(^{(4,11)}\). Actually determination of the resistance pattern can help in great deal to select the best antibiotic in such a situation.

Actually, a statistical significant difference (p<0.05) was found in the susceptibility profile between ESBL producers and non ESBL producing E. coli for amikacin, nitrofurantoin, levofloxacin, kanamicin, nalidixic acid, gentamicin, ticarcilin, piperacillin, and cefotaxime. These findings support the hypothesis that extended spectrum ESBL producing strains of E. coli are more likely to have diminished susceptibility to non beta-lactam antibiotics compared to non beta-lactamase producing E. coli\(^{(21)}\). Hence, the antimicrobial susceptibility profile of the individual isolates should be used to guide treatment.

Penicillins are bactericidal agents that inhibit the bacterial cell wall synthesis. In this study...
the resistance of the isolated E. coli to penicillin and ampicillin was 100%. Similar results were reported too by other studies[6] particularly among the β-lactamase producers. This low susceptibility to penicillins may be due to the continuous use of these drugs for many years. Moreover, earlier other studies[22] reported that ampicillin has no more effect on urinary tract pathogens.

The cephalosporins particularly second and third generations are generally used for the treatment of E.coli infections. Sensitivity to ceftriaxone in this study was detected only in 16.2% which was lower than that reported in other studies (28.1%, 50% and 24%) [4,5,17]. Furthermore the resistance to cefotaxime and cefalexin was 94.1% in non β-lactamase producers, while in β-lactamase producers it was 100%, which is in agreement with the result of another work by Jirachai, et al[23]. This high resistance to cephalosporins could be explained by the fact that in our locality these drugs are easily available from pharmacy without doctor prescription and are relatively inexpensive antibiotics. Also, inadequate doses of these agents are sometimes used for treatment of different types of infections which may result in the development of high degree of resistance.

Flouroquinolones are particularly useful for the treatment of UTI because a high concentration of the drug in the urine can be achieved. The sensitivity to ciprofloxacin in the present work was observed in 67.6 % of the isolated E. coli which was in agreement with other studies[4,5,17,23]. The sensitivity to levofloxacin among β-lactamase non-producers was 75% which is similar to that reported by Jirachai and his co-workers (73%) [23].

Concerning aminoglycosides, they are generally prescribed against infections caused by gram negative bacilli. Amikacin really showed a high sensitivity percentage (85%), which is in agreement with the findings of other researchers[1,5,11,18,20], while other studies[10,17,24] revealed a lower sensitivity which may be due to the extensive use of this drug in those localities. Also, the sensitivity to gentamicin was 61.7% which is similar to that reported by other studies[1,23,25].

In conclusion, E. coli isolates recovered from clinical specimens in this region produced B-lactamase in high percentage, they are resistant to penicillins and most cephalosporins and the MDR was higher among the B-lactamase producers. Therefore strict antibiotic policy should be adopted in hospitals to estimate the impact of higher resistance in bacteria and to take steps for reducing this resistance. Knowledge of the resistance pattern in a geographical area will help to guide appropriate antibiotic use, and screening for ESBL production as a routine procedure in clinical laboratories which may give a valuable information to the clinician in appropriate selection of antibiotics.

References
7. Methee C, Pichai J, Anuwat K, et al. Epidemiology of ESBL producing Gram negative Bacilli at Siriraj Hospital,


The abnormal urodynamic findings as a predictor of complete response to treatment in patients with complicated nocturnal enuresis

Nooman H. Saeed*, Ziad M. Awwad**

* Department of Surgery, College of Medicine, University of Mosul; ** Special Surgery Department (Urology), Jordan University Hospital, Amman, Jordan.

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ABSTRACT

Objective: Patients with complicated nocturnal enuresis might show poor response to various forms of treatments, the purpose of this study is to urodynamically assess their bladder function and capacity aiming to predict the underlying cause for their problem and to assess their response to treatment with desmopressin and anticholinergics(oxybutynin).

Patients and methods: A retrospective study of 63 patients (50 female and 13 male). Their age ranged between 5-14 years, complaining of complicated nocturnal enuresis. Conducted at the urology clinic in Jordan University Hospital in Amman. All patients underwent urodynamic study, their data were analyzed, and treatment was established accordingly. Response was compared between patients having bladder instability and those with stable bladder, also between those with normal cystometric capacity and those with reduced cystometric capacity.

Results: Bladder instability was reported in 55 patients (87.3%) as compared to 8 patients (12.7%) with stable bladder. Also 52 patients (82.5%) had reduced bladder capacity and 11 patients (17.5%) had normal bladder capacity. Of 52 patients with reduced cystometric capacity, 42 patients (80.7%) had partial or no response to treatment.

Conclusion: Management of patients with complicated nocturnal enuresis is challenging, and needs an elaborate efforts to settle the cause(s). Bladder instability and reduced bladder capacity represent significant part of the underlying etiologies. The reduced bladder capacity is a reliable predictor of response to treatment with desmopressin and anticholinergics in this group of patients.

Keywords: Complicated nocturnal enuresis, urodynamics.
Octurnal enuresis is defined as involuntary voiding that occurs during sleeping at night\(^1\). Approximately 15% of children are still wetting their beds at night at 5 years of age and 1% at 15 years of age\(^1\). When it is not associated with day time symptoms it is called monosymptomatic or uncomplicated nocturnal enuresis, when day time symptoms such as frequency, urgency, incontinence, or chronic constipation are present it is called polysymptomatic or complicated nocturnal enuresis\(^1\). About 20% of nocturnal enuresis is of complicated type\(^1\). There is close relationship between complicated nocturnal enuresis and various types of bladder dysfunctions\(^2\)-\(^6\). Some authors agreed that in neurologically normal enuretic children, with the absence of urinary tract abnormalities and urinary tract infections, complicated nocturnal enuresis is closely related to detrusor instability\(^7\)-\(^10\). The aim of this study is to evaluate bladder function and capacity in children with complicated nocturnal enuresis who are neurologically normal by using urodynamics, and to predict their response to treatment with desmopressin and anticholinergics (oxybutynin).

**Patients and methods**

A retrospective study that was conducted by reviewing the records of 63 patients who were referred to the urology clinic at Jordan University Hospital, complaining of complicated nocturnal enuresis, between January 2002-December 2006. The clinical and urodynamic records were reviewed including history and detailed enuretic diary, physical examination, investigations including urine analysis, culture and sensitivity, renal function test, urinary tract ultrasound, IVU, and MCUG confirming the absence of urinary tract abnormalities. Those children who were found to have urinary tract infection were treated with antibiotics before performing the urodynamic study to avoid the effect of urinary tract infection on urodynamic results.

Urodynamic study was performed as an out patient procedure using Duet computerized urodynamic system, conventional fill cystometric study was used. All urodynamic study records were analyzed, and bladder function assessed with respect to detrusor activity during filling phase, pattern of contractions, bladder capacity, presence of leak during filling phase, and presence of post void residue. Detrusor overactivity was defined as abnormal detrusor contractions with pressure that exceeded 10 cm H\(_2\)O that the patient could not suppress them during filling phase\(^11\). Bladder capacity referred to the maximum cystometric capacity achieved where the patient could not afford any more due to severe urgency, pain, or when urine leak has started\(^1\). Bladder capacity was considered reduced if it is less than the predicted capacity for age calculated by using Koffs formula\(^8\)((age+2)\times 30). Patients were started on desmopressin 0.2 mg tablet once per day at bed time and anticholinergics (oxybutynin 0.2 mg/kg/dose t.i.d.). The patients were followed up monthly to start with, then the intervals increased up to every three months according to patients responses. Follow up period ranged from 12-60 months (mean 21 months), and it included urinary diary, repeating urodynamic study to evaluate patients response to treatment. The patients were observed to have three types of response:

I- No response: including those who did not show any clinical response to treatment.

II- Partial or incomplete response: including those who showed improvement of nocturnal enuresis and/or improvement of day time symptoms but did not become totally asymptomatic.
III- Complete response: including those who became totally dry at night and asymptomatic at day time.

In those who became totally asymptomatic gradual termination of the drugs was performed over few months, while those with incomplete or no response to treatment more prolonged therapy was attempted together with frequent repetition of urodynamics and more sophisticated studies and therapy regimens.

Results

The study included 63 patients (50 female (79.4%) and 13 male (20.6%)). Their age ranged between 5-14 years with mean age of 8.87 years (age was recorded at time of first consultation), there was no significant difference in age between male and female patients. At urodynamic study evaluation the patients were classified into those who had stable bladder (8 patients) and those with bladder instability (55 patients)(Figure 1). Also patients were grouped into those who had normal cystometric capacity (11 patients) and those with reduced cystometric capacity (52 patients)(Figure 2). The overall response rate was shown in table 1. Regarding response rate in relation to bladder stability (table 2), those patients who had stable bladder showed complete response in 4 patients (50%), and partial response in 4 patients (50%). Whereas those with bladder instability had complete response in 16 patients (29%), partial response in 32 patients (58%), and no response in 7 patients (13%). There was no statistically significant correlation between bladder instability and response rate (P= 0.35).

On evaluation of bladder capacity and response rate (table 3), the patients who had normal bladder capacity showed complete response in 10 of them (90.9%), and partial response in 1 patient only (9.1%). As compared to patients who had reduced cystometric capacity where response rate was complete in 10 patients (19.2%), partial in 35 patients (67.3%) and no response in 7 patients (13.5%). So of the 52 patients (82.5%) who had reduced cystometric capacity, 42 patients (80.7%) had partial or no response to treatment, which was statistically significant (P= 0.001).

Table (1): Overall response in all patients.

<table>
<thead>
<tr>
<th>Complete</th>
<th>20 patients</th>
<th>(31.7%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial</td>
<td>36 patients</td>
<td>(57.1%)</td>
</tr>
<tr>
<td>No response</td>
<td>7 patients</td>
<td>(11.1%)</td>
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</table>

Table (2): Response rate in relation to bladder stability.

<table>
<thead>
<tr>
<th>Patients</th>
<th>No Response (%)</th>
<th>Partial Response (%)</th>
<th>Complete Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>20 (11.1%)</td>
<td>36 (57.1%)</td>
<td>20 (31.7%)</td>
</tr>
<tr>
<td>Stable</td>
<td>0 (0%)</td>
<td>4 (6.3%)</td>
<td>4 (6.3%)</td>
</tr>
<tr>
<td>Instable</td>
<td>7 (11.1%)</td>
<td>32 (50.8%)</td>
<td>16 (25.4%)</td>
</tr>
</tbody>
</table>
Table (3): Response rate in relation to bladder capacity.

<table>
<thead>
<tr>
<th>Patients</th>
<th>No Response (%)</th>
<th>Partial Response (%)</th>
<th>Complete Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>7 (11.1%)</td>
<td>36 (57.1%)</td>
<td>20 (31.7%)</td>
</tr>
<tr>
<td>Normal capacity</td>
<td>0 (0%)</td>
<td>1 (1.6%)</td>
<td>10 (15.9%)</td>
</tr>
<tr>
<td>Reduced capacity</td>
<td>7 (11.1%)</td>
<td>35 (55.6%)</td>
<td>10 (15.9%)</td>
</tr>
</tbody>
</table>

Discussion

Nocturnal enuresis is a common problem that can affect healthy children\(^\text{(1)}\). It causes multiple psychological problems being a social stigma. Despite extensive research and advances in urology, still there is a significant controversy regarding its etiology and pathophysiology, and now it is generally accepted that multiple pathologic factors are probably involved\(^\text{(1)}\).

In this study bladder instability was found in 87.3% of the patients, this result was higher than what was found by Medel et al. where bladder instability was found in 79% of their patients\(^\text{(11)}\), and this is expected in cases of complicated nocturnal enuresis where bladder dysfunctions are predicted in quite large percentage of them, and it is higher than what is found in monosymptomatic nocturnal enuresis, where bladder dysfunctions in general are expected in around (49%) of cases\(^\text{(11)}\).

Also 82.5% of the patients were found to have lower bladder capacity than what is predicted for their age. Yeung et al studied preselected children with refractory monosymptomatic nocturnal enuresis, all of their patients were found to have low functional bladder capacity, and the majority had no nocturnal polyuria\(^\text{(2)}\). The theory that small functional bladder capacity can be a cause of nocturnal enuresis is not new, although published studies may have contradictory evidences, where some studies showed children with nocturnal enuresis have small functional bladder capacity, while others found it to be entirely normal\(^\text{(3,4)}\). Furthermore, some of previous studies included relatively few patients, and although there might have been a trend for smaller bladder capacity the differences might have not reached statistical significance\(^\text{(12,13)}\).

It is not clear whether the abnormal bladder contractions resulting in low bladder capacity. Interestingly Medel et al found children with larger than normal bladder capacity had a similar incidence of detrusor instability whether they had monosymptomatic or complicated nocturnal enuresis\(^\text{(11)}\).

Bladder instability was not significantly correlated with response to treatment and therefore could not predict the possibility of symptoms control. It was the reduced bladder capacity that showed significant correlation with poor response to treatment and predicted well the possibility of symptoms control. This finding is comparable with what was found in previous studies, but only desmopressin was used in monosymptomatic nocturnal enuresis\(^\text{(14)}\).

Treatment of complicated nocturnal enuresis needs to target the underlying pathology(s). Partial response to treatment was encountered in 57.1% of the included patients, some of those patients became totally free of symptoms at day time but still they had nocturnal enuresis, it seems that those patients have some of their underlying ongoing multipathologies responded to treatment while others not. Yeung et al studied children with refractory nocturnal enuresis, they found them to have various patterns of bladder dysfunctions, and classified those patients into two main groups, including those with functional bladder capacity that only become reduced with the appearance of detrusor instability during sleep, and those with reduced functional bladder capacity at day and night\(^\text{(15)}\).

It might be that those patients in the first group may explain why some patient with partial response to treatment became asymptomatic at daytime while continued to have nocturnal enuresis, and these differences in response to treatment again prove the multiple underlying pathologies for complicated nocturnal enuresis. Monitoring of those children at night by using continuous ambulatory urodynamics probably will cast further light on more hidden
underlying pathologies. Cystometric monitoring during sleep in those patients revealed that sleeping disturbances and bladder dysfunction are common\(^{16-21}\). Nocturnal polyuria resulting from deranged circadian rhythm of ADH secretion occurs in around 70% of enuretic children in general\(^{16-18}\). Interestingly most of those enuretic children with refractory symptoms do not have abnormal diurnal/nocturnal rhythm of ADH secretion and no significant nocturnal polyuria\(^{18-20}\). Whether it is an increased urine production or a reduced bladder capacity that results in the mismatch between nocturnal bladder capacity and the amount of urine produced during sleep at night, there must be a simultaneous arousal failure in response to bladder fullness before bedwetting occurs\(^1\). These findings cast further interest on looking for more specific theories tailored to target the underling etiologies in dealing with refractory complicated nocturnal enuresis.

**Conclusion**

Complicated nocturnal enuresis belongs to a heterogeneous group of underlying etiologies. It is important to say: those patients are not only complaining of social problems but also they have disorders which need appropriate management to control their symptoms. Bladder instability and reduced cystometric capacity are significant offending findings. The bladder capacity is a reliable predictor of response to treatment with desmopressin and anticholinergics.

**References**

15. Yeung CK, Sit FK, To LK, Chiu HN, Sihoe JD, Lee E, Wong C. Reduction in nocturnal functional bladder capacity is a common factor in the pathogenesis of
Case report:

Ovarian cavernous hemangioma in two years old female with repeated UTI; a case report and literature review

Zaid S. Khudher*, Ammar Abdulsalaam Hamid**
*Department of Surgery, College of Medicine, University of Mosul,
**Cardiovascular Surgeon, Aljumhouri Teaching Hospital, Moaul.

ABSTRACT

A two years old female child with history of a trivial trauma to the lower abdomen was complaining from repeated UTI.

Ultrasound and MRI examination of the abdomen and pelvis revealed 48x30 mm solid pelvic mass. Excision of the mass was done through laparotomy. Histopathological examination revealed ovarian cavernous hemangioma.

Although the ovary itself is a highly vascularized organ hemangioma of the ovary is a very rare lesion (1). Such benign tumours of the blood vessels are rare in ovaries during childhood (2). The first case was reported by Payne in 1869 Although often an incidental finding at operation, ovarian hemangioma may rarely be associated with gynecologic cancers. (3)

Case report

A two years old female patient presented with repeated attacks of urinary tract infections as frequency, difficulty in micturition over 1 year duration. History of a trivial trauma to the lower abdomen also reported.

Clinically there was no significant finding elicited neither on general nor on abdominal examination.

General urine examination was repeated three times during the period of complaint and revealed pus cells and bacteria. The last was two weeks before surgery.

Urine culture and sensitivity revealed E. coli sensitive to gentamicin, cephalexin, ceftriaxone and ciprofloxacin. Appropriate antibiotic was prescribed according to the result during attacks with good clinical response.

A retro-vesical solid mass measuring 48X30 mm was discovered by ultrasound examination of the abdomen and pelvis pressing on the urinary bladder, otherwise normal both kidneys. Same lesion in the retrovesical region causing pressure on the bladder was confirmed by MRI examination of abdomen and pelvis (fig.1).

Chest x-ray and plane abdominal radiograph were normal.
Through lower midline incision an exploratory laparotomy revealed a hemorrhagic mass at the left ovary, oophorectomy was done and sent for histopathology. (fig. 2)

The post operative period was uneventful (fig. 3). Histopathological examination revealed ovarian cavernous hemangioma.

**Discussion**

Vascular tumors of the female genital tract are rare especially those of the ovary. Alvarez and Cerezo\(^4\) reported a case of cavernous hemangioma in 68 years old lady, unlike what was reported in this paper.

Extensive punctuate calcification in cavernous hemangioma was reported by Kim et al \(^5\); the present tumor was devoid of calcification probably because of early presentation and surgery.

Vague abdominal and pelvic pain were the presenting features in an 11 and 32 years old patients reported by Correa-Rivas et al \(^6\) and Cormio et al \(^7\) respectively. The second was due to a big pelvic tumor, while repeated (UTI which may be coincidental or due to pressure effect of the mass) was the leading feature at the time of presentation in our case. Akbulut et al\(^3\) reported coexistence of the hemangioma with a serous papillary carcinoma of the ovary and an endometrial polyp in a 65 years old woman unlike our case which was a child and no such an association.

Disseminated vascular tumors involving both ovaries, both lungs and pleura, pericardium, and mediastinum was reported by Miyauchi et al \(^8\).

**Conclusion**

Despite the rarity of this tumor, it is advised to be taken into consideration in the differential diagnosis of ovarian tumors.

Recurrent UTI is one way of presentation in children, in addition to pressure symptoms and vague pelvic or abdominal pain in adults.

**References**

ال afterEach الاستشارة
المجلة طب الموصل

هيئة التحرير

الرئيس

الأعضاء

التحرير

الإدارة

서울로 710

전화: 02-581-7900

팩스: 02-581-7901

E-mail: annalsmosul@yahoo.com