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# IRAQI JOURNAL OF MEDICAL SCIENCES

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# Iraqi Journal of Medical Sciences

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**Iraqi Journal of Medical Sciences** is published by College of Medicine, Al-Nahrain University. It is a quarterly multidisciplinary medical journal. High quality papers written in English, dealing with aspects of clinical, academic or investigative medicine or research will be welcomed. Emphasis is placed on matters relating to medicine in Iraq in particular and the Middle East in general, though articles are welcomed from anywhere in the world.

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# Iraqi JMS FORMAT

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Iraqi Journal of Medical Sciences (Iraqi JMS) is a periodic, peer-reviewed journal published quarterly by College of Medicine, Al-Nahrain University. Iraqi JMS publishes manuscripts in all fields of health and medicine written in English.

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## Examples

1. Standard Journal Article: use et al when the number of authors exceeds 3.

Halliwell B, Gutteridge JMC. Oxygen toxicity, Oxygen radicals, transition metals and disease. Biochem J. 1984; 219: 1-14.

2. Books: Mann JI, Pyorala K, and Teuscher A. Diabetes in epidemiological perspective. 3rd ed. London: Churchill Livingstone. 1983.

3. Chapter in book: Phillips SJ, and Whisnant JP. Hypertension and strock. In: Laragh JH, and Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

- **Tables**: Each table should be typed on a separate page double-spaced, including all headings, number all tables with English numerals and include a short title. Vertical lines between columns are to be avoided.
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Proof Reading will be done by the secretarial office of the journal. The principal author will receive a copy of the journal. The authors are responsible for accuracy of all statements, data, and references included in the manuscript.

- After the manuscript has been accepted for publication, authors are required to supply the final version of the manuscript on CD in MS word version 6 or later.

# Iraqi Journal of Medical Sciences

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## Issued Quarterly

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# The Concept of Plagiarism

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## What is Plagiarism?

According to the Merriam-Webster Online Dictionary, to "plagiarize" means

1. to steal and pass off (the ideas or words of another) as one's own.
2. to use (another's production) without crediting the source.
3. to commit literary theft.
4. to present as new and original an idea or product derived from an existing source.

In other words, plagiarism is an act of fraud. It involves both stealing someone else's work and lying about it afterward [(1)].

In higher education institutions and universities, plagiarism is defined in multiple ways. To name a few: Stanford sees plagiarism as "use, without giving reasonable and appropriate credit to or acknowledging the author or source, of another person's original work, whether such work is made up of code, formulas, ideas, language, research, strategies, writing or other form"[(2)]; Yale views plagiarism as "the use of another's work, words, or ideas without attribution" which included "using a source's language without quoting, using information from a source without attribution, and paraphrasing a source in a form that stays too close to the original" [(3)]; Princeton perceives plagiarism as the deliberate use of "someone else's language, ideas, or other original (not common-knowledge) material without acknowledging its source" [(4)]; Oxford characterizes plagiarism as the use of "a writer's ideas or phraseology without giving due credit"[(5)]; and Brown explains plagiarism to be "appropriating another person's ideas or words (spoken or written) without attributing those word or ideas to their true source" [(6)].

## Types of Plagiarism

Anyone who has written or graded a paper knows that plagiarism is not always a black and white issue. The boundary between plagiarism and research is often unclear. Learning to recognize the various forms of plagiarism, especially the more ambiguous ones, is an important step towards effective prevention. Many people think of plagiarism as copying another's work, or borrowing someone else's original ideas. But terms like "copying" and "borrowing" can disguise the seriousness of the offense [(7)]:

## Sources Not Cited

1. **The Ghost Writer** "The writer turns in another's work, word-for-word, as his or her own".
2. **The Photocopy** "The writer copies significant portions of text straight from a single source, without alteration".
3. **The Potluck Paper** "The writer tries to disguise plagiarism by copying from several different sources, tweaking the sentences to

make them fit together while retaining most of the original phrasing".

4. **The Poor Disguise** "Although the writer has retained the essential content of the source, he or she has altered the paper's appearance slightly by changing key words and phrases".
5. **The Labor of Laziness** "The writer takes the time to paraphrase most of the paper from other sources and make it all fit together, instead of spending the same effort on original work".
6. **The Self-Stealer** "The writer "borrows" generously from his or her previous work, violating policies concerning the expectation of originality adopted by most academic institutions".

## Sources Cited (But Still Plagiarized)

1. **The Forgotten Footnote** "The writer mentions an author's name for a source, but neglects to include specific information on the location of the material referenced. This often masks other forms of plagiarism by obscuring source locations".
2. **The Misinformer** "The writer provides inaccurate information regarding the sources, making it impossible to find them".
3. **The Too-Perfect Paraphrase** "The writer properly cites a source, but neglects to put in quotation marks text that has been copied word-for-word, or close to it. Although attributing the basic ideas to the source, the writer is falsely claiming original presentation and interpretation of the information".
4. **The Resourceful Citer** "The writer properly cites all sources, paraphrasing and using quotations appropriately. The catch? The paper contains almost no original work! It is sometimes difficult to spot this form of plagiarism because it looks like any other well-researched document".
5. **The Perfect Crime** "The writer properly quotes and cites sources in some places, but goes on to paraphrase other arguments from those sources without citation. This way, the writer tries to pass off the paraphrased material as his or her own analysis of the cited material".

## Intentional Plagiarism

Just like hacking into websites, plagiarizing papers can be something of a thrill in itself. For many students it becomes a question of ingenuity: "can I sneak a plagiarized paper past my professor?" But there is usually more behind intentional plagiarism than just the thrill of deception.

## Self-plagiarism

Self-plagiarism (also known as "recycling fraud"[8]) is the reuse of significant, identical, or nearly identical portions of one's own work without acknowledging that one is doing so or without citing the original work. Articles of this nature are often referred to as duplicate or multiple publications. In addition to the ethical issue, this can be illegal if copyright of the prior work has been transferred to another entity. Typically, self-plagiarism is only considered to be a serious ethical issue in settings where a publication is asserted to consist of new material, such as in academic publishing or educational assignments [9]. It does not apply (except in the legal sense) to public-interest texts, such as social, professional, and cultural opinions usually published in newspapers and magazines.

In academic fields, self-plagiarism occurs when an author reuses portions of his own published and copyrighted work in subsequent publications, but without attributing the previous publication [10]. Identifying self-plagiarism is often difficult because limited reuse of material is both legally accepted (as fair use) and ethically accepted [11].

It is common for university researchers to rephrase and republish their own work, tailoring it for different academic journals and newspaper articles, to disseminate their work to the widest possible interested public. However, it must be borne in mind that these researchers also obey limits: If half an article is the same as a previous one, it will usually be rejected. One of the

functions of the process of peer review in academic writing is to prevent this type of "recycling".

### Why Students Plagiarize

There are many reasons students plagiarize. Sometimes deadlines come around more quickly than expected, sometimes assignments feel overwhelming, and sometimes the boundaries of plagiarism and research just get confused.

Teach your students that the real skills they need to learn are interpretation and analysis -- how to process the information they find. Tell them that anyone with some basic knowledge can find information on the internet -- it's what they do with that information that is important.

### Poor Planning

Some students might think, "Why sweat over producing an analysis that has already been done better, by someone who knows more?" Students may also be intimidated by the quality of work found online, thinking their own work cannot compare.

Students are not always the best judges of how much time their assignments will take. They may not be aware of the extent of work involved in a research paper, or may simply be overwhelmed by the task and put it off until the last minute, leaving them with no time for original work of their own.

Scheduling stages of progress on their papers is a very effective way to deal with this. Having them submit bibliographies, outlines, thesis statements, or drafts on specified dates before the final draft is due will give them a good idea of the amount of work involved. It will also help them organize their time and make the task seem less overwhelming.

### Plagiarism vs. Paraphrasing

Many students have trouble knowing when they are paraphrasing and when they are plagiarizing. In an effort to make their work seem "more original" by "putting things in their own words," students may often inadvertently plagiarize by changing the original too much or, sometimes, not enough.

### Searching vs. Researching

Today's students learn quickly that finding and manipulating data on the Internet is a valuable skill. With the wealth of information available online, the production of original analysis and interpretation may seem like "busy work" compared to finding the best or most obscure sources [(12)].

### Plagiarism and the World Wide Web

The World Wide Web has become a more popular source of information for student papers, and many questions have arisen about how to avoid plagiarizing these sources. In most cases, the same rules apply as to a printed source: when a writer must refer to ideas or quote from a WWW site, she must cite that source.

If a writer wants to use visual information from a WWW site, many of the same rules apply. Copying visual information or graphics from a WWW site (or from a printed source) is very similar to quoting information, and the source of the visual information or graphic must be cited. These rules also apply to other uses of textual or visual information from WWW sites.

### Strategies for Avoiding Plagiarism

1. **Give credit** whenever you use another person’s idea, opinion, or theory, any facts, statistics, graphs, drawings—any pieces of information—that are not common knowledge.
2. Put in **quotations** everything that comes directly from the text especially when taking notes like another person’s actual spoken or written words.
3. **Paraphrase**, but be sure you are not just rearranging or replacing a few words. Instead, read over what you want to paraphrase carefully; cover up the text with your hand, or close the text so you can’t see any of it (and

so aren't tempted to use the text as a "guide"). Write out the idea in your own words without peeking.

4. **Check your paraphrase** against the original text to be sure you have not accidentally used the same phrases or words, and that the information is accurate.
5. **Tell your students that what interests you most is:**
   a) Seeing how they understand the assigned topic.
   b) How they develop their own style and voice.
   c) Explain to them that you know writing is a learning process.
   d) You do not expect them to be as brilliant as experts who have devoted years to the subject.
   e) Their experiences and the context of your class give them a unique perspective that may give them a far more interesting angle on the issues than those of the "experts."
   f) Explain that your students must retain the essential ideas of the original, but significantly change the style and grammatical structure to fit in the context of their argument.

## Legal aspects

Though plagiarism in some contexts is considered theft or stealing, it does not exist in a legal sense. "Plagiarism" is not mentioned in any current statute, either criminal or civil [13]. Some cases may be treated as unfair competition or a violation of the doctrine of moral rights [13]. The increased availability of intellectual property due to a rise in technology has furthered the debate as to whether copyright offences are criminal.

Plagiarism is not the same as copyright infringement. While both terms may apply to a particular act, they are different concepts. Copyright infringement is a violation of the rights of a copyright holder, when material restricted by copyright is used without consent. On the other hand, the moral concept of plagiarism is concerned with the unearned increment to the plagiarizing author's reputation that is achieved through false claims of authorship. Plagiarism is not illegal towards the author, but towards the reader, patron or teacher. Even when copyright has expired, false claims of authorship may still constitute plagiarism.

For professors and researchers, plagiarism is punished by sanctions ranging from suspension to termination, along with the loss of credibility and perceived integrity [14,15]. Charges of plagiarism against students and professors are typically heard by internal disciplinary committees, which students and professors have agreed to be bound by [16].

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# A Simultaneous Coupling Azo-dye Method for the Quantitative Assay of Esterases: Biochemical Characterization

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## Abstract

**Background** Enzyme activity is a subject of continuous research. Comparison of data obtained from various quantitative methods needs standardization of techniques in order to verify the results of histochemical and biochemical assays utilized in the study of tissue enzyme activity.

**Objective** Establishment of a biochemical method for the quantification of enzyme activity in α-naphthyl acetate esterases (ANAE) containing solution using hexazotized pararoseaniline (HP) as a coupling agent.

**Methods** Wavelength of maximum absorbance ($\lambda_{max}$) of coupled HP in solution was analyzed spectrophotometrically based on the simultaneous-coupling method of ANAE demonstration.

**Results** $\lambda_{max}$ of the coupled HP was found to be at 425 nm. The relationship between the optical density of the final reaction product (FRP) and the enzyme concentration was linear with the use of azo dye in solution.

**Conclusion** Data obtained from the biochemical assay of ANAE activity was in agreement with those documented by the histochemical methods in use. Thus, characterization of enzyme activity may be standardized when studying tissue sections and tissue homogenates.

**Keywords** Esterases, Biochemical assay, Histochemistry, Spectrophotometry

## Introduction

The activity of enzymes is regulated at different cellular levels. Many variables determine the activity of an enzyme, which may be localized at one region of the cell but not the other. This concept of integrating the location and function of enzymes has revitalized their study during the last decade, although such functions of enzymes do not necessarily correlate with their location [(1)].

In analysis of enzyme activity, it is often important to compare the results obtained with different quantitative methods. Work with esterases (EC 3.1.1.x), a diverse group of hydrolases catalyzing the reaction on ester bonds in the metabolic pathways [(2)], has detailed various histochemical and biochemical techniques employing the substrate α-naphthyl acetate with different types of diazonium salts suitable for one assay but not for the others [(3)].

Nachlas and Seligman were the first to describe a post-coupling azo dye method for the demonstration of nonspecific esterases using β-naphthyl acetate as a substrate [(4)]. Later works used α-naphthyl acetate as results were found to be more sensitive [(5)]. These researchers developed methods for biochemical measurement of esterases activity, using a simultaneous-coupling azo dye reaction with two different diazonium salts, *p*-Nitrobenzenediazonium tetrafluoroborate (*p*-NBDTFB) and Fast Violet B. Production of the azo

dye then could be measured with a spectrophotometer at the wavelength of maximum absorbance ($\lambda_{max}$) of the azo dye. This was found to be a sensitive assay allowing direct comparison between biochemical and histochemical data with one of the most widely used histochemical substrates for esterases, α-naphthyl acetate. Yet, the problem with the use of such diazonium salts was that they require optimum pH levels different from those present in vivo (pH 5.0 for *p*-NBDTFB, pH 8.0 for Fast Violet B) [5].

On the contrary, hexazotized pararoseaniline (HP), another diazonium salt widely used in histochemistry for staining of fresh frozen sections, was proved to couple effectively with α-naphthol, the product of α-naphthyl acetate hydrolysis, to produce the colored final reaction product (FRP) at physiological pH of 7.4 [3].

Generally, there are few quantitative methods utilizing substrates entirely satisfactory for histochemical work with α-naphthyl acetate. Conventional methods to determine esterase activity in tissue homogenate make the enzyme hydrolyzes α-naphthyl acetate and then a Fast Blue B dye complex is formed with α-naphthol [6]. Other spectrophotometric assays, which are limited to α-naphthyl ester substrates, continuously record esterase activity at 510 nm by monitoring absorbance changes due to the formation of an azo dye complex with Fast Blue RR salt [7]. Thus, most of the quantitative histochemical techniques used for the detection of α-naphthyl acetate esterases (ANAE) activity are either limited to the spectrophotometric assay performed on tissue sections [8], or they have used different types of chromogens other than the HP [9-11].

In addition, some trials for the use of ultraviolet (UV) absorbance in the determination of α-naphthol release in tissue homogenate have reported insensitivity because of the high background UV absorbance. These methods depend on the direct measurement of the product of enzymatic reaction rather than the quantification of the amount of the colored FRP. Thus, they are liable for the overlap in absorption spectra of α-naphthyl acetate and α-naphthol [5].

This work tries to establish a standard biochemical method for the detection and quantification of ANAE activity in solution, using HP as a coupling agent at physiological pH, so that enzyme activity can be accurately measured in tissue homogenates.

## Methods

Reagents were obtained from ADWIC, basic fuchsin (C.I. 42510) from SD Fine-Chem, sodium nitrite from United Company for Chemicals, α-naphthol from Nice Chemicals, and hog liver esterase (EC 3.1.1.1.) from Sigma.

### *Buffers*

Phosphate buffer 0.2 M (pH 7.4) was prepared by dissolving 11.4 g of $Na_2HPO_4$ and 2.7 g of $KH_2PO_4$ in distilled water. Volume was completed to 500 ml and the pH was adjusted with 1 N HCl [12].

### *Bovine serum albumin*

Stock solution of bovine serum albumin (BSA) (10 mg/ml) was prepared and stored in refrigerator (+4 $^{o}$C). A final concentration of 0.67 mg/ml of BSA in the assay medium was adequate for maintaining the azo dye in solution [5,13].

### *Diazonium salt*

Preparation of the diazonium salt was based on the method of demonstration of α-naphthyl acetate esterases [4,12]. Pararoseaniline was made by dissolving 1 g of basic fuchsin in distilled water. Then, 5 ml of concentrated HCl was added and the solution was warmed slowly, cooled to room temperature, filtered and stored at 4 $^{o}$C. Hexazotization of the uncoupled diazonium was done by adding 1 ml of pararoseaniline to 1 ml of freshly prepared 4% sodium nitrite. The mixture was stirred and put in ice-cooled bath.

The absorption spectra of the pararoseaniline and the uncoupled HP were measured as follows: a mixture of 4 ml phosphate buffer, 0.1

ml acetone, 0.32 ml pararoseaniline or HP, and 0.293 ml BSA was prepared. 3 ml of the mixture was pipetted in a quartz cuvette and the absorbance was recorded with Centra 5 UV-Vis Double-Beam Spectrometer at 350-650 nm wavelengths with 5 nm intervals. Control solution in which distilled water substituted the pararoseaniline or the HP was used. Measurements were done within 5 minutes of making the mixture.

***Determination of $\lambda_{max}$ of the azo dye***

A standard solution of α-naphthol was made by dissolving 144 mg in 25 ml 99% acetone to give a final concentration of 40 mM. Then, 0.1 ml of this solution was added to a 3 ml quartz cuvette containing 2.2 ml buffer, 0.2 ml BSA and 0.5 ml HP. The wavelength absorbance was adjusted to zero immediately before addition of α-naphthol and the solution was then read at 350-650 nm. An absorption spectrum is thus obtained and the $\lambda_{max}$ of the coupled HP is determined automatically.

***Relationship between α-naphthol concentration and optical density of FRP***

To make a standard curve, a series of various concentrations of α-naphthol were prepared (5-80 mM) and the absorbance was read immediately (that is, within 5 minutes of addition of the diazonium salt) as it is likely that if the azo dye is kept over extended periods of time the spectral characteristics would be altered [5]. Regression analysis is performed using Microsoft Excel 2010 tool in order to define a regression equation and the line of best-fit.

***Effect of enzyme concentration on α-naphthol release***

The detection of the enzymatically released α-naphthol was done by mixing 2 ml buffer, 0.2 ml BSA (10 mg/ml), 0.5 ml HP and 0.2 ml enzyme solution to fill in the 3 ml cuvette. Commercial hog liver esterase of original package at a concentration of 10 mg/ml was used after 1:1000 dilution in distilled water and kept on ice until use. Volumes between zero and 100 µl (0-1.0 µg/ml) were assayed with HP. The solution was incubated at 25°C and the reaction was initiated by the addition of 0.1 ml α-naphthyl acetate (8 mg/ml). The change in absorbance at $\lambda_{max}$ was recorded continuously over a period of 1 minute [5] and the regression analysis was done accordingly.

## Results

The measurements of the optical density of the pararoseaniline stock solution and the uncoupled HP are shown in figure1; the absorption spectra show higher optical density values of the non-hexazotized pararoseaniline in comparison to the uncoupled HP at wavelengths shorter than 425 nm, at which point both spectra intercept each other.

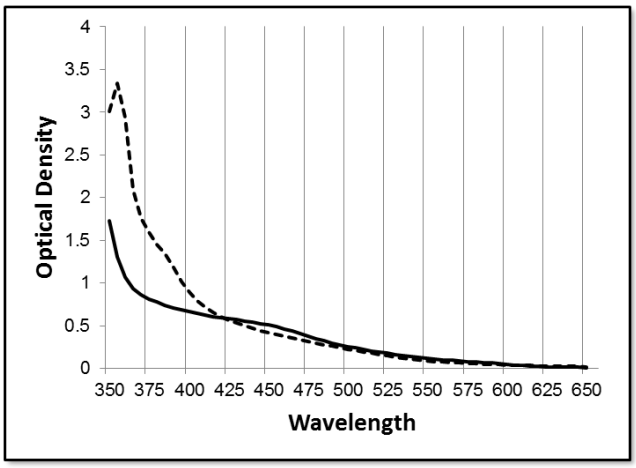



**Figure 1. Absorption spectra of non-hexazotized pararoseaniline solution ( _ _ _ _ ) and uncoupled HP ( ____ ) mixture. Both spectra slope down at higher wavelengths with an interception at 425 nm.**

Coupling with 40 mM α-naphthol is illustrated in figure 2. The absorption spectrum of azo dye formed upon coupling of HP to α-naphthol revealed peak absorption ($\lambda_{max}$) at 425 nm.

A standard curve with best-fit correlation and regression equation of various concentrations (5-80 mM) of α-naphthol in relation to the optical density of the coupled azo dye at 425 nm is seen in figure 3. There was a linear correlation

between α-naphthol concentration and the optical density of the FRP.

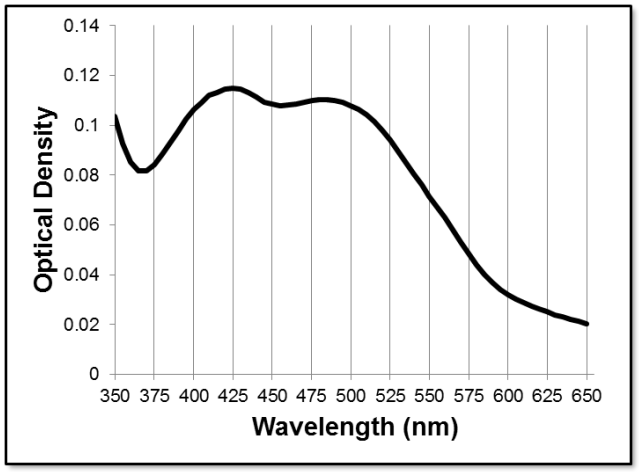



**Figure 2. Absorption spectrum of azo dye formed upon coupling of HP to 40 mM α-naphthol, demonstrating peak absorption ($\lambda_{max}$) at 425 nm.**

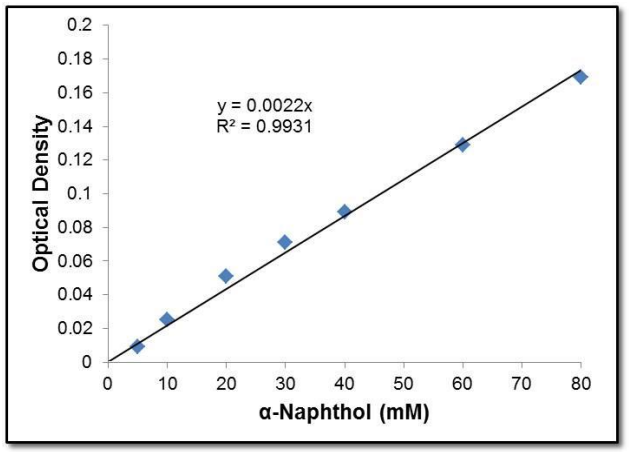



**Figure 3. Standard curve and regression equation of various concentrations (5-80 mM) of α-naphthol in relation to the optical density of the coupled HP at 425 nm**

The effect of enzyme concentration on the hydrolysis of α-naphthyl acetate to α-naphthol, measured by the optical density of the FRP produced upon coupling with the HP, is demonstrated in figure 4; the reaction showed linear relationship with enzyme concentration.

## Discussion

The simultaneous coupling azo dye methods are well documented to be more sensitive especially at lower enzyme activity; the histochemical information obtained from esteratic staining of tissue sections depend mainly on the use of HP, because of the high efficiency of its coupling reaction with α-naphthol [3].

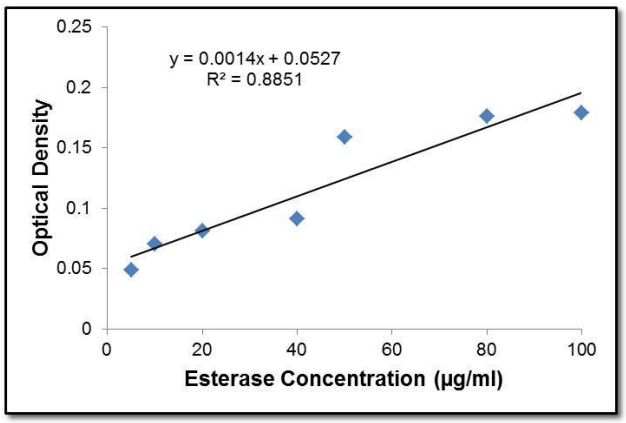
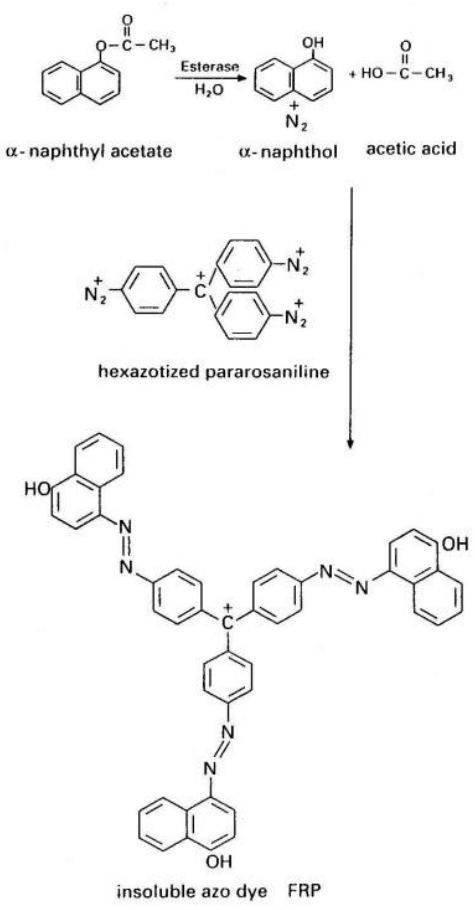



**Figure 4. Optical density of the FRP as a function of esterase concentration. Measurements were done within 1 minute upon coupling with HP at its $\lambda_{max}$ (425 nm). There is linear relationship within the range of enzyme concentration under study. The *y* intercept represents the amount of auto-hydrolysis**

In figure 1, the absorption spectra show higher optical density of the non-hexazotized pararoseaniline in comparison to the uncoupled HP at wavelengths shorter than 425 nm, at which point both spectra intercept each other. The lower optical density readings of the uncoupled HP might be due to change in the molecular configuration after hexazotization of pararoseaniline according to the following chemical formula [12] showing below.

The absorption spectrum of azo dye formed upon coupling of HP to α-naphthol (figure 2) revealed peak absorption ($\lambda_{max}$) at 425 nm. This is in concordance with the documented $\lambda_{max}$ for azo dye staining method in tissue sections [8]. In

other words, the use of BSA for maintaining the azo dye in solution did not alter the wavelength absorption characteristics of the incubation medium. This is also evident from the linearity of the plot of α-naphthol concentrations versus optical density measurements (figure 3), and from the linear relationship between the enzyme concentration and the optical density of the FRP (figure 4); both plots (figures 3 and 4) were measured at the assigned $\lambda_{max}$ for azo dye (425 nm). Besides, this linearity reveals that the inhibitory effect of diazonium salts on enzymes did not present an overwhelming problem with the use of HP in biochemical assays, as it adheres to the Beer-Lambert Law at the concentrations measured, similar to what have been noticed in previous works with other azo dyes [14].




Upon comparing the benefit of the use of HP in the detection of ANAE activity, this azo dye works optimally at the physiological pH of 7.4, which is an important advantage in this study against other diazonium salts such as *p*-NBDTFB and Fast Violet B that work best at alkaline or acidic pH, respectively [5]. In other words, HP staining method in solution or tissue homogenate does not affect the physiological environment needed for the optimum activity of the enzyme.

In contrast to the UV assay methods, tissue homogenates with components that possess high UV absorbance can be assayed using HP without the complicating factor of a distorting background because wavelength absorption falls within the range of the visible spectrum (350-650 nm). In addition, the simultaneous coupling in tissue homogenate solutions is a continuous assay, enabling initial reaction velocities to be measured over very short periods of time. Thus, problems arising from instability of diazonium salts and azo dyes are minimized [5].

In this study, esterase-catalyzed α-naphthol hydrolysis demonstrated straight lines of the reaction progress, indicating that a steady-state was already reached at the beginning of the measurements. An intercept with the *y*-axis above zero may point to a relief from the steady-state attributed to auto-hydrolysis of the substrate (figure 4). The *y* intercept is, therefore, a measure of the amount of auto-hydrolysis.

This auto-hydrolysis may raise a question of non-enzymatic catalyzed staining of tissue sections, that is, there might be some degree of azo dye staining not due to the presence of the enzyme, rather, due to the auto-hydrolysis of the substrate α-naphthyl acetate. Therefore, the biochemical method used in this work for measuring ANAE activity in solution or tissue homogenate could eliminate an artifact inevitably present when determining enzyme activity in tissue sections.

Despite the finding that the $\lambda_{max}$ of coupled HP in solution is similar to what has been recorded for tissue sections [8], some authors believe that living cell or tissue imaging, in which cells and tissues are kept intact during analysis, is the best approach to generating data on the activity of an enzyme [1]. Justification depends on the reflection of the in vivo situation, because activity measurements of a purified enzyme both in diluted solutions and in tissue or cell homogenates probably do not imitate

the activity of that enzyme in the crowded compartmentalized cell [15].

According to above mentioned perspective, it would be advisable to consider the segregation of cellular compartments by differential fragmentation and centrifugation in order to isolate the desired cellular fraction that contains the enzyme under study.

A debate concerning the work on biochemical assays may arise when considering enzyme histochemistry as the oldest histochemical modus operandi, with an approach that has minimized its impact in the '80s and '90s of the last century with the growing immunohistochemistry and in situ hybridization techniques. Recently, genes and gene expression have received almost all the attention. Studying enzyme activity was considered old-fashioned until scientists realized that gene expression tells us little about function; in the first decade of this century, enzyme activity localization and imaging had a strong revival because they focus on function. Imaging of living cells and tissues, in combination with localization of enzyme activity, became a strong match as metabolic mapping [1].

Yet, studying enzyme activity in solution or whole tissue homogenate is still a field of interest and the future of such work looks bright as continuous attention is paid to the innovation of novel methods for the detection of enzyme activity instead of the older procedures [16-18]. Therefore, it was reasonable to develop a simultaneous coupling method that utilizes HP for the detection of esterases activity in tissue homogenates solutions. This work adds to the methods used for such detection utilizing one of the widely used azo dyes, the HP, with technical parameters comparable to those applied in studying enzyme activity in tissue sections. Further studies comparing data obtained from tissue sections and tissue homogenates are recommended.

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# Possible Anthropometric Explanation of Age-related Changes in Splenic Volume in a Sample of Healthy Iraqi Individuals Using Ultrasonography

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## Abstract

**Background** Spleen is a hemopoietic organ which is capable of supporting elements of different systems. Determination of spleen size is important in diagnosing small, normal or enlarged spleens. Songraphy can rapidly and reliably help to determine the size of the spleen.

**Objective** The purpose of this study was to analyze the changes in splenic volume with respect to age using anthropometric measurents (body mass index (BMI), body surface area (BSA)) and to find the predicting equation of spleen volume.

**Methods** 215 Iraqi subjects (103 females with age range (6-74) years and 112 males with age range (7-75) years) without conditions that can affect the spleen or splenic abnormalities were evaluated with ultrasonography. Spleen length, width and thickness were measured and spleen volume was calculated. Spleen size was correlated with age, gender, BMI and BSA.

**Results** The mean splenic volume was significantly variable in different age groups.The spleen volume increases starting from childhood to reach a peak in a young adults (20-49) years and then declines in the middle age and elderly subjects (age with greater than 50 years). Splenic volume for males was significantly higher than females. BSA had a stronger linear correlation with splenic volume than BMI. Multiple regression analysis indicated that BSA had significant positive association with spleen volume in subjects younger than 50 years.

**Conclusion** Spleen volume was significantly variable among different age groups. Spleen volume affected by BSA especially in subjects younger than 50 years. These results illustrate that the normal value of spleen volume affected by different factors 1) age 2) sex 3) BMI 4) BSA.

**Key words** Spleen volume, Ultrasonography, Age, BMI, BSA

## Introduction

The spleen is an intra abdominal organ; many diseases can affect its size ranging from infective processes to malignant disorders. Spleen size varies widely according to age. The estimation of splenic size in vivo is often important in the diagnosis, treatment and prognosis of a variety of disorders. Palpation and percussion are the standard bedside techniques to document spleen size, but are far from accurate to detect small increase in size [(1)]. The spleen has to be enlarged two to three times its normal to be clinically palpable [(2,3)]. Therefore the imaging has become essential for the accurate measurement of the splenic size, the serial monitoring of the splenic size over the

course of the patients illness and development of the guidelines for return to play. Several prior studies have sought to develop the standards for the splenic size such as Computed Tomography scan, Scintigraphy, Magnetic Resonance Image and Sonography. The conventional sonography was found to be a well-established, widely used relatively inexpensive mean of assessing the splenic size without ionizing radiation. Radiography expose patient to x-ray and radionuclide studies expose the patient to gamma radiation [2,4-6]. Sonography is routinely used to determine the internal structure of the body because the examination is real time, independent of organ function and no risk of ionizing radiation. Ultrasonography is a non-invasive, established, safe, quick and accurate method for the measurement of spleen size [6]. It allows a physician to see inside a patient without resorting to surgery. Several published studies have examined the range of spleen dimensions and volume in normal adults and their correlation with age, gender and body habitus [7-11].

The present study was designed to measure three dimensional ultrasonography of the spleen in healthy Iraqi subjects of different age group and correlates them with the anthropometric body mass index (BMI) and body surface area (BSA) measurement.

## Methods

Spleen measurements were performed for 215 individuals prospectively recruited from individuals referred for US at Al-Kadhimiya Teaching Hospital for the period March 2009 to March 2010. The exclusion criteria were recent febrile illness (within the last 6 months), upper abdominal symptoms, liver disease, history of splenectomy, malignancy, malaria or sickle cell anaemia or trait, and those whose examination showed focal splenic lesions. Pregnant females were also excluded as spleen size has been shown increase during pregnancy [12].

All examination were performed by same radiologist experienced in abdominal US using a commercially available real time US scanner Siemen sonoline versa Pro with a 3.5-5 MHz sector transducer.

Images were obtained with the subject lying in the supine or slightly right lateral decbitus position, and through an oblique intercostal approach. Following suspended deep inspiration all measurements were made on images through the splenic hilum using electronic calipers .In each examination, three sequential measurements of the splenic length, thickness and width were obtained, and the mean value of each dimension was recorded to ensure minimal intraobserver variation. All measurements were recorded to the nearest millimeter.

Maximal splenic length was defined as the maximum distance between the most superior margin and inferior margin of the spleen on a longitudinal coronal sonogram. Maximal splenic thickness was defined as the maximal distance between the medial and lateral borders of the spleen on a transverse sonogram. Maximal splenic width was defined as the maximal anterior to posterior diameter of the spleen on a transverse sonogram [13].

Spleen volume was determined for each subject according to the formula for a prolated ellipse (0.524×width×thickness×length), which has been shown to be an accurate method for the calculation of spleen volume [14-16]. Height and weight were obtained to calculate the body mass index kg/$m^2$. Subjects were categorized into three groups; Group I (first quartile) consisted of subjects with BMI of ≤ 23.1 kg/$m^2$, group II (interquartile) subjects with BMI of (23.2-27.9) kg/ $m^2$ and group III (fourth quartile) subjects with BMI of ≥ 28 kg/$m^2$. Body surface area (BSA) was obtained based on the formula of Dubois [17].

BSA= ($W^{0.425}$×$H^{0.725}$) ×0.007184 where W=weight in kg, H=height in cm, factor unit =cm/kg. Subjects were categorized into three group .group I (first quartile consisted of subjects with BSA of ≤ 1.68 $m^2$), group II (inter quartile) subjects with BSA of (1.69 -1.93) $m^2$ and group III (fourth quartile) were individuals BSA ≥ 1.94 $m^2$.

## Statistical Analysis

Analysis was done by using the statistical package for social sciences (SPSS) version 13 software. The mean difference between the two groups was assessed for statistical significance by independent samples t-test. Bivariate correlation was performed calculating Pearsons linear correlation coefficient to assess the direction strength and statistical significance of linear correlation between two quantitative variables. Analysis of variance (ANOVA) was used to test for differences in the mean of outcome variables between more than two groups. The 95% confidence interval for mean was used to predict the mean in reference population with 95% confidence. Multiple linear regression model was used to assess the independent association of a set of explanatory variables on a quantitative outcome variable. $p< 0.05$ was considered statistically significant.

## Results

The study included 215 healthy subjects (103 females with mean age 37.4 years (range 6-74) years and 112 males with mean age 37.3 years, (range 7-75) years). The spleen length, height and width of them as shown in table 1.

**Table 1. Splenic dimensions (length, height & width) in the studied group**

| Dimension | Females N = 103 | | | Males N = 112 | | | Total N = 215 | | |
|---|---|---|---|---|---|---|---|---|---|
| | mean±SD | Range | SE | mean±SD | Range | SE | mean±SD | Range | SE |
| Splenic length | 10.6±1 | 7-12.6 | 0.1 | 11.2±1 | 6.7-12.9 | 0.1 | 10.9±1.1 | 6.7-12.9 | 0.07 |
| Splenic height | 4.9±0.7 | 3.1-6.8 | 0.07 | 5.4±0.8 | 2.8-7.8 | 0.08 | 5.1±0.8 | 2.8-7.8 | 0.06 |
| Splenic width | 4.1±0.5 | 2.8-5 | 0.05 | 4.5±0.6 | 2.6-5.9 | 0.05 | 4.3±0.6 | 2.6-5.9 | 0.04 |
| Splenic volume | 115.8±34.2 | 35.9-210.4 | 3.37 | 143.7±41.1 | 27.4-248.9 | 3.89 | 130.3±40.4 | 27.4-248.9 | 2.75 |

Splenic length, height, and width measured in cm. Splenic volume measured in $cm^3$

Splenic volume correlates in a curve linear fashion to age. It was shown to increase starting from childhood to reach a peak in a young adults (20-49 years) and then decline in middle age and elderly subjects(age with greater than 50 years ). Figure 1 shows that quadratic, cubic model provided the best fit for the observed data.

The mean of splenic volume was significantly variable in different age groups.Splenic volume for males was significantly higher (143.7 $cm^3$) than females (115.8 $cm^3$) as shown in table 2.

**Table 2. Correlations between splenic volume, age and gender**

| Character | | Spleen volume ($cm^3$) | | | |
|---|---|---|---|---|---|
| | | No. | Range | mean±SE | Mean±1.96SD (95% CI) |
| Age (years) | ≤ 10 | 6 | 27.4-64.7 | 45.5±6.16 | 15.9 to 75.1 |
| | 11-20 | 18 | 59-152.7 | 107.4±7.37 | 46.1 to 168.7 |
| | 21-30 | 42 | 77.6-185.8 | 127.4±4.1 | 75.3 to 179.5 |
| | 31-40 | 71 | 69.2-248.9 | 139.7±4.68 | 62.5 to 216.9 |
| | 41-50 | 41 | 48.3-245.2 | 148.1±6.59 | 65.4 to 230.8 |
| | 51-60 | 118 | 63.5-192.8 | 123.9±8.13 | 56.3 to 191.5 |
| | 61-70 | 13 | 61.1-174.6 | 122.5±10.58 | 47.6 to 197.4 |
| | ≥71 | 6 | 70.1-154.6 | 107.2±15.35 | 33.5 to 180.9 |
| Gender | Female | 103 | 35.9-210.4 | 115.8±3.37 | 48.8 to 182.8 |
| | Male | 112 | 27.4-248.9 | 143.7±3.89 | 63.1 to 224.3 |

P = <0.001 (ANOVA) for age, P = <0.001 (t-test) for gender

To study the changes in splenic volume for males and females separately, we grouped the readings to three important age groups: (< 20, 20-49, and ≥50). We see a growing in splenic volume for teenagers (age < 20 years), the volume remains same in size as a plateau for young adults (age between 20-49 years) then, the splenic volume decline in elderly group (age ≥50 years) (Figure 2).

Table 3 shows that the mean of spleen volume has a significant increases as BMI increase from ≤ 23.1 to greater than 28 kg/$m^2$ (p=0.001) and higher significant increase with the BSA from ≤ 1.68 to greater than 1.9 $m^2$ (p<0.001); this is more illustrated with correlation to sex of patient. The BSA had a slightly stronger linear correlation with splenic volume than BMI in males and females (r=0.574, p<0.001 for females; r=0.5, p<0.001 for males).

**Table 3. Correlations between spleen volume, BMI and BSA**

| Parameter | | Spleen volume ($cm^3$) | | | |
|---|---|---|---|---|---|
| | | No. | Range | Mean±SD | SE |
| **BMI (kg/$m^2$)** | First quartile (≤ 23.1) | 57 | 27.4-219.7 | 117.7±41.3 | 5.47 |
| | Interquartile range (23.2 - 27.9) | 105 | 59-241.8 | 129.6±36.9 | 3.6 |
| | Fourth quartile ( ≥28.0) | 53 | 77.6-248.9 | 145.2±41.8 | 5.74 |
| **BSA ($m^2$)** | First quartile (≤1.68) | 54 | 27.4-174 | 99.2±33.9 | 4.62 |
| | Interquartile range (1.69 - 1.93) | 108 | 63.5-241.8 | 135.4±31.7 | 3.05 |
| | Fourth quartile (≥1.94) | 53 | 69.2-248.9 | 151.7±44 | 6.04 |

BMI: body mass index, BSA: body surface area, for BMI (r=0.35, p<0.001 for females; r=0.32, p<0.001 for males}, for BSA {r=0.574, p<0.001 for females; r=0.5, p<0.001 for males); ANOVA p=0.001 for BMI, p<0.001 for BSA

As shown in table 4 part A, age had the strongest independent association with the spleen volume in age group less than 20 years. The spleen volume is expected to increase by 7 $cm^3$ for each year after adjusted for gender .Male had significantly higher spleen volume by about 25.9 $cm^3$ as compared to females after adjusting the age in age group less than 20 years .The overall model was statistically significant and able to explain 71% of variation and cause variable, we had found regression equation which formulated as spleen volume ($cm^3$) = -21.3+ (7×age) + (25.9×gender).

Gender was the most important variable in explaining changes in spleen volume in age group between 20-49 years. Males had significantly higher in spleen volume by about 26.3 $cm^3$ as compared to females after adjusting age. Age is associated with a statistically significant increase in spleen volume in age group between 20-49 years, for each year; the spleen volume is expected to increase by about 1.1 $cm^3$ after adjusting for gender. The overall model was statistically significant & able to explain 17% of variation &cause variable, we had found regression equation which formulated as spleen volume ($cm^3$) = 87.5 + (1.1×age) + (26.3×gender) (Table 4 part B).

As shown in table 4 part C, male gender is associated with a statistically significant increase in spleen volume by 36.3 $cm^3$ compared to females at age group ≥50 years after adjusting age. Age is associated with a statistically significant decrease in spleen volume, for each one year increase in age, the spleen volume expected to decrease by 1.4 $cm^3$ after adjusting the gender. The overall model was statistically significant & able to explain 33% of variation and cause variable, we had found regression equation which formulated as spleen volume ($cm^3$) = 187.9+ (-1.4×age) + (36.3 ×gender).

**Table 4. Multiple liner regression models with spleen volume as the dependent (response) variable and age and gender as independent (explanatory) variables (stratified by age group)**

| | Age Group (Years) | Partial regression coefficient | Standardized coefficient | P value |
|---|---|---|---|---|
| **(A) <20** | Constant | -21.3 | | 0.22 |
| | Age in years | 7 | 0.748 | <0.001 |
| | Male compared to female | 25.9 | 0.334 | 0.014 |
| P (Model) <0.001, R2 = 0.71 | | | | |
| Regression equation: spleen volume = -21.3+(7×age)+(25.9×gender) (where male gender =1 and female gender =0) | | | | |
| **(B) 20-49** | Constant | 87.5 | | <0.001 |
| | Age in years | 1.1 | 0.204 | 0.007 |
| | Male compared to female | 26.3 | 0.35 | <0.001 |
| P (Model) <0.001, R2 = 0.17 | | | | |
| Regression equation: spleen volume = 87.5 +(1.1×age)+(26.3×gender) (where male gender =1 and female gender =0) | | | | |
| **(C) ≥ 50** | Constant | 187.9 | | <0.001 |
| | Age in years | -1.4 | -0.308 | 0.03 |
| | Male compared to female | 36.3 | 0.511 | 0.001 |
| P (Model) <0.001, R2 = 0.33 | | | | |
| Regression equation: spleen volume = 187.9+(-1.4×age)+(36.3 ×gender) (where male gender =1 and female gender =0) | | | | |

Body surface area had the strongest independent association with the spleen volume at age group less than 20 years old, for each one square meter increase in body surface area, the spleen volume increase by about 67.6 $cm^3$ at age group less than 20 years old after adjusting the age, gender and BMI. Male gender is associated with a statistically significant increase in spleen volume by about 20.5 $cm^3$ compared to female after adjusting the age and anthropometry measurements (BMI and BSA). BMI was statistically significant increase in spleen volume at age <20 years. For each one $kg/m^2$ increase in BMI, the spleen volume is expected to increase by 2.7 $cm^3$ after adjusting the age, gender and BSA as shown in table 5 part A. The overall model was statistically significant and able to explain 82% of variation and cause variable, we had found regression equation which formulated as spleen volume ($cm^3$) = -101.1 + (1.6×age)+ (20.5×gender)+(2.7× BMI )+(67.7×BSA).

In table 5 part B, BSA had the strongest independent association with the spleen volume at age group between 20-49 years. For each 1 $m^2$ increase in BSA, the spleen volume expected to increase by 70.9 $cm^3$ after adjusting the age, gender and BMI. Male gender is associated with a statistically significant increase in spleen volume by about 18 $cm^3$ compared to female after adjusting the age and anthropometric measurements the overall model was statistically significant and able to explain 27% of variation and cause variable, we had found regression equation which formulated as spleen volume ($cm^3$) = -37.8 + (0.7×age) + (18×gender) + (0.5× BMI)+(70.9×BSA).

Male gender is associated with a statistically significant increase in spleen volume by about 37 $cm^3$ compared to female at age group ≥50 years after adjusting the age and anthropometric measurements. Splenic volume decreased at age group ≥ 50 years (statistically not significant). BMI and BSA had no effect or statistically significant association with spleen volume when used in a multivariation model, we had found regression equation which formulated as spleen volume ($cm^3$)=165.1+(-1.3×age)+(37×gender)+(0.8×BMI)+(-3.2×BSA),table 5 part C.

**Table 5. Multiple linear regression models with spleen volume as the dependent (response) variable and age, gender, BMI and BSA as independent (explanatory) variables (stratified by age group)**

| Age Group (Years) | | Partial regression coefficient | Standardized coefficient | P value |
|---|---|---|---|---|
| **(A) <20** | Constant | -101.1 | | 0.004 |
| | Age in years | 1.6 | 0.173 | 0.51 |
| | Male compared to female | 20.5 | 0.264 | 0.026 |
| | BMI (kg/m$^2$) | 2.7 | 0.256 | 0.038 |
| | Body surface area (m$^2$) | 67.7 | 0.55 | 0.044 |
| **P (Model) <0.001, R2 = 0.82** | | | | |
| **Regression equation :spleen volume = -101.1+(1.6×age)+(20.5×gender)+( 2.7× BMI )+(67.7×BSA) where male gender =1 and female gender =0** | | | | |
| **(B) 0-49** | Constant | -37.8 | | 0.24 |
| | Age in years | 0.7 | 0.131 | 0.07 |
| | Male compared to female | 18 | 0.239 | 0.003 |
| | BMI (kg/m$^2$) | 0.5 | 0.062 | 0.48 |
| | Body surface area (m$^2$) | 70.9 | 0.306 | 0.001 |
| **P (Model) <0.001, R2=0.27** | | | | |
| **Regression equation :spleen volume = -37.8+(0.7×age)+( 18×gender)+( 0.5× BMI )+( 70.9×BSA) where male gender =1 and female gender =0** | | | | |
| **(C) ≥ 50** | Constant | 165.1 | | 0.09 |
| | Age in years | -1.3 | -0.279 | 0.1 |
| | Male compared to female | 37 | 0.52 | 0.003 |
| | BMI (kg/m$^2$) | 0.8 | 0.066 | 0.73 |
| | Body surface area (m$^2$) | -3.2 | -0.014 | 0.94 |
| **P (Model) =0.007, R2=0.33** | | | | |
| **Regression equation :spleen volume = 165.1+(-1.3×age)+( 37×gender)+(0.8× BMI )+(-3.2×BSA) where male gender =1 and female gender =0** | | | | |

## Discussion

The spleen is a large organ to the left of the stomach and below the diaphragm, serving to store blood, disintegrate old blood cells, filter foreign substances from the blood, and produce lymphocytes (white blood cells). In this study, we analyzed the changes in splenic volume with age using anthropometric measurement (BMI and BSA).

As shown in the results of this study, there is a significant increase in splenic volume from childhood and teenager to middle age and significant decrease at older age among all these subjects in both sexes. These results were in agreement with a previous study [18] where the splenic length and width of both sexes increases linearly with age until the middle age with a maximum of 12 cm, and thereafter undergoes gradual diminution, and a phenomena documented by Bisset et al [19]. Sharkawy et al [20] found all the measured parameters increased with age till 20 years. Previously reported [21] showed a rapid growth in splenic length up to age 20 years, followed by a mild decrease. Some authors showed a decrease in spleen diameter with increasing age [7,11] as well as the study of Arore et al [22] showed that the splenic length, width and thickness decreased with increased age in both males and females. Hoefs et al [23] found a linear correlation of splenic volume with

age and suggested formula. The results of our study partly agreed with the study of Adil et al [24] who found that a splenic volume also moderately correlated with age. While other studies [8,13] found no correlation of spleen size with the age of the subjects.

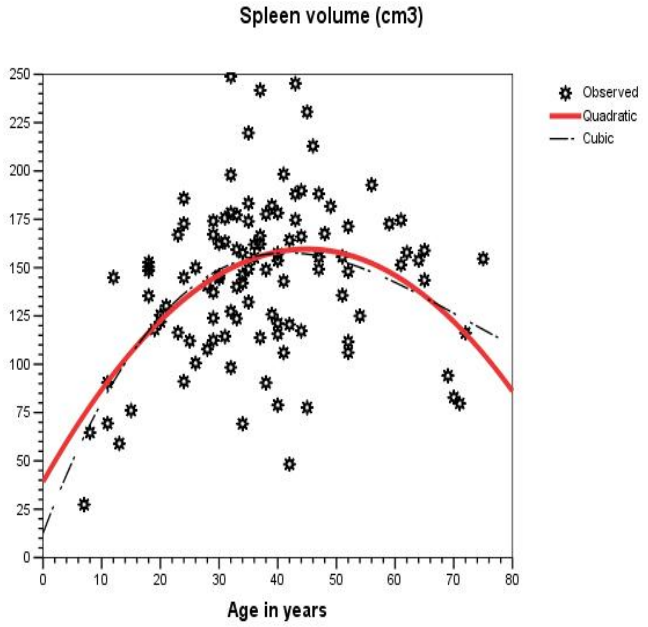


**Figure 1. Correlation between the splenic volume and age**

In the current study spleen volume is found to be larger in males than in females .The results come in concordance with some studies [10,25-28]. Results of a previous study [10] found statistical significant differences between male and female subjects from about 15-40 years with spleen of males being about 0.5 cm longer. Perhaps this may be due to the differences in height, weight, surface area and the genetic factors. This was different when compared with findings of prassopulos et al [9], kanekoj et al [11], Elsharkawy et al [20], Hoefs et al [23] and Ramak [29]. They observed that there are no differences in spleen size between genders.

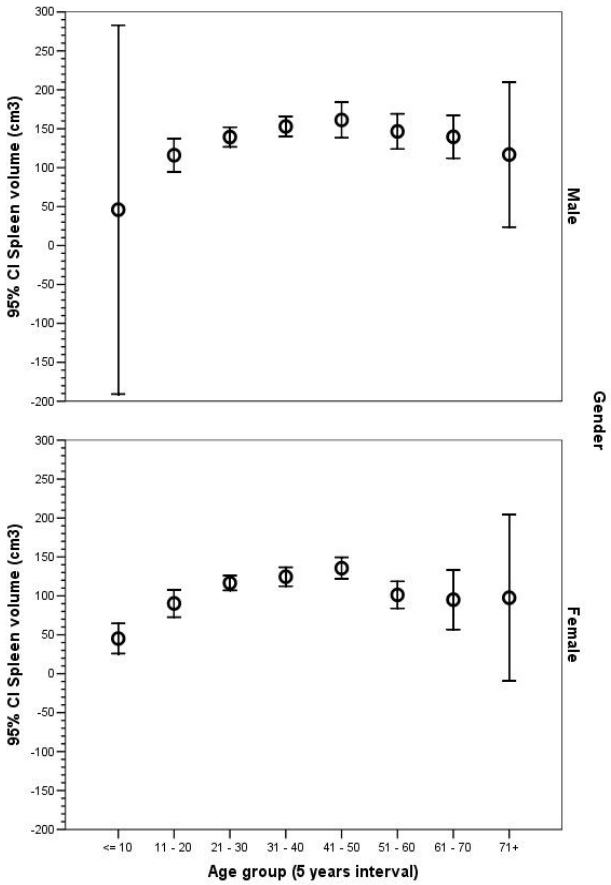


**Figure 2. The splenic volume by age after controlling for gender.**

Body surface area is a function of both height and weight either of which may vary separately. The results of our study showed that BSA had the strongest independent association with the spleen volume in subjects younger than 50 years, BMI showed statistically significant positive association with spleen volume at age <20 years .The relationship between spleen volume, BMI and BSA has been reported in literature [30]. The splenic volume correlated with BMI and BSA. The formulae spleen volume =BSA [278 *age (-0.36)] was derived and can be used to estimate the splenic volume. Spleen volume shows a strong correlation to its length as reported by previous studies [31,32]. Therefore spleen volume can be replaced its length deduction reasoning. Results in a prior study [33] found that the spleen length was highly

correlated with BSA. Multiple regression analysis indicated that BSA had significant positive association with spleen length in a sample with age range 1 day-17 years. This strong relationship between spleen volume and BSA is not surprising because for may be physiologic & clinical purposes and BSA is a better indicator of metabolic mass than body weight because its less associate with excessive body fat. On the other hand a study of Mustapha et al [13] didn't find a significant correlation between BMI and spleen volume, other study found the spleen volume was no significantly correlated with BSA [25]. This may be due to nutritional factors or environmental factors.

There were few limitations in this study. **First**, although depending on the use of a single investigator and the same equipment to perform the measurements in all the participants to increase the reproducibility (reliability) we were unable to assess interobserver variability .We used the mean of three measurements for each of the spleen parameters investigated to mitigate concerns about reproducibility and intraobserver variability. In one study, the mean splenic length measurements were shown to have a higher reproducibility than maximal splenic length measurements [34]. It would be more ideal if two observers were included which would allow the calculation of an interobserver error.

**second** we used the formula for a prolated ellipse to calculated the spleen volume as it is widely accepted in the published literature for the estimation of volume from unidimensional measurements .However, its recognized that the spleen can often be irregularly shaped, and the estimated volume using this formula is less accurate nevertheless, its wide use in the published literature allows us to perform meaningful comparison with our data.

In conclusion, splenic volume was significantly variable in different age groups. We see a growing in splenic volume in teenager (age <20 years), the splenic volume remain in the same size as a plateau in young adult (age between 20-49 years) then, the splenic volume decline in elderly group (age with greater than 50 years). Splenic volume for males was significantly higher than females. BSA had a significant positive association with spleen volume in subjects younger than 50 years. These results illustrate that the normal value of spleen volume affected by different factors 1) age 2) sex 3) BMI 4) BSA.

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# Lead Exposure Effects on Batteries Manufacturing Factory Workers in Baghdad

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## Abstract

**Background** Lead is an environmentally persistent toxin that causes pathologies and induced oxidative stress by reactive oxygen species (ROS) causing reduction of antioxidants and a weakening of defense system of the cell.

**Objective** To evaluate the occupational lead level and its impact on workers in Batteries Manufacturing Factory / Baghdad.

**Method** Blood, hair and urine samples were taken from 45 occupational lead exposed workers in Batteries Manufacturing Factory in Baghdad with age ranged (25-63) years during the period from October 2010 to the end of January 2011. Flame and flameless Atomic Absorption spectrophotometer were used in the measurements of blood lead and hair lead concentrations, HPLC was used in the measurement of vitamin E concentration, and ELISA was used for the determination of 8-Hydroxydeoxyguansin concentration.

**Results** The results in this study showed a high concentration of lead in blood and hair for exposed workers in comparison with the normal corresponding values for the control. The results also showed that there was a significant decrease in δ –Aminolevulinic acid dehydratase activity, a low level of vitamin E in the serum and an increase in the level of 8-Hydroxydeoxyguansin in urine of exposed workers.

**Conclusion** The correlation between oxidative stress parameters and clinical indices implies that there is a disrupted antioxidant balance which might contribute to lead induced toxicity in erythrocytes.

**Keywords** Lead exposure, δ–Aminolevulinic acid dehydratase, 8- Hydroxydeoxyguansin, Antioxidants, Lead battery.

## Introduction

Lead is a dangerous heavy metal which is widely spread in the environment. Lead content in the air, food and tap water has increased several folds during recent years due to extensive use of this metal in petrol, paints, battery and other industries [(1)]. The high lead levels in the blood of exposed workers are expected to produce clinical symptoms of lead intoxication such as anorexia, muscular pain and headache. Despite of many attempts to reduce the exposure to this metal, there are still some reports of cases with severe lead toxicity [(2-4)].

The pathogenesis of lead toxicity is multifactorial as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body [(5)].

Lead affects directly the hair because lead is an electrophile that forms covalent bonds with sulfhydryl group of cysteine in proteins. Keratin in hair contains a high fraction of cysteine relative to the other amino acids and strongly binds to lead [(6)]. Lead toxicity leads to free radical damage via two separate, although

related, pathways (A) The generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide. (B) The direct depletion of antioxidant reserves [5].

In any biological system where ROS production increases, antioxidant reserves are depleted. In this situation, the negative effects on the human systems ability to deal with increased oxidative stress occur via independent pathways, the body can limit and repair the damage of these species by some enzymes like ceruloplasmin (Cp), superoxide dismutase (SOD), glutathione peroxidase (GPx), Catalase (CAT), as well as by some vitamins such as vitamin A, vitamin E and vitamin C [7].

In a study of 137 lead exposed workers, those with high blood lead levels (over 40 µg/dl) had significant reductions in blood GPx that correlated with elevated erythrocyte malondialdehyde (MDA) level (a clinical marker of oxidative stress). Those with lower lead exposure (25-40 µg/dl) had elevated levels of GPx, a suggested compensatory reaction for increased lipid peroxidation [8].

On the other hand there are some studies which suggest that the supplement of vitamin E reduce the effect of oxidative stress which is induced by lead levels increasing SOD and catalase activity [9-11].

Nakao *et al* found that the erythrocyte δ–Aminolevulinic acid dehydratase (δ–ALA-D) activities of exposed workers ranged from 10 to 58 mmol/ml of ertherocytes/hr which is lower than normal [12].

Urinary δ–AminolevulinicAcid (δ–ALA) and (δ–ALA-D) activity in blood were convenient indicators with a better specificity if compared with other indicators such as porphobilinogen (PBG), and zinc protoporphyrinogen (ZPP), for screening occupational lead exposure [13]. δ–ALA-D concentration is currently regarded as the most reliable index of exposure to lead [12,14,15].

8-Hydroxydeoxyguanosine (8OHdG) is a modified base that occurs in DNA due to attack by hydroxyl radicals that are formed as by-products and intermediates of aerobic metabolism and during oxidative stress. 8OHdG has become increasingly popular as a sensitive, stable and integral marker of oxidative damage in cellular DNA.

A significant increase in the concentration of 8-OHdG is caused by exposure to tobacco smoke, heavy metals and ionizing radiation. 8-OHdG is correlated with oxidative stress and damage to DNA, which leads to the development of an antibody [16], this antibody can be used in the measurement of (8-OHdG) in urine using immunoassays technique, so (8-OHdG) can serve as a biomarker of oxidative stress [17].

## Methods

*Chemicals and Instruments*

During the work of this study a large number of chemicals has been used, and many instruments, therefore it has been decided to mention these chemicals and instruments with their origin in the text of procedure instead of having a list of these chemicals and instruments.

***Methods***

This study was carried out in Batteries Manufacturing Factory in Baghdad and the laboratory investigations were conducted in the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University within 6 months period starting from the first of October 2010 till the end of January 2011. The occupational lead exposed workers were 45 total, 30 were males while 15 were females; their age between (25-63) years and mean of 43.2±8.9 years. They were in contact with lead in different parts of the factory.

Forty healthy volunteers with matching age and Body Mass Index (BMI) were chosen as control. Prior to biological specimens' collection, occupational and clinical information were collected from the exposed subjects and control group using questionnaire and interviews. Dietary intake and food habits of subjects were also recorded. Workers suffering from major chronic diseases such as diabetic mellitus, heart or kidney diseases were excluded from this study. Random urine samples were collected to

avoid the errors from the inadequate collection of 24hr urine samples.

***Determination of lead concentration***

● In whole blood

After the dilution of samples and standards (1:10) with distilled water, the blood was homogenized by (25ml) of 10% triton X-100 (Riedel-deHaen). This step was followed by the addition of 5ml of 20% ammonium dihydrogen phosphate (Merk-Darmstdt), with one ml of concentrated nitric acid (BDH) then, the volume was completed to 500ml with distilled water, and the measurements were carried out at 283 nm, using flameless atomic absorption spectrophotometer (Graphite furnace) (GFAS), (GFA-EX7i –Shimadzu).

●In hair

After the digestion of the hair by concentrated nitric acid (BDH) then the solution was diluted (1:50) with deionized water. The calibration curve was plotted automatically by the instrument itself. Flame atomic absorption spectrophotometer (Shimadzu-6200) was used in this determination.

***Determination of urinary δ-ALA concentration and the activity of δ-ALA dehydratase***

A spectrophotometer (UV/VIS), (biotech-UV-2601-UK), was used in the measurement of urinary ALA concentration by method of Wada *et al.* 1969 [12]. The elevated urinary ALA concentration was indicated by reddish color in chloroform, while normal concentration usually gives faint yellow or faint red color at 556nm.

The activity of δ-Aminolevuilinic acid dehydratase in erythrocytes was estimated using Helen's method [18]. The principle of method to determine δ-Aminolevulinic acid dehydratase activity in blood is to adequately control the pH of the enzyme substrate solution at the optimum throughout the incubation period. This control of pH was to improve the sensitivity and reliability of the assay. N-ethylmaleimide (Fluka Company) has been substituted for a potentially hazardous mercury salt, used to remove sulfhydryl groups before color development with a modified Ehrlich's reagent (Merk-Darmstdt).

***Evaluation of vitamin E level in serum***

High Performance Liquid Chromatography (HPLC), (shimadazu–Japan), was used for this purpose. The serum was deproteinized by mixing with 15% sulphosalicylic acid (Fisher Scientfic UK limit). The mixture was then centrifuged, diluted, and analyzed at 285 nm.

***Estimation of the 8-Hydroxydeoxyguansin level in urine***

Urinary 8-hydroxydeoxyguansin level was measured using, Enzyme Linked Immunosorbent Assay (ELISA), (Biotech-ELx800/England), according to the procedure of the kit cayman USA (Item No. 58920).

## Results

Results in table 1 show the lead levels in the blood and hair of exposed workers which reflects the different duration of exposure.

Table 1 shows also a high level of lead in the hair of exposed workers when compared with that of control. This high level of lead in hair becomes even higher with longer duration of exposure.

Figure 1 show the correlation between the lead level in the blood for exposed workers and lead level in their hair (r=0.4302).

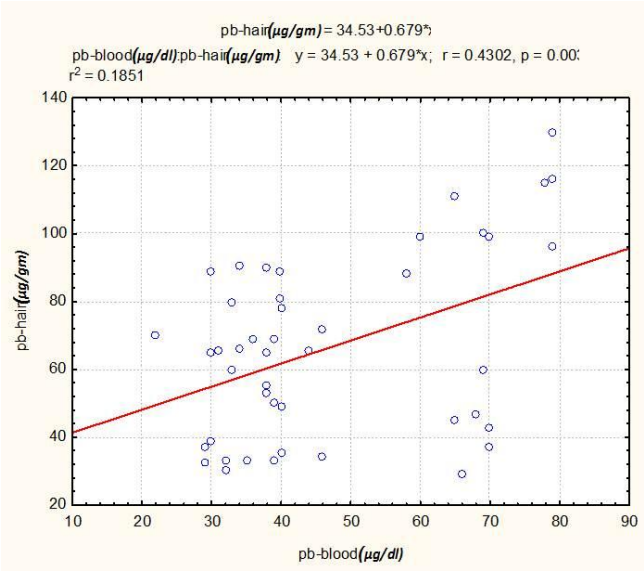


**Figure 1. Correlation between pb-hair and pb-blood in EW**

The results in table 2 show the activities of δ-Aminolevulinic acid dehydratase in the blood of exposed workers and the control group. The results in table 2 show the concentrations of δ-Aminolevulinic acid (ALA) in the urine of the exposed workers and the control group. The results in table 3 show the concentrations of 8-OHdG in the urine of exposed workers and the control group.

**Table 1. The effect of exposure duration on the concentrations of lead in blood and lead in hair for exposed workers (EW)**

| Groups | | Sample size | Pb-blood (µg/dl) Mean±SD | Pb-hair (µg/gm) Mean±SD |
|---|---|---|---|---|
| NEC | | 40 | 13.15±5.64 | 16.3±9.15 |
| EW | 0-10 years | 8 | 34.3±7.28$^{a*}$ | 59.6±22.89$^{a*}$ |
| | 11-20 years | 23 | 44.5±14.52$^{a*,b}$ | 56.9±20.5$^{a*,b}$ |
| | 21-30 years | 9 | 52.97±18.79$^{a*,b*,c}$ | 74.4±26.0$^{a*,b,c,}$ |
| | 31-40 years | 5 | 69.0±15.18$^{a*,b*,c*,d}$ | 107.5±24.46$^{a*,b*c*,d†}$ |

NEC: non exposed control, EW: exposed workers, †= P<0.01, * = P<0.0001, a = comparison between EW exposure duration and control group, b = comparison between exposure duration for 0-10 years period and other subgroups, c = comparison between exposure duration for 11-20 years, 21-30 years period and 31-40 years period, d = comparison between exposure duration for 21-30 years period with 31-40 period.

**Table 2. The effect of lead exposure on the U δ-ALA concentration and δ-ALAD activity in the blood for exposed workers**

| Groups | | No. | Urineδ- ALA Mean±SD (µmol/L) | δ-ALA dehydratase Mean values (mmol/ml of erythrocytes/hr) | p-value |
|---|---|---|---|---|---|
| NEC | | 40 | 3.6±o.65 | 97.4±10.5 | |
| EW | 0-10 years | 8 | 4.6±1.64a* | 60.7±14.8a* | $t^a$ -p<0.0001 |
| | 11-20 years | 23 | 7.3±1.2a*,b* | 47.1±9.9a*,b | $t^b$ - p<0.0001 |
| | 21-30 years | 9 | 9.2±1.4a*,b*,c*, | 33.9±12.0a*,b†,c*, | $t^c$ - p<0.0001 |
| | 31-40 years | 5 | 13.2±0.69a*,b*c*,d* (ns) | 31.1±7.2a*,b†,c*,d | $t^d$ -p<0.0001 |

NEC: non exposed control, EW: exposed worker, U-δ-ALA: urinary δ-Aminolevulinic acid, δ-ALA-D: δ-Aminolevulinic acid dehydratase, † = P <0.01, * = P<0.0001, a = comparison between EW exposure duration and control group, b = comparison between exposure duration for 0-10 years period and other subgroups, c = comparison between exposure duration for 11-20 years, 21-30 years period and 31-40 years period, d = comparison between exposure duration for 21-30 years period with 31-40 period.

**Table 3. The effect of exposure duration on the mean values of the urine 8-OHdG concentrations of the exposed workers (EW)**

| Groups | | Sample size | Urine level of 8-OHdG Mean±SD (ng/ml) |
|---|---|---|---|
| NEC | | 40 | 100.1±16.33 |
| EW | 0-10 years | 8 | 131.4±6.0$^{a*}$ |
| | 11-20 years | 23 | 160.7±17.4$^{a*,b*}$ |
| | 21-30 years | 9 | 178.0±15.6$^{a*,b*,c*,}$ |
| | 31-40 years | 5 | 186.0±10.3$^{a*,b*c**,d}$ |

NEC: non exposed control, EW: exposed workers, 8-OHdG: 8-hydroxydeoxyguansin, † = P<0.01, * = P<0.0001, a = comparison between 8-OHdG concentration for EW and control group, b = comparison between exposure duration for 0-10 years period and other subgroups, c = comparison between exposure duration for 11-20 years, 21-30 years period and 31-40 years period, d = comparison between exposure duration for 21-30 years period with 31-40 period.

**Table 4. The mean value of vitamin E concentrations (mg/dl) in serum of exposed workers (EW) according to exposure duration**

| Groups | | Sample size | Serum level of vitamin E Mean±SD (mg/dl) |
|---|---|---|---|
| NEC | | 40 | 1.0±0.36 |
| EW | 0-10 years | 8 | $0.47\pm0.11^{a*}$ |
| | 11-20 years | 23 | $0.28\pm0.13^{a*,b*}$ |
| | 21-30 years | 9 | $0.23\pm0.09^{a*,b,c,}$ |
| | 31-40 years | 5 | $0.15\pm0.07^{a*,b*,c*,d}$ |

NEC: non exposed control, W: exposed worker, * = P<0.0001, a = comparison between EW and control group, b = comparison between exposure duration for 0-10 years period and other subgroups, c = comparison between exposure duration for 11-20 years, 21-30 years period and 31-40 years period, d = comparison between exposure duration for 21-30 years period with 31-40 period.

The results in table 4 show the concentrations of vitamin E for exposed and control group. Figure 2 shows the correlation between the concentrations of lead in blood and urinary δ – ALA for the exposed workers. Figure 3 shows the correlation between the concentrations of lead in the blood and the activities of δ-ALA-D in the blood for the exposed workers. Figure 4 gives the correlation between vitamin E values in serum and 8-OHdG in urine for the exposed worker.

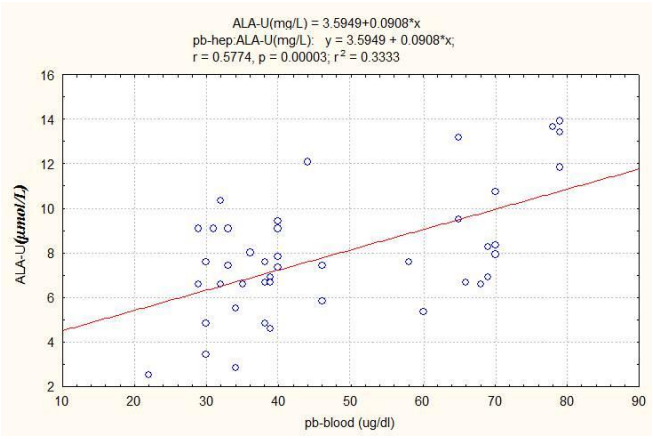


**Figure 2. Correlation between ALA-U and pb-blood in EW.**

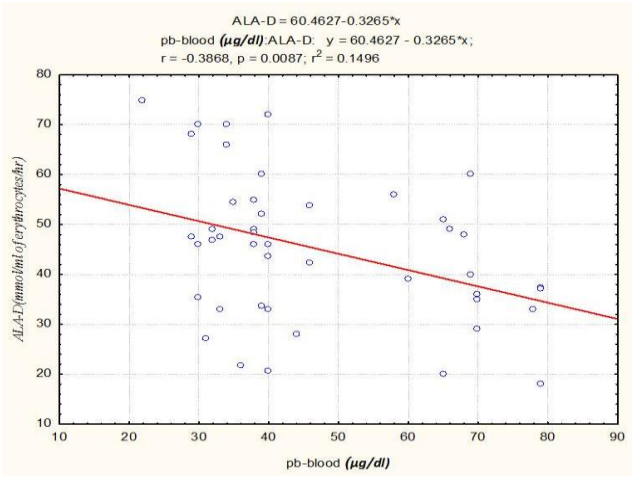


**Figure 3. Correlation between ALA-D and pb-blood in EW**

## Discussion

The results in table 1 prove the expectation of pollution in this factory and show clearly that this group of exposed workers having a high concentration of lead in their body represented by their blood and hair. The measured values of the lead blood levels depend on an equilibrium between the lead that is stored in the different parts of the body and that which is excreted outside the body [19].

The longer duration of lead exposure caused an increase in the concentration of lead in the blood and hair, and the correlation between blood lead levels and that of hair lead was positive for exposed workers, (r=0.4302, p=0.0032).

Results in table 2 revealed that there is a decrease in the activity of δ-Aminolevulinic acid dehydratase (δ-ALA-D) and this decrease become even more with the increased duration when compared with that of the control group. The reason behind this decrease in the activity of

δ-Aminolevulinic acid dehydratase enzyme is the inhibition by lead. The enzyme of δ-ALA-D is the most sensitive enzyme to the toxic effects of lead [20], therefore it has been selected for this purpose.

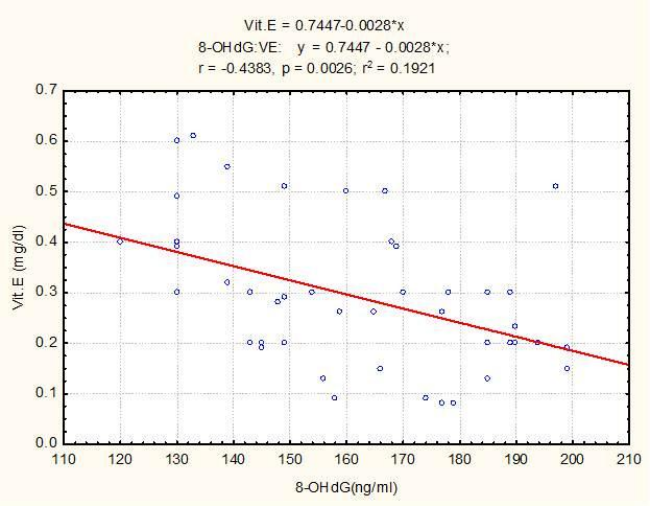


**Figure 4. Correlation between vitamin E and 8-OHdG in EW groups**

Table 2 shows also that the concentration of δ-Aminolevulinic acid (δ-ALA) in urine was higher than that of the control group and increases with increased duration. This increase in δ-Aminolevulinic acid concentration in urine can be explained as follows: Under normal physiological conditions, 90-95% of the ALA that is filtered is reabsorbed in the tubules. The reabsorption rate of ALA decreased with increasing serum ALA concentration [21]. The toxic damage to the renal tubules caused by the high concentration of lead may contribute to the decreased reabsorption rate [22]. The urinary δ-ALA and δ-ALA-D activity in the blood could be considered as biomarkers for the lead toxicity.

There is a positive correlation between the concentration of lead in blood and urinary δ-ALA for exposed workers with (r=0.5774,p=0.00003) as shown in figure 2, while figure 3 show a negative correlation between the concentration of blood lead and the activity of δ-ALA-D in the blood for the exposed workers where (r= -0.3868, p= 0.0087).

The results that have been obtained in table 3 show an increase in the concentration of 8-HydroxyDeoxyguanosine (8-OHdG) in urine and this increase become even more with the increase in the duration of exposure. The increase of 8-OHdG in urine could be explained depending on the fact that lead is inducing the oxidative stress which has been identified as the primary contributory agent in the pathogenesis of lead poisoning[23].

Results obtained in table 4 show a reduction in the level of vitamin E and this reduction increased even more with increasing duration of exposure. Vitamin E (Alpha-tocopherol, active form) protects the membrane lipoproteins of RBC from oxidative damage that is caused by lead toxicity through the lowering of lipid peroxide levels and increasing the activity of Superoxide dismutase (SOD) and catalase [24]. Therefore, this decrease in vitamin E enhances the susceptibility of RBC to the hemolytic effect of lead poisoning [25].

Figure 4 indicated a negative correlation between vitamin E in the serum and 8-OHdG in the urine for exposed workers (r= - 0.438, p= 0.00026).

The results of this study indicated that there is a definite oxidative stress due to lead pollution. This increased concentration of 8-OHdG in urine and impaired antioxidant such as vitamin E could be attributed to the increased blood lead concentration which induce oxidative stress.

Also, blood lead levels were considerably elevated in battery manufacturing factory workers, and the degree of elevation was increased with increasing their duration to lead exposure.

The determination of lead in hair could be considered as a useful screening test in estimating occupational lead exposure and it is a short term, inexpensive and non – invasive method.

The biomarkers urine ALA and erythrocyte δ-ALA-D activities levels are most suitable and convenient markers to screen and confirm occupational lead exposure.

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# Medico-legal Study of Fatal Incised Wounds in Baghdad

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## Abstract

**Background** Sharp force injuries and fatalities have been reported to be the most common crimes of violence in several countries, predominantly in those where access to firearms is restricted. Death due to sharp force violence is the most common cause of homicidal deaths in Sweden and in many other countries in Europe, Africa and Asia. However incised wounds are less common in homicide.

**Objective**

**Method** A medico-legal descriptive study conducted on 18 autopsies during 6 months period. Cases were studied according to their age, sex, scene of incident, number of injuries, presence of other types, suggestive manner of injury and anatomical regions affected. Blood sample was taken for alcohol detection then complete classical autopsy was done.

**Results** Incised wound cases were about a third of total sharp wound cases. Males were 5 times more frequently involved than females with an age range 36.4±29.1. Indoor and outdoor scene of incidents was almost equal. Most of them were multiple with mixed types of sharp wounds. Suggestive homicidal manner of death was seen. The neck was the most common anatomical region affected. There was no role of alcohol in causation of such injuries.

**Conclusion** Incised wounds are infrequently encountered in medico-legal autopsy practice. Males seem to be more prone to such injuries with multiple and mixed types in the majority of cases. Homicidal manner was suggested in all cases and neck was the preferable site for the perpetrator.

**Keywords** Incised wound, sharp wound, autopsy, Homicide.

## Introduction

A wound or injury can best be defined as damage to any part of the body due the application of mechanical force[(1)].Sharp force injuries span the spectrum of incised and stab wounds [(2)]. These injuries are generally limited to the path of the object with no significant component of transmitted force [(3)].

Wounds caused by pointed and sharp-edged weapons can be divided into four categories:

1. Stab wounds.
2. Incised wounds (cuts).
3. Chop wounds.
4. Therapeutic/diagnostic wounds [(4)].

Incised wounds are clean cut wounds through the tissues, caused by a sharp-edged instrument. Their length is longer than their depth [(5)]. They are frequently caused through slashing movements by bladed weapons such as knives and razors [(6)]. Direction of the wound is established by the phenomena of tailing of the wound. According to this, all incised wounds are deeper at point of commencement and shallower at termination. The deeper end is called *head of the wound* and the shallower end called *tail of the wound* [(7)]. Fatal incised wounds are usually suicidal, then homicidal; only occasionally they are accidental [(8)].

Incised wounds are less dangerous than stabs, as the relative shallowness of the wounds is less likely to affect vital organs, in as much as the arms and the face are the common targets [1].Bleeding is the most serious complication of any incised wound, though it will be external and more amenable to immediate treatment than the hidden internal bleeding of a stab wound [1].

Cutting injuries are generally located at the neck and upper extremities (flexor side of the wrist and antecubital fossa) with varying depths [9]. They are superficial in tentative marks of suicide and deep in fatal homicidal injuries (sometimes reaching vertebral bodies for the throat cuts) [10].

Fatal incised wounds are usually suicidal, then homicidal and only occasionally accidental. In suicide, fatal wounds are usually present in front of neck, wrists, groin and chest or back of legs, and the clothes are spared, in addition to the presence of hesitation or tentative cuts [5].

While in homicide, the wounds may be on any part of the body, usually more multiple, severe and at more than one site. The clothes are not spared. They are associated with defensive wounds on the body [8].

The major cause of death in victims with incised wound injury is massive sudden external hemorrhage leading to hypovolemic shock and finally death (loss of about 1/3 of total blood). Infection of the wound may cause toxemia or septicemia and death by shock (tetanus may also cause death) [8]. Death from incised wounds of the neck may be due not only to exsanguinations, but also massive air embolus [4].

Death due to sharp force violence is the most common cause of homicidal deaths in Sweden and in many other countries in Europe, Africa and Asia [11]. A retrospective study was carried out on 125 homicide victims autopsied from 2006 to 2010 at the Forensic Medicine Center in Dammam, Saudi Arabia. Of these cases 83.2% were males and 16.8% were females, and in 77.6% the victims were between the ages of 21 and 50 years. Most of the homicides occurred during daytime (80.1%), and summer was the most common season for the occurrence of such incidents. Among this sample the second most common type of injury was caused by sharp force (34.4%) [12].

Another retrospective study was carried out on 152 deaths due to sharp force injuries from 1993 to 2004 at the department of forensic medicine, Regional Institute of Medical Sciences, Imphal (India), revealing that sharp force injuries represent the second major class of injuries with an overall incidence of 3.07%. Stab wounds accounted for 40.79%, chop wounds 34.21% and cut throat injuries 25%. The most vulnerable age groups were between 16-30 years (46%) followed by 31-45 years (32.9%). Brain was the most commonly involved vital organ followed by the great blood vessels (especially neck vessels). These injuries were found to be more common among males than females [13].

## Methods

A descriptive prospective study was carried out on 18 medico-legal cases referred to the Medico-legal Institute in Baghdad from the first of January 2011 till the end of June 2011. Of those cases, 15 were males and only 3 cases were females. Their age ranged from14-70 years.

The cases were examined with respect to their sex, age, the weapons used, number and type of wounds found on the body and anatomical location of injuries. Furthermore, alcohol analyses were conducted during the autopsies of the cases studied.

Information regarding each case was obtained from investigation authority, police reports, and close relatives of the victim, eye witnesses and past medical history. This information include the age, sex, time and date of injury ,time and date of death, history of chronic diseases and psychiatric history.

External examination for each case was performed, which include examination of the clothes for any marks of damage, stain, and other legal evidences, and correlation with the site of injury on the body [14].

Digital photograph was taken prior to the removal of the clothes, then gross examination

of the naked body which includes any sign of bruises or abrasions and other wounds and their location in addition to incised wounds number, site, and presence of other wound types. Search was also done for the presence of any hesitation or tentative wounds, as well as examination for wounds viability by looking for evidence of reactive inflammation at wound edges grossly [15]. Any associated injuries (like bullet, burn, blunt force injury, etc.) were also reported, and then another digital photograph was taken to the naked body. X-ray survey was also done to detect any piece of weapon which might be present inside the body and to detect other bone injuries. It is also helpful in detection of air embolism in cases with incised wound injury to the neck in addition to the presence of haemothorax and pneumothorax due to other types of injuries.

Blood samples from the femoral vessels (5 ml) were collected in tubes by disposable syringes and preserved with 1% of sodium or potassium fluoride for detection of alcohol in the blood, then stored in a refrigerator for future analysis [16]using GC for alcohol.

## Results

Fifteen of 18 cases were males and only 3 were females, a ratio of 5: 1. Their age range was between 14-70 years with a mean of 36.4±29.1 years.

Incised wound as a cause of death was representing 0.7% only from total autopsy cases during the period of the study (2409) and 1.2% from total violent causes of death (1557) as shown in figures 1 and 2 respectively.

They were representing 35% from the total sharp wound cases (51) during that period while the remaining 65% were other types like stab puncture and chop wounds as it is shown in figure 3.

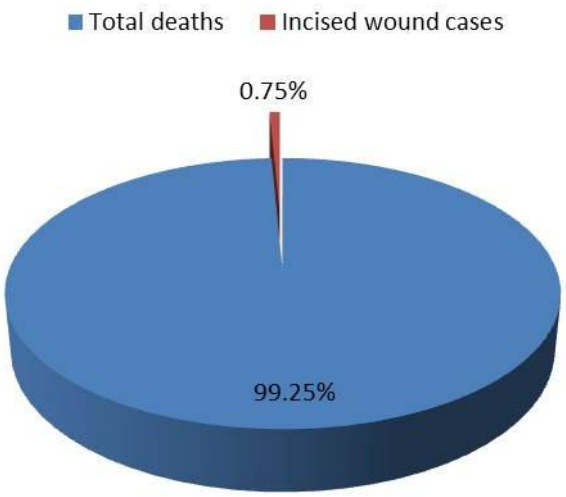


**Figure 1: A pie-chart showing relative frequency of incised wound cases to the total deaths no.**

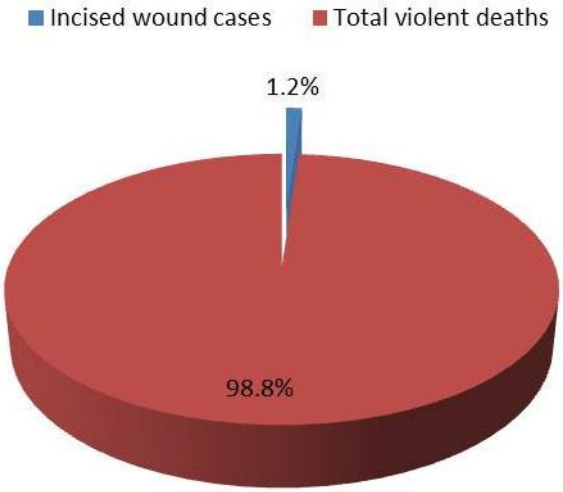


**Figure 2: A pie-chart showing relative frequency of incised wound cases to the total violent deaths number**

Figure 4 shows that 8of incidents occurred indoors and 7 were outdoors while 3 were with unknown scene of incident.

Five cases sustained single incised wound (cut throat type) and the remaining 13 were with multiple sharp force injuries as shown in figure 5. The number of wounds in each case ranged between 1 to 31 sharp wound as most of the cases were with mixed types of incised and stab wounds.

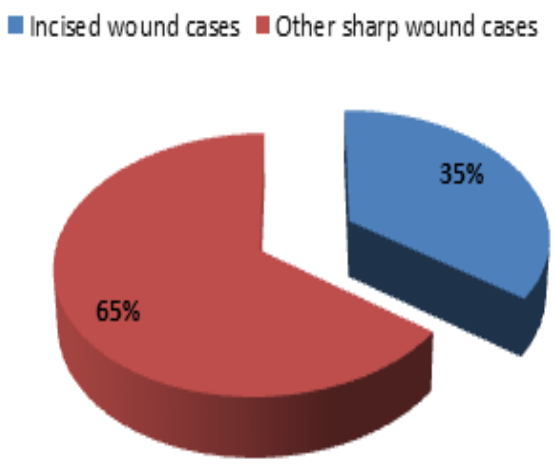


**Figure 3: A pie-chart showing relative frequency of incised wound cases to the total sharp wound cases**

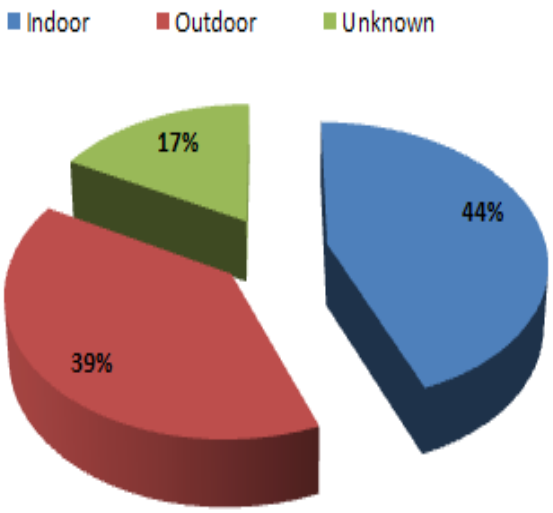


**Figure 4: Frequency distribution of incised wound cases according to the scene of injuries**

Homicidal manner of death was suggested in all cases examined in spite of the absence of any signs of struggle and resistance.

Almost all the wounds were viable and signs of bleeding with inflammatory reactions were seen at wound edges; only 2 nonviable wounds were noticed in 2 different cases with multiple and mixed types of sharp wounds.

The Neck was the most common anatomical site of injuries followed by the head and chest equally as shown in figure 6.

Alcohol detection was negative in all blood samples examined in the study.

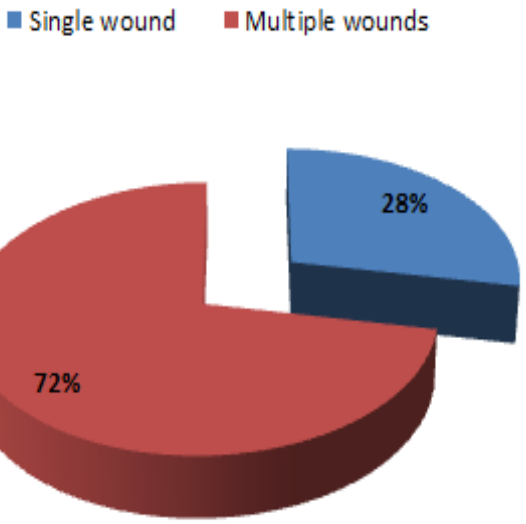


**Figure 5: Frequency distribution of incised wound cases according to the multiplicity of wounds.**

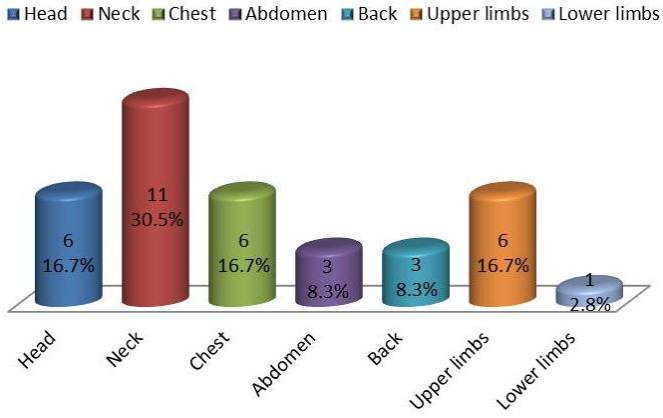


**Figure 6: A bar chart showing the distribution of incised wounds among anatomical regions**

## Discussion

Sharp force injuries in general represent the 8th class of major injuries as shown in this study preceded by firearm injuries, road traffic accidents (RTA), burns, electrical shock, blunt force injuries, asphyxias and explosions respectively (many victims of explosions are not referred to the Medico-Legal Institute). This frequency disagrees with 2similar retrospective studies carried out in India and USA [13,17]. These low figures in this study compared to the higher figures in the previous studies may be due to the easiness to get firearms and the unstable security situation in Baghdad has resulted in

wide use of firearms in committing crimes instead of sharp weapons.

**Figure 7: Incised woundof the chest**

As a result, fatal incised wounds constituted only a smaller percent of total sharp wound types as the perpetrator prefer to inflect fatal injuries by stabbing his victims as much as he can to kill him (Figure 3).Similar results regarding genderwhere males were more frequently affected than females and this is in agreement with a study on homicide by sharp force performed in Scandinavia with same percentages and almost similar sex ratio [(18)].

Regarding the scene of injuries, almost there was equality between indoor and outdoor site of injuries (Figure 4). This result is in disagreement with the result of another study done in India, in which most of victims were killed outdoors [(19)]. This disagreement may be due to different cultural, environmental and socio-economic backgrounds.

Most of the cases (13) were with multiple incised types of injuries mixed with other types (Figure 5).This result differs from that of another study which was carried out in France in whichthe total number of all types of sharp wounds was much higher with a range of 1 to 65 injuries per case[(18)], which is slightly higher than our result. This could be due to larger sample size in their study, as well as the differences in the motives in committing crimes between the two studies.

The Neck was the most common anatomical region affected by incised injuries while the chest comes next (Figure 6). These results disagreed with that of a study carried out in India, in which the chest was the region most often involved followed by the abdomen [(19)].

The difference between the results of these two studies could be because of the different manner of death as it was 100% homicide in this study and different motives of the crimes, as the main motive of crime in the present study was armed robbery for the victim`s car (most of the male victims were taxi drivers) according to the information. So the exposed areas of the victim`s body (the driver) to the offenders (sitting usually in the front and back seats while driving) were the neck, chest and head (the main targets).

No role of alcohol was found in the study, unlike its significant role in a study done in UK; this could be explained by differences in the religious and cultural backgrounds between the two countries [(20)].

**Figure 8: Defense wound of the hand**

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# Role of Ectopic Prolactin on Thyroid Hormones Level in a Sample of Iraqi Infertile Women with Uterine Fibroids

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## Abstract

**Background** A large number of traditional investigations and bioassay of hormones have been practiced in the diagnosis and management of infertility for a long time. Measurements of prolactin and thyroid stimulating hormone have been considered important components of the evaluation of women presenting with infertility.

**Objectives** To study the effect of prolactin hormone produced from uterine fibroid(s) on thyroid hormones levels and role of these hormones.

**Methods** One hundred three women with uterine fibroid were entered to the Obstetrics and Gynecology Department in Al-Kadhimyia Teaching Hospital in Baghdad-Iraq from the 15th of June 2007 to December 30th 2010. Fasting serum prolactin, total $T_3$, total $T_4$, and thyroid stimulating hormone (TSH) were estimated using MiniVIDAS [ELFA (Enzyme Linked Fluorescent Assay)] kits.

**Results** Forty five out of 103 (43.69%) women were found with primary infertility and the rest 58 (56.31%) with secondary infertility. Serum thyroid hormones mean ± standard deviations were found normal before and after surgery in both primary and secondary infertile women. Level of serum prolactin was found elevated about 9 folds in primary infertile women and 8 folds in those with secondry infertility before surgery more than their levels after. No significant difference was found between thyroid hormones before and after surgery in both infertile groups, unlike their prolactin which was found highly significant with p value <0.001 in both groups.

**Conclusion** It can be concluded that, first, the increase in prolactin level was due to an ectopic production from uterine fibroid(s), and second, there is no effect or role of this prolactin on their thyroid hormones function. Third, their infertility could be due to the presence of prolactin secreting fibroid(s).

**Keywords** Infertility, Ectopic prolactin, Thyroid hormones, Uterine fibroids

## Introduction

Infertility is defined as the failure of a couple to achieve a pregnancy despite one year regular unprotected sexual intercourse. A large number of traditional investigations and bioassay of hormones have been practiced in the diagnosis and management of infertility for a long time. By extensive studies it has been proved that for normal sexual function, thyroid secretion of $T_3$, $T_4$ needs to be approximately normal. The actions of thyroid hormones cannot be pinpointed to a specific function but probably result from a combination of direct metabolic effects on gonads and excitatory and inhibitory effects operating through anterior pituitary hormones that control sexual functions [(1)].

Prolactin is a polypeptide hormone secreted by the anterior pituitary gland, whose main role is the stimulation of lactation in the postpartum period. The increase in prolactin secretion can

be physiological (pregnancy and lactation) or pathological (hypothalamic and pituitary diseases, iatrogenic, uterine fibroids, *etc.*). Hyperprolactinemia is usually associated with menstrual and ovulatory disorders like amenorrhea, oligomenorrhea, an ovulation, ovulatory cycles with short or inadequate luteal phase, and galactorrhea [(2)].

Traditionally, measurements of prolactin and thyroid stimulating hormone have been considered important components of the evaluation of women presenting with infertility [(3)].

Uterine fibroids are one of the most common benign tumors of the female genital tract, occurring in 20-50% of women of reproductive age. The presence of uterine fibroids has been connected to fertility problems and many studies have been performed in order to define the correlation between fibroids and infertility. Unfortunately, still nobody can draw definitive conclusions on this matter [(4)].

Although initially identified as a pituitary gland hormone, several studies have demonstrated that prolactin is also produced by uterine tissues, including the endometrium, myometrium, and uterine leiomyomas. The significance of prolactin production in leiomyomas is not yet well defined [(5)]. In this study, we aimed to investigate the relation between thyroid hormones and prolactin produced from uterine fibroid(s) of infertile women, and their role on their infertility.

## Methods

One hundred three women with uterine fibroid entered to the Obstetrics and Gynecology Department in Al-Kadhimyia Teaching Hospital in Baghdad-Iraq from the 15$^{th}$ of June 2007 to December 30$^{th}$ 2010 suffering from symptoms associated with their uterine fibroid(s). They were at their reproductive age. Forty five out of 103 (43.69%) women were found with primary infertility and the rest 58 (56.31%) with secondary infertility. All other possible types of infertility were excluded from the study.

Patients with a history of previous operation or medical illness such as diabetes mellitus, thyroid diseases including chronic autoimmune thyroiditis, hypertension, renal disease, pituitary adenoma or those who were taking any medication which might affect their hormonal level or using contraceptives and smoking cigarette were also excluded from this study. All this information in addition to diagnosis whether they were primary or secondary infertility was under the supervision of a specialized gynecologist. Ultrasound was used to confirm the presence of uterine fibroid(s). They were undergone either for hysterectomy or myomectomy after they finished their final phase of the menstrual cycle. Fibroids were benign leiomyoma depending on their histopathological results. Women with serum prolactin levels >742 µIU/ml (normal prolactin reference range 106-742 µIU/ml) were advised CT scan or MRI to exclude the presence of pituitary adenoma under the supervision of specialized radiologist. They were considered as hyperprolactinemic.

Blood samples were drawn under sterile conditions from patients prior to operation, left to clot, and centrifuged at 800 x g for 10 minutes at room temperature. The circulating levels of PRL, total $T_3$, total $T_4$, and thyroid stimulating hormone (TSH) were determined by means of mini-VIDAS (ELFA; Enzyme Linked Fluorescent Assay) using a Prolactin Kit (Biomerieux Inc., Durham, North Carolina, USA).

The patients were followed up after their surgical treatment, and serum PRL, total $T_3$, total $T_4$, and thyroid stimulating hormone (TSH) were re-estimated again 3 weeks after surgery to avoid the effect of operation stress and to compare their levels with those estimated before. The same kits and instruments were used to measure all samples.

Their weight (Kg) and height ($m^2$) were measured to calculate their body mass index (BMI) in Kg/$m^2$. Ethical approval and patient permission were obtained prior surgery for the local ethics committee and to conduct the study.

**Statistical Analysis**

Statistical analysis was performed using the Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, IL., USA). Descriptive statistics such as mean, standard deviation (SD) and one-way ANOVA were used to evaluate the significance (*p*-value) between study variables before and after surgery. A *p*-value of <0.01 was considered statistically highly significant.

## Results

Patient's serum total $T_3$, $T_4$, TSH and prolactin were estimated before and after surgery. Thyroid hormones level (mean ± standard deviations) were found normal before and after surgery in both primary and secondary infertility groups. Serum prolactin level was found elevated about 9 folds in primary infertile women and 8 folds in those with secondry infertility before surgery more than after. This elevated level returns to its normal range after removing fibroids from their uterus. There mean± standard of body mass index was within the overweight range(25-29.9 Kg/$m^2$) as shown in table-1.

**Table 1. Clinical presentation of study patients before and after surgery**

| | | Infertility | | P value |
|---|---|---|---|---|
| | | Primary (n=45) | Secondary (n=58) | |
| Age (years) | | 26.2±2.35 | 25.5±1.16 | |
| BMI(Kg/m) ▲ | | 25.37±1.71 | 29.74±0.89 | |
| Serum total $T_3$ (nmol/l) | B | 0.98±0.95 | 0.97± 0.95 | NS |
| NRR=(0.95-2.5) | A | 0.97±0.93 | 0.97± 0.94 | |
| Serum total $T_4$(nmol/l) | B | 88.12± 1.5 | 91.01±1.1 | NS |
| NRR=(60-120) | A | 84.93± 0.97 | 89.12±1.2 | |
| Serum TSH(µIU/ml) | B | 1.1 ±0.14 | 0.91±0.16 | NS |
| NRR=(0.25-5) | A | 1.0±0.21 | 1.02±0.11 | |
| Serum PRL(µIU/ml)** | B | 3785.5±1796.5 | 2921.8± 756.4 | <0.001 |
| NRR=(106-742) | A | 400.9± 44.3 | 361.2± 36.66 | |

▲Reference range for BMI (normal BMI=18.5-24.9Kg/$m^2$, Over weight BMI=25-29.9 Kg/$m^2$, Obese BMI=30-34.9 Kg/$m^2$), B= before surgery, A=after surgery, NRR= normal reference range, NS=not significant, **= P<0.001.

No significant difference was found between thyroid hormones before and after surgery in both infertile groups, unlike prolactin hormone which was found increased with highly significant p value (<0.001) in both infertile groups.

## Discussion

Fibroids are benign (that is, non-cancerous) tumors of the uterus. They are also called uterine leiomyomas, or simply myomas. They grow from the muscle cells of the uterus and may protrude from the inside or outside surface of the uterus or they may be contained within the muscular wall. They may cause infertility in a number of different ways. Some studies have suggested that fibroids in the muscle portion of the uterus may cause an alteration or reduction of blood flow to the uterine lining making it more difficult for an implanted embryo to grow and develop. Another theory suggests that even small fibroids that grow inside of the uterine cavity may act as a foreign body and result in an inflammatory reaction that makes the uterine environment hostile for an embryo to implant [(6)]. It is known that hyperprolactinemia is a common problem encountered in reproductive disorders. The understanding that prolactin hypersecretion

not only causes galactorrhea and amenorrhea but also gonadal dysfunction and infertility led to the wider use of prolactin estimations [7]. Indinnimeo *et al* [8] reported that serum concentrations of PRL, which is a trophic hormone produced by the pituitary gland, have been shown to be raised in certain groups of patients with cancer. It was detected in 0-20% of the colon cancer by immunohistochemistry and in the plasma in 6-53% of the patients. Also, Hsu *et al* [9] concluded that surgical removal of cervical carcinoma resulted in normalization of serum PRL concentrations. This explains the increase of PRL concentration level in patient's sera, and its decline to its normal level after surgery. It should be mentioned that all patients in this study were examined by MRI and/or CT scan to exclude the presence of pituitary adenoma. This step was important for each patient before removing her fibroid(s).

In addition to its production by the pituitary gland and the decidualized endometrium of the late luteal phase, the human myometrium has been proposed as a second source of uterine PRL, since immunoreactive PRL was found in supernatants from myometrial explants cultures [10]. Prolactin can function as a circulating hormone and as a cytokine. The explanation of this function is based on PRL production and its distinct regulation in extrapituitary sites, it's binding to membrane receptors of the cytokine receptor super family, and activation of signaling pathways that promote cell growth and survival. Many studies showed increasing evidence of PRL and said that PRL plays a role in several types of cancer in the reproductive and non-reproductive tissues via local production, or accumulation. Considering PRL as an active participant in tumorigenesis should inspire and encourage the development of novel therapies aimed at reducing tumor growth by suppressing PRL production, or by blocking its receptors [11]. In a previous study, it was found that prolactin level in patients with uterine fibroids was increased with a significant positive correlation with fibroid's size [12]. Another study done by Abdulla and Baban 2010 reported that the highest serum prolactin mean was found among patients with uterine fibroids presented with site of pain while tissue prolactin was found at its highest mean among women presented with irregular menstrual flow and menorrhagia while asymptomatic were 15% and 25% of them presented with infertility [13].

Hyperprolactinemia adversely affects fertility potential by impairing GnRH pulsatility and thereby ovarian function [14,15]. Most obstetricians and gynecologists check serum levels of TSH and PRL in every female patient undergoing an infertility evaluation regardless of their menstrual rhythm [16].

It has been suggested that hypogonadism seen in hyperprolactinemic women is due to high circulating levels of prolactin interfering with the action of the gonadotrophin at the ovarian level and impaired gonadal steroid secretion, which in turn alters positive feedback effects at the hypothalamic and pituitary levels. This leads to lack of gonadotrophin cyclicity and to infertility [7]. Hyperprolactinemia in this study is due to the ectopic production of prolactin from patient's fibroid(s), and this ectopic prolactin has different profiles and bioactivity. So, the theory of interference which causes infertility [7] cannot be supposed in this study. Because of absence of pituitary adenoma, the main reason for their hyperprolactinemia was the fibroids and this cause when removed the level of their prolactin returned to normal. This explanation agreed with several studies that demonstrated prolactin production by uterine tissues in addition to pituitary gland, including the endometrium, myometrium, and uterine fibroids. The interest in this hormone has been stimulated by the finding that prolactin acts as a mitogen for vascular smooth muscle [17].

Measurement of prolactin and thyroid hormones, especially thyroid stimulating hormone (TSH), has been considered an important component of infertility work up in women [18]. Several articles have highlighted the association of hyperthyroidism or

hypothyroidism with menstrual disturbance, anovulatory cycles, decreased fecundity and increased morbidity during pregnancy. For example, Goswami *et al*. 2009 mentioned that a hormonal disorder of female reproductive system is comprised of a number of problems resulting from aberrant dysfunction of hypo-thalamic-pituitary-ovarian axis [(19)]. These relatively common disorders often lead to infertility. While Nasima 2009 reported that although her study results reflect some clinical findings of women with primary and secondary infertility such as irregular menstruation, oligomenorrhea, acne, hirsutism, obesity, goiter, and poly cystic ovary, but the difference was not statistically significant, and no correlation was observed between TSH and PRL levels [(16)].

During our articles survey, no one has studied thyroid hormones and TSH levels in patients with uterine fibroids. All articles explained the relation between infertility and thyroid dysfunction [(20,21)].

Since thyroid hormones and TSH were within the normal ranges before and after surgery in the present study, and the increase in patient's prolactin before surgery is due to the presence of fibroid(s), it can be concluded that, first, the increase in prolactin level was due to an ectopic production from uterine fibroid(s), and second, there was no effect or role of this prolactin on their thyroid hormones function. Third, their infertility could be due to the presence of prolactin secreting fibroid(s).

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# Histopathological Changes of the Mice Placenta Exposed to Lead Acetate

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## Abstract

**Background** Lead is one of the most dangerous metals and could be incorporated in various body tissues and thus exposure to it stills a major medical problem in both environmental and occupational setting.

**Objectives** To detect the deleterious and toxic effects of the lead acetate on the weight and histological features of mice placenta at different dosages.

**Methods** A prospective study including eighty mature pregnant mice were divided into two groups (experimental and control groups). Forty pregnant mice were divided into two major experimental groups (G1and G2) according to the level of the dose. The other forty animals with same age divided at the same way as in the previous experimental groups considered as control groups (C). Injections of lead acetate 0.1, 0.4 mg/kg body weight /day intraperitoneally (G1, G2 respectively) were started at the first day of gestation and continued for (17 or 20 days). When the female in each experimental and control groups reach day 17 of gestation 10 animals of each group were scarified, whereas the other 10 animals were left to be scarified at 20 day post coitum (dpc). Weight of placentas was recorded, in addition, histological study for these placentas were done.

**Results** Prenatal lead acetate injection to pregnant mice intraperitoneally for 17 day cause a significant reduction ($P<0.05$) in placental weight and a highly significant reduction ($P<0.01$) in placental weight was recorded at 20 dpc experimental groups (G2) but there was no significant decrease in weight of placenta for 17 and 20 dpc in experimental groups (G1) In addition placentas belongs to (G1 and G2) mothers revealed histopathological alterations in the various component of the placenta.

**Conclusion** Administration of low dose of lead acetate intraperitoneally to pregnant female mice causes significant decrease in weight of their placenta. Lead acetate also causes adverse effects on the histological features of fetal placenta.

**Key words** Lead, Placenta, Placental transport toxicology, Toxic trace elements, Metallothionein, Reproductive toxicology

## Introduction

During recent decades concerns have been raised about human infertility that might stem from exposure to environmental contamination [(1)]. Lead (Pb) is one of the most environmental pollutant [(2)], known to be a poisonous compounds for centuries [(3)], it is consistently observed to be a reproductive toxicant, causing decreased fertility and pregnancy loss [(4)]. Furthermore, life in utero as a developing embryo and fetus may be the most vulnerable period for lead toxicity to occur [(5)].

The placenta is generally described as the interface between the mother and the outside world and the developing fetus [(6)], it anchors the developing embryo to the uterine wall and connects it to the maternal blood stream, thus supplying the embryo with ions and metabolites and providing a waste-removal mechanism for the embryo [(7)]. More reliable evidence for the

permeability of the human placenta to lead began to appear in the 1930's, when chemical analytical techniques for detecting lead in blood and other tissues were developed [8]. The study of Georgieff MK et al clearly demonstrated the presence of lead in fetal tissues and left little doubt that the human placenta was permeable to this toxic trace metal [9]. Abundant early evidence of this hazard came from reports that women working in lead exhibited unusually high rates of sterility, spontaneous abortion, and stillbirths [10]. A study of Hertz-Picciotto et al. which was conducted on apparently normal humans, indicated that placental transfer of lead began as early as the 12$^{th}$ week of gestation and that the total lead content in fetal tissues increased throughout pregnancy [11]. Furthermore, maternal blood lead concentration was highly correlated with umbilical cord lead suggesting the transplacental movement of lead to the fetus, which crosses the placenta by passive diffusion and it has measured in the fetal brain as early as the end of first trimester (13 weeks). The high $Pb^{++}$ levels in the umbilical cord blood propose that the essential placental barrier for the $Pb^{++}$ passage to the fetus does not exist [6]. Moreover the mean concentration of lead in women was 2.56 µg/d and in umbilical cord blood was 2.01µg/d. A positive correlation was noted between lead concentration in maternal and umbilical cord blood (r=0.59) [12].

The toxic action of $Pb^{++}$ on the fetus is expressed by decreased intra-uterine growth, low birth weight [13], chromosomal aberration, macro-cephaly, miscarriages, still birth and early death of offspring [14]. A study by Bressler et al showed that during early gestation inorganic lead crosses the placental membranes rapidly and in significant amounts, even at very low maternal blood levels, and the yolk sac placenta is the primary transfer site for lead [15]. In addition, the generalized distribution of radioactivity observed in the embryos indicated that all major organ systems are exposed to lead ions during this very critical period of development [6]. In this study, the histopathological changes of the developing placenta, and the weight of the placenta of mice will be studied after injecting the pregnant mice with different dose of lead acetate.

## Methods

This experimental study was carried out at high Institute for infertility diagnosis and assisted reproductive technologies, Al-Nahrain University, A total of 80 mature female Swiss-Webster mice, weighing 25-30 g and age of about 8-10 weeks were divided equally into two groups (experimental and control groups). Forty animals were divided into two major experimental groups (G1and G2) according to the level of the dose (20 animal/group). Each major group subdivided into two minor experimental groups (10 animals/group) according to different periods for killing during gestation period (day17, and day20). The other 40 animals with same age divided at the same way as in the previous experimental groups considered as control groups (C) injected intraperitoneally by normal saline only. Vaginal smear were performed to all the adult female mice to detect heat stage for mating. Females in the estrous phase were left with mature healthy males for mating (1 male/1 female). In mice, day 1 of pregnancy was designated by the presence of a copulatory plug in the vagina [16].

The pregnant female was transferred into separate cage. Injection of lead acetate (0.1, 0.4 mg/kg body weight/day intraperitoneally for the experimental groups G1, G2 respectively were started at the first day of gestation and continued for ( 17 or 20 days) while the two parallel control groups were injected normal saline with the same rout and dose as that used in the experimental groups. At 17 and 20 days of gestation, 10 animals of each group were scarified at each of these intervals. The embryos were dissected into placental corn and embryonic portions using a scissor. Placentas were removed and placed in a dish containing worm normal saline. The numbers of placentas were recorded washed and weighed using sensitive electrical balance, then fixed in Bouin's solution, paraffin sections with 5 micron thickness were prepared and stained with hematoxylin eosin stain for histological study [17]. Data from treated and

control groups are expressed as mean ± standard error (SEM) and analyzed using student t-test to compare values from experimental and control groups at individual time points. Differences between groups were considered significant at (P<0.05) and highly significant at (P<0.01) [18].

## Results

A significant decrease (P<0.05) in weights of placenta belongs to mothers injected with 0.4 mg/kg body weight (b.w.) of lead acetate (G2) at 17 dpc while highly significant reduction (P<0.01) was recorded at 20 dpc, in comparison with that of control group (C) as shown in figure 2 and table 1, while the result showed no significant decrease in weights of placenta belongs to mothers injected with 0.1 mg/kg b wt. of lead acetate at 17 and 20dpc,in comparison with that of control group as shown in figure 1 and table 1.

**Table 1. Changes in weight of placenta associated with administration of (0.1, 0.4 mg/kg b.w.) (G1) and (G2) of lead acetate to pregnant female mice for 17 and 20 dpc**

| Placenta weight | Control | | Treatment | | | |
|---|---|---|---|---|---|---|
| | 17 day | 20 day | 0.4mg/k.b.w. 17 day | 0.4mg/k.b.w. 20 day | 0.1mg/k.b.w. 17 day | 0.1mg/k.b.w. 20 day |
| Mean | 0.10284 | 0.11145 | 0.05831* | 0.00988** | 0.11736 | 0.09928 |
| SD | 0.02874 | 0.03050 | 0.04404 | 0.02786 | 0.06121 | 0.01545 |
| SE | 0.0079 | 0.00847 | 0.01223 | 0.00774 | 0.01700 | 0.00429 |

k.b.w.: Kilogram body weight, * = p<0.05, ** = P<0.01

### Histological observations

1. Non-treated experimental group (Control group) [C].
   The histological sections of placenta belong to mothers from control group showed huge numbers of villi. Each villous contain a mesenchymal core, containing fetal capillaries. The villous surface area exposed to the lacuna filled with maternal blood. Between the villous capillaries and maternal blood is a continuous layer of syncytiotrophoblast supported by a layer of proliferating cytotrophoblast cells (Figures 3-5).
2. Experimental group female mice treated with 0.1 mg/kg b.w. of lead acetate at 17 and 20 dpc (G1); The histology of placenta belong to these mothers showed a reduction in the thickness and distruption of trophoplastic septa, necrotic area in the labrinth zone and deciduas, cystic degeneration of glycogen cells, appoptosis of trophoblast and stromal cells of placental tissue, irregular dilatation of maternal sinusoid, fibrin depostion around villi and calcification and hyalinized villous spots (Figures 6-9).
3. Experimental group female mice treated with 0.4 mg/kg b.w. of lead acetate at 17 and 20dpc (G2); the histology of placenta in this experimental group showed the same histological observations as in G1 but more extensive and promanent (Figures 10-12).

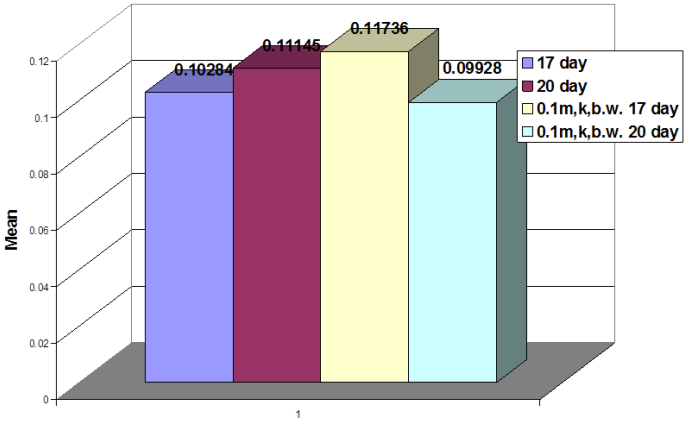


**Figure 1. Changes in weight of placenta associated with administration of (0.1 mg/kg b wt.) (G1) of lead acetate to pregnant female mice for 17 and 20 dpc**

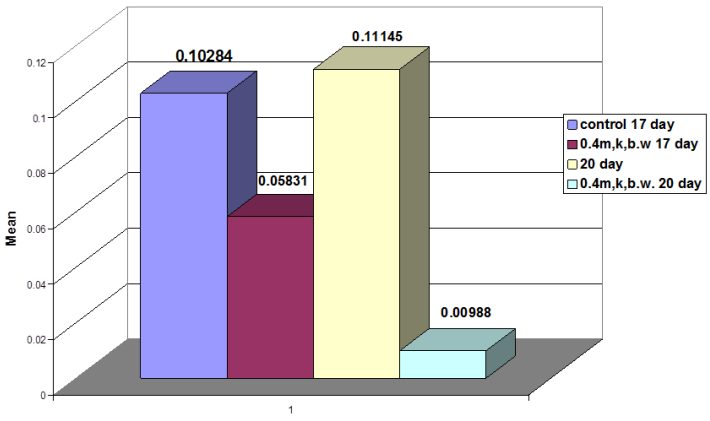


**Figure 2. Changes in weight of placenta associated with administration of (0.4 mg/kg b.w.) (G2) of lead acetate to pregnant female mice for 17 and 20 dpc**

## Discussion

In the current study the results revealed that injection of lead acetate to the mothers at dose (0.1 mg/kg b.w.) for 17 and 20 dpc (day post coitum) did not affect significantly the weight of placenta but the results demonstrated that the placental weight at dose (0.4 mg/kg b.w.) at 17 dpc was significantly decreased ($P<0.05$) and highly significant decreased ($P<0.01$) in 20 dpc compared with control group, these finding is agree with the studies of Wang Yun-Ying et al [19] who cited that fetal body weight, body length and placental weight were significantly lower ($P<0.05$) in the lead exposed rats.

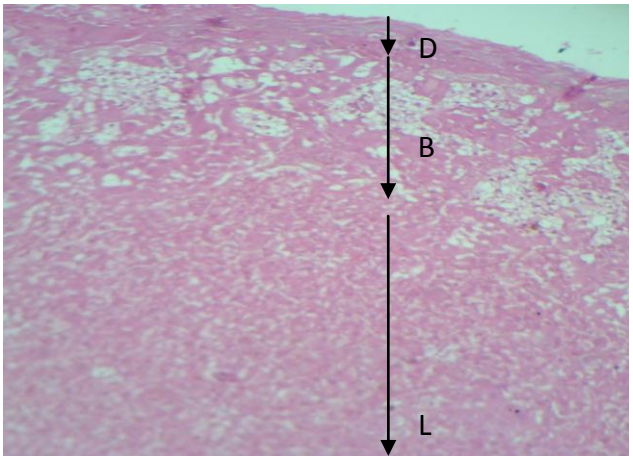


**Figure 3. Micrograph illustrate normal structure of placenta from full term mice fetus (control group, 20dpc), showed the labyrinth zone (L) wich contain the maternal sinusoid and the trophoblastic septa. The basal zone (Junctional zone) (B) wich comprised of spongiotrophoblast cells, trophoblastic gaint cells and glycogen cells. Decidual zone (D) (Maternal part of placenta), wich is a vascular densely packed with decidual cells..(10X, H&E)**

The decrease in placental weight may be attributed to the fact that trophpblastic cells are specialized for nutrient transfer, energy storage, hormone production and invasion [20]. Moreover tissue oxygen levels regulate the proliferation and differentiation of human trophpblast cells [21]. Lead readily crosses the placental-fetal barrier [22], during different gestational period and has a traumatic effect on the trophpblast, leading to interference of nutrition and oxygen exchange between mother and fetuses. The blood supply to the placenta was also interfered, leading to reduction of placental weight and retardation of development of fetuses [23]. In lead-treated animals, placental blood flow per embryo weight is reduced [24].

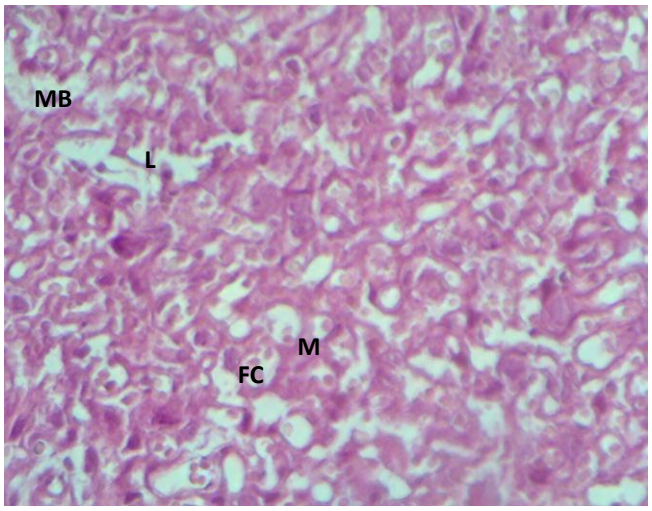


**Figure 4. Micrograph illustrate placenta from full term mice fetus,(control group, 17dpc), the villi are seen to have a highly cellular core of mesenchyme (M). Maternal blood (MB) in the surrounding lacuna (L). The trophoblas is reduced to a thin layer of syncytiotrophoblast only and the fetal capillaries (FC) tend to be located in the periphery of the core (40X, H&E)**

In addition placental growth, development and aging are crucial to the overall being well of the fetus and are controlled by many of endocrine signals, including steroids and growth factors [25]. Trophopblast giant cells are situated at the maternal-placental interface and are one of the major endocrine cells of the placenta, they synthesize and secret steroid and peptide hormones [16]. Estrogen is known as an inhibitor of placental growth [26]. The regulation of

estrogen biosynthesis in the placenta is very important for human embryos because altering placental function can cause permanent effects on embryos [27]. Lead exposures in utero significantly reduce steroid production [28], and have antiestrogenic activity [29].

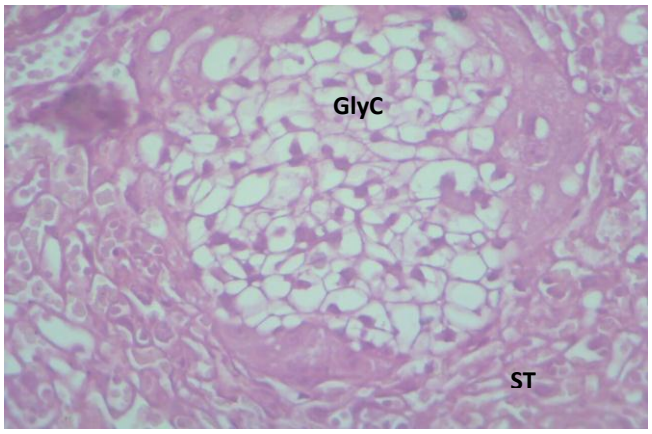


**Figure 5. Micrograph revealed the basal zone of placenta belong to mother from control group (20 dpc). The histological section showed a well developed spongiotrophoblasts (ST) around clusters of glycogen cells (GlyC) (40X, H&E)**

The decrease in placental weight may also be attributed to the mitotic inhibition, apoptosis, degeneration and/or necrosis of trophoblast, which are induced by direct placental injury or nonspecific effects associated with conditions of an excessively unfavorable maternal environment [30], leads to a lack of cells populations required for later normal histogenesis, resulting in small placenta [23]. Furthermore injury to cytoblasmic organelles may interfere with the nutrition and oxygen exchanges between mother and fetus, and may contribute to abnormal pregnancy outcome [19], and low birth weight [31].

The histological sections of placenta of the fetuses belong to mothers from experimental groups showed alterations in the various components.

In the lead-exposed placenta, expression of lead toxicity was detected in the necrotic area in the labyrinth zone at 17 and 20 dpc in G1 and G2 compared to control group (Figure 8, Figure 10, Figure 12) this observation agree with the studies of Satoshi et al [32], who cited that histological examination of the placenta revealed that there was apparent damage and patchy necrosis of the villous syncytiotrophoblastic cells in the study group in comparison to the control group.

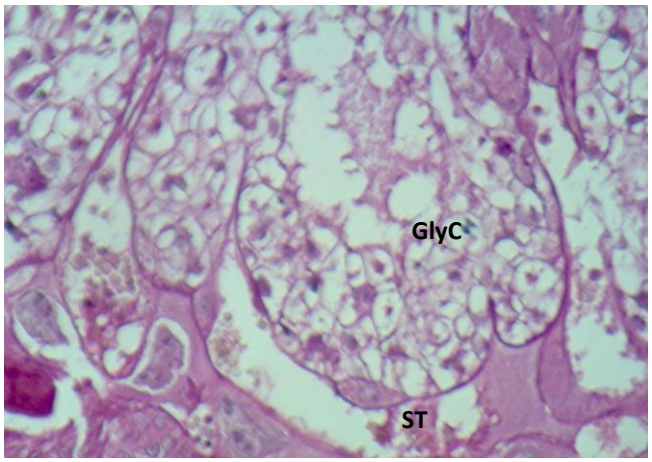


**Figure 6. A histological section of placenta belong to mother treated with 0.1 mg/kg b.w. of lead acetate at 17 dpc, showed cystic degeneration of glycogen cells (GlyC) and apoptosis of spongiotrophoblast cells (ST) in basal zone. (40X, H&E)**

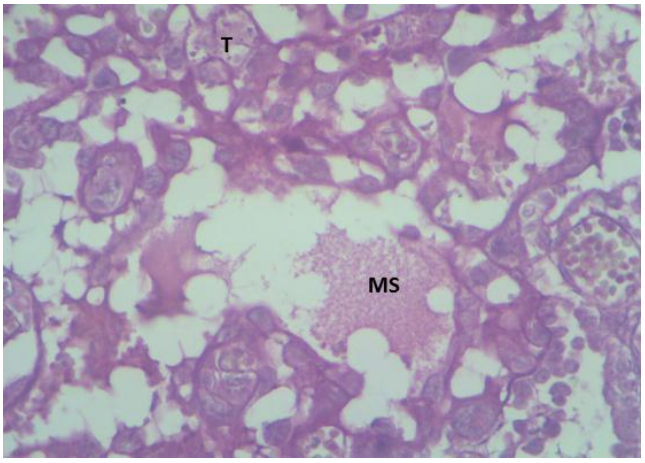


**Figure 7. A histological section of placenta belong to mother from prenatal exposed to lead acetate at dose 0.1 mg/kg b.w. at 17 dpc, showed vacuolar degeneration and apoptosis of trophoblasts (T) with fibrin deposition around the villi and irregular dilatation of maternal sinusoid (MS) in the labyrinth zone. (40X, H&E).**

This is also agreement with the previous studies conducted by Zhonghua et al who cited that in the experimental group of lead poisoning rats, the placenta showed focus necrosis in the deciduas and increased the trophoblast giant cells [23].

Moreover, trophoblast in the fetal part of the placenta are a common toxicological target tissue for some drugs and chemicals, because they have high proliferative activity and constitute a major structural component of the fetal part of the placenta [32].

Histological observations of basal zone in this study revealed a cystic degeneration of glycogen cells in both experimental groups (G1 and G2) (Figure 6), which is a condition describing abnormal retention of extensive cytoblasmic vacuolation within glycogen cells.

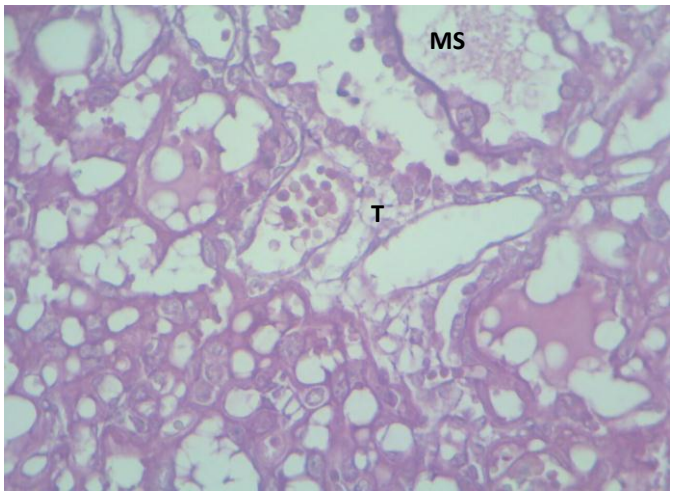


**Figure 8. The micrograph illustrate placenta belong to mother treated with 0.1 mg/kg b.w. lead acetate at (20dpc), revealed villus necrosis (arrow), vacuolar degeneration of trophoblasts (T), fibrin deposition and irregular dilaation of maternal sinusoid (MS) in the labyrinth zone. (40X, H&E)**

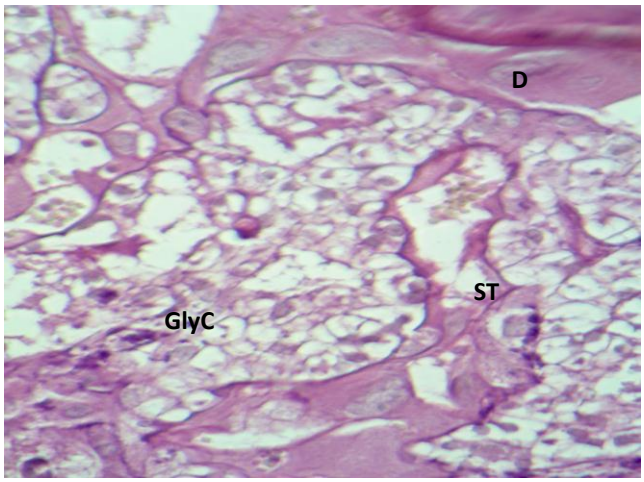


**Figure 9. The micrograph revealed the basal zone of placenta belong to mother treated with 0.1 mg/kg b.w. lead acetate at (20dpc), The histological section showed apoptosis of spongiotrophoblasts (ST) around clusters of degenerative glycogen cells (GlyC). Note fibrin deposition in ducidual zone (D). (40X, H&E)**

The degenerative cells undergo cytolysis and subsequently coalesce into multiple large cysts that are filled with multiple clusters of residual glycogen cells and cell debris. Placental apoptosis was also clearly obvious in both endothelial cells, trophoblast and stromal cells of placental tissue in both experimental groups (G1 and G2) (Figure 7, Figure 9, Figure 11), this correspond to work of Wang Yun-Ying et al [19], who cited that cell cycle arrest and DNA damage in trophoblast leads to apoptosis and mitotic inhibition in the labyrinth zone inducing growth arrest. Moreover, DNA fragmentation that was indicative of apoptosis was clearly present in basal and labyrinth zone of the placenta at each stage of gestation. This may be attributed to some lead-induced damages that may occur as a consequence of its propensity for disrupting the delicate pro-oxidant/antioxidant balance that exists within mammalian cells. The mechanism for lead induced oxidative stress includes the effect of lead on membrane, DNA, and antioxidant defense system of cells [33]. Lead induced oxidative stress might result from accumulation of 5-amino levulinc acid (ALA), a potential endogenous source of free radicals, induced by inhibition of lead to ALA dehydratase [34].

Additionally, direct interaction of lead with biological membranes may induce lipid peroxidation. Lead exposure might also induce decrease in activities of free radicals scavenging enzymes. This is mainly attributed to high affinity of lead to sulfhydryl-groups in these enzymes [35]. Moreover lead and other heavy metals have high affinities for glutathione (GSH), which is the primary intracellular antioxidant [36]. Furthermore lead can be concentrated in the cell nucleus thus perturbing cell proliferation and DNA synthesis [19]. Beside syncytiotrophoblast has been shown to be the site of metal-lothionein synthesis, a protein that binds lead, a

mechanism for sequestering in mature tissues [37].

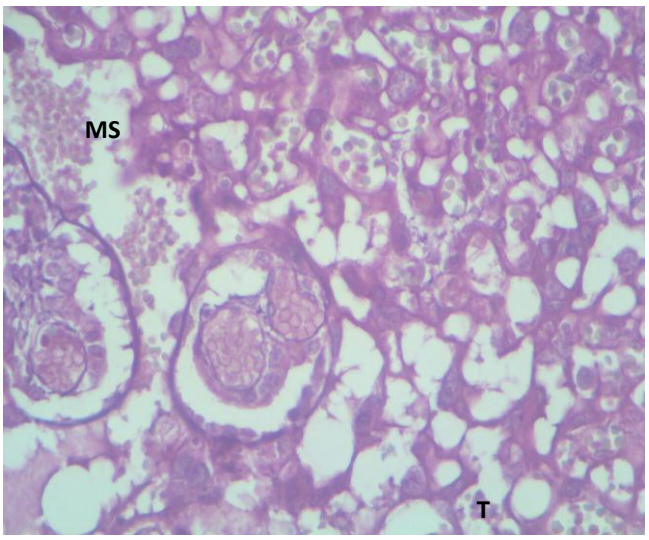


**Figure 10. Micrograph illustrate placenta belong to mother treated with 0.4 mg/kg b.w. lead acetate at (17 dpc), showed extensive degeneration and necrosis of trophoblast (T), severe cavitation, irregular dilatation of maternal sinusoid (MS) and calcium deposition in the labyrinth zone. (40X, H&E)**

In the labyrinth zone, a multiple cystic dilatation of maternal sinusoid was observed in some placentas on gestation day 17 and 20 in both experimental groups (G1 and G2) (Figure 6, Figure 10, and Figure 12).

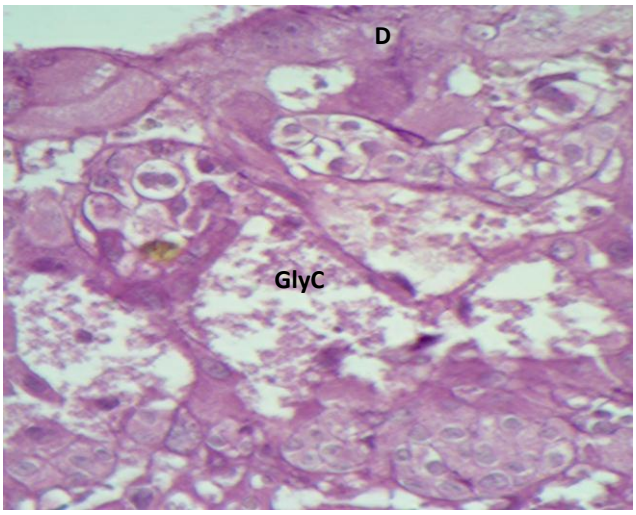


**Figure 11. Micrograph illustrate placenta belong to mother treated with 0.4 mg/kg b.w. lead acetate at (20dpc), showed marked apoptosis of glycogen cells (GlyC), with extensive fibrin deposition and calcification in basal and dicidual zone (D).(40X, H&E)**

There was also a deposition of fibrin in the labyrinth zone in experimental groups, (G1 and G2) (Figure 7, Figure 8, Figure 9, and Figure 11). This observation is agree with the work of Zhonghua et al [23] who found that the trophoblast in the labyrinth and trophospongium showed degeneration, fibrin deposition around the villi, and heavy deposition of fibrin in deciduas (Figure 9).

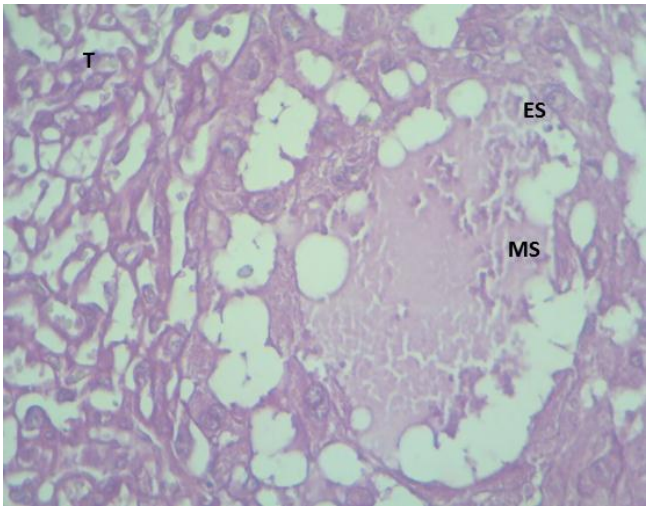


**Figure 12. Micrograph illustrate placenta belong to mother treated with 0.4 mg/kg b.w. lead acetate at (20 dpc), showed severe placental changes, generalized stromal necrosis and terminal villous deficiancy, trophoblastic (T) hypoplasia, and irregular dilatation of maternal sinusoid (MS) with disrupting of endothelial cells (EC) along with cavitation in the labyrinth zone (arrow). (40X, H&E)**

Calcified and hyalinized villous spots were observed in the experimental groups (G1 and G2) in comparison to the control group (Figure 10 and Figure 11) this is correspond with work of Satoshi et al [32], who proved that, since the placenta is equivalent to blood level, lead is precipitated in the term placenta along with calcium. As villi age they become necrotic, scarred with fibrous tissue, and may contain foci of calcium deposition. It is suggested, however, that fetal tissue levels may be influenced by calcium transport and intracellular calcium metabolism. On the other hand, lead may alter calcium-mediated cellular processes, producing toxicity [11]. A comparison of placental transfer of toxic metals by Tsuyoshi [27], found that lead

levels in the placenta were strongly correlated with levels of elements related to bone metabolism suggesting that placental lead may be associated with calcification and mean number of calcified and hyalinized areas per low power field were significantly higher (P> 0.01) in experimental group than in control group.

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# A Hierarchical Model for Defining Priorities of Environmental and Non-Environmental Risk Factors of Childhood Diarrhea

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## Abstract

**Background** Acute diarrhea constitutes a major cause of morbidity and mortality, especially among the children.

**Objective** To identify and determine the contribution of each of the risk factors associated with diarrhea occurrence in children in the commercial capital of Yemen, city of Aden.

**Methods** This outpatient-based case-control and questionnaire study was conducted during the first half of 2010, on random population of a total 304 cases of diarrhea and 517 controls. The explanatory variables were grouped according to the conceptual model, and analyzed by using a hierarchical approach, to provide a more dynamic view of the transmission characteristics of childhood diarrhea. Non-conditional logistic regression was used, and odds ratio and population-attributable fractions were estimated.

**Results** Factors related to food handling made a smaller contribution to diarrhea occurrence, compare to much more contribution by environmental contamination (fecal-oral transmission), and contact with one has diarrhea/ high density of housing. However the most determinant for diarrhea occurrence was the contribution of the socioeconomic factors.

**Conclusion** The data indicates that factors from all hierarchical levels were implicated in spreading of diarrhea, with relative high role for environmental contamination, and contact / crowding in the transmission. This is compatible with a predominance of the parasitic over the viral, spread by these routes. Diarrhea control strategies must emphasize on policies that improving the socioeconomic circumstances, and reducing the environmental contamination for the prevention of diarrhea.

**Keywords** Diarrhea, risk factors, children, Yemen, hierarchical model

## Introduction

Although the majority of diarrhea in children is a result of infectious agents, the etiological profile and the annual incidence of diarrheal in different populations may vary with several risk factors. Determinants for diarrhea in children therefore is complex and the relative contribution of each, varies as a function of complex interactions between the etiological agents and the risk factors. These factors could be grouped as socioeconomic, environmental, and related to contacts [1-3].

The factors can also be classified according to their place in a chain of causation, or a causal network as distal for socioeconomic conditions as that lead to lack of a refrigerator, or proximal as for consumption of food that was not kept in a refrigerator, and according to whether they are long lasting as due to sewage disposal, or transitory as due contact with someone with diarrhea, although the 'long lasting' factors may change, for example, as a result of improvements in the sewerage network. Such events of exposure are repeatable of a clustered data. Multilevel models or hierarchical network

[4] can be applied when events are repeatable to allow for correlation between the durations to events experienced by the same individual, or when individuals are clustered into higher- level units. Over the past twenty years multilevel modeling has become a standard approach in the analysis of clustered data. This study attempted to apply the hierarchical model in analyzing of the studied risk factors of diarrhea of children at Aden city.

## Methods

This was an outpatient-based case-control design study. Performed during the first half period of 2010, on random population includes the cases and controls of children less than ten years of age who were living in the commercial capital of Yemen, the city of Aden (in the southwest, at the tip of the Arabian Peninsula near the entrance to the Red Sea, with about half million population), and seen during their visit, suffering from diarrheal episode, at the outpatient-clinics at day working period to five governmental hospitals and health centers, and two private clinics, distributed among the governorate.

Cases were considered if the main reason for patients to attend the clinic was diarrhea, and when shows three or more of diarrheal episodes during the last 24 hours; not excluded if they had taken antibiotics prior to being seen in the clinic. Controls were children presenting at the same clinics for reasons other than diarrhea and included, healthy children presenting to the well baby clinic and immunization services; children attending for mild, non-chronic diseases; orthopedic procedures, pre- or post-operative assessments relating to small surgical procedures, or dermatological problems known not to be related with hygiene and sanitation. Cases, who reported any episode of diarrhea during the preceding 3 weeks, were considered as recurrent cases.

Recurrent diarrhea in the questionnaire which then excluded from estimation therefore include the cases of acute diarrhea occurs within a month period of last episode, persistent diarrhea that was last longer than two weeks, and cases which diagnosed as a chronic cases. Control who reported any episode of diarrhea during the preceding 3 months, were rejected. Controls were frequently matched to cases only on the base of sex and age variables, but not on how they paid for the consultation, since no big difference concerning the cost of the consultation specially that of the medication between the stated and private clinics in Yemen generally.

The information collected was from a physician and laboratory examination and from the person responsible for the child, through answering a standard, pre-coded questionnaire. The questionnaire was designed to investigate the diarrhea and the potential roles of multiple risk factors of the diarrhea, and include seven sections; 1st, on personal data, includes; Patient`s name, age, sex, address, and type of payment; 2nd, on socioeconomic characteristics, includes; Patient`s mother age, learning level, race, and marital status, the presence or absence of father, employment situation of the guardian, type of accommodation (house, apartment or shack), family income, and number of household goods owned by the family, includes; stove, fridge, mobile, television, air-condition, washing-machine, car and PC; 3rd, on the environmental conditions, includes; characteristics of used water and toilet, regularity of water supply, and storage type of water, sewage disposal and frequent of garbage collection, presence of animals, feces, or garbage, and occurrence of flooding at or around the house; 4th, on food handling, includes; source of child`s milk, presence or absence of a person who cook the food, food cook at separate kitchen, food stored in a refrigerator, and quality of consumed foods and liquids; 5th, on the contacts of the child, includes; contacts with someone has diarrhea, time spent by child outside of the home, crowding of the family/room, and number of children under five years old /room; 6th, on a child's health, includes; vaccinations, birth weight, day care attendance and mother's age at childbirth, and;

7th, on the diarrhea characteristics, diagnosis, and treatment. Include questions about the diarrhea type, episode, and starting date, symptoms, macroscopically and microscopically analysis of feces, and treatment. The total study children were 821, of whom 304 cases and 517 controls, distributed according to their ages into seven age groups; 0-5, 6-11, 12-23, 24-35, 36-47, 48-59, and 60 up to 120 months.

## Data Analysis

SPSS version 15.0 software, USA, was used for the univariate analysis for overall sample size, to explore the association between two variables, by calculating the odd ratio (OR) of Confidence Intervals (CI) 95% with the statistical significance at P<0.05. The variables that presented *P* <0.05 in the univariate analyses were then included in the next stage, which was an intra-block multivariate analysis. Multivariate analysis was used to find out whether (or not) the factors, which were significantly identified in univariate analysis, remain independently associated with the risk of diarrhea. Univariate analysis and multivariate non-conditional logistic analysis were conducted, always keeping in the models the age and sex variables that being used in the frequency matching.

Finally, the set of variables with *P* <0.05 from the multivariate analysis in each block was input to hierarchical analysis. The model defined two hierarchical levels: level-1 (distal) includes the socioeconomic variables, and level-2 (proximal), includes the variables of the three block; environmental conditions, person to person contact, and food handling. Because we were interested in the effect of the distal level variables, our final estimate for the effect of distal variables is that before the introduction of proximal variables, whereas estimates of effect of the variables at the proximal level must be made after introducing the variables at distal level into the model, excluding variables with *P* > 0.05.

For the distal variables, the population attributable fraction (PAF) was calculated in two ways: using the OR for the level alone, to reflect the fraction attributed to the variable whether mediated by the proximal variable or not, and using the OR after including the proximal variables, to reflect the proportion of cases attributed to the effect of the variable, which is not mediated by the proximal variables included in the final model. The proportion of cases in each age group caused by each variable PAF was calculated, using the formula: [(% of exposed cases) x (OR–1)/OR]. Age was tested as a possible interaction variable by means of the likelihood ratio test (Wald test). The analyses were carried out using the STATA statistical package, version 9.0 (STATA Corporation, 2003).

## Results

The total population of this investigation, that included and corroborated into the data analysis, was 814, of which 304 acute diarrheal cases and 517 controls. Irrespective of sex, the data in figure 1 shows the distribution of the control and the case children of the total population on the base of the age groups, were respectively, 28 (5.4%) and 32 (10.5%) less than 6 months; 42 (8.1%) and 69 (22.7%) between 6 and11-; 72 (13.9%) and 41 (13.5%) between 12 and 23-; 62 (12.0%) and 42 (13.8%) between 24 and 35-; 59 (11.4%) and 36 (11.8%) between 36 and 47-; 53 (10.3%) and 26 (8.6%) between 48 and 59-; and 201 (38.9%) and 58 (19.1%) between 60 and 120- months old. In all of the cases, three or more evacuations were reported on at least 1 day (Figure1).

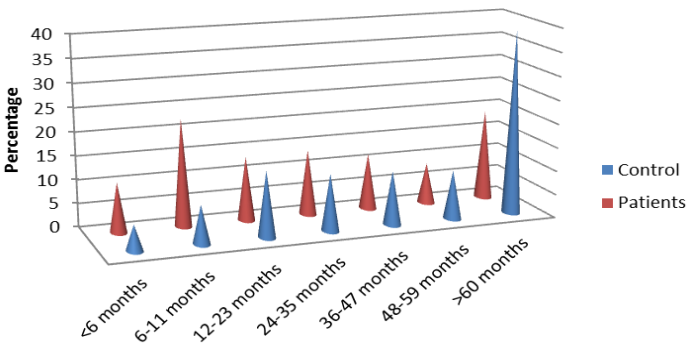


**Figure 1. Age group distribution of the total population into control subjects and those with acute diarrhea**

Risk Factors for Diarrhea, list of variables that were analyzed for their possible significant association with diarrhea in a univariate analysis, ($P \leq 0.05$), are shown in table 1, for the socioeconomic variables, ranked as level 1. Table 1 shows that the factors of the children`s mothers who were illiterate, < 18 years old, and of Somalia race, the housing type, and the family who had have less than- enough income and 4 items analyzed, were significantly associated with diarrhea in the univariate analysis, ($P \leq 0.05$). Both low educational attainment and living in a shack increased the likelihood that the child had diarrhea. Living in a shack had the strongest association (OR=3.11; 95%, CI=1.93-4.97). These factors remained significantly associated in the intra-block multivariate analysis. In the intra-block multivariate analysis, inadequate housing type remained with the highest OR (3.05; 95%, CI=1.81–4.55).

List of variables that were analyzed for their possible significant association with diarrhea in a univariate analysis, ($P \leq 0.05$), are shown in table 2, for the exposure risk factors of environment, contacts, and food quality, ranked as level 2. The part A of table 2 revealed that most of the environmental exposure risk factors were associated with diarrhea in univariate analysis ($P \leq 0.05$), with the exclusion of the variables relating to the type of toilet, and regularity of water supply. In the intra-block multivariate analysis, the following variables remained with $P \leq 0.05$; presence of feces in the backyard, sewage disposal coverage system, and two garbage-related variables: frequency of garbage collection, and closeness to open sewage ditch/garbage dump.

**Table 1. OR for the univariate[a] and multivariate[b] associations between diarrhea and the level 1 variable in the block of socioeconomic factors**

| Level 1: Socioeconomic Factor | | No. of Patients | No. of Controls | OR[a] | 95%CI | OR[b] | 95%CI |
|---|---|---|---|---|---|---|---|
| **Mother's Race** | Arabic | 272 | 475 | 2.32 | 2.09-2.61 | 2.28 | 2.04-2.54 |
| | Asian | 06 | 12 | | | | |
| | Somalia | 26 | 30 | | | | |
| **Mother's education** | Not educated | 290 | 295 | 2.56 | 2.37-2.70 | 2.39 | 2.2-2.63 |
| | 1ry education | 10 | 186 | | | | |
| | High education | 04 | 56 | | | | |
| **Mother's Age** | ≥18 | 16 | 12 | 2.28 | 2.12-2.4 | 2.09 | 1.89-2.51 |
| | <18 | 288 | 505 | | | | |
| **Marital Status** | Married | 296 | 494 | | | | |
| | Single | 08 | 22 | | | | |
| | Others | 0 | 01 | | | | |
| **Biological Father** | Present | 286 | 488 | | | | |
| | Not | 18 | 29 | | | | |
| **Family Income** | Excellent | 10 | 18 | 2.45 | 2.13-2.81 | 2.08 | 2.01-2.29 |
| | Fair | 148 | 293 | | | | |
| | Poor | 110 | 206 | | | | |
| **Total Goods Owned** | > 4 items | 149 | 297 | 2.49 | 2.09-2.93 | 2.3 | 2.0-2.63 |
| | Up to 4 items | 155 | 220 | | | | |
| **Housing** | House | 204 | 372 | 3.11 | 1.93-4.97 | 3.05 | 1.81-4.55 |
| | Apartment | 80 | 119 | | | | |
| | Shack | 20 | 26 | | | | |
| **Mother Work** | Not working | 270 | 451 | | | | |
| | At home | 01 | 03 | | | | |
| | Outside home | 33 | 63 | | | | |

[a] Adjusted for sex, and age. [b] Odds ratio from the intra-block multiple regression.

Among the contact factors, crowding at room/home and contact with a person with diarrhea, were significantly associated ($P$<0.05) in the univariate and the multivariate analysis (Table 2B). Although a three of the exposures related to food handling variables, includes; the type of water offered to the child; the child having eaten outside the home, and the children having had formula milk or readymade porridge containing milk powder, were associated with diarrhea in the univariate, only later two variables were associated with diarrhea in the intra-block multivariate analysis (Table 2C).

**Table 2a. OR for the univariate[a] and multivariate[b] associations between diarrhea and the level 2 variables in the blocks of environmental, contact, and food handling factors**

| Level 2a. Environmental Factors | | No. of patients | No. of Controls | OR[a] | 95%CL | OR[b] | 95%CL |
|---|---|---|---|---|---|---|---|
| **Open Sewage Ditch/Garbage Nearby** | Yes<br>No | 135<br>169 | 215<br>302 | 2.48 | 2.07-2.92 | 2.04 | 1.73-2.58 |
| **Feces in the Backyard** | Yes<br>No | 260<br>44 | 441<br>76 | 2.88 | 2.24-2.75 | 2.49 | 2.09-3.01 |
| **Sewage Disposal Cover System** | Sewage network<br>Open/other | 227<br>77 | 349<br>168 | 1.91 | 1.04-1.98 | 1.19 | 1.01-1.49 |
| **Frequency Of Garbage Collection** | Daily/every 2nd day<br>Others | 200<br>104 | 275<br>242 | 2.80 | 2.27-3.41 | 2.51 | 2.09-2.99 |
| **Toilet Characteristics** | With water flush<br>Others | 145<br>159 | 220<br>297 | | | | |
| **Presence of Animals at Home** | Yes<br>No | 116<br>188 | 221<br>296 | 2.82 | 2.50-3.46 | 2.39 | 2.07-2.82 |
| **Water Supply** | Piped<br>others | 275<br>29 | 445<br>72 | | | | |
| **Regularity of Water Supply** | At least daily<br><once a day | 148<br>156 | 195<br>322 | | | | |
| **Storage Vessels** | With lid<br>without | 178<br>126 | 266<br>252 | | | | |
| **Flooding in the Household** | Never<br>sometimes | 132<br>172 | 247<br>270 | 2.45 | 2.05-2.88 | 1.99 | 1.63-2.33 |

| Level 2b. Contact Factors | | No. of patients | No. of Controls | OR[a] | 95%CL | OR[b] | 95%CL |
|---|---|---|---|---|---|---|---|
| **Child in contact with one has diarrhea** | Yes<br>No | 49<br>255 | 56<br>460 | 2.90 | 2.53-3.54 | 2.90 | 2.98-2.87 |
| **Crowding (people/room)** | Up to 2 persons<br>2 or more child | 182<br>122 | 298<br>129 | 1. 93 | 1.55-2.46 | 1.94 | 1.77-2.28 |
| **No. of children under 5 years old/house** | Up to one child<br>2 or more child | 192<br>112 | 339<br>178 | 1.38 | 1.19-1.59 | 1.13 | 1.00-1.37 |
| **Child spend > 1 day outside house** | Yes<br>No | 40<br>264 | 43<br>474 | | | | |

| Level 2c. Food handling Factors | | No. of patients | No. of Controls | OR[a] | 95%CL | OR[b] | 95%CL |
|---|---|---|---|---|---|---|---|
| **Child on milk** | Breastfed<br>Can`s milk/other | 131<br>173 | 175<br>342 | 2.42 | 2.00-2.89 | 1.82 | 1.58-2.26 |
| **Type of water offered to the child** | Filtered/boiled /minerals<br>others | 128<br>176 | 195<br>322 | | | | |
| **Child consumed food not stored in a refrigerator** | Yes<br>No | 208<br>96 | 367<br>150 | | | | |
| **Child consumed food outside home** | Yes<br>No | 87<br>217 | 181<br>336 | 2.46 | 2.07-2.90 | 2.40 | 2.20-3.63 |
| **Child consumed food from floor** | Yes<br>No | 52<br>252 | 57<br>460 | 1.97 | 1.50-2.38 | 1.93 | 1.76-2.13 |
| **Absence of person routinely prepares food** | Yes<br>No | | | | | | |
| **Separate kitchen** | Yes<br>No | 273<br>31 | 470<br>47 | | | | |

[a] Adjusted for sex, and age. [b] Odds ratio from the intra-block multiple regression

Table 3 shows the results from the final multivariate hierarchical analysis. The estimated values for socioeconomic variables are those who shown significant association in the final intra-block model, and for the proximal levels in the model including the distal level. The effects of house type, illiterate mother, owning of up to four items, sewage disposal system, were greater in groups from 3 to 10 years old the effect of presence nearby of sewage ditch/garbage was greatest in children between 2 and 5 years old. The variables that increased the risk of diarrhea by >50% were: shack-type housing (OR=3.01), contact with people with diarrhea (OR=2.9), and sewage disposal system (OR=2.8). The variables with the strongest effect were not those responsible for the largest number of cases. The PAF for socioeconomic variables was 43%, and the large proportional contribution towards this was from low educating level for the mother (16%) and ownership of less than four household items (14%), and shack-type housing (10%). The block of variables relating to person-to-person contact presented a total PAF of 28%, predominantly made up of contact with people with diarrhea in the previous 10 days, and overcrowding, with PAFs of 16% and 9%, respectively, while that related to environmental conditions and food handling were 17% and 12% respectively.

**Table 3. OR & PAF derived from hierarchical multiple regression [a] on the risk factors for diarrhea.**

| | Level 1 | OR | CI 95% | PAF(%) [b] |
|---|---|---|---|---|
| **Socioeconomic Factors** | Mother with no education | 2.31 | 2.02-2.64 | 16 |
| | Family of low income | 2.34 | 2.15-2.56 | 14 |
| | Shack-type housing | 3.01 | 1.69-4.57 | 10 |
| | **Level 2** | **OR** | **CI 95%** | **PAF(%) [b]** |
| **Environmental contamination** | Present nearby of swage ditch/garbage Dump | 2.43 | 2.07-2.90 | 09 |
| | Open sewage disposal system/Others | 2.80 | 2.08-3.64 | 08 |
| **Food preparation** | Child ate food outside of the home | 2.28 | 2.08-2.51 | 0.9 |
| | Child not on breastfeed | 2.65 | 2.42-2.89 | 0.8 |
| **Contact/crowding** | Contact with one has diarrhea | 2.90 | 2.28-3.39 | 16 |
| | Three or more people per room/home | 2.31 | 2.02-2.70 | 09 |

[a] Adjusted for sex, and age . [b] Population attributable fraction.

## Discussion

This case–control study, examined risk factors for diarrhea among children less than 10 years old in the city of Aden/Yemen. The present analysis addresses diarrhea as a public health issue, without presenting here the data from analysis of; child`s health section, current treatment approaches; the complex interactions between the different etiological agents and the several diarrheal risk factors, as well as the part of data concerning the univariate analysis of exposure to diarrhea under the influence of the studied variables, since it has been carried out before [5].

The presentation of this study that almost all of the risk exposure factors which associated with diarrhea in univariate analysis, were remained associated with diarrhea in the multivariate analysis ($P< 0.05$), and that the multivariate hierarchical model of benefit in presenting the obtained new resultant values which mediating the effects, on the diarrhea burden, of the socioeconomic variables following to the interrelation in this model between the socioeconomic variables of level 1, with the different risk factors of level 2 (environmental contamination, food handling, and person-to-person contact), may interpret the implication of various infectious agents (parasitic, bacterial or viral) into the etiology of diarrhea, since each of these pathological agents have different transmission mechanisms and depend on complex social and environmental factors, particularly in large cities.

The distribution of diarrhea according to the pathogenic agents in Aden as one of the cities of the developing countries is not resembled to what occurs in the context of the developed countries [5]. In the developed countries, the viral etiological agents predominate over the bacterial or parasitic agents; a context where the importance of direct contact between people has been consistently demonstrated. This emphasizes the importance of direct contact between children for the transmission of pathogenic agents in these environments and accords with the growing importance of viral agents, beside also other agents spreading by interpersonal routes including *Shigella*, *Giardia* and *Cryptosporidium*, in the pathogenesis of diarrhea [6,7].

The presenting by Rashid and Abbas [5] in children of Aden city, of higher frequency of diarrheal cases due to the parasitic etiology than that of the viral cause, may interpreted in view of our finding that the variables of a relatively high PAF such as contact with one has diarrhea and three or more people per room (28%), presence nearby of sewage ditch/garbage dump and sewerage disposal system (17%), and child ate food outside of the home (9%), which collectively increased the risk of diarrhea by >50%, may therefore considered a strong possible transmission route of diarrhea of parasitic etiology.

Contact with human excreta can be primary, when defecating, or secondary due to contact with released sewage in children's playing sites and passages, and sanitation is the most powerful tools in controlling primary or secondary contact with sewage. However, availability of a sewerage system does not always necessarily mean a low incidence in acute diarrheal diseases [6]. Undoubtedly, individual sanitation and the supervision role of the parents are the most powerful tools in controlling primary or secondary contact with sewage. What corroborate this explanation, is the finding of significant ($P<0.05$) association with diarrhea of children of age groups 2-5 years old who where were closeness to open sewage ditch/garbage dump [5], and also the notion that Giardiasis is common in other societies like Salvador, where 13.7% of young children were found to be infected, and that gastrointestinal pathogens (e.g. bacteria or parasite) are predominantly transmitted by food or water, whereas person-to-person transmission is a major route for the transmission of viral diarrhea among children [6-8].

Regarding food, we found an association between diarrhea and the child having eaten outside of the home, and also an association with child`s not on breastfeed. These may be

related to the quality of the care taken in preparing the food offered to the child. There is evidence that food prepared at home carried a lower risk of diarrhea, while the introduction of new foods to young children increases the risk of diarrhea [3,9]. Of the exposures traditionally associated with diarrhea, such as deficient water supply and inadequate sewage disposal [10], only the later appear in the final model. This is in a partial agreement with other study [7], which showed that both were not appear in the final model. Irregularity in water supply (may reduce the frequency of good hygiene practices like washing hands and objects…etc, or could even lead to direct contamination of the water) showed no association with diarrhea [11]. Appearance of inadequate sewage disposal in the final model of our study is in a good agreement with the longitudinal study carried out in Salvador [12], suggesting that access to a sewerage system may have a strong influence on the incidence of diarrhea.

Since the risk factors with high ORs in our data were very limited, the PAF therefore may be more useful for defining priorities for interventions that would have a greater impact in reducing the disease burden. Our results show that socioeconomic factors include shack type-housing, low schooling of mother, and ownership of less than four items (level 1) were responsible for a large proportion of the diarrhea burden, with PAF of 49% that reduced to 43% after controlling for the exposures studied (level 2). This is in consistent with the finding of other studies [7].

In conclusion, diarrhea is a complex multifactorial process related to the precarious living conditions, and to transitory factors occurred shortly before the episode. The risk factors identified here do not include water (traditional risk factors for diarrhea) but sanitation, probably reflecting the needs for investment in establishing of sanitation network to begin in Aden city as soon as possible. Improvement of environmental sanitation and domestic hygiene and raising the socioeconomic status including the education of the population will contribute to the elimination of the underlying causes of acute diarrhea.

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# Clinical Association of Depression in a Group of Iraqi Patients with Parkinson's Disease

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## Abstract

**Introduction** Parkinson's disease (PD) is the second most common form of a group of progressive neurodegenerative disorders. Depression is more common in Parkinson's disease than in the general population.

**Objective** To evaluate the depressive features that accompanies Parkinson's disease and its relation to different parameter of this disease.

**Methods** This cross sectional study examined 30 patients with idiopathic Parkinson's disease neurologically and psychiatrically, searching for depression and its association with different features of idiopathic Parkinson's disease. The American diagnostic and statistical manual (DSM-IV) and the international classification of the disease (ICD10) were used for diagnosis of depression.

**Results** 53% of Parkinson's disease patients were found to be depressed and their depression significantly correlated with autonomic system infliction, dysphagia and insomnia. This finding was not correlated with duration or severity of the Parkinson's disease or the late complications of the disease.

**Conclusion** We found depression to be very common among Parkinson's disease patients; however, no specific type of depression could be identified in those patients. There is significant association between depression and autonomic involvement, insomnia and dysphagia in Parkinson's disease. Depression in Parkinson's disease was not found to be related to the age of onset or to the duration of the disease.

**Keywords** Parkinson, Depression

## Introduction

Parkinson's disease (PD) is the second most common neurologic degenerative disorder after Alzheimer's disease. Pathophysiologically there is neuronal loss within the substantia nigra of the midbrain and the neocortex [(1)]. Parkinson's disease is a variety of Parkinsonism occurs without obvious cause this idiopathic form is called Parkinson's disease or paralysis agitans [(2)]. This common disease, known since ancient times, was first cogently described by James Parkinson in 1817 [(3)]. The disease is of unknown etiology, but loss of Dopaminergic neuron at the substantia nigra is the primary pathological process that leads to upsetting the normal balance between inhibitory dopamine effects and the excitatory acetylcholine effects on the motor neuron in the motor cortical area [(2)].

Depression is more common in Parkinson's disease than in the general population, ranging from 10% to 25% in epidemiological studies to 40% in clinics specialized in movement disorders [(4)]. Serotonergic, noradrenergic, and dopaminergic mechanisms are all involved in its onset [(5)].

The diversity of etiological factors had led to different classification systems including endogenous-reactive, primary- secondary and unipolar- bipolar and so on. In order to avoid this classification confusion most researchers use the American diagnostic and statistical manual (DSM-IV) and the international classification of the disease (ICD10) for diagnosis of depression [6]. Depression symptoms are defined as those that impair social, occupational, or other important areas of functioning [7]. The aim of this study is to evaluate the depressive features that accompany the Parkinson's disease and its relation to different parameter of this disease.

## Methods

In our cross-sectional study, thirty patients with Parkinson's disease attending consultation clinic at Al-Kindy Teaching Hospital between September 2005 and April 2006 were included. Twenty five were males and five were females; the diagnosis of Parkinson's disease fulfilled the UK Parkinson's disease society brain bank clinical diagnostic criteria [1]. Patients with secondary Parkinsonism, patients with chronic medical disease and patients with Parkinson plus diseases were excluded from this study. The patients were asked about sleep, sphincter control, postural dizziness and swallowing deficit. Each patient was examined medically and neurologically by a neurologist.

The patients assessed by a psychiatrist, the DSM4 criteria for major depression was applied on the patients to diagnose depressive illness (Table 1).

The diagnosis of minor depression is based on the same symptoms, but only requires presence of two to five symptoms in the DSM IV-TR classification, and dysthymia requires that depressed mood has been present on most days for at least two years with at least two out of six other symptoms [8].

**Table 1. DSM – 4 criteria for diagnosis of major depression [6]**

| | |
|---|---|
| **A** | **Five or more of following present during the same 2 weeks (at least one of the symptom is either 1 or 2)** |
| 1 | Depressed mood most or the day, every day |
| 2 | Diminished interest or pleasure in all or almost all activities most of the day, nearly every day |
| 3 | Significant weight loss when not dieting or weight gain or decrease or increase appetite nearly every day |
| 4 | Insomnia or hypersomnia nearly every day |
| 5 | Psychomotor retardation or agitation nearly every day |
| 6 | Fatigue or loss of energy nearly every day |
| 7 | Feelings of worthlessness or excessive or inappropriate guilt nearly every day |
| 8 | Diminished ability to think or concentrate nearly every day |
| 9 | Recurrent thoughts of death, recurrent suicidal idea and suicidal attempt |
| **B** | **symptoms do not meet criteria for mixed episode** |
| **C** | **the symptom cause clinically significant distress or impairment in social, occupational, or other areas of functioning** |
| **D** | **the symptoms are not due to drug or general medical disease** |
| **E** | **the symptom are not better accounted for by bereavement** |

## Results

Out of the 30 patients with Parkinson's disease, 16 were proved to have depression according to (DSM 4). Out of those 16 patients, 12 were males and four were females. Four females out of 5(80%) Parkinson's disease patients had depression; while 12 males out of 25 (48%) were having depression (Table 2). Six of the patients were between 50-59 years, six between 60-69 years and four between 70-79 years. We did not have any patient developing depression that was above 80 years and below 50 years (table3). The duration of Parkinson's disease of one year was recorded in three patients out of the 16 who

have depression. Moreover, two years duration were in three patients, three years duration in two patients, four years duration in four patients and finally, five years duration in were in four patients (Table 4) .

All patients with depression reported some autonomic involvement, insomnia and dysphagia. Five patients out of the 16 had on – off phenomena and only one patient was bed bound state. All the patients between 60-79 years showed severe depression, while only 50% of those between 50- 59 years and 33% of those between 60-69 years, in total 62% were having severe depression. Anxiety/agitation was prevalent among 75% of patients, while stressful life events were seen among 62.5% of patients; both symptoms demonstrating female predominance. The prevalence of the low mood and loss of interest were in 10 out of the 16 patients (62.5%) as shown in Table 5.

**Table 2. Parkinson's disease patients who have depression**

| Patients | | Male | Female | Total |
|---|---|---|---|---|
| Depression | **Yes** | 12 | 4 | 16 |
| | **No** | 13 | 1 | 14 |
| Total | | 25 | 5 | 30 |

**Table 3. Age distribution of Parkinson's disease patients who have depression**

| Patients | Age (years) | | | |
|---|---|---|---|---|
| | 50-59 | 60-69 | 70-79 | Total |
| Females | 4 | 5 | 3 | 12 |
| Males | 2 | 1 | 1 | 4 |
| Total | 6 | 6 | 4 | 16 |

**Table 4. Duration of Parkinson's disease patients who have depression**

| No. of Patients | Duration (Years) | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **Total** |
| | 3 | 3 | 2 | 4 | 4 | 16 |

**Table 5. Different characteristics of the study group distributed across age categories**

| Character | | Age group (years) | | | Total |
|---|---|---|---|---|---|
| | | 50-59 | 60-69 | 70-79 | |
| Depression | Severe | 3 | 2 | 4 | 9 |
| | Mild | | | | |
| Anxiety/ Agitation | Males | 1 | 0 | 1 | 2 |
| | Females | 3 | 3 | 4 | 10 |
| Stress | Males | 1 | 1 | 1 | 3 |
| | Female | 2 | 2 | 3 | 7 |
| Depressive Features | Low mood | 3 | 3 | 4 | 10 |
| | Guilt feeling | 3 | 1 | 1 | 3 |
| | Suicidal thoughts | 1 | 0 | 1 | 2 |

## Discussion

When James Parkinson's wrote his monograph describing the shaking palsy (as he termed) in 1817; he mentioned that the disease process left the senses and intellect uninjured. This opinion was repeatedly analyzed by other neurologist and researchers; until 1923 when Lewy proved that mental disturbances are so common in Parkinson's disease [(9)]. In a textbook published in 1996 it was postulated the depression occurring in association with Parkinson disease could be a reaction to the disease and its disabilities or could be an intrinsic feature of the Parkinson's disease [(10)].

Our study showed that 53% of the enrolled Parkinson's disease patients had depression. This was very close to the 51% prevalence seen in Reiff [(7)], and in old literature of Sano et al clinic /hospital based study [(11)]. Marsden mentioned in many review articles that one third of the Parkinson's disease patients have depression [(2)]. This is the same rate in the Harding series [(12)]. Brown et al, found depression in 40% in their series of Parkinson's disease patients [(13)]. A review of literature reveals prevalence rate of depression from (12-90%) [(14)]. This wide range differences among all these studies could be due to using different methodological scales for diagnosis of depression. Sano et al had used Hamilton criteria for depression diagnosis while the present study used the DSM 4 criteria which are more widely used at the present time to diagnose the depression, and considered the most sensitive and precise depression diagnostic scale [(6)].

The present study showed that 80% of the Parkinson's disease females had depressive features and only 50% of the Parkinson's disease males had depressive features. These different gender rates of depression with higher female percentage in the present study were in consistent with those of previous studies which found that women with Parkinson's disease reported lower levels of quality of life, another index of social functioning, and more depression than men [14-19]. These finding disagree with old literatures of Horn [9] and Majons [20] who found no sex difference in the depression rate. The differences between the old and newer literatures may be related to better understanding of numerous correlates or possible risk factors for depression in Parkinson's disease, including female sex and psychiatric comorbidity [21]. One possible source of differences in cognition, and a growing area of research, is the effect of estrogen on dopaminergic neurons and pathways in the brain [15].

The study showed a high correlation with history of stressful life events. Thus the depression can be reactive to Parkinson's disease consequences and to the life events as well. Nevertheless the stressful life events were unrelated to the anxiety. These results support the fact that the depression not seems to correlate with disease duration or disease severity, would support the argument that depression is intrinsic feature of the disease [22].

We found no relation or specific pattern between depression and the duration of the Parkinson's disease. This was in agreement with Reichmann's findings who found no correlation with the severity of motor impairment [23] as well as Horn [9], and Sano [11]. Shiba [24] found that depression may precede motor symptoms by years. The apparent lack of relation of depression to disease duration supports the opinion that classifies depression in Parkinson's disease as an intrinsic feature of the Parkinson's disease and not as a reaction to the disease and its disabilities. The study showed that all patients with depression were having autonomic involvement, insomnia, and dysphagia. Five patients out of the 16 had on-off phenomena and only one patient was bed bound state. It cannot be excluded that depression in Parkinson's reflects more advanced and widespread neuro-degeneration [25] rather than severity of the disease [26].

We could not find any Parkinson's disease patient who was below 50 and above 80 years of age with depression. This may be a result of the small sample of this study. We could not see a pattern relating the age of the Parkinson's patients and the development of depression. This result was agreeing Sano [11], Mjons [20] and Horn [9] studies, but not Brown's study. Brown found depression to be more common before age of 58 years. This suggests that the disease threat of disability and loss of social status may become relevant for many people as adaptation to the disease results which leads to return to normal mood. The results agreed with this suggestion as we noted no significant difference of affection by depression with the duration of the disease.

The present study found more severe symptoms of depression in late ages and in those between 50-59 years. These results agreed with Brown [13], Sano [11] and Horn [9]. The depression of intrinsic factor, disabilities and the complications of the Parkinson's disease can be the possible causes for the more severe depression in Parkinson's disease in older patients. We also identified high prevalence anxiety and agitation in depressed Parkinson patients; but no clear evidence of its importance. These results need to be compared to the isolated depression to confirm whether they are significant or not.

There is inconsistent evidence that depression of Parkinson's disease is distinct from non-Parkinson's disease depression, with some studies reporting higher rates of anxiety, pessimism, suicide ideation without suicidal behavior, and less guilt and self-reproach [27]. Our study showed very low incidence of suicidal thoughts. This was in agreement with the Burn [28] who found depression is mostly of mild to

moderate intensity and suicide is very rare in Parkinson's disease.

In conclusion, depression to be very common among Parkinson's disease patients; however, no specific type of depression could be identified in those patients. There is an association between depression and autonomic involvement, insomnia and dysphagia in Parkinson's disease. In our study, depression in Parkinson's disease was not found to be related to the age of onset or to the duration of the disease. The strength of our study was mainly that only few previous studies assessed depression as an intrinsic feature of Parkinson's disease, as opposed to simply assuming depression to be reactive to Parkinson's disease. Our findings that the prevalence was not correlated with the duration of the disease were evidence that supports this theory.

Limitations of our study can be that we used the cross-sectional design. This is mainly because in Iraq, we are faced with many challenges when conducting studies on patients, as many will refuse to be followed up, or to repeatedly appear at the hospital for security reasons. Also, we could not identify comparable controls. Gives rise to many potential confounding factors that may have obscured our vision. For example, females are known to have higher prevalence of depression Moreover, socioeconomic status, life style, medications, and comorbidities might all have played a role in over or underestimating the prevalence of depression on those patients. We would therefore recommend future studies to compare a large sample of Parkinson patients to controls that are matched for age, gender, stage of Parkinson's disease, comorbidities, and medication use. This can yield results that are potentially less biased. Finally, study designs like cohort or case control can aid strengthen future research. It would also be interesting to conduct research investigating the effect of antidepressant medications use on depression in Parkinson's disease.

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# Spontaneous Cerebellar Hematoma: Review of 20 Cases

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## Abstract

**Background** Haemorrhages into structure of the posterior fossa pose unique risks due to limited space in this compartment and risk of hydrocephalus from compression of fourth ventricle. Most are neurosurgical emergencies requiring close monitoring and/or immediate evacuation.

**Objectives** To analyze therapy results obtained for surgically and conservatively treated patients. As well as to find prognostic parameters that can predict patient's outcome.

**Method** Retrospective study conducted at the Neurosurgical Hospital in Baghdad, and Al-Shaheed Ghazi Al-Hariri for Surgical Specialties Hospital. All the information were collected from hospital records between December 2000 and December 2002 for 20 patients with spontaneous cerebellar hematoma and included Clinical data like the onset of symptoms and signs, durations, progression of clinical condition, past medical history. Records of vital signs and neurological signs. Records of laboratory studies include complete blood picture, blood grouping, blood biochemistry, prothrombin time, partial thromboplastin time, bleeding and clotting time). Records of CT-scan analyzed according to the site, size, and presence of associated pathology. Size of the hematoma estimated by the maximum axial diameter.

**Results** Age of patients in this study ranged from 6-76 Years .There was 12(60%) male and 8(40%) female patients with male to female ratio 1.5:1. Mortality in the conservatively treated group is 33%, while it is 54% in the surgically treated group. Hydrocephalus was present in 100% of patients in the surgical group, while it is not present in the conservatively treated patients.

**Conclusions** The most important prognostic factors were GCS score on admission, interval between hemorrhage onset and treatment, size and site of hematoma, presence of hydrocephalus, and fourth ventricular extension. Suboccipital craniectomy to evacuate the hematoma is the most effective procedure where surgical treatment is indicated.

**Keywords** Cerebellar hematoma, suboccipital craniectomy, ventriculostomy.

## Introduction

The cerebellum occupies the posterior fossa, where it lies posterior to the brain stem. It consists of two hemispheres united in the midline by a portion of cerebellar substance known as the vermis [1]. Cerebellar hemorrhages account for about 10% of ICH [2,3]. Most of them attributable to hypertension, although vascular malformation and coagulopathy are also important cause [4]. The clot usually originates in the region of dentate nucleus, an area supplied by branches of the superior and anterior inferior cerebellar arteries [2,3]. They are usually confined to a single hemisphere, but they may extend into the 4th ventricle. Cerebellar hemorrhage is usually classified into mild and severe, depending on the size of the hematoma. The mild type is less than 3cm in diameter and the severe type 3cm or more [5].

CT scan: The initial radiologic study to be requested in the workup is unenhanced CT-scan [2]. The clot can be well visualized [6]. The CT scan

may also provide additional valuable anatomical information, such as size, site, blood in the 4th ventricle, brain stem distortion, obstructive hydrocephalus, calcification of vascular lesion [2, 4]. CT scan may reveal structural abnormality like tumor, AVM [2,4,7,8]. CT infusion scanning can confirm the presence or absence of an aneurysm within short period of time [11]. CT scan may reveal structural abnormality like tumor, AVM [4,7,9-11]. CT infusion scanning can confirm the presence or absence of an aneurysm within short period of time [4,12,13]. MRI provides superior resolution during the subacute and chronic stages can identify the underlying associated pathologies. MRI is superior to CT scan and angiography in detecting cavernous angiomas [7]. Angiography may be employed if an AVM or other specified lesion is suspected, particularly in a young patient without history of hypertension [9]. It confirms the diagnosis of lesions such as tumor, aneurysm, or AVM [4,14,15]. The aims of the study are to assess therapeutic approaches and outcome for surgically and conservatively treated patients and to find prognostic parameters that can predict patient's outcome.

## Method

This is a retrospective study conducted at the Neurosurgical Hospital in Baghdad, and Al-Shaheed Ghazi Al-Hariri for Surgical Specialties Hospital between December 2000 and December 2002 on 20 patients with spontaneous cerebellar hematoma. All the information was collected from hospital records and follow up during period of hospitalization. Clinical data collected include records the onset of symptoms and signs, durations, progression of clinical condition, past medical diseases, especially systemic hypertension, drug history as past history of anticoagulant taking. Records of systemic examinations including vital signs, neurological examinations. Laboratory studies include complete blood picture, blood grouping, blood biochemistry prothrombin time (PT) partial thromboplastin time (PTT), bleeding and clotting time. Radiological evaluation included CT scan as early as possible for all patients. Size of the hematoma was estimated by the maximum axial diameter. Magnetic resonance imaging (MRI) and MRA were done for patients. Digital subtraction angiography was done for Patients. Medical treatment for all patients, therapy includes control of blood pressure, intravenous fluids, early nutritional support, Dexamethazon 4 mg 4 times daily, H2 blockers. 9(45%) patients treated conservatively and 11(55%) patients treated surgically in form of suboccipital craniectomy and evacuation of the hematomas in 7 (35%) patients or external ventricular drain in 4 (20%) patients. Indications of surgery depended on large size hematoma (≥3 cm in maximal axial diameter), altered level of consciousness, and presence of hydrocephalus.

## Results

### *Age and gender*

Total number of patients enrolled in the study was 20. Age of patients in this study ranged from 6-76 Years (mean±SD = 55± 18years). There was 12(60%) male and 8(40%) female patients with male to female ratio 1.5:1 (Figure 1).

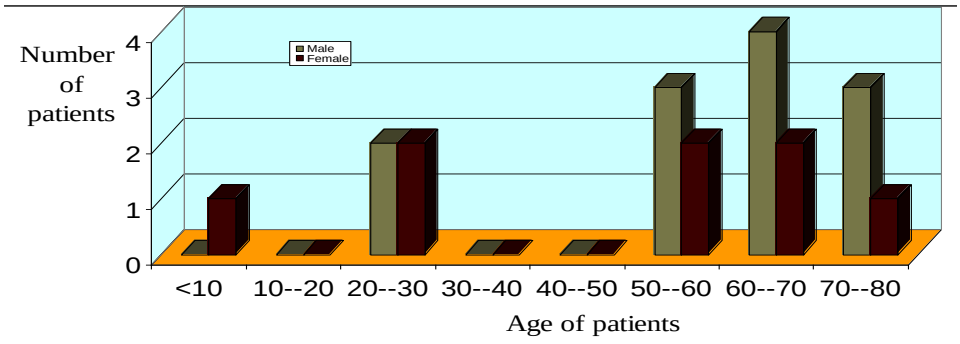


**Figure 1. Age and gender distribution**

Regarding the Interval between hemorrhage onset and treatment, only 2(10%) patients have been managed in the first 6 hours, 8(40%) patients treated 6-24 hours, 10(50%) patients treated after 24 hours (Figure 2).

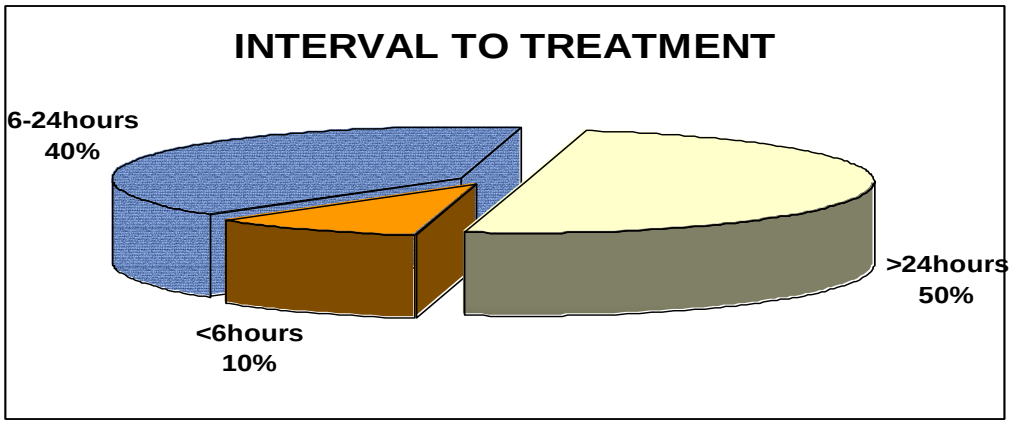


**Figure 2. Interval between hemorrhage onset and treatment**

Table 1 showed that patients with G.C.S. score ≤ 7.7 (100%) patients died while all patients with G.C.S. score ≥ 12 survived. Mortality in patients with hematoma size ≥ 3 cm in diameter was 9 (75%), while all patients with hematoma size < 3 cm in diameter survived.

**Table 1. Mortality according to the size of hematoma and G.C.S**

| Hematoma size | | <7 | 8-11 | 12-13 | ≥14 | All grades |
|---|---|---|---|---|---|---|
| Hematoma <3cm | No. of patients | 0 | 2 | 1 | 5 | 8 |
| | No. of deaths | 0 | 0 | 0 | 0 | 0 |
| Hematoma ≥3cm | No. of patients | 7 | 4 | 1 | 0 | 12 |
| | No. of deaths | 7 | 2 | 0 | 0 | 9 |
| | % | 100% | 50% | 0% | 0% | 75% |
| Total | No. of patients | 7 | 6 | 2 | 5 | 20 |
| | No. of deaths | 7 | 2 | 0 | 0 | 9 |
| | % | 100% | 33% | 0% | 0% | 45% |

Table 2 displays the main differences in between both treatment groups:

1- Mean size of clot in maximum axial diameter in conservative and surgical group were 2.8 cm and 3.6 cm respectively.
2- Hydrocephalus was present in 100% of patients in the surgical group, while it is not present in the conservatively treated patients.
3- 67% of patients in the conservatively treated group had G.C.S ≥ 12, while none of the patients in the surgically treated group had G.C.S ≥ 12.
4- Mortality in the conservatively treated group is 33%, while it is 54% in the surgically treated group.

**Table 2. Comparison between treatment groups**

| Conservative | | Surgical | | Character | |
|---|---|---|---|---|---|
| No. of patients | | 11 | 55% | 9 | 45% |
| Mean age (years) ±SD | | 58.3±(16.1) | | 56.1±(21.2) | |
| Pre-existing hypertension (no.) (%) | | 8 | 73% | 7 | 78% |
| Site of hematoma | Hemispheric | 8 | 73% | 8 | 89% |
| | Vermian | 3 | 27% | 1 | 11% |
| Mean diameter of clot (cm) | | 3.6 | | 2.8 | |
| Size ≥3 cm | | 10 | 90% | 2 | 22% |
| GCS≥12 | | 0 | 0% | 6 | 67% |
| Presence of hydrocephalus | | 11 | 100% | 0 | 0% |
| 4th ventricular extension | | 2 | 18% | 2 | 22% |
| Survivors | | 5 | 45% | 6 | 67% |
| Dead | | 6 | 54% | 3 | 33% |

The highest mortality was among the patients who underwent ventriculostomy (75%). The mortality in patients treated with suboccipital craniectomy was (43%). The least mortality was among the medically treated group (Table 3).

**Table 3. Mortality according to treatment**

| Treatment | Total No. of patients | Deaths (no.) | Survivors (no.) | Mortality (%) |
|---|---|---|---|---|
| Medical | 9 | 3 | 6 | 33% |
| Suboccipital craniectomy | 7 | 3 | 4 | 43% |
| Ventriculostomy | 4 | 3 | 1 | 75% |

## Discussion

In this study ages of patients ranged from 6-76 years, exhibiting peak incidence between 50-70 years of age. There were 60% males and 40% females patients with male to female ratio 1.5:1. Kaufman reported that the highest frequency of spontaneous cerebellar hematoma is in the sixth through eighth decades of life and these occur more commonly in males [4]. Hematoma size considered as an important prognostic factor because patients with hematoma size ≥3cm have 75% mortality while none of the patients with hematoma size < 3 cm had died (Table 1). Lois et al considered hematoma size > 3 cm as a radiologic predictor for poor outcome [16]. Rial and Uno also reported that the degree of consciousness disturbance, hematoma size ≥ 3 cm, hydrocephalus, age, and intraventricular extension are predictors of prognosis [9,10]. In this study only 10% of all patients have been treated in the first 6 hours, 40% treated within 6-24 hours, and 50% treated after 24 hours. There was delay in the treatment because many patients were referred from distant areas, and from other hospitals, and also because of the time needed to complete the diagnostic investigations of patients.

In this study we found mortality rate for patients treated by suboccipital craniectomy and external ventricular drain were 43%, 75% respectively (Table 3). Kanno et al reported that suboccipital craniectomy to evacuate the hematoma is the most effective procedure where surgical treatment is indicated [5]. The main differences between the two groups were in; the maximal axial diameter of clot, the presence of hydrocephalus, level of consciousness (Table 2). These differences noticed because in our study we regard the above features as factors that indicate surgical treatment.

We conclude that the age and preexisting hypertension were the major risk factor, the important prognostic factors were GCS score on admission, hematoma size and location, presence of hydrocephalus, and intraventricular extension and suboccipital craniectomy to evacuate the hematoma is the most effective procedure where surgical treatment is indicated.

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# Perineal Ultrasound for Evaluating Bladder Neck and Urethra in Stress Urinary Incontinence

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## Abstract

**Background** Urinary incontinence is a silent epidemic severly affecting the quality of life of women. Urodynamic study is the gold standard investigation for assessing women with urinary incontinence. However it is invasive and unavailable in some hospitals. Ultrasound is safe, noninvasive and available in most units.

**Objective** To determine the role of perineal ultrasound for assessing the bladder neck mobility, pubo urethral angle and retrovesical angle during rest and straining in normal women and in those with stress urinary incontinence.

**Methods** Twenty patients with urodynamic stress urinary incontinence and twenty age-matched control patients were included in the study. Perineal sonography was carried out in both groups to evaluate the role of this technique in the diagnosis of stress urinary incontinence. By using the posterior edge of the symphysis pubis as a reference point, posterior urethra-vesical angle (PUVA) and the angle between the vertical axis and urethral axis (alpha angle) were measured at rest and on straining. Bladder neck mobility was evaluated only at the cephalocaudal plane by measuring the desensus diameter.

**Results** Posterior urethro-vesical angle (PUVA) was found to be significantly different between the study and control groups both at rest and on straining ($P < 0.05$). The pubo urethral angle (alpha angle) was found to be significantly different between study and control groups only on straining ($P < 0.05$).Cephalocaudal distance (desensus diameter) of urethra was longer in patients with stress urinary incontinence ($P < 0.05$).

**Conclusion** Perineal sonography has an important role in diagnosing patients with stress urinary incontinence.

**Key words** Perineal ultrasound, stress urinary incontinence, bladder neck

## Introduction

Urinary Incontinence defined as a complaint of involuntary loss of urine. It is distressing condition, although rarely life threatening, severely affecting all aspects of women's life and psychologically [(1)].

Ultrasonography is a safe, non-invasive, acceptable and available tool in most units as well as the expertise to use it [(2)]. The results of ultrasound examination of a female's lower urinary tract comprise quantitative and qualitative findings. Quantitative parameters are measurement of the retrovesical angle, alpha angle (angle between vertical axis of symphysis and urethral axis), bladder neck descends and the position of internal urethral orifice, while the qualitative parameters to determine and describe the funneling of bladder neck and the position and mobility (fixed or hypermobile) of urethra and bladder base (vertical, rotational, or no descent) [(3)]. It's use in urogynecology for the assessment of bladder neck mobility and funneling of the internal urethral meatus both of which are important in women with urinary incontinence. The position of the bladder neck is determined relative to inferio-posterior margin

of the symphysis pubis or relative to a system of coordinates based on the central axis of the symphysis pubis. Measurements are taken at rest and on maximal Valsalva, and the yields difference is the numerical value for bladder neck descent. Comparative studies have shown good correlations with radiological methods [4]. The reproducibility of measurements of bladder neck mobility is high [5]. On Valsalva, the proximal urethra will be seen to rotate in a posteroinferior direction to a greater or lesser degree, due to the fact that the urethra and anterior vaginal wall are tethered to the symphysis pubis and the pelvic sidewall.

Aims of this study is to determine the role of perineal ultrasound in the diagnosis of genuine stress incontinence by assessing the reproducibility of an electronic sonographic technique for measurement of (bladder neck mobility, posterior urethrovesical angle and urethral angle) and comparing these ultrasound variables in women with stress urinary incontinence and in controls.

## Methods

This is observational cross sectional study included 40 women were selected from patients attending outpatient clinic of Obstetrics and Gynecology at Al-Kadhimiya Teaching Hospital during the period from the first of May 2010 till the end of June 2011.

Participants: 20 women with urodynamically proven genuine stress urinary incontinence constituted the study group and 20 women without stress urinary incontinence. The control group where selected from outpatient clinic and medical staff volunteers.

Inclusion criteria: Women with stress urinary incontinence proved by urodynamic study.

Exclusion criteria: Women with history of urge incontinence plus sign of Detrusor over activity on urodynamic study or other type of urinary incontence e.g. mixed incontinence, those with neurological disease (e.g. diabetic neuropathy, multiple sclerosis, spinal cord injury. etc), Pregnant patients or within 6 weeks postpartum, those With previous surgical treatment for urinary incontinence and uterovaginal prolapse, pelvic cancer and Patients with recurrent urinary tract infection.

The participants in the study were informed about the nature of study and its benefits, and an informed consent was obtained from each participant.

Detailed history and physical examination were performed including age, parity, past medical, obstetrical, gynecological, drug and surgical history, urogynecological symptoms (e.g. dysuria, frequency, nocturia, feeling of lump, condition which precipitating stress urinary incontinence like coughing, sneezing, lifting heavy weight, study of symptom, it's duration, severity, number of incontinence episodes, did she need pads).

For all women urine was sent for microscopic examination, culture and sensitivity and UTI were treated if present.

Incontinent patient underwent standard urodynamic investigation to establish the diagnosis of genuine stress urinary incontinence and absence of Detrusor overactivity.

***Perineal Utrasongraphy:*** Perineal sonography was done to evaluate the urethrovesical junction to all participants. patient was asked to come in comfortably filled bladder, in dorso-lithotomy position, Perineal ultrasound was performed using Siemens Versa ultrasound machine, the 3.5 MHz probe, covered with sterile glove, was placed on sagittal axis of perineum after gel application. The image was frozen and placed on one side of screen when inferior edge of symphysis pubis, the bladder, urethro-vesical junction, and the urethra were visualized. The patient was asked to strain and again the image was frozen and placed on the other half of screen.

The intersection point of coronal plane passing through the urethro-vesical junction and the horizontal plane passing below the symphysis pubis was marked during both rest and stress position to measure the desensus diameter.

All the sonographic parameters were measured by the same senior sonographist.

Perineal ultrasonography was carried out in both

groups (study and control) to evaluate the role of this technique in the diagnosis of stress urinary incontinence. By using the posterior edge of the symphysis pubis as reference point***, the following parameters were measured at rest and during straining:***

***1-The posterior urethro-vesical angle (PUVA)*** described as the angle between the urethral axis and the floor of the bladder axis one-third closer to the urethra.

**2-** ***The alpha angle*** described as the angle between the vertical axis and urethral axis.

**3-** ***Desensus diameter*** to evaluate bladder neck mobility at cephalocaudal plane.

For each parameter two measurements were taken and the average was calculated.

The correlation of bladder neck and symphysis pubis during the resting phase and stress in patient with stress urinary incontinence is shown in figure 1.

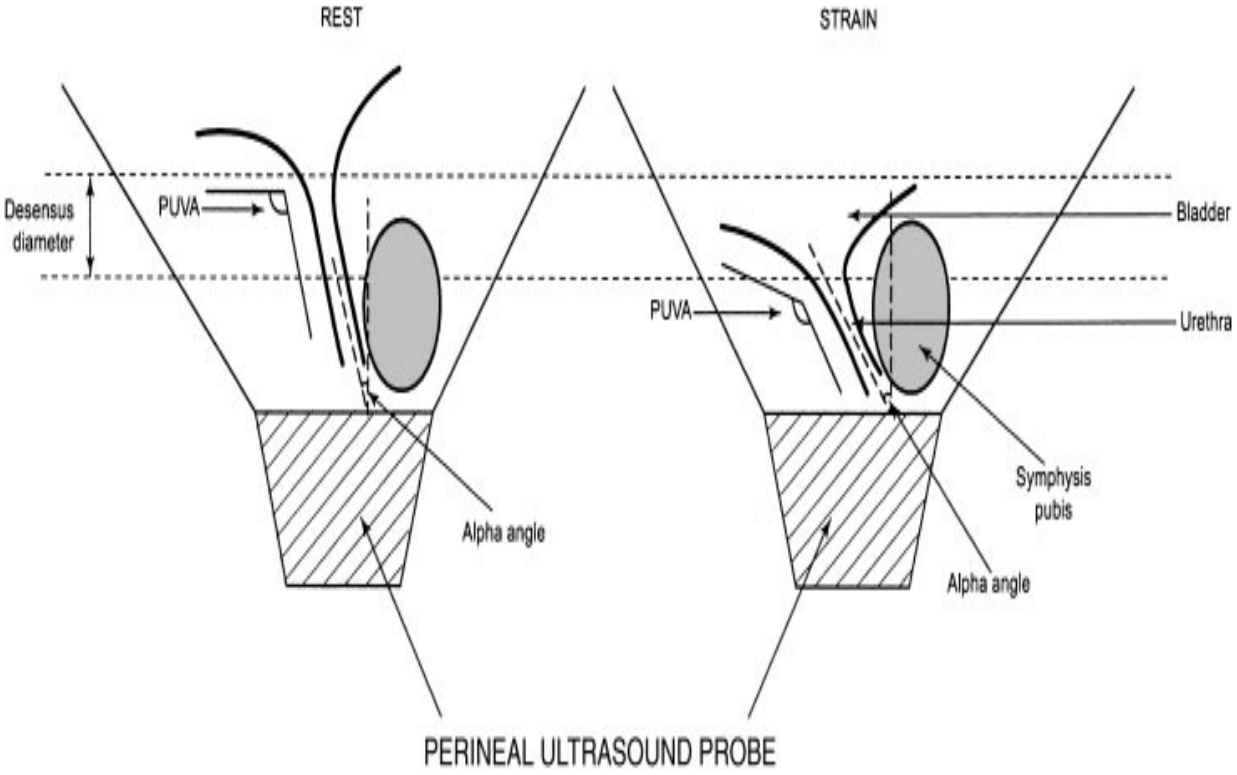


**Figure 1. Diagram showing measurement taken by perineal ultrasound**

- ***The posterior urethro-vesical angle (PUVA)*** is the angle between the urethral axis and the floor of the bladder axis one –third closer to the urethra.
- ***The alpha angle*** described as the angle between the vertical axis and urethral axis.
- ***Desensus diameter***: the intersection point of coronal plane passing through the urethro-vesical junction and the horizontal plane passing below the symphysis pubis measured at rest and during Valsalva maneuver and the difference represent the desensus diameter.

**Statistical analysis**

Data were analyzed using SPSS version 16 and Microsoft office Excel 2007. Numeric data were expressed as mean ±SD. Student t-test was used to compare between numeric data. P-value less than 0.05 were considered significant.

## Results

The study group included 20 women with urodynamically proven stress urinary incontinence and a control group included 20 age matched healthy women without stress urinary incontinence.

There was statistically significant difference regarding the parity and weight between the two groups with P value (0.001, < 0.001) respectively, with parity range was 2-7 in the study group and 1-4 in the control group. The age range in the study group was 38-54 year and 40-51 year in the control group. There was no significant difference regarding the age. As shown in table 1 and figure 1.

**Table1. Age, parity, and weight in the study (with stress urinary incontinence) and control groups**

| Characteristics | Study group (n =20) | Control group (n=20) | *P-value* |
|---|---|---|---|
| Age | 46.90±7.66 | 45.42±5.66 | 0.08 |
| Parity | 5.15±2.412 | 2.90±1.30 | 0.001 |
| Weight | 78.45±8.83 | 67.90±4.80 | <0.001 |

Statistically significant difference is found in all three sonographic parameters as shown in table 2.

**Table 2. Comparison of Perineal sonographic parameters between the study group and control group**

| US findings | Study group (n =20) | Control group (n=20) | *P-value* |
|---|---|---|---|
| Desensus diameter(mm) | 26.32±1.70 | 10.90±4.69 | <0.001 |
| PUVA (at rest) | 127.82°±8.10° | 108.28°±4.35° | <0.001 |
| PUVA (at Valsalva maneuver) | 170.67°±15.08° | 113.97°±32.18° | <0.001 |
| Alpha angle (at rest) | 20.16°±3.50° | 18.55°±3.50° | 0.222 |
| Alpha angle (at Valsalva maneuver) | 52.87°±5.27° | 28.46°±6.28° | <0.001 |

PUVA =posterior urethro-vesical angle

Table 3 represents comparison of perineal sonographic parameters between Patients with or without cystocele in the study group. There was no significant difference in desensus diameter, PUVA and alpha angle at straining between two groups. There was statistically significant difference in PUVA at rest between those with and without cystocele.

**Table 3. Perineal sonographic parameters in patients with or without cystocele in the study group**

| US findings | Cystocele absent (n =8) | Cystocele present (n=12) | *P-value* |
|---|---|---|---|
| Desensus diameter(mm) | 21.51±2.75 | 23.38±8.97 | 0.510 |
| PUVA (at rest) | 124.59°±9.66° | 129.98°±6.42° | 0.009 |
| PUVA (at Valsalva maneuver) | 164.20°±14.60° | 174.15°±11.42° | 0.128 |
| Alpha angle (at rest) | 18.26°±2.61° | 21.43°±3.52° | 0.051 |
| Alpha angle (at Valsalva maneuver) | 50.08°±5.64° | 52.07°±6.50° | 0.491 |

Table 4 represents Comparison of Perineal sonographic parameters in pre- and postmenopausal patients in the study group where all sonographic parameter (PUVA, alpha

angles both at rest and Valsalva maneuver) were not significantly different except the desensus diameter which was significantly higher in postmenopausal than premenopausal women.

**Table 4. Perineal sonographic parameters in pre- and postmenopausal patients in the study group**

| US findings | Premenopausal (n =9) | Postmenopausal (n=11) | *P-value* |
|---|---|---|---|
| Desensus diameter(mm) | 24.08±4.90 | 29.15±3.80 | 0.048 |
| PUVA (at rest) | 124.74°±10.29° | 126.34°±4.94° | 0.119 |
| PUVA (at Valsalva maneuver) | 162.26°±15.74° | 1.77.55°±10.85° | 0.520 |
| Alpha angle (at rest) | 19.58°±4.08° | 20.63°±3.06° | 0.445 |
| Alpha angle (at Valsalva maneuver) | 51.84°±6.96° | 53.71°±3.52° | 0.051 |

**Table 5. Sensitivity, specificity, positive and negative predictive values for stress incontinence, when the desensus diameter was >15 mm and the posterior urethra-vesical angle (PUVA) was >120°.**

| Parameter | Desensus diameter >15 mm | PUVA (rest) >120° | Mean PUVA >130° | Beta angle (valsalva) >140° |
|---|---|---|---|---|
| Sensitivity% | 95 | 95 | 95 | 95 |
| Specificity % | 98.5 | 97.4 | 97.45 | 97.5 |
| Positive predictive value % | 98.3 | 98.2 | 97.95 | 97.7 |
| Negative predictive value% | 95.45 | 95.45 | 95.45 | 95.45 |

## Discussion

Perineal sonography is a simple, non-invasive technique for the objective assessment of the lower urinary tract in patients with urinary incontinence. In contrast to the radiological techniques, perineal sonography produces direct dynamic images of the continence mechanism without exposure to X-ray. Evaluation of the urethro-vesical junction in stress urinary incontinence is essential. For this reason the Q-tip test, a clinical test with debatable specificity; lateral cystourethrography, a conventional method; and videourethrocystography, a sophisticated method, have been used. As sonography is inexpensive, reliable, easy to apply and free of any contrast material and X-ray exposure, it has practically replaced all the former methods in the evaluation of the urethro-vesical junction in stress urinary incontinence patients within the last decade [6].

Several studies have been published regarding the reliability of perineal ultrasound for diagnosis of genuine stress urinary incontinence all over the world [3,7-9].

On one line with our study, Kolbi et al (Vienna) 1988 compare Perineal ultrasound with urethrocystography in 30 patients with genuine stress incontinence they found that the Perineal sonography had similar result of urethrocystography where the B angle was (129.1±23), alpha angle was (20.8±13.2) both at rest, B angle was (151.3±21.9) and alpha angle was (38±19.1) both at straining where P value was significant ($p < 0.001$) which is agree with our result where alpha angle, B angle at rest, alpha angle and B angle at valsalva maneuver (20.16±3.50), (127.82±8.1), (52.87±5.27), (170.6 ±15.08) respectively where P value<0.001 (10) .

Shah et al 2007 again reanalyzed sonographic variable (retrovesical angle or PUVA) in comparism to lateral cystourethrography who concluded that perineal ultrasound was superior than lateral cystouethrography and within the routine evaluation of women suffering from

incontinence, the lateral cysturethrogram can be replaced by Perineal ultrasound without any limitations of the diagnostic value [11].

Alper et al (Turkey) 2001 investigated the role of sonographic imaging in the evaluation of stress incontinence by measuring PUVA angle at both rest and straining by Perineal and Transvaginal route, he concludes that the Perineal sonography is superior to Transvaginal route in the evaluation of stress urinary incontinence [8].

In a study by Gungor *et al*.1997, suggested that posterior urethro-vesical angle (PUVA) of more 120 on straining assessed by Transvaginal Ultrasound, correlated with poor support of the bladder neck, stress urinary incontinence, and posterior urethro-vesical angle of less than 120° on straining, correlated well with good support to the urethro-vesical junction [12]. While in comparison to our result PUVA was >140° associated poor urethro-vesical junction support. this minor difference may be the difference of ultrasound route where the present study the route of ultrasound was transperineal which was superior than Transvaginal as mentioned by Alper's study 2001[8], where there are two problem in transvaginal approach first it prevent free movement of bladder, specially in patient with marked descent of bladder neck. Second problem is that probe themselves move during stress, giving false impression of motion, thus distortions occurring during stress may be partially art factual.

Other finding of our study, Cephalocaudal distance (desensus diameter) was longer in patients with stress urinary incontinence ($P$ < 0.001).

Weil et al (Netherlands) 1993 measured the cephalocaudal and ventro-dorsal components of urethro-vesical junction mobility by transrectal sonography in 33 incontinent and 22 continent patients. The cephalocaudal mobility was statistically significant in contrast to the ventrodorsal mobility [13].

Demirci *et al*. carried out perineal sonographic measurements of the cephalocaudal and the ventro-dorsal components of urethro-vesical junction movement at rest and at stress in 35 patients with stress urinary incontinence and 20 continent controls. The cephalocaudal distance of the urethro-vesical junction, from the pubis at rest position, was similar in both Continent and incontinent groups, but there was a significant difference during stress. The cephalocaudal mobility and the ventro-dorsal distance from the pubis were markedly different between the two groups both at rest and during stress. However, the ventro-dorsal mobility was similar in both groups. It was concluded that the urethro-vesical junction mobility of stress incontinence cases was higher on the cephalocaudal axis than the ventro-dorsal axis. The distance between urethro-vesical junction and the pubis was greater on the ventro-dorsal axis as compared to the control group and urethro-vesical junction passed down the pubic symphysis in 63% of stress incontinence cases during stress [14].

Pregazzi *et al* (Italy) 2002 concluded that Bladder neck mobility can be demonstrated by Perineal or vaginal ultrasound and measured using the symphysis pubis as the immobile reference; Perineal utrasonography show a significant difference in ultrasound variable (bladder neck-symphysis pubis distance) both at rest and valsalva maneuver between stress incontinent and continent control group, where p value was <0.001[7], which consistent with our study where p value also was <0.001.

Brandt *et al* (Brazil) 2006 found that hypermobility of urethro-vesical junction with significant elongation of proximal urethral length over 14 mm by perineal ultrasound in 36 women complaining stress urinary incontinence [15]. This study is consistent with study done by Delancey *et al* (USA) 2007evaluated 240 women but in primiparous who found that the vesical neck movement measured during cough with translabial ultrasound with stress incontinence was 15±6.2 versus 10.9 ±6.2 in primiparous continent women P< 0.001[16], which agree with result of our study where cut off point of bladder neck mobility was 15mm.

The result of our observation was in agreement with result of Minardi *et al* ( Italy) 2007 they

assess the patients with stress incontinence by Perineal ultrasound with 3 point scale( the posterior urethro-vesical angle, the angle of urethral inclination, and the proximal pubourethral distance) were significantly different under stress compared to the resting phase where p value=0.028 [17].

Di pietto *et al* (Italy) 2008 study used Perineal ultrasound to assess urethral mobility they proposed a physiological range of pubic–urethral distance under stress in young women between 10 and15 mm, and in post-menopause women who establish a non-rigid range of mobility in relation to age and parity: between 15 and 18 mm [9] .

In a study by Gungor *et al*. 1997, suggested that urethro-vesical junction descent of more than 1 cm on straining assessed by Transvaginal ultrasound, correlated with poor support to bladder neck, stress urinary incontinence and urethro-vesical junction drop of less than 1 cm correlated well with good support to the urethro-vesical junction [12]. While in comparison to our result desensus diameter > 15 mm associated poor urethro-vesical junction support this difference may be due to using of Transvaginal approach in Gungor's study which prevents free movement of bladder, especially in patient with marked descent of bladder neck as mentioned above.

On the contrary to our study, Farah study (Baghdad) 2006 show perineal ultrasound had low sensitivity and specificity in assessing bladder neck mobility in 18 women with genuine stress incontinence where the sensitivity, the specificity and the positive predictive value was 36.4%, 50%, 66.7% respectively [18], which agree with Gaupp et al (USA) 2009 who was evaluating bladder neck mobility in 73 women, 31 were with stress incontinence and 42 continent as control group of similar age and parity by Perineal ultrasound by 5 experts blinded to continence status, the sensitivity, the specificity, ppv and npv (negative predictive value) was 53.0±8.8 %, 61.2±12.4 %, 48.8±8.2%, 65±7.3% respectively[19].

In conclusion, perineal sonography has an important role in diagnosing patients with stress urinary incontinence. In particular, a posterior urethro-vesical angle >140° and a desensus diameter >15 mm correlates with poor support of bladder neck in patients with stress incontinence.

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# Serum Trace Elements (Zinc, Copper and Magnesium) in Iraqi Patients with Thalassemia Major Receiving Desferrioxamine and its Relation with Growth State

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## Abstract

**Background** Patients with β-thalassemia major (TM) require periodic blood transfusion and iron-chelating therapy for all their life and they frequently show complications like trace elements abnormalities.

**Objectives** To evaluate the levels of zinc, copper and magnesium in these patients and to study the relation of these elements with growth state.

**Methods** Fifty-four patients with TM, with mean age (15.4 years) and age range (7-31) years, and thirty healthy subjects as controls, their mean age (14.7 years) and age range (8-30 years) were included in this case – control study. Each group divided into children and adult groups. Serum levels of zinc, copper and magnesium of all groups were measured calorimetrically. Serum levels of ferritin were measured depending on ELFA technique, in addition to height and weight of all patients and subjects.

**Results** Mean serum levels of ferritin were significantly elevated in TM patients as compared to controls, while mean serum levels of zinc and magnesium of both patients groups were significantly decreased as compared with control subjects. Mean serum levels of both patients groups were significantly elevated compared to control subjects groups.

**Conclusion** Patients with TM were suffering from hypozincamia and hypomagnesmia and required additional adjustment, the patients also showed hypercupremia, and there was obvious growth defect in which may be a result of hypozincamia.

**Keywords** Thalassemia, zinc, height, short-stature

## Introduction

Beta thalassemia major is an inherited disease resulting from reduction or total lack of beta globin chains, thalassemic patients need repeated blood transfusion for survival [(1)], and recurrent blood transfusion lead to accumulation of excess iron in the body tissues [(2)] causing progressive organ dysfunction that is fatal without chelation therapy [(3)]. Desferrioxamine (DF) has been the major iron-chelating treatment [(4)]. DF has a low general toxicity, perhaps because of its low lipid solubility [(5)]. Different mechanisms of possible DF toxicity include blockade of critical iron-dependent enzymes and reduction in critical trace elements other than ferric (copper, zinc, magnesium and calcium) [(6)].

Short stature, low weight and sex development delay are common in children with beta-thalassemia, this may be related to iron overload [(7)]. DF-induced dysplasia is associated with height reduction and can be seen in patients receiving DF chelation therapy at doses less than 50 mg/kg/day [(8)]. Iron chelation has been correlated with growth failure and bone abnormalities, and high DF dosage has been associated with cartilage alterations [(9)].

Zinc is one of the essential micronutrients in human and plays a particular role in human growth and development [10]. Patients with thalassmia had shown chronic zinc deficiency. Zinc deficiency may cause hyperzincuria, high ferritin levels, hepatic iron overload and hepatic dysfunction [11]. Short stature, low body weight, aneroxia and hypogonadism were found in the zinc deficient patients and also in most of the thalassemic patients [12]. It is well known that somatomedins mediate growth by contributing to the effect of growth hormone and they require zinc to be synthesized in the liver [13].

The serum concentration of copper in patients with TM depends on several factors including the amount of copper intake in daily diet, intestinal uptake of copper, iron accumulation, kidney function, copper to zinc ratio and administration of DF [14]. Magnesium may be reduced in a number of anemias, including β-thalassemia [15], hypomagnesemia may occur due to hypoparthyoidism [16]. In this study we aimed to evaluate the levels of trace elements (zinc, copper and magnesium) in Iraqi patients with TM and to study the relation between these minerals levels and growth state in these patients. In this study CDC growth charts were regarded as reference for evaluation of growth status [17], as these charts are recommended at inherited blood diseases center at AL-Karamma Teaching Hospital for evaluation of growth status of each patient. By using these charts, the measurements like height, weight can be compared with that of person of the same gender and age. Each chart has smoothed curves or lines that represent growth percentiles, these curves (percentiles) serves as a reference for comparison. The height or weight will be plotted on the grid and then compared to these percentiles [17].

## Method

Fifty-four patients with TM [34 male (62.9%), 20 female (37%)] with mean age (15.4 years) and age range (7-31) years who were attending the inherited blood diseases center at AL-Karamma Teaching Hospital periodically, and 30 apparently healthy subjects (18 males, 12 females) with mean age (14.7 years) and age range of (8-30) years as controls were included in the study after obtaining their informed consents. Twenty patients (37.1%) were below 12 years age (children); thirty-four patients (60.9%) were above 12 years age (adult). For control subjects 12 subjects (40%) were below 12 years age (children) and 18 subjects (60%) were above 12 years age (adult). TM diagnosis was based on the clinical, hematological and hemoglobin electrophoresis profiles.

All patients were on periodic blood transfusion (every fourteen days) and daily desferrioxamine at dose of 40 mg/kg body weight given subcutaneously. Exclusion criteria included patients with hepatitis and patients receiving minerals supplements. Blood drawing was done at tenth day of blood transfusion. Eight ml blood samples were collected from each patient and control by vein puncture after 12 hours fast, transferred to 10ml sterile plane tube, allowed to clot for 30 minutes at room temperature and centrifuged at 3000 rpm for 5 minutes to obtain serum. Serum aliquots (about 2 ml) were divided into four 1ml eppendroffs tubes for ferritin, zinc, copper and magnesium estimation. Height of each patient and control subject was evaluated at standing position without head or foot gear with a stadiometer instrument (measuring board with a movable headboard), while weight was calculated with beam balance scale.

Body mass index (BMI) had been estimated from person's weight and height; it was calculated by dividing weight (in kilograms) by height (in square meters). Assessment of growth was made depending on CDC growth charts, which were also used in this center to evaluate growth of the patients, as shown on figure 4, figure 5 and figure 6. Child (patient or control subject) with height for age percentile below third were classified as having short stature, and those with weight for age percentile below third were classified as having underweight [17]. For adult groups, patient or control subject with BMI for age percentile below third were regarded as underweight. Serum ferritin level was measured

by kit from bioMérieux (France) which depends on assay combines a one-step enzyme immunoassay sandwich method with a final Fluorescent detection (ELFA), using VIDAS instrument. Serum zinc and copper were measured by kit from LTA Company (Italy). Serum magnesium was measured by kit from LiNEAR Chemicals Company (Spain).

**Statistical analysis**

Data were translated into a computerized database structure .An expert statistical advice was sought for statistical analysis using SPSS version 12 computer software. Data in this study were presented as mean ± standard deviation (mean ±SD). ANOVA and student's t-test were used to compare the group means. The linear regression test was applied for the correlation between different parameters, and the significance of the r value was checked using t-test. The P value <0.05 was considered to be statistically significant.

## Results

Table 1 shows the demographic characteristics of the subjects. There was non-significant difference between the control subjects and patients regarding gender, age. Mean age of patients group was (15.4 years) and age range (7-31) years, mean age of children patients group was (8.95 years), while mean age of adult patients group was (19.2 years). Mean age of control subjects group was (14.7 years) and age range (8-30 years), mean age of children controls group was (9 years), while mean age of adult controls group was (18.5 years). Serum analysis showed significantly (P <0.05) elevated levels of ferritin in patient group (1725.5±558.1 ng/ml), as compared with that of the control subjects 126.8 ±43.5 ng/ml.

**Table 1. Demographic data of the studied groups**

| Characteristic | Patients group | | | Controls group | | |
|---|---|---|---|---|---|---|
| | children | Adult | Total | Children | Adult | Total |
| Number | 20 | 34 | 54 | 12 | 18 | 30 |
| Gender F/M | 6/14 | 14/20 | 20/34 | 4/8 | 6/12 | 12/18 |
| Age (years) | 8.95±1.1 | 19.2±5.8 | 15.4±2 | 9±0.85 | 18.55±5.6 | 14.7±6.4 |
| Ferritin (ng/ml) | - | - | 1725.5± 558.1 | - | - | 126.8± 43.5 |

Serum zinc levels were significantly decreased (P <0.05) in children and adult patients groups (60.25±15.1 µg/dl, 55.17±12.5 µg/dl) as compared to children and adult control subjects (91.6±9.03 µg/dl, 90.6±8.13 µg/dl), respectively. Serum copper levels were significantly elevated (P <0.05) in children and adult patients groups (135.7±45.5 µg/dl) (139.3±46.8 µg/dl) as compared to children and adult control subjects (89.8.8±11.8 µg/dl, 95.2±9.17 µg/dl, respectively). Serum magnesium levels of patients were significantly decreased (P <0.05) in children and adult patients groups (1.69±0.31 mg/dl, 1.77±0.38 mg/dl), as compared to children and adult control subjects (2.03 ±0.19 mg/dl, 2.04±0.21 mg/dl, respectively).

For adult groups, mean of BMI of patients group (19.9±6.2 kg/$m^2$) was non-significantly lower than that of control subjects (21.76±3 kg/$m^2$). Twenty from thirty-four patients (85%) having BMI for age percentile below third, compared to none of control subjects.

For children groups, height of patients was non-significantly decreased (113.3±21.4cm), as compared to control subjects (122.8±4.76cm). Sixteen out of 20 patients (80%) having height for age percentile below third, compared to 2 from 12 control subjects (16.6%). Weight of patients were non-significantly decreased (22.9±3.98 kg), as compared to control subjects (25±2.08 kg). Weight for age and weight for height percentiles of all control subjects were above third compared in 7 from 20 patients (35%) and 1 from 20 patient (5%) patients, respectively (Table 2).

**Table 2. Age, serum levels of zinc, copper and magnesium, height, weight and BMI of patients groups and control subjects groups.**

| Parameter | Children | | | Adults | | |
|---|---|---|---|---|---|---|
| | Patients | Control | P value | Patients | Control | P value |
| **Age** | 8.95±1.14 | 9±0.85 | 0.897 | 19.2±5.8 | 18.5±5.6 | 0.701 |
| **Zn (µg/dl)** | 60.2±15.1 | 91.6±9.03 | 0.000 | 55.1±12.5 | 90.6±8.1 | 0.000 |
| **Cu (µg/dl)** | 135.7±45.5 | 89.8±11.8 | 0.003 | 139.3±46.8 | 95.2±9.1 | 0.000 |
| **Mg (mg/dl)** | 1.69±0.31 | 2.03±0.19 | 0.002 | 1.77±0.38 | 2.04±0.2 | 0.009 |
| **Weight (kg)** | 22.9±3.98 | 25±2.08 | 0.103 | - | - | - |
| **Height (cm)** | 113.3±21.4 | 122.8±4.7 | 0.144 | - | - | - |
| **BMI (kg/m$^2$)** | - | - | - | 19.9±6.2 | 21.7±3 | 0.247 |

Zn: zinc, Cu: copper, Mg: magnesium, BMI: body mass index

The results of the present study revealed a significant correlation among age, height and weight in children patients and control group (P <0.05). Also there is significant correlation among age and BMI in adult patients and control group (P <0.05).

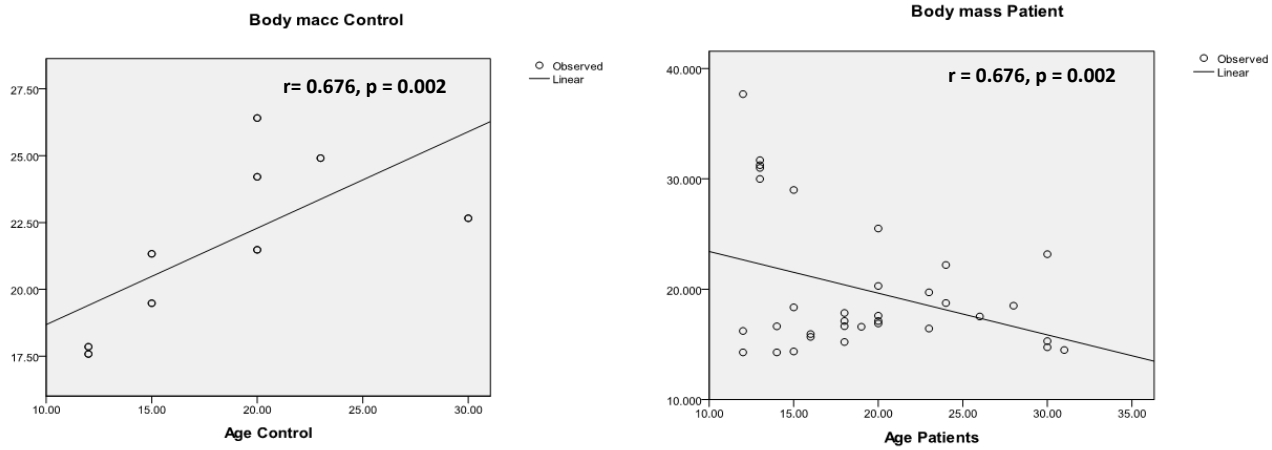


**Figure 1. Correlation between body mass index and age in adult controls (left), adult patients (right)**

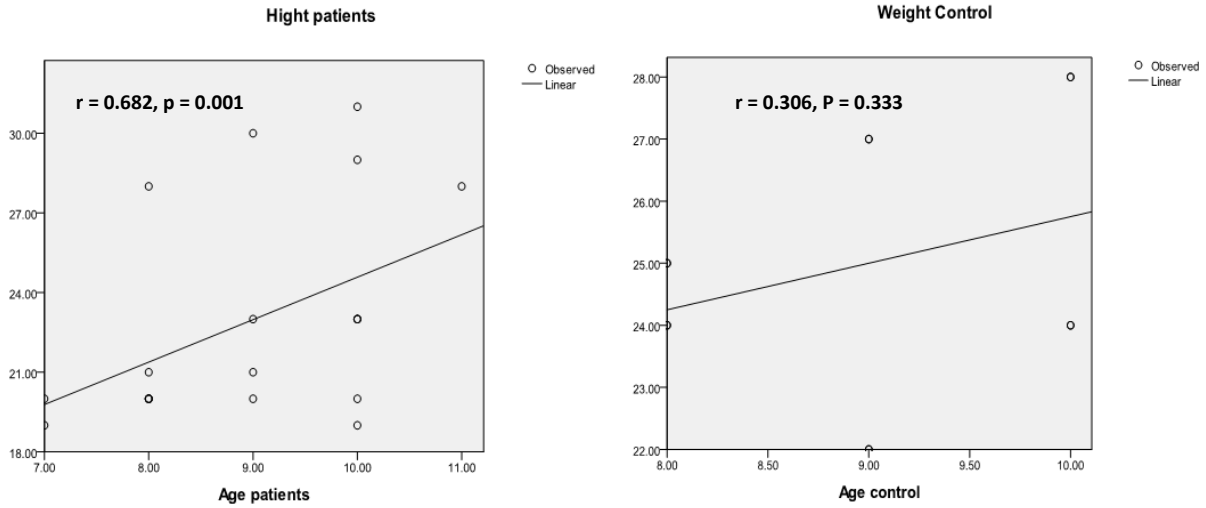


**Figure 2. Correlation between weight and age in children patients (left), and children controls (right)**

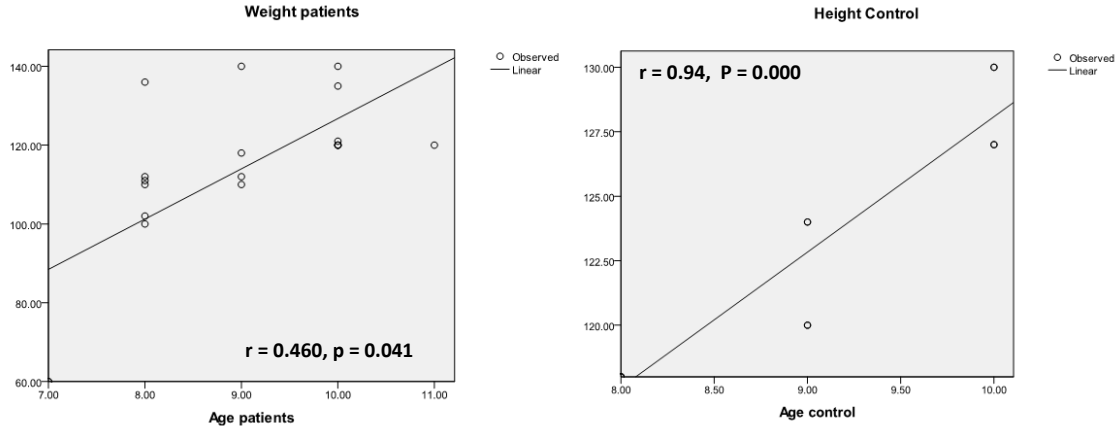


**Figure 3- correlation between height and age in children patients (left), and children controls (right)**

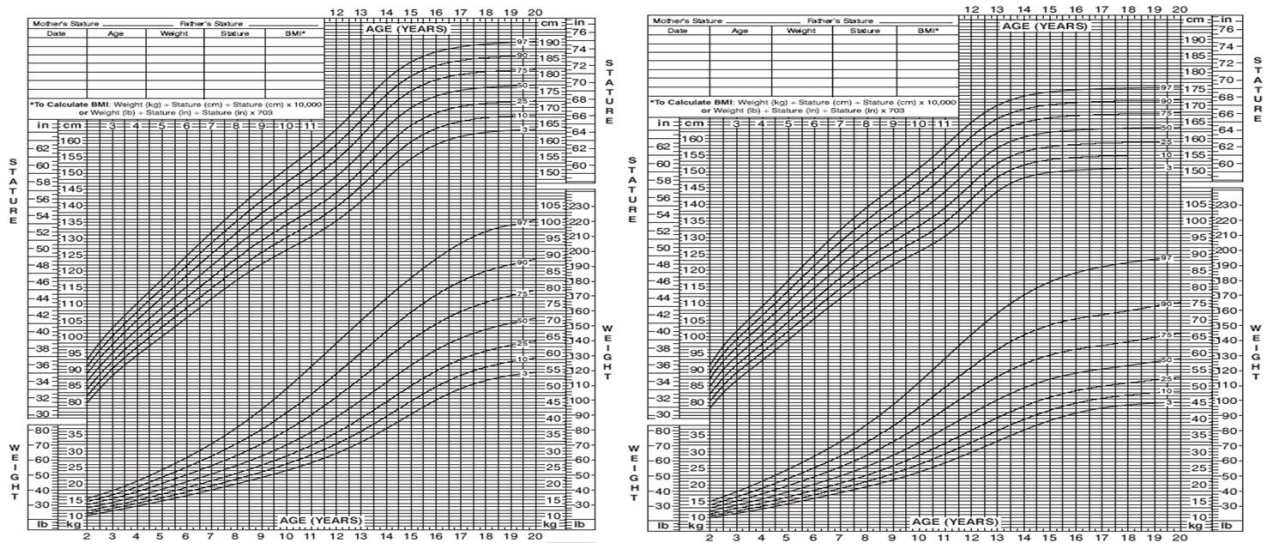


**Figure 4. CDC growth chart -age-to weight and age –to stature percentiles (2-20 years) for boys (left), for girls (right).**

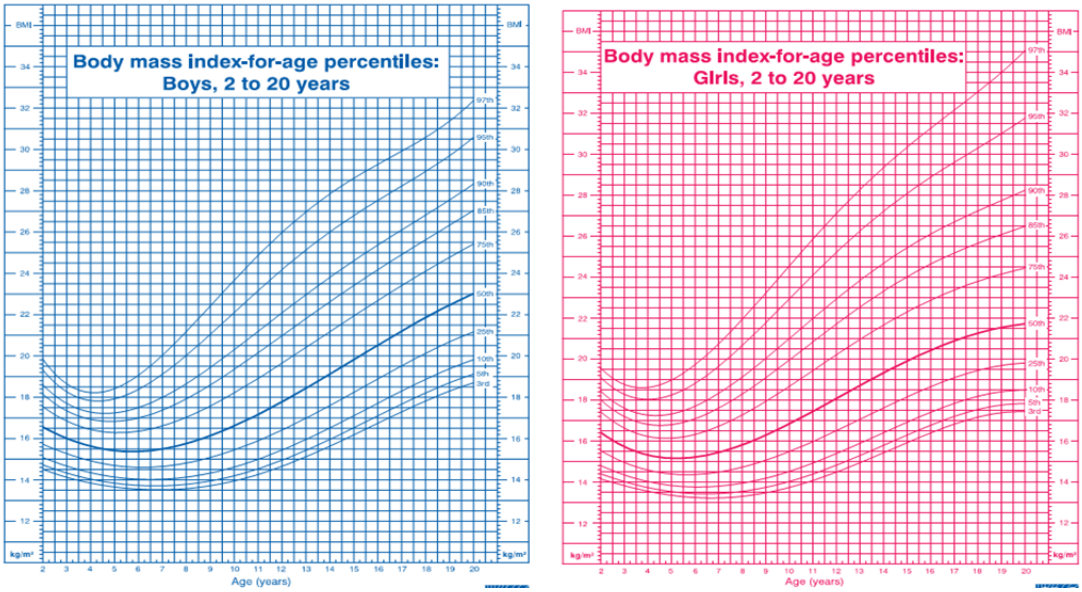


**Figure 5. CDC growth - chart body mass index-for - age percentiles (2-20 years) for boy (left), girl (right).**

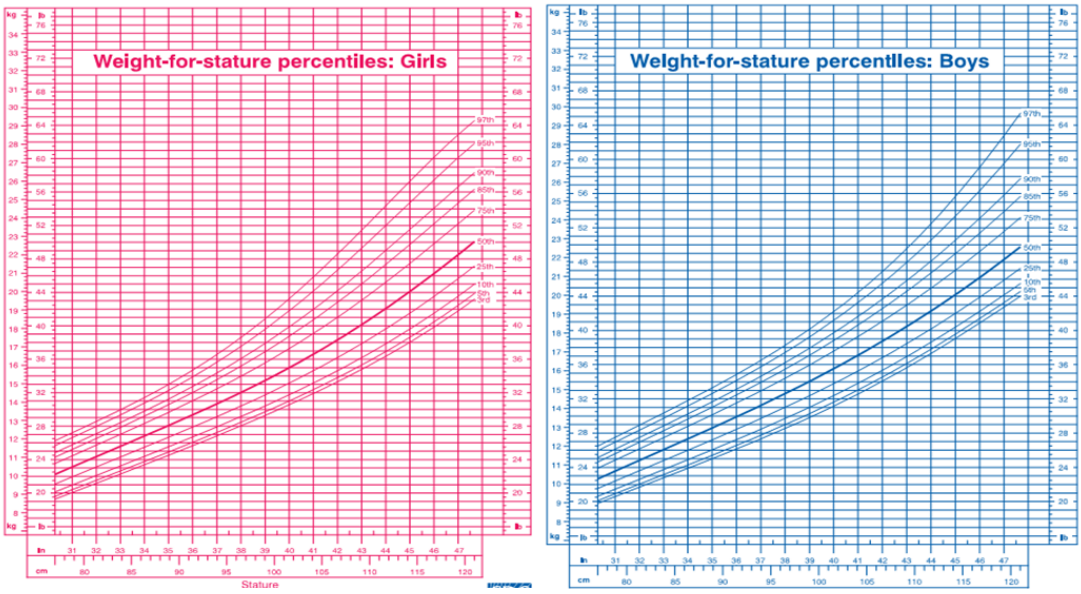


**Figure 6. CDC growth chart – weight-for-stature percentiles (2-20 years) for girls (left), for boys (right).**

## Discussion

In this study, patients with TM showed lower levels of serum zinc as compared to that of control subjects ($p<0.05$), which indicates that most of patients had hypozincemia; this may be related to dietary insufficiency of zinc in those patients in addition to the effects of disease and desferrioxamine administration without dose adjustment for each patient. This result correlates with results of many previous studies [11,14,18,19]. Mehdizadeh et al have reported that mean serum zinc level was significantly higher in thalassemia patient group, and concluded that zinc deficiency was rare in thalassemia [20], Kosarian *et al* reported normal serum zinc level in major thalassemia patients and control, thus they were not affected by zinc deficiency [21]. Previous studies have shown that zinc deficiency is a growth-limiting factor in thalassemia since linear growth in the patients who received zinc supplementation is equal to that of normal healthy children [22]. The causes of zinc deficiency in thalassemia patients may be related to insufficient amount of zinc in daily meals, abnormality in urinary excretion of zinc, kidney dysfunction, disturbance in zinc metabolism and higher level of zinc excretion in sweat [23]. In this study zinc deficiency and iron overload as observed from the elevated levels of serum ferritin of patients can be regarded among factors led to the observed short stature (height percentile below third) and low body weight (weight percentile below third) among children patient and decreased BMI among adult patients, this is similar to that of other studies where short stature was more prevalent in patients above the age of 10 years [24], also in other study in which patients older than twenty years, 75% of girl and 62% of boy having height percentile below third [25].

The levels of serum copper of TM patients were significantly higher than that found in control subjects which correlate with the results of many other studies [16,26], while the study by Kassab-Chekir showed no change in serum copper concentration in thalassemia patients [19]. Bekheirnia [27], Tabatabae [28] and Naser [18] revealed reduction in serum copper concentration. Al-Samarrai et al concluded that the etiology of hypercupremia is hemochromatosis which is principal complication of thalassemia [16]. Hypercupremia in another study in patients with beta thalassemic explained to result from defective erythropoieses and excess denaturation of α and

β globin chains that generate free radicals which lead to oxidative damage [29].

The levels of serum magnesium of patients were significantly decreased when compared to that of control subjects, the decrease in magnesium levels may result from dietary insufficiency of magnesium and the inappropriately high dose of desferrioxamine (40 mg/kg). Previous study showed that magnesium levels were within normal levels [30]. In another study, magnesium depletion in thalassemia patients was documented by low serum magnesium levels, abnormal magnesium tolerance tests, and low symptoms responsive to magnesium therapy [17]. We suggest for further work to estimate the levels of these minerals in patients receiving new classes of treatment used recently in this center.

From the above results we conclude that thalassemic patients suffer from trace minerals abnormalities as they showed hypozincemia, hypomagnesemia and hypercupremia and these abnormalities associated growth defect.

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# Significance of Platelet Indices in Patients with Acute Ischemic Stroke

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## Abstract

**Background** Platelets play an important role in the development of intravascular thrombosis. Platelet size has been considered to reflect platelet activity. Platelet indices had been studied as an independent predictor of acute ischemic stroke (IS).

**Objectives** Assessing the relation of acute IS with different platelet indices.

**Methods** Fifty patients were enrolled: 25 of them had first acute IS (mean age 64 years, 12 (48%) were males) [group 1], while the rest 25 patients were those with more than one IS (mean age 68 years, 16 (64%) were males) [group 2] in comparison with the control group (20) subjects (mean age 57 years, 10 (50%) males). Work up included history and clinical examination in addition to brain CT scan and platelet indices which are: mean platelet count (MPC), mean platelet volume (MPV), platelet large cell ratio (P-LCR), and platelet distribution width (PDW) within first 24 hours of patient presentation.

**Results** The mean platelet count (MPC) was found to show significant difference between group 2 versus group 1 and control (P=0.012, P=0.023 respectively), while no statistically significant differences were reported with the other indices (MPV, PDW or P-LCR). Linear negative correlation was demonstrated between MPC and MPV, PDW and P-LCR in group 1, such correlation wasn't found in group 2.

**Conclusion** Mean platelet count (MPC) differs significantly in cases of multiple stokes in comparison with those having first stroke or the control group; therefore, the significance of this finding needs further studies.

**Keywords** Platelet indices, platelet count, mean platelet volume, platelet distribution width, platelet-large cell ratio, ischemic stroke.

## Introduction

Stroke is a sudden loss of neurologic function resulting from focal disturbance of cerebral blood flow due to ischemia or hemorrhage [(1)]. Platelets play an important role in the pathophysiology of ischemic stroke by developing intravascular thrombus after erosion or rupture of atherosclerotic plaques [(2)].

Platelet volume is a marker of platelet function and activation that is readily measured as mean platelet volume (MPV) and positively associated with platelet reactivity [(3-7)]. Larger platelets contain more dense granules and are metabolically more active than small platelets and having higher thrombotic potential [(8-14)]. In steady-state platelet production, there is an inverse but nonlinear relationship between MPV and MPC [(3, 15-19)].

The aim of this study is to investigate whether MPC, MPV, platelet distribution width (PDW) and platelet large cell ratio (P-LCR) have any association with ischemic stroke and possibility of being independent risk factors for stroke among the other conventional risk factors.

## Methods

In cross-sectional study; fifty consecutive patients presented with clinical manifestations

of acute ischemic stroke were enrolled in the period between November, 2010 and March, 2011 as they were received at Al-Kadhimiya Teaching Hospital.

For every patient, medical evaluation conducted including history, clinical examination, evaluation of risk factors, and drug therapy. Venous blood samples were collected within first twenty-four hours of hospitalization and before any medical or therapeutic intervention.

Blood samples were taken and analyzed by a Sysmex KX- 21N auto-analyzer at same hospital laboratory. For all patients; brain computed tomography and neurological opinion were taken. Those patients with infection, inflammatory disease, trauma, underlying hematological disease, autoimmune disease, malignancy and patients with clear source of emboli showering were excluded from the study. In this study (50) Eligible patients were included and distributed into two groups each group include (25) patients:

1. Group 1: subjects with the evidence of first-ever ischemic stroke (IS).
2. Group 2: subjects with more than one stroke.

There were (20) control subjects with similar baseline characteristics and exclusion criteria, who confirmed their stable healthy status by direct interviewing, used for comparison of the same parameters in this study. They had been met during their routine follow up at cardiology clinic.

All patients and control were informed and agreed to participate in this study in addition to local medical ethical committee license.

The normal value of the studied platelets parameters were reported as follows:

- MPC: (128-434×$10^9$/L) for females and (134-377×$10^9$/L) for males,
- MPV: (8.5-12.8 fL) for females and (8.1-12.4 fL) for males,
- PDW: (9.4-18.1 fL) for females and (9.8-18.0 fL) for males,
- P-LCR: (14.3-44 %) for females and (10.7-45.0 %) for males.[20]

Statistical Analysis included ANOVA (for demographic differences among the 3 groups), Chi-square (for differences in each parameter between groups), and Pearson correlation coefficient (for correlation analysis), by the programs SPSS 16 and Microsoft office Excel 2007, considering P≤ 0.05 as statistically significant difference.

## Results

The mean age of patients in group 1 was 64.72±8.50 years, ranging from 46 to 80 years. The mean age of patients in group 2 was 68.72±6.10 year, ranging from 60 to 83 years, while the mean age of control subjects was 62.4±9.5 years, ranging from 46 to 73 years and those were matched with no statistical differences with either group 1 (p= 0.1), or group 2 (p=0.09). There was no significant difference in the mean age between patients in group 1 and patients in group 2 (p=0.222) as shown in table 1.

**Table 1. Demographic characteristics of patients and control subjects**

| Feature | Control group | Group 1 | *P* | Group 2 | *P* | *P!* |
|---|---|---|---|---|---|---|
| No | 20 | 25 | | 25 | | |
| Age (years) | 62.4±9.5 | 64.72±8.50 | 0.1 | 68.72±6.10 | 0.09 | 0.222 |
| Male, **No.** (%) | 10 (50) | 12 (48) | 1 | 16 (64) | 0.474 | 0.254 |
| Hypertension, **No.** (%) | 14(70) | 17 (68) | 1 | 18 (72) | 0.606 | 0.758 |
| DM, **No.** (%) | 14(70) | 14 (56) | 0.704 | 12 (48) | 0.212 | 0.571 |
| Current smoking, **No.** (%) | 10 (50) | 11 (44) | 0.519 | 10 (40) | 0.712 | 0.774 |
| IHD, **No.** (%) | 20(100) | 4 (16) | <0.001 | 4 (16%) | <0.001 | 1 |

*!* Comparison between group 1 and group 2, * <0.05, ** <0.001

In group 1 patients, 12 (48%) were males while group 2 patients whom presented with more than one IS, 16 were (64%) males in comparison with the control group in which 10 (50%) were males. The three groups were matched with respect to gender (p=0.493) as noticed in table 1.

The risk factors like smoking, hypertension, diabetes mellitus and ischemic heart disease (IHD) were reviewed also, but with no statistical significance differences reported among the 3 groups except for the history of IHD that presented more in the control group as demonstrated in table 1.

Mean platelet count (MPC) for group 1 was 253.72±76.35×$10^9$/L and in group 2 was 341.04±133.52×$10^9$/L while for control 234.60±60.36×$10^9$/L. There is statistically significant difference in MPC of group 2 in comparison to control group and group 1 (p = 0.023 and 0.012 respectively) but there is no similar statistically significant difference between group 1 and control group (Table 2).

**Table 2. Mean platelet count (MPC) among the 3 groups**

| Parameter | Groups | mean±SD | Range | P value | | |
|---|---|---|---|---|---|---|
| | | | | Control *Vs* group 1 | Control *Vs* group 2 | Group 1 *Vs* group 2 |
| MPC (x$10^9$/L) | Control | 234.60±60.36 | 156-328 | 1.000 | 0.023* | 0.012* |
| | Group 1 | 253.72±76.35 | 125-404 | | | |
| | Group 2 | 341.04±133.52 | 202-759 | | | |

MPC = mean platelet count, * = P<0.05

No statistically significant difference reported in mean platelet volume (MPV) among the 3 groups (p = 1.000, 0.801 and 1.000 respectively) (Table 3) and similar results concerning mean platelet distribution width (PDW) among these 3 groups (p = 1.000, 0.614 and 0.465 respectively) were reported (Table 4) also as well as for platelet large cell ratio (P-LCR) (P values= 1.000, 1.000 and 0.862; respectively) as shown in table 5.

**Table 3. Mean platelet volume (MPV) among the 3 groups**

| Parameter | Groups | mean±SD | Range | P value | | |
|---|---|---|---|---|---|---|
| | | | | Control *Vs* group 1 | Control *Vs* group 2 | Group 1 *Vs* group 2 |
| MP**V** (**f**/L) | Control | 11.10±0.98 | 10.0-12.3 | 1.000 | 0.801 | 1.000 |
| | Group 1 | 10.94±1.31 | 8.6-13.9 | | | |
| | Group 2 | 10.63±0.93 | 9.3-12.7 | | | |

MPV = mean platelet volume

**Table 4. Mean platelet distribution width (PDW) among the 3 groups**

| Parameter | Groups | mean±SD | Range | P value | | |
|---|---|---|---|---|---|---|
| | | | | Control *Vs* group 1 | Control *Vs* group 2 | Group 1 *Vs* group 2 |
| **PDW** (**f**L) | Control | 15.3±1.8 | 12.3-18.0 | 1.000 | 0.614 | 0.465 |
| | Group 1 | 15.12±3.1 | 10.9-23.8 | | | |
| | Group 2 | 14.1±19.3 | 11.6-19.1 | | | |

MPD = mean platelet distribution

**Table 5. Mean platelet large cell ratio (P-LCR) among the 3 groups**

| Parameter | Groups | mean±SD | Range | P value | | |
|---|---|---|---|---|---|---|
| | | | | Control *Vs* group 1 | Control *Vs* group 2 | Group 1 *Vs* group 2 |
| **PLCR (%)** | Control | 33.7±7.89 | 24.1-44.8 | 1.000 | 1.000 | 0.862 |
| | Group 1 | 33.38±9.81 | 17.8-39.4 | | | |
| | Group 2 | 30.78±7.36 | 20.4-46.6 | | | |

PLCR = platelet large cell ratio

Strong negative correlations were found in group 1 patients between MPC and PDW, MPV and P-LCR, ($p<0.001$) (Figures 1a, b and c) respectively whereas these correlations were found non-significant in group 2 patients ($p = 0.3$, 0.4, and 0.4 respectively) (Figures 2a, b and c)

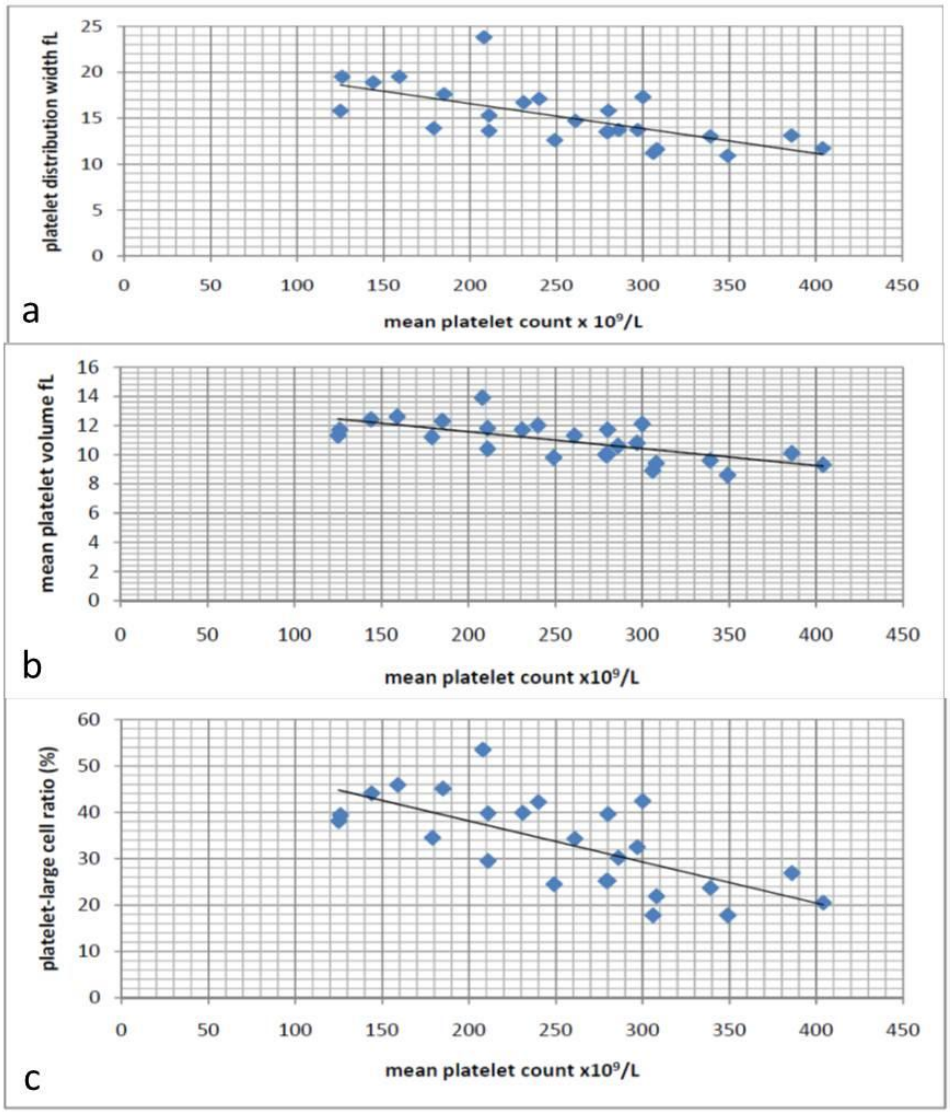


**Figure 1. The correlation between different platelet indices in group 1; (a) mean platelet count and platelet distribution width, (b) mean platelet count and mean platelet volume, (c) mean platelet count and platelet-large cell ratio**

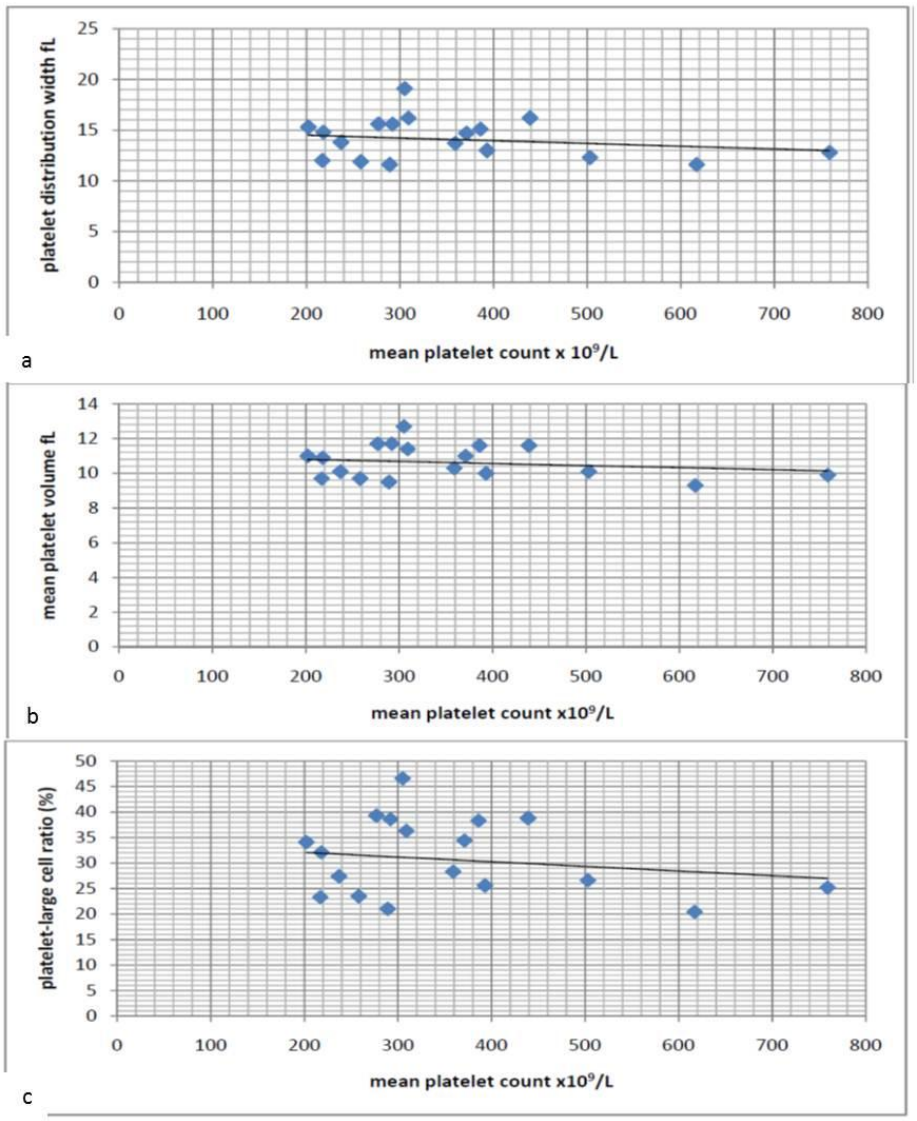


**Figure 2. The correlation between different platelet indices in group 2; (a) mean platelet count and platelet distribution width, (b) mean platelet count and mean platelet volume, (c) mean platelet count and platelet-large cell ratio**

## Discussion

Different platelet indices and especially MPV and MPC are considered as good indices of hemostasis and thrombosis [8,9]. It had been shown that there is association between MPV and ischemic stroke due to greater thrombopoitic reactivity of larger platelet [4,5, 15-19].

In this study, patients with ischemic stroke whether first-ever ischemic stroke or multiple stroke due to presence of similar risk factors (like hypertension, diabetes mellitus, smoking and atherosclerosis) were compared with control group who have similar risk factors but without history of stroke.

Both stroke patients groups were comparable from statistical point of view concerning age and risk factors as well as with control group.

It can be understood that increased MPC will increase the chance of arterial thrombosis and this had been shown also in this study as MPC was higher in group 2 patients when compared with group 1 or control (p= 0.012 ,0.023 respectively) similar to Numminen et al report [21] but in contrast to O'Brien et al [15], Butterworth et al [18] and McCabe et al [22] who

showed no differences in MPC while Nadar et al [23] and Tohgi et al [24] showed a decreased MPC in acute ischemic stroke secondary to platelet consumption. This increase in MPC in group 2 in comparison to control group and group 1 could be explained as part of inflammatory reaction [4,5].

MPV did not show any statistically significant association with development of ischemic stroke among both patients groups and control group in contrast to Greisenegger et al and others [2,13,15,18,19] who suggested that MPV is independent risk factor for development of ischemic stroke secondary to higher platelet thrombopoietic reactivity and this may be due to higher concentration of dense granules with excess release of procoagulant materials [15,18,19] but this couldn't be demonstrated in this study due to alteration in platelets size after storage at room temperature till the time of analysis or delayed presentation of patient to hospital [25]. Platelets may swell in a time dependent fashion after blood sampling in EDTA [25].

Both PDW &P LCR are related to MPV and therefore, they didn't show any difference as the latter didn't confirm any differences among the 3 groups [26].

Reduction of this linear correlation with the use of effective anti-platelet therapy didn't reduce the risk for subsequent stroke in this study which may indicate the existence of other uncontrolled risk factors that potentiate the risk of ischemic stroke.

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# Risk Factors of Abdominal Wound Dehiscence: Evaluation and Outcome

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## Abstract

**Background** Wound Dehiscence is the premature opening of a wound along surgical suture. It is a surgical complication that results from poor wound healing that can increase significantly the risk of morbidity and mortality. Risk factors in general are age, diabetes, obesity, cancers, emergency surgery, pulmonary diseases, malnutrition, weight loss, anemia, chemotherapy and radiotherapy.

**Objective** To identify the most important risk factors for wound dehiscence in an attempt to decrease the incidence, morbidity and mortality of wound dehiscence.

**Methods** This is a cross sectional study with convenient sample of 66 patients 48 males and 18 females with wound dehiscence after laparotomy in Al-Kindy Teaching Hospital, Baghdad, Iraq from February 2008 to May 2011. The medical records for all patients regarding risk factors responsible for delayed wound healing and burst abdomen of all patients were reviewed. Laparotomy done for all patients under general anesthesia. Retention through and through sutures inserted on both sides of the abdominal wall were used in all cases.

**Results** The commonest time for the wound to burst was on the 10$^{th}$ postoperative day. The average duration of hospital staying was 26 days. The most common primary diagnosis of those patients was intestinal obstruction (30.3%), and adhesolysis was the most common operative procedure (18.2%). Emergency laparotomy was the most frequent factor found (92.4%). The mortality rate in patients with wound dehiscence increased with an increment of the number of risk factors, it was 15.6% in Patients who had 2-4 risk factors and reached 100% for patients with 8 risk factors.

**Conclusion** The identification of the avoidable factors and their correction could reduce the risk of development postoperative burst abdomen and in turn decrease the mortality and morbidity rate.

**Keywords** Wound dehiscence, risk factors, laparotomy

## Introduction

Surgical wound dehiscence after laparotomy remains a serious complication. It presents a mechanical failure of wound healing of surgical incisions. Surgical incisions stimulate the healing process which in reality is a complex and continuous process with four different stages: Hemostasis, inflammation, proliferation, and maturation [(1)].

During hemostasis, platelets aggregate, degranulate and activate blood clotting. The clot is degrading, the capillaries dilate and fluids flow to the wound site, activating the complement cascade. Macrophages, lysis of cells and neutrophils are a source of cytokines and growth factors that are essential for normal wound healing [(2)].

The proliferation phase, which is the phase of granulation tissue forms in, the wound space begins in the 3$^{rd}$ postoperative day and lasts for several weeks. The most important factor in this phase is fibroblasts which move to the wound

and are responsible for the collagen synthesis. The maturation phase begins in the 7 postoperative day and lasts for 1 year or more, continued collagen deposition and remodeling contribute to the increased tensile strength of wounds [3].

It is important for the surgeon to know that wound healing demands oxygen consumption, normoglycemia and absence of toxic or septic factors, which reduce collagen synthesis and oxidative killing mechanisms of neutrophils [4]. Abdominal wound dehiscence (burst abdomen, fascial dehiscence) is a severe postoperative complication; with mortality rates as high as 45% [5]. The incidence, as described in the literatures, ranges from 0.4% to 3.5% [6-7].

Conditions associated with increased risk of wound dehiscence are anemia, hypoalbuminemia, malnutrition, malignancy, jaundice, obesity and diabetes, male gender, elderly patients and specific surgical procedures as colon surgery or emergency laparotomy which are associated with wound disruption [8]. Not all factors could be prevented, but the factors that could be prevented include pneumonia, wound infection, anemia, improper incisions and bad surgical technique [6]. Abdominal wound dehiscence can result in evisceration, requiring immediate treatment, prolonged hospital stay, high incidence of incisional hernia, and subsequent reoperations underline the severity of this complication [9].

Pre-operative conditions especially in elective operations should be recommended to reduce or eliminate the risk. No tobacco use, no steroid use prior to surgery, carefully controls of the patients comorbidity as anemia, malnutrition, obesity and cardiovascular or lung diseases. During the surgical procedures, measure to reduce the risk of infection and hypoxia in the tissue are the two most important factors for the postoperative wound healing process. The type of abdominal closure may play an important role. The tension free closure is recommended and a continuous closure is preferable [9].

Careful monitoring of patients with a predisposition to delayed healing is essential for prevention or mitigation of wound separation, especially between the fifth and twelfth postoperative days, when dehiscence most often occurs [9]. In about half the cases of dehiscence there is a noticeable increase in serosanguineous drainage on the wound dressing before separation of the outer layers becomes apparent. Patients also may report the feeling that something has “given way” in the wound [10].

The patient should be instructed to lie quietly and, if it is an abdominal wound, to try to avoid increasing intra-abdominal pressure by coughing or straining in any way. Should splinting an abdominal wound fails to prevent further separation and a spilling of the viscera through the opening, emergency surgery is imperative [10].

The intension of the current study is to identify the most important risk factors for wound dehiscence in an attempt to decrease the incidence, morbidity and mortality of wound dehiscence

## Methods

This is a cross sectional study of 66 patients with wound dehiscence after laparotomy at Al-Kindy Teaching Hospital in Baghdad from February 2008 to May 2011. The medical records for all patients regarding risk factors responsible for delayed wound healing and burst abdomen were reported age over 75 years, diagnosis of cancer, chronic obstructive pulmonary disease (medical history of COPD), malnutrition (total serum protein less than 3 mg/dl), postoperative coughing, distension and vomiting, emergency laparotomy, body weight loss more than 10 kg in the last 10 months, sepsis (intraoperative, postoperative and wound infection), obesity(BMI is more than 35), anemia (Hb less than 10 mg/dl), diabetes, use of steroids in the last 12 months, tobacco, use and previous administration of chemotherapy or radiotherapy, all were identified as risk factors.

The diagnosis of burst abdomen was made when all the abdominal layers gave way i.e. complete disruption of all layers of an abdominal wound.

Cases in which only the superficial layers gaped, are usually due to hematoma or sepsis, and cases in which it has recognized that the deep layers had parted but the skin remained intact, were excluded.

Wound disruption was more often observed on the 10th postoperative day (range from 1-18 days).

Laparotomy was done for all patients under general anesthesia. Washing of the peritoneal contents with warm saline was done, and retention through and through sutures inserted on both sides of the abdominal wall were used in all cases. The parietal peritoneum, posterior rectus sheath, and the anterior rectus sheath were all approximated (after refreshing the edges) by a single layer of continuous sutures of No. 1 monofilament nylon, mounted on a large half circle, rounded tip needle. Each suture was placed 1.5-2 cm away from the wound edge on either side, at an interval of about 1cm from each other. The skin was closed as a separate layer with silk or nylon and patients were followed postoperatively for one year.

## Results

The study included 66 patients with wound dehiscence; there were 48 (72.7%) males and 18 (27.3%) female. The mean age was 58 years (range from 32 to 85 yrs) SD (±8.45).

**Table 1. The age group of patients**

| Age group (years) | Number | % |
|---|---|---|
| 20-40 | 14 | 21.21 |
| 41-50 | 12 | 18.18 |
| 51-60 | 15 | 22.72 |
| 61-70 | 19 | 28.8 |
| >70 | 6 | 9.1 |
| Total | 66 | 100 |

The most commonly affected age group was between 61 to 70 years (28.8%). The commonest time for the wound to burst was on the tenth postoperative day (range from 5 to 18 days). The average duration of hospital stay 26 days (range from 20 to 35 days) SD (±4.18).

**Table 2. The primary diagnosis and initial operative procedures that caused wound dehiscence**

| Primary diagnosis | No. | % | Operative procedures | No. | % |
|---|---|---|---|---|---|
| **Perforated peptic ulcer** | 16 | 24.2 | Simple closure (Grahams patch) | 7 | 24.2 |
| **Perforated typhoid ulcer** | 10 | 15.6 | Simple closure | 7 | 10.6 |
| | | | Resection and anastomosis | 2 | 3.1 |
| | | | Ileostomy | 1 | 1.5 |
| **Colon cancer** | 10 | 15.15 | Rt. Hemicolectomy | 2 | 3.1 |
| | | | Resection and anastomosis | 6 | 9.1 |
| | | | Colostomy | 2 | 3.1 |
| **Intestinal obstruction** | 20 | 30.3 | Adhesolysis | 12 | 18.2 |
| | | | Resection and colostomy | 3 | 4.6 |
| | | | Colostomy | 5 | 7.8 |
| **Acute cholecystitis (empyema)** | 1 | 0.02 | Cholecystectomy | 1 | 1.5 |
| **Acute perforated appendicitis** | 1 | 0.02 | Appendicectomy | 1 | 1.5 |
| **Colonic injury** | 8 | 12.12 | Direct repair | 3 | 4.6 |
| | | | Colostomy | 55 | 7.8 |
| **Total** | 66 | 100 | | 66 | 100 |

The most common primary diagnosis was intestinal obstruction 30.3%of which adhesolysis was the most common operative procedure done in 12 patients (18.2%).

**Table 3. The types of incisions of primary operations**

| Type of incisions of primary operations | No. | % |
|---|---|---|
| Upper midline | 5 | 7.8 |
| **Lower midline** | 14 | 21.21 |
| **Upper and lower midline** | 32 | 48.5 |
| **Upper paramedian** | 8 | 12.12 |
| **Lower paramedian** | 6 | 9.1 |
| **Kocher** | 1 | 1.5 |
| Total | 66 | 100 |

The most frequent incision to bust was upper and lower midline 48.5%.

**Table 4. The proportion of risk factors**

| Risk factors | No. | % |
|---|---|---|
| Emergency laparotomy | 61 | 92.4 |
| Postoperative cough, vomiting, distension | 44 | 66.7 |
| Sepsis | 36 | 54.5 |
| Age over 65 | 28 | 42.4 |
| Malignancy | 19 | 28.8 |
| COPD, cardiopulmonary disease | 17 | 25.7 |
| Malnutrition, anemia | 15 | 22.7 |
| Obesity | 11 | 16.7 |
| Diabetes mellitus | 10 | 15.2 |
| Chronic diseases | 9 | 13.6 |
| Radiotherapy, chemotherapy | 3 | 4.5 |
| Prolong steroid therapy | 3 | 4.5 |

The emergency laparotomy was the most frequent factors found in 92.4% of the cases, the next was the postoperative cough, vomiting, and distension in 66.7%.

**Table 5. The number of risk factor for all patients and its relation to the final outcome (mortality and morbidity)**

| Patients and No. of risk factors | No. | % | Mortality No. | % | Morbidity No. | % |
|---|---|---|---|---|---|---|
| Patients with 2-4risk factors | 32 | 48.5 | 5 | 15.6 | - | |
| Patients with5-6 risk factors | 23 | 34.8 | 9 | 39.1 | 1 | |
| Patients with 7-8 risk factors | 8 | 12.1 | 5 | 62.5 | 1 | |
| Patients with > 8 risk factors | 3 | 4.6 | 3 | 100 | - | |
| **Total** | **66** | **100** | **22** | **33.3** | **2** | **3** |

All patients were re-operated after the wound dehiscence diagnosis and 22 of them (33.3 %) died due to postoperative complications of reoperation mostly due to respiratory, cardiovascular complications and septicemia. In 2 (3%) of them recurrence of wound dehiscence was observed.

The number of patients with wound dehiscence increased with an increase in the number of risk factors, reaching 100% for patients with 8 risk factors.

## Discussion

This study included 66 patients with wound dehiscence; there was higher rate of wound dehiscence in males 48 (72.7%) than females 18 (27.3%) and this is comparable to the study done by Gürleyik who found that males have a higher risk of developing abdominal wound dehiscence (76%) and this may be explained by the fact that men build up higher abdominal wall tension than females [(12)].

The most commonly affected age group in this study was in the 7$^{th}$ decade (28.8 %) and this goes with the study of Rodríguez-Hermosa who established that the mean age was 70 years [(13)]. This may be due to deterioration of the tissue repair mechanisms in the elderly especially during the first few days of wound healing process.

The commonest time for the wound to burst was on the tenth postoperative day (range from 5 to 18 days) when the dehiscence became apparent shortly after the skin sutures were removed and

this coincide with a study by John Spiliotis *et al* [11] who found that the wound dehiscence was more often observed on the 9th postoperative day (ranging from the 6th to 15th).

The average duration of hospital stay was 26 days (ranging from 20 to 35 days) in our study and this is similar to a study by Mazilu *et al* [14] who found that the wound dehiscence is associated with a high mortality and morbidity rates, and increased the costs and hospitalization period.

On the subject of the primary diagnosis this study showed that the most common primary diagnosis was emergency surgery (intestinal obstruction 30.3%, perforated peptic ulcer 24.2%) whereas Khan MN *et al* found that Malignant intestinal obstruction was the leading cause of wound dehiscence[15] and Col *et al* and Niggebrugge *et al* demonstrated a significantly higher incidence of postoperative wound dehiscence in emergency operations for perforated peptic ulcer and intestinal obstruction [16,17]. This may be due to fact that the patients are under bad nutritional state due to vomiting in these cases in addition to low immunity state because of underlying disease especially malignant tumor as a cause of intestinal obstruction.

As regards to the incisions of primary operations the present study showed that the most common incision was upper and lower midline incision 48.5% and the second one was lower midline incision 21.21% and this agrees with the study done by Mokela *et al* who established that vertical incision was reported as a risk factor compared with transverse incision [18].

Emergency laparotomy was the most frequent risk factor (92.4%) followed by postoperative cough, vomiting, and distension (66.7%) and sepsis (54.5%). This goes with the study of Heller *et al* who demonstrated a significantly higher incidence of postoperative wound dehiscence in emergency than in elective surgery [19]. This may be due to that the patients who undergo emergency surgery are generally not well prepared and in bad general condition and nutritional state and the chance of contamination of the surgical field is higher than in elective surgery. Moreover, the performance of the surgeon might be affected at night, which could lead to suboptimal closure of the abdomen at the end of the operation.

Regarding the mortality rate of this study was 33.3 %.in a study by Waqer *et al*, the mortality rate was 45% [20] and was 28% in the study of Rodríguez-Hermosa *et al* [21].

Concerning the number of the risk factors and its relation to the mortality and morbidity rate we found that the mortality rate increased with the increment of the number of risk factors, it was 15.6% in Patients who had 2-4 risk factors and reached 100% in patients with 8 risk factors, while Spiliotis *et al* found mortality of 75% in patients with 7 or more risk factors [11].

In conclusion, the identification of the avoidable factors like pneumonia, wound infection, anemia, improper incisions and bad surgical technique and their correction could reduce the risk of development postoperative burst abdomen and in turn decrease the mortality and morbidity rate.

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# Clinical Study of Respiratory Distress Syndrome in Al-Kadhimiya Teaching Hospital

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## Abstract

**Background** Respiratory distress syndrome (RDS) or hyaline membrane disease (HMD) is an acute lung disease of the newborn caused by surfactant deficiency. It is Seen primarily in neonates younger than 36-38 weeks of gestational age weighing less than 2500 gram.

**Objective** To find out the incidence, main risk factors of HMD, note the changes in serum calcium, potassium and sodium and to study the outcome of HMD during the period of the illness.

**Methods** A prospective study was performed on one hundred neonate admitted to the neonatal care unit in Al-Kadhimiya Teaching Hospital. Sex, gestational age, weight, mode of delivery, maternal illnesses were studied as risk factors. Serum calcium, Na and K were measured.

**Results** The incidence of RDS was 0.75% of total live births, 98(98%) of them were below 2.499 kilograms and less than 37 weeks of gestational age. Risk factors of RDS are male sex accounted for 61% of total cases. Cesarean sections were 28% of the total. Maternal diabetes mellitus represented 25%. Perinatal asphyxia was 22%. Familial predisposition had an incidence of 8% and finally prolonged rupture of membranes stood for 5%. Hypocalcaemia and hypokalemia were common with serum sodium was within the lower normal limits. The time of improvement for most babies (about 40%) was by day 4 of life. Mortality was 30% with the major percentage of death (which was 90%) was between day 1 and day 4.

**Conclusion** RDS occurs predominantly in premature babies as a main risk factor with male sex and cesarean sections. In addition to other risk factors such as maternal diabetes mellitus and perinatal asphyxia. Associated changes in serum electrolytes and serum calcium including hypocalcaemia, hypokalemia and lower normal serum sodium are common. The 4th day of life was the time of improvement for most babies while most of them who died did so by the first 4 days of life. Among the admitted babies, about third of them (actually 30%) died because of RDS.

**Key words** RDS, Gestational age, Mortality.

## Introduction

Respiratory distress syndrome (RDS), also known as hyaline membranedisease (HMD), is an acute lung disease of the newborn caused by surfactant deficiency (Surfactant is synthesized in type II pneumocytes or alveolar cells, It appears in the amniotic fluid between 28-32 weeks, but mature levels of pulmonary surfactant are usually present after 35 weeks' gestational age).

It is seen primarily in neonates younger that 36-38 weeks' gestational age and weighing less than 2500 gm [(1-4)].

The infant is often with one or more of the following risk factors, prematurity, male sex, familial predisposition, cesarean section, perinatal asphyxia, chorioamnionitis, hydrops, and maternal diabetes [(3,5)].

Most of the affected infants exhibit progressive signs of respiratory distress soon after birth that

include tachyapnea, expiratory grunting (from partial closure of glottis), subcostal and intercostals retractions, cyanosis and nasal flaring [1,2,5-7].

The natural course of the disease is that of increasing severity duringthe first 1-2 days before improvement occur during 4th or 5th day, which is heralded by spontaneous diuretic phase of RDS [8].

Radiological findings are correlated with the severity of clinical findings, as they are normal in the early period of the disease. Blood gas sampling can be obtained (mixed respiratory and metabolic acidosis, hypoxemia) [3,5]. Pulse oximetry is used as a non-invasive tool to monitor oxygen saturation, which should be maintained at 88-95% [1,3]. Serum electrolyte levels and calcium should be monitored every 12-24 hours for management of parenteral fluids [3]. Serum glucose level may be high or low initially and must be monitored closely to assess the adequacy of dextrose infusion. Hypoglycemia alone can lead to tachypnea and respiratory distress [3].

Administration of betamethasone to the women 48 hours before the delivery of the fetuses between 24-34 weeks of gestational age significantly reduces the incidence of RDS [1-3,5]. Repeated weakly doses of betamethasone until 32 weeks may reduceneonatal morbidity and duration of mechanical ventilation. By the usage of surfactant replacement therapy, the mortality rate of RDS decreases by about 50% [3,5,9].

Early supportive care of low birth weight (LBW) infants, Surfactant replacement, Oxygenation and continuous positive airway pressure (CPAP), Assisted ventilation, Antibiotic therapy [2,3,5,6]. The intensive observation and care of high-risk newborn infants can significantly reduce the morbidity and mortality of RDS [1].

Antenatal steroids, postnatal surfactant use, improved modes of ventilation, and developmentally appropriate care have resulted in low mortality from RDS (≈10%) [4]. Mortality increases with decreasing gestational age. Surfactant therapy reduces mortality about 40%. About 85-90% of all infants surviving RDS are normal. Infants weighing more than 1500g have much better outcomes.

The long term prognosis for normal pulmonary function is excellent [1,4,10].

The intension of the study is to find out the incidence, main risk factors of HMD, note the changes in serum calcium, potassium and sodium, and to study the outcome of HMD during the period of the illness in the neonatal care unit in Al- Kadhimiya Teaching Hospital.

## Methods

A prospective study was performed and one hundred babies admitted to the neonatal care unit in Al-Kadhimiya Teaching Hospital out of 13258 live births to mothers admitted to the obstetrics and gynecology department during a period from the first of July 2007 to the first of January 2008. The clinical diagnosis of RDS was based upon the following:-

1. Tachyapnea (respiratory rate>60/minute).
2. Expiratory grunting.
3. Sternal and intercostal recession.
4. Cyanosis in room air.
5. Delayed onset of respiration in very immature babies.
6. The radiological findings which include fine granular, ground glass appearance, and air bronchogram.

The following information was collected from obstetricians, mothers and relatives of the newborn; including type of delivery, prolonged rupture of membranes and maternal DM and family history of similar condition.

Then, full systematic examination was done to all admitted neonates and during their stay in neonatal care unit; they were followed carefully with special concern about their treatments, complications and their outcomes.

Portable chest X-ray, serum potassium, sodium and calcium were done to all of them immediately after admission. We assumed that the normal values as follow:

Serum sodium is 132-145 mmol/l, serum potassium is 4-7 mmol/l and serum calcium is 7.2-11.2 mg/dl [4,5]. Blood culture and other investigations were done according to specific

conditions and complications occurred. Neonates with transient tachyapnea of the newborn (TTN) and meconium aspiration, pneumonia were excluded from this study. The weight of the neonates was measured by scale, and gestational age assessed by expanded New Ballard Score.

Neonates were divided into six groups according to their birth weights and gestational ages as follows; (a) ≤ 0.999 kg and/or (≤ 28 wk), (b) 1-1.499 kg and/or (29-30 wk), (c) 1.500-1.699 kg and/or (31-32 wk), (d) 1.700-1.999 kg and/or (33-34 wk), (e) 2-2.499 kg and/or (35-36 wk), (f) ≥ 2.500 kg and/or (≥ 37 wk).

## Results

The number of live births during the period of the study in Al-Kadhimiya Teaching Hospital was (13258) and (100) out of (4782) admitted to the neonatal unit were suffering from RDS which equals to a percentage of (0.75%).

The highest incidence of RDS was in those who were 29-30 week of gestational age (35%) and mainly in male sex (22 out of 35) with the highest male: female ratio in those who were ≤ 28 week of gestational age which was (1.8:1); while there is equal ratio for those with ≥ 37 week of gestational age; these are shown in table 1.

**Table 1. Sex distribution of the patients with gestational age**

| Gestational age (weeks) | ♂ | ♀ | Total No. | % | M:F |
|---|---|---|---|---|---|
| ≤28 | 11 | 6 | 17 | 17 | 1.8:1 |
| 29-30 | 22 | 13 | 35 | 35 | 1.7:1 |
| 31-32 | 13 | 9 | 22 | 22 | 1.4:1 |
| 33-34 | 9 | 7 | 16 | 16 | 1.3:1 |
| 35-36 | 5 | 3 | 8 | 8 | 1.6:1 |
| ≥37 | 1 | 1 | 2 | 2 | 1:1 |
| Total | 61 | 39 | 100 | 100 | 1.5:1 |

There are various risk factors for RDS with different percentages, relationships and frequencies of occurrence. Preterm neonates were with the highest percentage which was 98(98%) while the second most frequent risk factor was male sex 61(61%), cesarean section had a high rate 28(28%) diabetic mothers had a risk to develop RDS in their newborn babies with a percentage of 25(25%), after that; the perinatal asphyxia comes with 22 cases (22%), finally; the other less important risk factors were familial predisposition 8 cases (8%) and prolonged rupture of membranes with chorioamnionitis 5 cases (5%). All the above risk factors are shown in table 2.

**Table 2. Various risk factors for RDS and their percentage**

| Risk factor | % |
|---|---|
| Preterm | 98 |
| Male | 61 |
| Cesarean section | 28 |
| Maternal DM | 25 |
| Perinatal asphysxia | 22 |
| Familial predisposition | 8 |
| Prolonged rupture of membranes | 5 |

Serum sodium values were in the normal range but actually most of the patients were on the lower limits of the range, while serum potassium values were swinging between the normal range and above it with the fact that most of patients were on the highest readings.

On the contrary, serum calcium values were swinging from below the normal range to the normal with the most of patients was on the lowest readings. These are best illustrated in table 3.

**Table 3. Serum sodium, calcium, and potassium in relation to gestational age**

| Gestational age (weeks) | Serum $Na^+$ | | Serum $Ca^{2+}$ | | Serum $K^+$ | |
|---|---|---|---|---|---|---|
| | Mean value | Range | Mean value | Range | Mean value | Range |
| **≤ 28** | 137.5 | 133-142 | 7.20 | 6.6-7.8 | 6.55 | 5.3-7.8 |
| **29-30** | 136.5 | 132-141 | 7.30 | 6.9-7.7 | 6.40 | 5.2-7.6 |
| **31-32** | 140.0 | 134-146 | 7.20 | 6.5-7.9 | 6.50 | 5.5-7.5 |
| **33-34** | 138.5 | 133-144 | 7.40 | 6.7-8.1 | 6.50 | 5.4-7.6 |
| **35-36** | 139.5 | 134-145 | 7.55 | 6.5-8.6 | 6.35 | 5.3-7.4 |
| **≥37** | 141.5 | 135-138 | 6.60 | 6.4-6.8 | 5.70 | 5.1-6.3 |

There is a specific relationship between the gestational age, body weight and time of improvement in days, as shown in table 4. The time of improvement fastens with increasing gestational age and most of the patients improved actually within the first 4 days within a range of 2-9 days.

**Table 4. Time of improvement in days according to body weight**

| Gestational age (years) | Time of improvement (days) | Mean value |
|---|---|---|
| **≤28** | 4-9 | 6.5 |
| **29-30** | 3-8 | 5.5 |
| **31-32** | 2-8 | 5.0 |
| **33-34** | 3-7 | 5.0 |
| **35-36** | 2-4 | 3.0 |
| **≥37** | 2-3 | 2.5 |

Another relationship is between the gestational age, body weight and time ofdeath in days, it also shows that the time of death in days is increased with increasing gestational age with a significant decline from the first to thefourth day within a range of 1-7 days. These are best shown by table 5.

About two thirds of the patients 70 (70%) survived and discharged home, whilethe other third had died with a percentage of 30 (30%).

## Discussion

The incidence of RDS in Al-Kadhimiya Teaching Hospital during the period of the study was 0.75% among all live birth deliveries. Al-Saraj (in Al-Mosul) reported the same incidence of 0.75% in his study [11], while Al-Ezzi (in Al-Ramadi) reported 0.72% [12]. In US the incidence was about 1% [13], about 0.6% of newborns had RDS (about 24,000 or 6 per 1,000 live births) [14] and finally, Peter (in United States) gave an incidence of 1% [2].

The gestational ages of 98% of the babies were ≤ 36 weeks while it was 97% in Al-Saraj study (11) and most cases are seen in babies born before 28 weeks. It is very uncommon in infants born full-term (at 40 weeks) [15], with the highest incidence was in those who were 29-30 weeks of gestational age, although it was 31-32 weeks of gestational age as Al-Saraj found [11].

**Table 5. Time of death according to body weight**

| Gestational age (years) | Time of death (days) | Mean value |
|---|---|---|
| **≤28** | 1-5 | 3.0 |
| **29-30** | 1-6 | 3.5 |
| **31-32** | 1-7 | 4.0 |
| **33-34** | 1-7 | 4.0 |
| **35-36** | 2-5 | 3.5 |
| **≥37** | ---- | --- |

Regarding the sex of the babies with RDS, 61% of the babies were males and 39% were females, while Al-Saraj claimed that 58% were males and 42% were females [11]. Al-Ezzi found that 60% were males and 40% were females [12]. Scope reported males' incidence as 59% and females' as 41% [14]. Other risk factors include multifetal pregnancies, maternal diabetes, and being male

and white [16]. Cesarean section as a risk factor was present in 28% as compared to 15% reported by Al-Saraj [11] and 20% of Al-Ezzi [12]. Maternal DM 25% and they are full term as compared with 31% claimed by Al-Saraj [11]. Perinatal asphyxia in 22% with a near percentage reported by Al-Saraj which was 20% [11]. Familial predisposition in 8% which is almost similar to that reported by Al-Saraj with his 8.5% [11] and Stoll and Kliegman (in United States) who claimed an increase in incidence of RDS with a familial predisposition [1]. Prolonged rupture of membranes was 5%, which is in consistent to that reported by Al-Saraj with 6% [11]. The risk of neonatal RDS may be decreased if the pregnant mother has chronic, pregnancy-related high blood pressure or prolonged rupture of membranes, because the stress of these situations can cause the infant's lungs to mature sooner [17], at the same time this is what Stoll and Kliegman had said [1].

Hypocalcaemia and hyperkalemia were common among neonates with RDSespecially those with more severe respiratory symptoms, infants of diabetic mothers, more premature and non-fed babies. While serum sodium was mostly within the lower normal results which may be due to inappropriate secretion of antidiuretic hormone (ADH) secondary to RDS. These findings are in consistent to that found by Peter [2] and Tricia et al (in United States) [3].

Forty percent of babies improved by day 4 with no more oxygen requirement after 4$^{th}$ day, and the average of improvement is between day 2 and day 9 the that is decreased with increasing gestational age. This is agreed by the study done by Al- Saraj with 42% improvement by day 4 and a range of 2-9 days [11], The condition often worsens for 2 to 4 days after birth with slow improvement thereafter [17] other study said that RDS resolves within 4 or 5 days [15].

The time of death in days declines exponentially from the 1st. day to the 4$^{th}$ day where 90% of deaths occur during this period with a range of 1-7 days. Some infants with severe respiratory distress syndrome will die, although this is rare on the first day of life. If it occurs, it usually happens between days 2 and 7 [17].

The mortality rate was 30% which lies within that reported by Al-Saraj being 35% [11] while it was 10% in other studies (in United States) [16] andThe overall mortality for newborns with documented RDS was 81/200 (41%), and was highest (70%) for babies weighing <1000g at birth [18] and Dinwiddie (in England) who said that mildly affected infants recover with no more than good nursing care, warmth, graduated oxygen therapy and minimal handling with an overall mortality of 10% [16] but a more pessimistic results were reported by Al-Ezzi of about 48% [12], in contrast to what had been claimed by Stoll and Kliegman as a 10% mortality rate [1].

By conclusion the incidence of RDS was 0.75% and the greatest incidence was in those who were 29-30 weeks of gestation, the main risk factors for RDS are prematurity, male sex and cesarean sections.

The mortality rate was 30% and most of patients die between day 1 and day 4. While the time of improvement for most babies is day 4.

Hypocalcemia and hyperkalemia were common while serum sodium within the lower normal limits. So we recommend improving the antenatal care of all pregnant and better selection of patients for cesarean sections. Nursing staff that works in neonatal unit should be well trained withperiodic educational courses. The nursery care units should be supplied with all facilities for mechanical ventilation and blood gas analysis with serum electrolytes and calcium should be available for early detection and follow up of complications. Availability of surfactant replacement is vital because it is effective for both prevention and treatment of RDS.

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# المجلة العراقية للعلوم الطبية



**عنون المراسلات إلى المجلة العراقية للعلوم الطبية، صندوق بريد 14222 بغداد، العراق. تلفون و فاكس (964-1-5224368).**
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