pISSN: 2070-1128
eISSN:2227-4081

MMJ

MUSTANSIRIYA MEDICAL JOURNAL

VOLUME 15 ISSUE 1 APRIL 2016

IN THIS ISSUE

Ammar et al : Otitis media with effusion in children

Jamil et al : Oral rehydration solution in Sulaimani

Zeena et al : Polycystic ovarian syndrome in reference to metabolic syndrome

Wasan et al: Non Palpable Breast Mass

Israa et al : HSV-2 in Pregnant Women

Henan et al: Maternal serum placental growth factor level in pre eclamptic women

Raed et al: Proximal isovelocity surface area in mitral valve stenosis

Hayder Q.: Segment posterior spinal fixation in thoracolumbar burst fractures

Hayder et al : Metformin on omentin-1 serum levels in a newly diagnosed type 2 DM

Walaa et al : Omentin-1 in Acute Coronary Syndrome

Sahar et al : Ultrasound guided needle True -Cut biopsy in diagnosis of breast lesions

Shilan : Effect of maternal age on the outcomes of in vitro fertilization Sulaimani

Huda et al : Apolipoproteins and Lipid Profile & Oral Diseases and Hypertension

Abbas : C-reactive protein in hypertensive patients

Abbas et al : Evaluation of patients with nodal osteoarthritis

PEER-REVIEWED SCIENTIFIC JOURNAL

THE OFFICIAL JOURNAL OF AL-MUSTANSIRIYA UNIVERSITY, COLLEGE OF MEDICINE.

MMJ

MUSTANSIRIYA MEDICAL JOURNAL

Volume 15 Issue 1 April 2016

PEER- REVIEWED SCIENTIFIC JOURNAL

THE OFFICIAL JOURNAL OF AL- MUSTANSIRIYA UNIVERSITY, COLLEGE OF MEDICINE

Contents

IV Instructions to Authors

1 Diagnostic accuracy of otitis media with effusion in children
Ammar Hadi Khmmas, Mohammed Radef Dawood, Abdulhussein Kareem , Yahya Ajeel Hammadi

7 Knowledge and attitude of mothers regarding oral rehydration solution in Sulaimani
Jamil Aziz, Adiba Ismaeel Ali, Ibrahim Khasraw Ghafoor , Adnan Mohammed Hasan

11 Assessment of oral health status, leptin, and inflammatory markers in serum and saliva of patients with polycystic ovarian syndrome in reference to metabolic syndrome
Zeena Raad Helmi, Reem Adnan Sabri, Ban Hadi Hameed

20 Non Palpable Breast Mass : Radiological & Pathological Evaluation
Wasan Ismail Al-Saadi, Basim Shehab Ahmed, Qahtan Adnan Mahdi, Israa Qader ,Yaarub Idrees

25 Infection with herpes simplex virus type 2 (HSV-2) in pregnant women
Israa Hashim Saadoon, Sumaya T. Saihood

30 The role of maternal serum placental growth factor level in predicting delivery within two weeks in pre eclamptic women
Henan Dh. Skheel Aljebory , Handi Shaker

38 Validation of proximal isovelocity surface area in Mitral valve stenosis
Raed S.Abed , Ghazi F. Haji , Ghassan M.Mahmood

45 Comparison between short and long segment posterior spinal fixation in thoracolumbar burst fractures
Hayder Qatran Raheem

49 Effects of metformin on omentin-1 serum levels in a newly diagnosed type 2 diabetes mellitus: Randomized, placebo controlled study
Hayder M. Al-Kuraishy, Miqat T. Hamada, Abdilkarim Y. Al-Samerraie

57 Measurement of Serum Omentin-1 in Patients with Acute Coronary Syndrome
Walaa Ahmed AL-Jedda, Abbas Naji H.Al-Sharifi, Mushtaq Abed H. AL-Taie

65 Diagnostic Sharpness of Ultrasound guided needle True -Cut biopsy in diagnosis of breast lesions
Sahar Basim Ahmed ,Theer Jwad Kadhim

70 The effect of maternal age on the outcomes of in vitro fertilization Sulaimani region
Shilan Hussein Karim

75 Apolipoproteins and lipid profile in patients with oral diseases and systemic arterial hypertension
Huda Shakir Ahmed , Noor Thair Tahir, Hind Shakir Ahmed

80 High –sensitivity c-reactive protein in hypertensive patients living in United Arab Emirates
Abbas AL-Sharifi

84 Evaluation of Serum Interleukin-6, Body Mass Index and bone mineral density in Patients with Nodal Osteoarthritis
Abbas Toma Joda, Adnan F. Al-Najar, Mustafa Saleam Khalaf

Instructions to Authors

Mustansiriya Medical Journal MMJ (quarterly peer-reviewed journal) is the official Journal of the College of Medicine, Al-Mustansiriya University. MMJ welcomes manuscripts on all aspects of medicine in the form of original articles, case reports, review articles, educational forms and letters to the editor. All the Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by International Committee of Medical Journal Editors (updated 2015). http://www.icmje.org/urm_full.pdf.

Types of Manuscripts

Original articles: Randomised controlled trials, intervention studies, studies of screening and diagnostic test, outcome studies, cost effectiveness analyses, case-control series, and surveys with high response rate.

Review Articles and Educational Forum: Review articles are written by researchers of considerable experience in the field concerned. The author should review the recent trend or advances in that field in the light of his own work. However, when an author has not done enough original work on a topic but nevertheless wants to share the knowledge on recent advances/trends which may be useful for post-graduate students, he may do so by writing an article for Educational Forum.

Case reports: New/interesting/very rare cases can be reported. Cases with clinical significance or implications will be given a priority; whereas, mere reporting of a rare case may not be considered. Up to 1000 words excluding references and abstract, and up to 10 references should be used.

Letter to the Editor: Should be short, decisive observation. They should not be preliminary observations that need a later paper for validation.

The Editorial Process

A manuscript will be reviewed for possible publication with the understanding that it is being submitted to **MMJ** alone on time and has not been published anywhere. Simultaneously submission, or already accepted paper for publication elsewhere would be rejected.

The journal expects that one of the related authors will be permitted to correspond with the Journal for all matters related to the manuscript. On submission, editors review all submitted manuscripts initially for suitability of formal review. Manuscripts with insufficient originality, serious scientific or technical flaws, or lack of a significant message are rejected before proceeding for formal peer-review.

Manuscripts that are found suitable for publication in **MMJ** are sent to two or more expert reviewers. The reviewers should not be affiliated with the same institutes as the contributor/s. However, the selection of these reviewers is at the sole discretion of the editor. The journal follows a double-blind review process, wherein the reviewers and authors are unaware of each other's identity.

Every manuscript is also assigned to a member of the editorial team, who based on the comments from the reviewers takes a final decision on the manuscript. The comments and suggestions (acceptance/rejection/ amendments in manuscript) received from reviewers are conveyed to the corresponding author. If required, the author is requested to provide a point by point response to reviewers' comments and submit a revised version of the manuscript. This process is repeated till reviewers and editors are satisfied with the manuscript.

Manuscripts accepted for publication are copy edited for grammar, punctuation, print style, and format. Page proofs are sent to the corresponding author. The corresponding author is expected to return the corrected proofs within short period. It may not be possible to incorporate corrections received after that period. The whole process of submission

of the manuscript to final decision and sending and receiving proofs is completed online.

Authorship Criteria and Ethical Considerations

Authorship credit should be based only on substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; drafting the article and/or revising it critically for important intellectual content. Acquisition of funding, the collection of data, or general supervision of the research group, by themselves, do not justify authorship.

Evidence for approval by a local Ethics Committee (for both human as well as animal studies) must be supplied by the authors on demand. Animal experimental procedures should be as humane as possible and the details of anesthetics and analgesics used should be clearly stated. The ethical standards of experiments must be in accordance with the guidelines provided by the CPCSEA and World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Humans and animals.

The journal will not consider any paper which is ethically unacceptable. A statement on ethics committee permission and ethical practices must be included in all research articles under the 'Materials and Methods' section.

Submission of the Manuscript

Manuscripts, or the essence of their content, must be previously unpublished and should not be under simultaneous consideration by another journal. The authors should also declare if any similar work has been submitted to or published by another journal. They should also declare that it has not been submitted/published elsewhere in the same form, in English or in any other language, without the written consent of the Publisher. In addition, it should be claimed that the paper is the original work of the author(s) and not copied (in whole or in part) from any other work.

By virtue of the submitted manuscript, the corresponding author acknowledges that all the co-authors have seen and approved the final version of the manuscript. The corresponding author should provide all co-authors with information regarding the manuscript, and obtain their approval before submitting any revisions. The submitted papers should be in Times New Roman font, double spaced (14 pt) for the titles and (12 pt) for the text. **Any paper not follow the instructions will not enter the editorial and peer-reviewing process.**

The submitted paper should be prepared as three main separate files:

a. **First Page File**: composed of the following:

1. **Covering letter**: include the type and title of the article, prior publications of the authors, support, conflicts of interest, permissions and address of the corresponding author.
2. **Contribution details**: contribution of each other to the concepts, design, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review.
3. **Title page:** this include the following:

Type of manuscript (Original/Review/Case); the title of the article, which should be concise, but informative; running title or short title not more than 50 characters; the name by which each contributor is known (Last name, First name and initials of middle name), with his or her highest academic degree(s) and institutional affiliation; the name of the department(s) and institution(s) to which the work should be attributed; the name, address, phone numbers, and email address of the contributor responsible for correspondence about the manuscript; the total number of pages, total number of photographs and word counts separately for abstract and for the text (excluding the references and abstract) and finally the Acknowledgements.

b. **The Article File:** this file composed of the Abstract and Key words, Introduction, Material and Methods, Results, Discussion and Conclusions, References, and finally Tables and Figures.

Blinded Article file: The manuscript must not contain any mention of the authors' names or initials or the institution at which the study was done or acknowledgements. Manuscripts not in compliance with The Journal's blinding policy will be returned to the corresponding author. The main text of the article, beginning from Abstract till References (including tables) should be in this file. Do not zip the files. Do not incorporate images in the file. The pages should be numbered consecutively, beginning with the first page of the blinded article file.

c. **Images**: Graphs and Contributors' Form assignment.

Detailed Description of the Article File

Abstract

- This page should contain the full title of the manuscript and an abstract.
- The abstract must be concise, clear and informative.
    - Not exceed 150 words for case reports or review articles (unstructured).

- Not exceed 250 words for original articles (structured).

- The abstract must be in a structured form (background, Aims, Materials and Methods, Results, and conclusions).
- The abstract should explain briefly what was intended, done, observed and concluded.
- The conclusions and recommendations not found in the text of the article should not be given in the abstract.
- If references must be cited in the abstract they must include the authors, journal title, year of publication, volume number and page range.
- The use of the abbreviations in the abstract should be avoided.
- **Key words**: Provide 3-5 keywords which will help readers or indexing agencies in cross-indexing the study (**the words found in title should not be given as key words)**.

Introduction

- It should start on a new page.
- The body of the research articles should start with a brief introduction, which outlines the historical origins of the study and stating the aim of the study and/or hypothesis to be tested.
- Essentially this section must introduce the subject and briefly say how the idea for research originated.
- Give a concise background of the study. Do not review literature extensively but provide the most recent work that has a direct bearing on the subject.
- Justification for research aims and objectives must be clearly mentioned without any ambiguity.
- The purpose of the study should be stated at the end.
- **The introduction should not discuss the results.**

Materials and Methods

- The methods section should be written such that another researcher is able to reproduce the research work.
- Important methodological aspects of your work should be described, even if such descriptions can also be found in prior publications.
- The number of subjects, the number of groups, the study design, sources of drugs with dosage regimen or instruments used, statistical methods and ethical aspects must be mentioned under this section.
- Give explicit descriptions of modifications or new methods so that the readers can judge their accuracy, reproducibility and reliability.
- Drugs and chemicals should be precisely identified using their non-proprietary names or generic names.
- If necessary, the proprietary or commercial name may be inserted once in parentheses. The first letter of the drug name should be small for generic name (e.g., dipyridamole, propranolol) but capitalized for proprietary names (e.g., Persantin, Inderal).
- **Statistical Analysis**: The details of statistical tests used and the level of significance should be stated. If more than one test is used it is important to indicate which groups and parameters have been subjected to which test.

Results

- The Results should contain both positive and negative results supported by valid statistical methods.
- The results should be stated concisely without comments.
- They should be presented in logical sequence in the text with appropriate reference to tables and/or figures.
- The data given in tables or figures should not be repeated in the text.
- The same data should not be presented in both tabular and graphic forms.
- Simple data may be given in the text itself instead of figures or tables. Avoid discussions and conclusions in the results section.

Discussion

- The Discussion could contain the summary of the major findings, (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); while avoiding repetition of the statements in Abstract or the Results.
- Citing literature supporting or defying the findings should be provided.
- The Discussion should be able to project a major advancement of scientific concept, method or effect.
- Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation) should be stated.
- Controversies raised by this study along with possible future research directions (for this particular research collaboration, underlying mechanisms, clinical research) could also be mentioned.
- Do not repeat in detail data or other material given in the Introduction or the Results section.
- **Conclusions** must be drawn considering the strengths and weaknesses of the study. They must be conveyed in the last paragraph under Discussion. Make sure conclusions drawn should count with the objectives stated under Introduction.

References

- Vancouver style should be followed for all the references.
- Only published manuscripts should be included in the reference list.
- Avoid using abstracts as references.
- References should be numbered consecutively in the order in which they are first mentioned in the text (not in alphabetic order).
- Identify references in text, tables, and legends by Arabic numerals in superscript with square bracket after the punctuation marks. Multiple citations within a single set of brackets should be separated by commas. Where there are three or more sequential citations, they should be given as a range. Example: "...has been shown previously. [1,4-6,22]"
- The titles of journals should be abbreviated according to the style used in Index Medicus or the PubMed. Use complete name of the journal for non-indexed journals.
- **Example of Journal Reference:**
  - Authors (use **et al.** after 6 authors, if there are more than six authors, complete names should not be written), article title (should be exact as existing), journal name (should be in standard PubMed abbreviations, full journal name should not be written), year, volume, page numbers (445-447 to be written as 445-47).
    - Singh JK, Bawa M, Kanojia RP, Ghai B, Menon P, Rao KL. Idiopathic simultaneous intussusceptions in a neonate. Pediatr Surg Int 2009;25:445-7.
- **Example of Book References:**
  - **Personal author(s):**

    Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.
  - **Editor(s), compiler(s) as author:**

    Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.
  - **Chapter in a book:**

    Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. pp. 465-78.

Tables

- Tables should be self-explanatory and should not duplicate textual material.
- Tables with more than 10 columns and 25 rows are not acceptable.
- Number tables, in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.
- Place explanatory matter in footnotes, not in the heading.
- Explain in footnotes all non-standard abbreviations that are used in each table.
- Obtain permission for all fully borrowed, adapted, and modified tables and provide a credit line in the footnote.
- For footnotes use the following symbols, in this sequence: *, , , , ||, , **, ,
- Tables with their legends should be provided at the end of the text after the references. (Should state in the main text the exact position of the tables and figures).

Figures

- Each figure must be numbered and a short descriptive caption must be provided. A computer drawn figure with good contrast is acceptable.
- Sometimes, raw data for graphs may be requested in Excel sheet when the article is accepted for publication.
- Graphic files for diagrams and figures may be converted to *.pcx, *.tiff, *.jpg format.

For further information, please contact the following email address: mmj.mcom@yahoo.com, or visit our website at: http://www.mcmiq.com/en/mmj

Research Article

Diagnostic accuracy of otitis media with effusion in children

Ammar Hadi Khmmas *FICMS*, **Mohammed Radef Dawood** *FICMS*, **Abdulhussein Kareem** *FICMS*, **Yahya Ajeel Hammadi** *FICMS*

Date Submitted: 2/7/2015
Date Accepted:8/10/2015

Address for Correspondence:
Dr. Abdulhussein Kareem
Department of Surgery, College of medicine, Al-Mustansiriya University

Abstract

Background: Otitis media with effusion is a common disease and causes hearing disability that necessitates early and accurate management.
Objectives: Comparison of standard otoscopic tympanic membrane finding, pneumatic otoscopy and tympanometric results with myringotomy finding.
Patients and Methods: A prospective study of 60 children (120) ears with a suspicion to have otitis media with effusion that have been done from 1st of Jan 2014 to the 1st of Jan 2015, at Otolaryngology Department in Al-Yarmouk Teaching Hospital-Baghdad, all patients were underwent a clinical assessment by standard otoscopy, pneumatic otoscopy and audiological assessment by tympanometry, then we compared all results with myringotomy finding as a reference standard for predicting the presence or absence of fluid within the middle ear.
Results: there were 53% males and 47% females. Among them 63% were in age group 5-8 years. The main presenting symptom was hearing impairment (90%). Retraction of the tympanic membrane was the main otoscopic findings in 83.5%, from which fluid at myringotomy found in 72%. Type B tympanogram in 47.5% of which 88% revealed fluid in taping. The Sensitivity, specificity and accuracy of tympanogram (A and B) were 89%, 63% and 83% respectively, and for type C1 and C2 tympanograms the results was variable, while for clinical assessment (otoscopy) they were 85.7%, 60.5% and 76.7% respectively, and in (pneumatic otoscopy) the results was 97%, 30% and 73% respectively, and in cases of a combined clinical and tympanometric assessment, the results was 97%, 52% and 86% respectively.
Conclusion: Conjunction of tympanometry and pneumatic otoscopy examination increase accuracy.

Key words: Otitis media with effusion; Otoscopy; Pneumatic otoscopy; Tympanometry; Myringotomy.

INTRODUCTION

Otitis media with effusion (OME) is the chronic accumulation of mucus within the middle ear and sometimes the mastoid air cell system, the time that the fluid has to be present for the condition to be chronic is usually taken as 12 weeks, in children it usually presents because of the associated hearing impairment, there were many synonyms have been and are used for this condition, these include: “glue ear”, and “chronic non purulent otitis media, its prevalence is bimodal with the first and largest peak of approximately 20% at two years of age and a second peak of approximately 16% at around five years of age, also it is around twice as many children being diagnosed with OME in the winter as opposed to the summer [1]. An untreated OME causes hearing impairment with potential subsequent delay in speech and cognitive development, especially in young children [2]. OME is difficult to diagnose by standard otoscopy alone and an adjunctive tool should be used to improve diagnostic accuracy, there are various tools have been proposed for the diagnosis of OME other than otoscopy in order to improve diagnostic accuracy, these include micro-otoscopy, tympanometry and pneumatic otoscopy, myringotomy[3]. The use of pneumatic otoscopy to demonstrate decreased mobility of the tympanic membrane is considered an important primary diagnostic method, other factors that help confirm the diagnosis include type B tympanogram (flat curve), but still tympanocentesis which is usually performed at the

time of myringotomy, remains the gold standard for diagnosing OME [4].

The aims of the study: 1) to assess the accuracy of clinical diagnosis (otoscopy and pneumatic otoscopy), 2) to assess the accuracy of different types of tympanograms, 3) to correlate between them and the myringotomy findings.

PATIENTS AND METHODS

This is prospective study of 60 patients (120 ears), aged 3- 12 years attended at Otolaryngology Department in Al-Yarmouk Teaching Hospital –Baghdad with suspension of having OME, in period from the 1st of January 2014 to the 1st of January 2015, all children were subjected for proper ENT examination using otoscopy, pneumatic otoscopy, audiological assessment by tympanometry and the data were documented on special formula prepared for that reason contained a detailed parameters such as: Otoscopic examination: like tympanic membrane (position, color, translucency, cone of light, and presence of air bubbles or fluid level), and the mobility of tympanic membrane was assessed with pneumatic otoscope (which has a magnification of 2.5 times convex lens) as either mobile, impaired or immobile tympanic membrane, then tympanometry was performed to all studied children regardless to the otoscopic examination, by a Macio M44 tympanometer of 226 Hz (low frequency pressure probe tone) , through using A Fiellau – Nikolajson [5] classification for tympanograms, as Peaked curves: Type A : maximum compliance the same or more than 0.2 ml with a middle ear pressure of (-99 to +200 dapa). Type C1: maximum compliance the same or more than 0.2 ml with a middle ear pressure of (-100 to -199 dapa). Type C2: maximum compliance the same or more than 0.2 ml with a middle ear pressure of (- 200 to - 400 dapa). Non-peaked curve: Type B: maximum compliance less than 0.2 ml or middle ear pressure less than (- 400 dapa), and for the purpose of analysis, type A and C1 curves were classified as normal, and type B and C2 curves were classified as abnormal [6]. This study was approved by the Ethic committee of our institution, and after explanation and taking the consent from their parents they underwent myringotomy under general anesthesia, as a part of other surgical procedures, and by using operating microscope (Carl Zeis- Germany/ using 250 mm lenses) the fluid was obtained through a radial incision which made in the anterior inferior quadrant of the tympanic membrane. The results of standard otoscopy, pneumatic otoscopy, and that of tympanometry were calculated, and their diagnostic accuracy was determined by the confirmation of middle ear effusion based on myringotomy findings as a referenced standard by applying the parameters as the sensitivity, specificity and accuracy values for the analysis.

RESULT

Among 60 children there were 32 boys (53%), and 28 girls (47%), the most common age group was 5-8 years, were their 38 children (63%), and the hearing impairment was the main presenting symptom as it was found in 54 children (90%).

1-Tympanic membrane examination:

Table 1 shows the findings during tympanic membrane examination by using otoscopy and pneumatic otoscopy and the findings at myringotomy by operating microscope.

The mobility of tympanic membrane which was detected by Siegel's pneumatic otoscopy, revealed that 15 ears (12.5%) with normal mobility, of those only 2ears (13%) had middle ear effusion, while 84 ears (70%) had impaired mobility, of which 59 ears (70%) had fluid in the middle ear and the rest 25 ears (30%) were dry. At the same time immobile tympanic membrane was detected in 21 ears (17.5%), of which 16 ears (76%) had effusion while 5 ears (24%) were dry.

Table (1) Results of tympanic membrane examination and myringotomy finding.

Tympanic membrane examination		NO	%	Myringotomy finding			
				dry	%	fluid	%
position	normal retracted pocket atelectasis	9 100 5 6	7.5 83.5 4 5	6 28 4 5	66.5 28 80 83	3 72 1 1	33.5 72 20 17
color	pale grey Amber Blue	80 37 3	66.5 31 2.5	36 7 0	45 19 0	44 30 3	55 81 100
translucency	dull translucent	83 37	69 31	17 26	20.5 70	66 11	79.5 30
cone of light	normal shuttered absent	0 99 21	0 82.5 17.5	- 30 13	- 30 62	- 69 8	- 70 38
air bubbles		8	6.5	0	0	8	100
fluid level		9	7.5	2	22	7	78
mobility	normal impaired immobile	15 84 21	12.5 70 17.5	13 25 5	87 30 24	2 59 16	13 70 76

2-Tympanogram types and myringotomy fluid:

In type A curve agreement to findings at myringotomy occurred in 12 ears (67%). Fifty seven ears (47.5%) demonstrated type B curve which suggest presence of effusion. Fifty ears (88%) of these cases were in agreement with myringotomy findings. There were 45 ears (37.5%) with type C1, and in C2 curves there were 21 ears (47%) revealed effusion, the relation between tympanometry curves and results of myringotomy, it shown in figure 1.

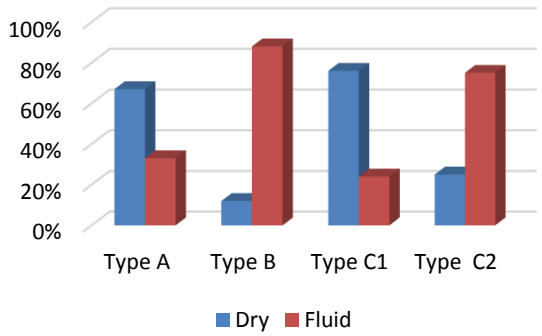


Figure (1) Comparison of tympanogram types and the myringotomy finding

3-Tympanometry sensitivity, specificity and accuracy:

By using a 2 x 2 matrix sensitivity and specificity calculated for the detection of OME with myringotomy findings as the reference standard, for type A and B curves the results were 89% and 63% respectively, and when add the transition type C tympanogram into analysis and consider them negative then sensitivity will be 65% and specificity will be 83.5%, but when consider C2 positive in addition to type B tympanogram then the result will be the sensitivity: 84% and specificity: 72%, as it shown in table 2.

Table (2) Tympanometry sensitivity and specificity in the detection of OME with surgical findings as the reference standard.

Tympanogram	Myringotomy (OME present)	Myringotomy (OME absent)
B	**True positive 50**	**False positive 7**
A	**False negative 6**	**True negative 12**
A+C	**False negative 27**	**True negative 36**
B+C2	**True positive 65**	**False positive 12**
A+C1	**False negative 12**	**True negative 31**

The accuracy of tympanometry was calculated in regard to type A and B curves only and found to be 83%.

4-Otoscopic sensitivity, specificity and accuracy:

Regarding standard otoscopic examination (translucent versus dull) we have sensitivity of (85.7%) and specificity of (60.5%) table 3.

Table (3) Otoscopic sensitivity and specificity in the detection of OME with surgical findings as the reference standard.

Otoscopy	Myringotomy (OME present)	Myringotomy (OME absent)
TM dull (OME suspect present)	**66**	**17**
TM translucent (OME suspect absent)	**11**	**26**

5-Pneomatic otoscopy sensitivity, specificity and accuracy:

Regarding pneumatic otoscopy: the sensitivity was 97%, and the specificity was 30%, as it shown in table 4

Table (4) Pneumatic otoscopy (TM Mobility) sensitivity and specificity in the detection of OME with surgical finding as the reference standard.

Pneumatic Otoscopy	Myringotomy (OME present)	Myringotomy (OME absent)
Impaired or immobile TM (OME suspect present.)	**True positive 75**	**False positive 30**
Mobile TM (OME suspect absent.)	**False negative 2**	**True negative 13**

6-Combined clinical and tympanometric sensitivity, specificity and accuracy:

The combination of clinical (pneumatic otoscopy) and tympanometry values as shown in table 5, this resulted in an increase in sensitivity: 97% and accuracy: 86% but a decrease in specificity: 52%.

Table (5) Combined pneumatic otoscopy and tympanometry sensitivity and specificity in the detection of OME with surgical findings as the reference standard.

Tympanogram & Pneumatic Otoscope	Myringotomy (OME present)	Myringotomy (OME absent)
B and C2 Impaired or immobile TM	**True positive 65**	**False positive 10**
A and C1 Mobile TM	**False negative 2**	**True negative 11**

DISCUSSION

The current study reported that, (63%) were 5-8 years of age, with a male predominance (53%). This agreed with other studies, as Rishi and Prakash A, study which reported that, most of the children (56.8%) were in 5-8

years of age [7]. Ahmad et al, study had shown that (54.4%) of children were males and (45.6%) were females [8], and Dong-Hee L and Sang-Won Y, had shown 33 males and 18 females [9], however it concluded that it is likely to be a little difference if any in risk for boys compared to girls [1], however the current study do not represent true prevalence of the disease as it was performed in only hospital. Regard to the most common presenting symptom, it was hearing impairment (90%), also Ahmad et al, study shown that, the main presenting symptom was hearing impairment (47.2%) [8], while Syed et al, study shown that, the common presenting symptom was fullness in the ear (50.3%) [10]. The hearing impairment was noticed by the parents or teacher due to scholastic retardation, or it suggested by seeming lack of attentiveness, failure to respond to normal conversational level speech.

1-Tympanic membrane examination:

Retracted tympanic membrane was found to be the commonest otoscopic sign (83%), this agrees with Ahmad et a, study, in which it was (91.7%) [8], and by Orji, FT and Mgbor NC study which showed that it was the most specific otologic finding in detection of OME [11], while Syed et al, study found that, the most common sign was dull eardrum (72.18%) [10].

The current study revealed that (72%) of retracted tympanic membrane, and (80%) of dull tympanic membrane showed fluid by myringotomy, these results were similar to the study done Hamed et al, which reported that (68%) of retracted TM, and (74%) of dull TM, showed fluid by myringotomy[12].

It is generally accepted that the middle ear fluid decreases the mobility of TM mainly due to increased mass on the TM, reduction middle ear air space, and abnormal pressure in the middle ear cavity [13].

Regard to the color of tympanic membrane, the current study indicated that, the color of the ear drum carry less diagnostic value, the same findings and conclusions was reported by a study done by Hamed et al [12], and by a study done by Daly KA et al, which confirmed that the color of the tympanic membrane is of lesser importance than the position and mobility [14],these observation was confirmed by a study done by De Melker RA, which reported that serious retraction of the eardrum and absence of mobility under positive pressure were the most predictive features but the color of the tympanum did not show any relation to effusion[15].

2- Sensitivity, Specificity and Accuracy of tympanic membrane examination:

Loss of translucency is a feature suggestive of OME, with sensitivity of (85.7%), specificity of (60.5%) and accuracy of (76.7%). in current study the standard otoscopic examination had high sensitivity but low specificity and these results were almost similar to that of Hamed et al, study that shown the sensitivity was (82%), specificity was (52%) and accuracy was (71%) [12], and Dong-Hee L and Sang-Won Y study, reported a sensitivity of (89.7%) and low specificity of (71.4%) [9].

Generally, most clinicians who use otoscope have good sensitivity for the presence of middle ear effusion, but being confident about the absence of effusion (specificity) takes experience and practice [16].

Regard to the cone of light, it was appeared to be shuttered in (82.5%) from which (70%) were contained fluid, and it was absent in (17.5%) of which (38%) contained fluid confirmed by myringotomy.

These results were comparable to that of study done Hamed et al, which reported that, the cone of light was found in (80%) from which (69%) contained fluid which being approved by taping, and it was absent in (20 %) of which (37.5%) confirmed to had fluid by myringotomy [12], so the current study found that cone of light assessment of less importance in diagnosis of OME. It reported that, the absence of the cone of light does not necessarily signify an ear disorder, as it could be due to the slope of the tympanic membrane or the shape of the external ear canal [17].

The presence of an air bubbles and fluid level carry important sign for detection of OME [12].

The calculations of the sensitivity, the specificity, and the accuracy for pneumatic otoscopy were (97%), (30%), and (73%) respectively. Dong-Hee L and Sang-Won Y shown in their study that sensitivity was (97.2%) and specificity was (38.5%) [9]. Finitzo et al,study found that, the sensitivity was (93%) and specificity was (58%) [18].

The current study found that the cloudiness and mobility of the tympanic membrane show the highest sensitivity and accuracy with variable specificity in contrast to other clinical findings, the same observation was found in a study done by De Melker RA which revealed that cloudiness and mobility of the tympanic membrane had the highest sensitivity and specificity [15].

3- Tympanometry types:

The most common cause of flattened, or type B tracing, with a low static admittance, when the ear canal volume is normal is decreased mobility of the tympanic membrane secondary to middle ear fluid [19], so type B tympanogram is the best diagnostic tool for predicting OME in the children [20]. The current study found that (88%) of type B curve and (33%) of type A curve were

revealed positive by taping, these results looks comparable to other studies, as study done by Hamed et al, which reported that (89.5%), of type B curve and (27%) of type A curve were positive by taping [12], and also by a study done Ahmad et al, which showed that in tympanograms of type B (92.2%), and type A (2.8%) revealed fluid which confirmed by myringotomy[8]. Fish B M et al, study concluded that not all of the ears which their tympanometer registered as having 'type B' traces could expected to had effusions, since (13 %) of type B tympanogram were dry at myringotomy, in these cases there had been displacement of fluid from the middle ear by N2O during anesthesia [21], and when a type A tympanogram was confirmed by the presence fluid at myringotomy was probably due to small amount of fluid was found [22].

The most probably reasons for dry tap by myringotomy, despite having fluid in middle ear cavity are very thick fluid, located in dependent areas, or by Nitrous Oxide action [7].

4- Tympanometry Sensitivity, Specificity and Accuracy:

Regard to type B tympanogram , and type A tympanogram the current study revealed that the sensitivity was (89%), specificity was (63%), and accuracy was (83%), these findings were close to the results of other studies, as finitzo et al, which revealed a results; sensitivity was (90%) and specificity was (86%) for tympanometry [18], study done by Palmu AA and Syrijanen R, had shown that, the sensitivity of type B tympanogram was (61%) and specificity (99%) [23], and study done by Shaio A S, and YC Guo, which reported that type B tympanogram with sensitivity of (96.6%) and specificity of (99%) [24].

Analyzing papers with findings at myringotomy as the reference standard, suggest that a type B tympnogram is frequently associated with OME, a type A is infrequently associated with OME and a type C falls in between [1]. It being a well-documented method for the diagnosis of OME, tympanometry has a sensitivity varying between (82–90%) and specificity between (68–98%) relative to findings in myringotomy [25, 26].

Analyzing the result of type B tympanogram versus type A and C tympanogram ,it shown that, the sensitivity was (65%) and the specificity was (83.5%) , these result looks comparable to those of other studies as Finitzo et al, were the sensitivity was (57%), and the specificity was (94%) [18], and when analyzing the result of type A and C1 tympanograms versus type B and C2 tympanograms, it shown that of a sensitivity was (84%) and a specificity was (72%), as a parameters in detecting OME, while on other hand, a study done by Miia K Laine et al. which grouped result of type C2 and B tympanograms (the positive test result) contrasted with type A and C1 tympanograms (the negative test result), as a parameters in excluding OME, it showed that, the sensitivity was (84%), and the specificity was (87%) [27].

This wide range of values can only be partly explained by the differing proportion of ears with OME and the fact that the anesthetic can itself aerate the middle ear giving a 'false' dry tap [1].

5- Sensitivity, Specificity and Accuracy of combined Clinical and Tympanometric results:

In combination of clinical (pneumatic otoscopy) and tympanometry, the results revealed a sensitivity of (97%), a specificity of (52%) and the accuracy of (86%), these findings was agreed with results of other studies, like Finitzo et al, which revealed both sensitivity and specificity above (90%) [14], and Harris et al, study which shown that, they were between (80% -100%) [28].

Using pneumatic otoscopy with tympanometry improves the accuracy of diagnosis because many abnormalities of the eardrum and ear canal that might cause an abnormal tracing can be visualized, determining the presence of obstructing cerumen in the canal, and characteristics of the tympanic membrane (e.g., color, mobility, position, and translucency) are helpful in correlating tympanometry findings with clinical disease, the two tests can be complementary, because pneumatic otoscopy provides a qualitative measure of tympanic membrane mobility (i.e., does the tympanic membrane move with insufflations) and tympanometry produces more quantitative information (e.g., numeric and graphic data about generated positive and negative pressures, absorption of acoustic energy by the middle ear system, ear canal volume) [29], although myringotomy is a 'gold-standard' method for confirmation (effusion or no effusion),but as it is an invasive procedure, its application is not possible in a primary care setting for ethical and practical reasons [15].

CONCLUSIONS

- Clinical diagnosis (otoscopy and pneumatic otoscopy) has good sensitivity and accuracy.
- Tympanogram type A and B carry highest accuracy among other types of tympanogram consider to myringtomy confirmation.
- Conjunction of tympanometry and pneumatic otoscopy examination increase accuracy.
- All tools are significant in diagnosis of OME, but accuracy found more in tympanometry than otoscopy and pneumatic otoscopy depending on sensitivity and specificity tests when compared with myringotomy results.

RECOMMENDATION

It is recommended for the availability of tools and devices required for examining the ear for purpose to diagnose OME in the health care centers preliminary as well as training on these devices for doctors working place.

REFERENCES

1. George Browning. Otitis media with effusion. In: Micheal G, George GB, Martin JB, editors. Scott-Brown's. Otorhinolaryngology, Head and Neck Surgery 7th ed. London: Edward Arnold; 2008. Vol. 1; 72: p. 877-884.
2. Olatoke, F., et al., The prevalence of hearing loss among schoolchildren with chronic suppurative otitis media in Nigeria, and its effect on academic performance. Ear, Nose, & Throat Journal, 2008. 87(12): p. 19.
3. Buchanan, C.M. and D.D. Pothier, Recognition of paediatric otopathology by General Practitioners. Int J Pediatr Otorhinolaryngol, 2008. 72(5): p. 669-73.
4. Rosenfeld RM, Culpepper L, Doyle KJ, et al. Clinical practice guideline: Otitis media with effusion. Otolaryngol Head Neck Surg. 2004; 130(5): 95-118.
5. Fiellau-Nikolajsen M. Tympanometry and secretory otitis media: Observations on diagnosis, epidemiology, treatment, prevention in prospective cohort studies of three-year-old children. Acta Otolaryngol (Stockh) 1983; 394: 62-63.
6. Engel J, Anteunis L, Chenault M, Marres E. Otoscopic findings in relation to tympanometry during infancy. Eur Arch Otorhinolaryngol 2000; 257: 366-71.
7. Rishi B and Prakash A. Correlation Between Tympanogram and Myringotomy Fluid in Pediatric Patients with Otitis Media with Effusion.Intl. Arch. Otorhinolaryngol. Sao Paulo 2008; 12: 2: 220-223.
8. Ahmad N. Al-Juboori1, Ameer A. Al-Aqeedee and Hussam D. Saeed.Otitis. Media with Effusion in Children: A Follow up Study in West Baghdad, Iraq. J of communication Disorders, Deaf Studies & Hearing Aids 2014; 2 (4): ISSN: 2375-4427 JCDSHA.
9. Dong-Hee L and Sang-Won Y. Clinical Diagnostic Accuracy of Otitis Media with Effusion in Children, and Significance of Myringotomy: Diagnostic or Therapeutic. J Korean Med Sci 2004; 19: 739-43.
10. Syed HI, Arif HB, Abu Yusuf F. Study on otitis media with effusion. Bangladesh J otolrhinolaryngol 2009; 15: 50-54M.
11. Orji FT and Mgbor NC. Otoscopy compaired with tympanometry: an evaluation of the accuracy of simple otoscopy. Niger J Med. 2007; 16 (1): 57-60.
12. Hamed MM, Hani MB, Mazin NF, et al. Clinical and Tympanometric Assessment of Middle Ear Effusion Versus Myringotomy Finding. J Fac Med Baghdad 2008; 50: 321-326.
13. Xiying Guan and Rong Z. Gan, Mechanisms of Tympanic Membrane and Incus Mobility Loss in Acute Otitis Media Model of Guinea Pig. J Assoc Res Otolaryngoll. 2013; 14(3): 295–307.
14. Daly KA, Hunter LL,Giebink GS. Chronic otitis media with effusion. Pediatr Rev. 1999; 20(3):85-93.
15. De Melker RA . Evaluation of the diagnostic value of pneumatic otoscopy in primary care using the results of tympanometry as a reference standard. British J of General Practice , January 1993 ; 43:22 – 24.
16. Gates GA. Acute Otitis Media and Otitis Media with Effusion. In: Cummings CW, Fredrichson JM, Harker LA, Krause CJ, Richardson MA, Schuller DE, editors. Otolaryngology Head & Neck Surgery. 3rd Ed. St.Louis: Mosby Inc; 1999. p. 461–477.
17. Robert Thayer Sataloff, Joseph Sataloff. Occuptional Hearing Loss. Taylor and Francic CRC editors. 3rd Ed.2006: p.38.
18. Finitzo T, Friel-Patti S, Chinn K,Brown O. Tympanometry and otoscopy prior to myringotomy: issues in diagnosis of otitis media. Int J Pediatr Otorhinolaryngol. 1992; 24(2): 101-10.
19. Edward Onusko. Tympanometry. Am Fam Physician 2004; 70(9): 1713-1720.
20. Ren DD, Wang WQ. Assessment of middle ear effusion and audiological characteristics in young children with adenoid hypertrophy. Chin Med J (Engl) 2012; 125: 1276-1281.
21. Fish B. M., A. R. Banerjee, C. R. Jennings, I. Frain, A. A. Narula. Effect of anaesthetic agents on tympanometry and middle-ear effusions. J of Laryngology & Otology 2000(114): 336–338.
22. Koivunen P, Alho OP, Uhari M, Niemelä M, Luotonen J. Minitympanometry in detecting middle ear fluid. J Pediatr 1997; 131: 419-22.
23. Palmu AA and Syrijanen R. Diagnostic value of tympanometry using subject-specific normative values.Int J Pediatr Otorhinolaryngol. 2005; 69(7): 965-71.
24. Shiao A S, and Y C Guo.A comparison assessment of videotelescopy for diagnosis of pediatric otitis media with effusion. Int J pediatr Otorhinolaryngol 2005; 69(11): 1479-502.
25. Iino Y, Nakamura Y, Koizumi T. Prognostic factors for persistent middle ear effusion after acute otitis media in children. Acta Otolaryngol. 2004;12: 23–24.
26. Spermo S, Markic Z, Kurblija. Clinical importance of tympanometry in the diagnosis of chronic secretory otitis. Srp Arh Celok Lek. 1998; 126(7-8): 242-7.
27. Miia K. Laine, Paula A.Tähtinen, Olli Ruuskanen, Eliisa Löyttyniemi & Aino Ruohola. Can nurses exclude middle-ear effusion without otoscopy in young asymptomatic children in primary care? ScandJ of Prim Health Care, 2015; 33: 115–120.
28. Harris PK, Hutchinson KM, Moravec J. The use of tympanometry and pneumatic otoscopt for prediting middle ear disease. Am J Audiol. 2005; 14: 3-13.
29. Kaleida PH, Fireman P. Diagnostic assessment of otitis media. Clin Allergy Immunol. 2000; 15:247–62.

Research Article

Knowledge and attitude of mothers regarding oral rehydration solution in Sulaimani

Jamil Aziz, Adiba Ismaeel Ali, Ibrahim Khasraw Ghafoor , Adnan Mohammed Hasan

Date Submitted: 10/8/2015
Date Accepted:28/10/2015

Abstract

Background: Diarrheal disease still one of the important problems among children in our governorate (Sulaymani) and ORS is an effective treatment for where they get mild to moderate dehydrations.
Objective: To assess the knowledge and attitudes of mothers regarding ORS.
Patients and methods: From July 1st 2012 to march 31st 2013, total of 200 mothers enrolled in this study. We collected information regarding their children's age, sex, residence, maternal education, water supply. Their knowledge and attitude evaluated on their awareness about ORS, using, source of education, procedure of preparation and giving it to their children. Those information collected by direct interview of those mothers.
Results: 99.5% of mothers were aware about ORS. Only 0.5% (1 mother) was not aware of it. There was significant correlation between both educational level of the mothers and water supply, and persons who gave advice to them on using ORS and drug use. But no significant correlation between mothers knowledge and source of information about ORS.
Conclusion: ORS is a very effective treatment for diarrhea in children. Mothers knowledge and attitude to be improved about it.

Key words: Mother's knowledge, Diarrhea, ORS.

INTRODUCTION

Diarrheal disorders in childhood account for a large proportion of childhood deaths, with an estimated 1.8 million deaths per year globally. The World Health Organization reports that there are more than 700 million episodes of diarrhea annually in children below 5 years of age in developing countries. While global mortality may be declining, the overall incidence of diarrhea remains unchanged at about 3.2 episodes per child per year. [(1)]

Dehydration resulting from diarrhea is a significant cause of death for young children in developing countries. Oral Rehydration Solution (ORS) is useful to replace fluid and electrolyte loss. Although ORS is effective in preventing and treating dehydration, its use in home treatment is not wide spread due to reluctance among mothers to use ORS in cases of acute diarrhea. [(2, 3)] Timely management of the children with ORS has substantially declined the mortality and morbidity from acute infectious diarrhea. [(3)]

An evaluation of global trends in diarrhea management from 1986 to 2003 showed minimal progress in ORS use and a decrease in the proportion of children with diarrhea given continued feeding[(4)]. A 2007 analysis of the two most recent Demographic and Health Surveys conducted in 34 countries found decline in ORS use for children below 3 years of age with diarrhea in 68% of those countries. Moreover, the proportion of children who had fluids withheld during diarrhea increase in 91%

of the countries included in the analysis. [5]It is commonly observed that most of the mothers neither can mix commercially available ORS properly nor are able to realize the significance of giving more fluids during acute diarrhea to their children. [6]

The aim of this study was to assess the knowledge and attitude of mothers towards the use of oral rehydration solution in the treatment of acute diarrhea in children in Sulaimani.

PATIENTS AND METHODS

This cross sectional survey was conducted at the Sulaimani Pediatric Teaching Hospital during the period July 1st 2012 to March 31st 2013. Total of 200 mothers of children with diarrhea were enrolled in the study, they were interviewed and information collected that included a detailed history highlighting their demographic data, presenting complaints, use of ORS, treatment given at home, mothers knowledge about ORS and drugs used for diarrhea, maternal education, water sources, feeding, and socioeconomic status.

Regarding knowledge and attitude about ORS a score was plotted from the following information: awareness about ORS, used ORS or not, equipment used for giving ORS, preparation of ORS, and assessing the adequacy of ORS used. When the response was correct the score of 2 was given and when response was incorrect a score of 0 was given. Total score of 10 would reflect good knowledge and a score of zero reflects very poor knowledge. Using ORS for diarrhea, equipments used for mothers' attitude regarding ORS

For statistical analysis SPSS (Statistical Package for the Social Sciences) version 17 was applied. Correlation between knowledge score and variables was done using chi-square test, P value less than or equal to 0.05 was regarded as significant.

RESULT

Table 1 shows the demographic characteristics of the group included in the study with the median age of children being 2 years, minimum was 0.3 years (4 months) and maximum was 8 years. The highest number of cases was in the age group 1 to 5 year. The male to female ratio was almost 1:1. More than half of mothers (105 mothers constituting 52.5%) had low educational level, and only 37 mothers had high level of education making 18.5% of the total, the rest did not have any educational background. There were different sources of water as in 103(51.5%) cases was from well, and in 63 (31.5%) was piped, and in 34 (17%) was water tankers.

Table 1: Characteristics of the sample

Variable	No.	%
Age in years		
< 1	25	12.5
1-5	160	80
> 5	15	7.5
Gender		
Male	101	50.5
Female	99	49.5
Residence		
Inside city	172	86
Outside city	28	14
Maternal Education		
Illiterate	58	29
Low	105	52.5
High	37	18.5
Water supply		
Well	103	51.5
Piped	63	31.5
Tanker	34	17
Total	200	100

Table 2 shows data regarding knowledge and attitude of the mothers. The vast majority 199 mothers (99.5%) were aware about ORS and only 1 (0.5%) was not aware about ORS, 183 (91.5%) of the mothers used ORS and 17 (8.5%) did not use ORS. Among mothers who used ORS 7 (3.5%) used it depending on their own decision, and 42 (21%) advice was given by another family member, while 85 (42.5%) advice given by a physician, and 49 (24.5%) advice made by: paramedical staff). Among mothers who used ORS, in 139 (69.5%) it was given by cup and spoon, in 44 (22%) it was given by bottle. The amount of ORS given was adequate by 109 mothers (54.5%) and 74 (37%) was inadequate, while 107 mothers (53.5%) prepared ORS correctly and 76 mothers (38%) preparation of ORS were not correct. Of all mothers 172 (86%) had used drugs and of 28 (14%) had not used drugs. Among those who used drugs 21 (10.5%) was self decision and 151 (75.5%) prescription was made by family physician.

When correlation done between knowledge score and demographic characteristics of the sample, there was significant correlation with educational level of mother and the source of water supply (p= 0.0001). In making correlation between knowledge score and mothers source of information about ORS, P value was not significant (0.226). But correlation between knowledge score and the person giving advice to the mothers on using ORS, P value was (0.0001) significant with the physician having the greatest influence, so also correlation done between knowledge score and the person who advised mothers on drug use P value was (0.0001) significant.

Table 2: Mothers' knowledge and attitude regarding Oral Rehydration Solution

Variable	Number	%
Aware of ORS		
Yes	199	99.5
No	1	0.5
Total	200	100
Used ORS		
Yes	183	91.5
No	17	8.5
Total	200	100
Advised to use ORS by		
Self	7	3.9
Family member	42	22.9
Physician	85	46.5
Health worker	49	26.7
Total	183	100
Give ORS via		
Cup and spoon	139	76
Bottle	44	24
Total	183	100
Used adequate amount of ORS		
Adequate	109	59.5
Inadequate	74	40.5
Total	183	100
Preparation of ORS		
Correct	107	58.5
Incorrect	76	41.5
Total	183	100
Used Antidiarrheal Drugs		
Yes	172	86
No	28	14
Total	200	100
Decision for using antidiarrheal drugs by		
Self	21	12.2
Physician	151	87.8
Total	172	100

DISCUSSION

Among children, mortality and morbidity in acute infectious diarrhea have dramatically reduced due to oral rehydration therapy and early realimentation [7]. Numerous studies have documented that knowledge about oral rehydration solution has increased. [8], Despite the fact that availability of ORS can substantially reduce the mortality and morbidity resulting from diarrhea, poor knowledge pertaining to diarrhea and its management has posed the third world countries with diarrhea associated deaths and ill health among children[9]

In this study 99.5% mothers were aware of oral rehydration solution, this finding is similar to that of Jha N et al study[10], 97.6% of mothers had information about ORS and also its usefulness in the management of dehydration due to diarrhea.

About half of the mothers in our study 105 (52.5%) had low level of education, 58(29%) of them were illiterate and 37(18.5%) had high educational level while in Attaya P et al study[11], 52% of mothers had elementary school or lower and 62% were housewives.

In present study 85(42.5%) patients were given oral rehydration solution on advice of family physician, 42 (21%) on advice of family member, 49 (24.5%) on advice of health worker and 7 (3.5%) on their own knowledgewhile Seyal et al study [12] reported that 27% used ORS by their own knowledge, 28% used on advice of general practitioners, 10% by pediatricians, 3% by medical officers and 27% from other sources could be due to improvement of health services in our region.

As far as preparation of oral rehydration solution is concerned, in our study 107 (53.5%) of mothers correctly recalled the preparation of oral rehydration solution whereas in a study by Taha AZ [15] was 64%**,**

In our study 172(86%) used drugs in diarrhea and 28(14%) did not use drugs, and of them 151(75.5%) were advised by family physician while **in** This high percentage is probably due to success of our physicians in applying ORS program in our society.

In conclusion; although most of the mothers had satisfactory knowledge about ORS and used it when needed, their attitude regarding oral rehydration solution was inadequate.

REFERENCES

1. Bhutto ZA. Acute gastroenteritis in children .In: Kliegman RM,Behrman RE,Jenson HB,Stanton BF(eds). Nelson Textbook of Pediatrics. Philadeiphia ;Saunders Elsevier 2008, 18th edition; 337:1605.(we donot have have this new edition)
2. Mac Donald SE, Moralejo MN, Matthews MK. Maternal Understanding of Diarrhea-Related dehydration and its Influence on Oral Rehydration Solution Use in Indonesia. Asia Pac J Public Health. 2007;19(1):34-39
3. Cezard JP, Bellaiche M, Viala J, Hugot JP. Medication in Infectious Acute Diarrhea in Children. Arch Pediatr. 2007; 14 Supp 3: S169-75
4. Forsberg BC, Petzold MG, Tomson G, Allebeck P. Diarrhea case management in low- and middle-income countries–an unfinished agenda. Bull World Health Organ. 2007; 85:42–48
5. Ram PK, Choi M, Blum LS, Wamae AW, Mintz ED, Bartlett AV. Declines in case management of diarrhea among children less than five years old. Bull World Health Organ. Am J Trop Med Hyg. 2011; 85(6): 1134–40.
6. Seyal T, Hanif A. Knowledge, attitude and practices of the mothers and doctors regarding feeding, oral rehydration solution (ORS) and use of drugs in children during acute diarrhea. Annals King Edward Med Coll, 2009; 15(1): 3841.
7. Cezard JP, Bellaiche M, Viala J, Hugot JP. Medication in infectious acute diarrhea in children. Arch Pediatr 2007;14(3):S169-75.
8. Ahmed A, Malik IA, Iqbal M, Nawaz M, Azim S, Bukhtiari N, et al. The use ORS (Nimkol) in management of childhood diarrhea by mothers in the suburbs of Rawalpindi -Islamabad.

9. Datta V, John R, Singh VP, Chaturvedi P. Maternal knowledge, attitude and practices towards diarrhea and oral rehydration therapy in rural Maharashtra. Indian J Pediatric 2001;68(11):1035-37.
10. Jha N, Singh R, Baral D. Knowledge, attitude and practices of mothers regarding home management of acute diarrhea in Sunsari,Nepal.Nepal Medical College Journal 2006;8(1):27-30.
11. Attaya P, jatuporn T, Chareon T. Knowledge attitude and practice on the use of oral rehydration solution of People in a Highly Dense inner-City Community. Administrat Thai Pharm Health Sci J 2006;1(2):96-103
12. Seyal T, Hanif A. Knowledge, attitude and practices of the mothers and doctors regarding feeding, oral rehydration solution and use of drugs in children during acute diarrhea. Annals 2009;15(1):38-41.
13. Taha AZ. Assessment of mother's knowledge and practice in use of oral rehydration solution for diarrhea in rural Bangladesh. Saudi Med J 2002; 23(8):904-8.

Table 3. Correlation of variables with knowledge and attitude scores

Variable		Average score						P value
		0	2	4	6	8	10	
Age	Less than 1year	0(0%)	6(3%)	1(0.5%)	3(1.5%)	6(3%)	9(4.5%)	0.462
	1-5 year	1(0.5%)	20(10%)	23(11.5%)	35(17.5%)	14(7%)	67(33.5%)	
	More than 5 year	0(0%)	1(0.5%)	2(1%)	0(0%)	3(1.5%)	9(4.5%)	
	Total	1(0.5%)	27(13.5%)	26(13%)	38(19%)	23(11.5%)	85(42.5%)	
Gender	Male	0(0%)	12(6%)	8(4%)	26(13%)	14(7%)	41(20.5%)	0.42
	Female	1(0.5%)	15(7.5%)	18(9%)	12(6%)	9(4.5%)	44(22%)	
	Total	1(0.5%)	27(13.5%)	26(13%)	38(19%)	23(11.5%)	85(42.5%)	
Mothers education	Illiterate	1(0.5%)	14(7%)	12(6%)	16(8%)	7(3.5%)	8(4%)	0.0001
	Low	0(0%)	12(6%)	13(6.5%)	22(11%)	14(7%)	44(22%)	
	High	0(0%)	1(0.5%)	1(0.5%)	0(0%)	2(1%)	33(16.5%)	
	Total	1(0.5%)	27(13.5%)	26(13%)	38(19%)	23(11.5%)	85(42.5%)	
Residence	Inside Sulaimani	0(0%)	23(11.5%)	19(9.5%)	31(15.5%)	20(10%)	79(39.5%)	0.17
	Outside Sulaimani	1(0.5%)	4(2%)	7(3.5%)	7(3.5%)	3(1.5%)	6(3%)	
	Total	1(0.5%)	27(13.5%)	26(13%)	38(19%)	23(11.5%)	85(42.5%)	
Source of water	Well	0(0%)	10(5%)	5(2.5%)	11(5.5%)	11(5.5%)	66(33%)	0.0001
	Piped	1(0.5%)	10(5%)	16(8%)	20(10%)	5(2.5%)	11(5.5%)	
	Tank	0(0%)	7(3.5%)	5(2.5%)	7(3.5%)	7(3.5%)	8(4%)	
	Total	1(0.5%)	27(13.5%)	26(13%)	38(19%)	23(11.5%)	85(42.5%)	
Heard about O.R.S.	Yes	1(0.5%)	26(13%)	26(13%)	38(19%)	23(11.5%)	85(42.5%)	0.266
	No	0(0%)	1(0.5%)	0(0%)	0(0%)	0(0%)	0(0%)	
	Total	1(0.5%)	27(13.5%)	26(13%)	38(19%)	23(11.5%)	85(42.5%)	
Who advise on O.R.S.	Self	0(0%)	1(0.5%)	2(1%)	1(0.5%)	0(0%)	3(1.5%)	0.0001
	Family	0(0%)	6(3%)	12(6%)	17(8.5%)	3(1.5%)	4(2%)	
	Physician	0(0%)	1(0.5%)	1(0.5%)	5(2.5%)	13(6.5%)	65(32.5%)	
	Health worker	0(0%)	3(1.5%)	11(5.5%)	15(7.5%)	7(3.5%)	13(6.5%)	
	Total	0(0%)	11(5.5%)	26(13%)	38(19%)	23(11.5%)	85(42.5%)	
Who advise on drug	Self	0(0%)	0(0%)	8(4%)	9(4.5%)	2(1%)	2(1%)	0.0001
	Physician	0(0%)	16(8%)	12(6%)	27(13.5%)	13(6.5%)	83(41.5%)	
	Total	0(0%)	16(8%)	20(10%)	36(18%)	15(7.5%)	85(42.5%)	

Research Article

Assessment of oral health status, leptin, and inflammatory markers in serum and saliva of patients with polycystic ovarian syndrome in reference to metabolic syndrome

Zeena Raad Helmi ***F.I.C.O.G,*** **Reem Adnan Sabri,** ***M.Sc,*** **Ban Hadi Hameed,** ***F.I.C.O.G***

Date Submitted: 27/7/2015
Date Accepted:9/11/2015

Address for Correspondence:
Dr. Zeena R. Helmi, Department of Obstetrics and Gynecology College of Medicine, University of Al-Mustansiriya

Abstract

Background: Polycystic ovarian syndrome was first described in1935 by Stein and Leventhal as classical triad of amenorrhea, obesity and hirsutism

Aims of the study: Assessment of oral health status including gingival inflammation by (GI), periodontal situation by (PI), dental status by (DMFT), and salivary flow rate (SFR) and their relationship with the severity of symptoms, and evidence of metabolic syndrome in PCOS patients and assessing the serum and salivary levels of leptin, CRP, and fibrinogen in PCOS patients and find out the associations with the oral health and metabolic syndrome.

Patients and Methods: Clinical examination had been done for (42) PCOS women and (30), age and BMI, matched control subjects for blood pressure (systolic and diastolic) and anthropometric measurements (BMI, waist to hip, and waist to height ratios). Oral clinical examination including an assessment of gingival inflammation (GI), periodontal situation (PI), dental status (DMFT), and salivary flow (SFR) had also been done. Samples of blood and whole unstimulated (resting) saliva were collected from all participants then investigated for the level of leptin, CRP, and fibrinogen, lipids (HDL and triglyceride), and FBG had also been measured.

Results: Oral health measurements including: GI, PI, and DMFT were significantly higher in PCOS patients, while SFR were less in PCOS compared to controls. Leptin and fibrinogen (in blood and saliva) of the PCOS women were higher than that of controls. Whereas (26.19%) serum and (40.47%) salivary CRP levels >6mg/L, but no one of the controls exhibited high CRP levels. Significant correlation between serum and salivary leptin were resulted in both PCOS and controls, and the same story for CRP and fibrinogen. Elevated levels of the rest variables in both blood and saliva were significantly correlated with MS. GI, PI, and SFR were also significantly correlated with MS, while DMFT was not. GI, PI, and DMFT had significant correlation with BMI in PCOS and controls. Severe gingival inflammation and established destructive periodontitis were significantly correlated with salivary: CRP and leptin, but it were non-significant with salivary fibrinogen and SFR.

Conclusion: Saliva was found to be a useful alternative to serum for the determination of leptin, CRP, and fibrinogen in PCOS. The susceptibility for dental carries, gingivitis, and periodontitis may significantly increase in PCOS that gingivitis and periodontitis are common finding in patients with PCOS, and that was supported by significant decrease in salivary flow rate. Salivary leptin and CRP have become important markers for inflammation related to oral health and in assessing the risk of developing cardiovascular disease.

Key words: Oral health, Metabolic syndrome, Leptin, CRP, Fibrinogen.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorder affecting women in reproductive age; it is a genetic disorder that can be inherited from either parents [(1)]. PCOS can be divided into two main types: insulin-resistant (obese PCOS) and non-insulin-resistant. In 2003 the Rotterdam criteria for the diagnosis of PCOS states 2 of the 3 features were needed to be present to make the diagnosis. These features include: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries [(2)]. It would appear that many women with PCOS fulfill the criteria for the metabolic syndrome in view of a higher reported incidence of hypertension, dyslipidemia, visceral obesity, insulin resistance and hyperinsulinemia [(3)].

Leptin is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism [(4)]. Most obese individuals are thought to be leptin resistant [(5)]. CRP is (115 kDa) protein synthesized by the liver. Many studies reveal elevated serum CRP levels in women with PCOS. Recent research has shown that fibrin plays a key role in the inflammatory response [(6)]. Oral health is integral to general health. Because of interest in the link between oral and general health clinicians are increasingly using salivary analyses to diagnose systemic disease and to monitor general health. There has also been an increase the association between periodontal inflammation and PCOS [(7)]. In recent times there has been increasing interest in saliva-based analyses, because saliva collection methods are simple and noninvasive. Oral fluid sampling is safe for both the operator and the patient, and has easy and low-cost storage [(8)]. Since the saliva was put forth as a potential diagnostic tool, its use for surveillance of disease and general health has become a highly desirable goal in healthcare and medical research [(9)].

MATERIALS AND METHODS

Forty-two patients having PCOS, based on Rotterdam criteria, and thirty healthy women were matched in age and BMI.

Exclusion criteria: smoking, thyroid dysfunction, adrenal dysfunction, diabetes mellitus, hypertension, and pregnancy.

History with examination were performed which included: age, onset of disease, family history, weight, height, systolic and diastolic blood pressure, collection of blood and saliva, other anthropometric (BMI, WHR, and WHtR), metabolic (leptin, CRP, and fibrinogen), and oral health (DMFT, GI, PI, and SFR) measurements. Venous blood was obtained and the serum was separated and kept in deep freeze for further analysis. To avoid circadian variations, saliva sample was collected between 9 AM and 11 AM into small plastic polyethylene cups. Whole unstimulated saliva for 10 minutes was collected from participants. Then centrifuged and the supernatant is kept at -20ºC until use for further analysis including:

1-Leptin by Sandwich ELISA kit(DRG),catalog no. 2395 (Germany).

2-C-reactive protein(LTA) by qualitative determination kit catalog no. AK00111 (Italy).

3.Fibrinogen (Spinreact) Clauss method by kit catalog no. 1709211 (Spain) .

Statistical Analysis: Statistical tests were achieved by using Microsoft "Excel 2010" statistical package which run under "windows" operating system and a computerized program, the statistical package for social sciences (SPSS). T- student's test was used for comparison with control group.

RESULTS

Table (1) shows comparable mean, standard deviation and median values of clinical characteristic and anthropometric measurements (age, onset of disease, family history, weight, height, systolic and diastolic blood pressure, other anthropometric (BMI, WHR, and WHtR).

Table (2) shows Lipid profiles including (TG, HDL, and TG/HDL) and FBG.

Oral health measurements

Table (3): shows the clinical oral examination of PCOS and controls that include the GI and PI: each group was subdivided into subgroups according to the severity of inflammation in the gingival tissue and periodontal tissue respectively (with the expression of no.%) for each grade of inflammation within the subgroup). DMFT: calculation of the number and percentage of decayed, missed, and filled teeth of each group and mean±SD of salivary flow rate were less in PCOS than that of controls.

Oral health measures, inflammatory biomarkers and leptin

Table (4) shows mean ± SD, and the correlation between the components' number on one hand and the elevated salivary and serum leptin, salivary and serum

CRP, salivary and plasma fibrinogen, GI, PI, DMFT, and SFR in PCOS on the other hand. Salivary and serum leptin had significant linear correlation with all components (P <0.05). Whereas the rest had significant linear correlation only with the (4 and 5 components) (P <0.05) except for DMFT which is non-significant for all components.

Table 1. Clinical characteristics and anthropometric measurements of study subjects

Subject	Control (30)	PCOS (42)
Age (year) Mean ± SD Median	 27.66 ± 5.81 27.5	 27.95 ± 5.67 28.5
Onset of disease (year) Mean ± SD Median	_	 3.93 ± 2.71 3
Family history No. (%)	_	 12 (28.5%)
Weight (kg) Mean ± SD Median	 76.27 ± 17.59 71.35	 78.41 ± 16.25 77.65
Height (cm) Mean ± SD Median	 161.9 ± 5.28 163	 160.9 ± 7.95 161.5
BMI (kg/m²) Mean ± SD Median 18.5 - 24.9 No.(%) 25 - 29.9 No.(%) ≥ 30 No.(%)	 29.38 ± 5.88 28.95 6 (20%) 10 (33.3%) 14 (46.7%)	 30.39 ± 5.96 29.73 8 (19%) 14 (33%) 20 (47.6%)
Waist (cm) Mean ± SD Median	 92.33 ± 11.50 90	 93.02 ± 15.07 91.5
Hip (cm) Mean ± SD Median	115.8 ± 6.04 115	112.4 ± 10.26 110
Waist/hip ratio Mean ± SD Median ≥ 0.8 No.(%)	 0.78 ± 0.06 0.78 13 (43.3%)	 0.82 ± 0.09 0.83 27 (64.3%)
Waist/height ratio Mean ± SD Median ≥ 0.5 No.(%)	 0.55 ± 0.08 0.53 19 (45.3%)	 0.57 ± 0.09 0.57 35 (83.3%)
Systolic BP (mmHg) (Mean ± SD) ≥ 130 No. (%)	 119.76 ± 6.8 3 (10%)	 124.38 ± 6.49 10 (23%)
Diastolic BP (mmHg) Mean ± SD ≥ 85 No. (%)	 78.83 ± 3.37 1 (3.3%)	 81.59 ± 3.90 7 (16.6%)
Mean BP (mmHg) Mean ± SD	 92.47 ± 3.82	 95.85 ± 4.57

The results are expressed as mean±SD, number (%) and median. BMI: body mass index, BP: blood pressure, SD: standard deviation, No.: number, %: percentage.

Relationship of leptin and inflammatory markers levels to endocrine-metabolic parameters

In figure (1,2) the correlations between salivary and serum leptin levels for PCOS and controls were strong linear statistically highly significant (t-test =7.256, P < 0.001) for controls and (t-test =5.938, P < 0.001) for PCOS, While salivary and plasma fibrinogen levels for PCOS and controls had a weak linear correlation statistically non-significant for controls (t-test =0.806, P > 0.05) and positively correlated (linear correlation) statistically significant for PCOS (t-test =5.243, P < 0.05).

In figure (3A)PCOS and controls matched for BMI but salivary leptin levels for PCOS differ widely from those of controls and had strong positive linear correlation with BMI statistically highly significant (t-test = 5.23, P < 0.001) for PCOS and had positive linear correlation statistically significant for controls (t-test = 2.21, P < 0.05). Figure (3B) shows that serum leptin levels for PCOS differ from those of controls and were strongly correlated (positive linear correlation) with BMI statistically highly significant (t-test = 4.58, P < 0.001) for PCOS and had positive linear correlation statistically significant in controls (t-test = 2.33, P > 0.05).

In figure (4A)Salivary leptin levels for PCOS differ from those of controls and had positive linear correlation with W/H Ratio statistically significant in both PCOS and controls (t-test = 2.86, 1.99, P < 0.05) respectively. In figure (4B) serum leptin levels for PCOS differ from those of controls and had strongly positive linear correlation with W/H Ratio statistically highly significant in both PCOS and controls (t-test = 3.47, 3.06, P < 0.001) respectively.

Table 2. Lipid profile and fasting blood glucoseof PCOS patients.

Subject	PCOS (42)	Min – Max
TG (mmol/L) Mean ± SD ≥ 1.78 No. (%)	 1.31 ± 0.62 8 (19%)	 0.7 – 2.95 2.1 – 2.95
HDL (mg/dl) Mean ± SD ≤ 50 No. (%)	54.60 ± 7.38 11 (26.1%)	 32.5 – 67 32.5 – 50
TG/HDL (American units) Mean ± SD > 2 No. (%) ≥ 4 No. (%) ≥ 6 No. (%)	2.39 ± 1.67 11 (26.1%) 3 (7.1%) 3 (7.1%)	1.031 – 6.84 2.21 – 3.76 4.09 – 4.33 6.53 – 6.84
Fasting blood glucose (mmol/L) Mean ± SD ≥ 5.6 No. (%)	4.68 ± 0.82 5 (11.9%)	3.5 – 6.9 5.7 – 6.9

The results are expressed as mean±SD, no. (%), and min-max. Min: minimum, Max: maximum, TG: triglyceride, HDL: high density lipoprotein, SD: standard deviation.

Relationship of oral health measures with BMI

PCOS and controls were matched for BMI but gingival index (GI) scores for PCOS were higher than that of

controls and had strong positive linear correlation with BMI statistically highly significant in PCOS and controls (t-test = 3.081, 3.215, P < 0.001) respectively. (Figure 5A)

Periodontal index (PI) scores for PCOS were higher than that of controls and had strong positive linear correlation with BMI statistically highly significant (t-test = 3.385, P < 0.001) for PCOS and significantly correlated in controls (t-test = 2.807, P < 0.05). (Figure 5B)

Table 3. Oral health measurements of PCOS patients and controls.

		Control (30)	PCOS (42)
GI No. (%)	Normal	8 (26.66%)	-
	Mild	18 (60.13%)	16 (38.09%)
	Moderate	4 (11.72%)	16 (38.09%)
	Severe	-	10 (23.8%)
PI No. (%)	Normal PDT	12 (40%)	-
	Simple Gingivitis	13 (43.33%)	9 (21.42%)
	Beginning Destructive	5 (16.66%)	19 (45.23%)
	Established Destructive	-	14 (33.33%)
	Terminal	-	-
DMFT %		(33.69%)	(39.88%)
DT %		(8.8%)	(16.75%)
MT %		(2.02%)	(4.67%)
FT %		(19.16%)	(18.45%)
SFR (ml/min) (Mean ± SD)		0.394 ± 0.144	0.346 ± 0.079

The results are expressed as no. (%) for the indices, and mean ± standard deviation for the salivary flow rate. GI: gingival index, PI: periodontal index, PDT: periodontal tissue, DMFT: decayed, missed, and filled teeth, DT: decayed tooth, MT: missed tooth, FT: filled tooth, SFR: Salivary flow rate.

DMFT index scores for PCOS had a little difference from those of controls and had strong positive linear correlation with BMI statistically significant t-test for controls and PCOS = 3.869, 2.786, P < 0.05 respectively. (Figure 5C)

DMFT index was shown to have a weak inverse linear correlation with SFR, salivary CRP, salivary fibrinogen, and salivary leptin in PCOS statistically non-significant (t-test = 0.903, 1.83, 0.821, 2.01, P >0.05) respectively. (Figure 6 A, B, C, and D).

Gingival index (GI) had inverse correlation with mean values of salivary flow rate in all grades of inflammation (mild, moderate, and severe) statistically non-significant (P >0.05) (Figure 7A). There was linear correlation between scores of gingival index (degree of inflammation) and mean values of salivary leptin and CRP statistically significant in severe inflammation only (P <0.05). (Figure 7B and C) But it was non-significant with mean values of salivary fibrinogen (P >0.05) in all grades of inflammation (Figure 11D).

Periodontal index (PI) had inverse correlation with mean values of salivary flow rate in all grades of inflammation (simple gingivitis, beginning destructive, established destructive) statistically non-significant (P >0.05) (Figure 8A). There was a linear correlation between grades of periodontal index (degree of inflammation) and mean values of salivary leptin and CRP statistically significant (P <0.05) with the last grade of inflammation only (Figure 8B and C) But it was non-significant with mean values of salivary fibrinogen (P >0.05). (Figure 8D)

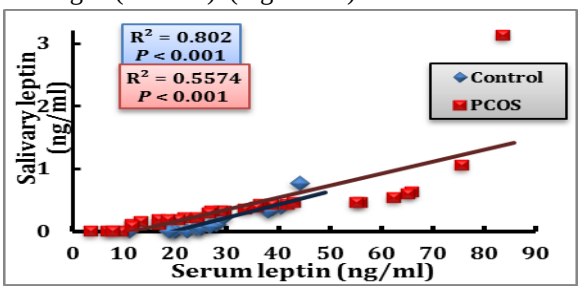


Figure 1. Regression line showing the strength of correlation between serum and salivary leptin concentration in both PCOS and controls.

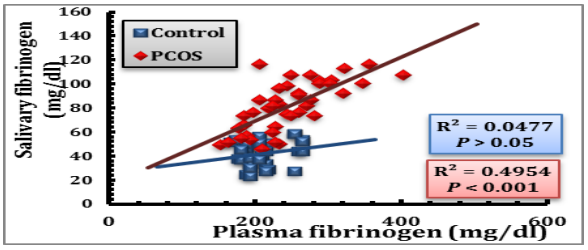


Figure 2. Regression line showing the strength of correlation between salivary and plasma fibrinogen concentration in both PCOS and controls.

DISCUSSION

In this study, examination of salivary leptin and salivary fibrinogen levels, salivary CRP in PCOS, DMFT in PCOS, the association between the oral health status and the metabolic syndrome in PCOS, the association between serum and salivary leptin, CRP, and fibrinogen and the metabolic syndrome in PCOS were done.

In the last ten years, several epidemiological studies have assessed the association between oral infection and systemic diseases that oral infections may confer independent risks for different systemic conditions (e.g. cardiovascular diseases). This study suggests an increased susceptibility for dental caries, gingivitis and periodontitis in women with PCOS compared with

healthy women in agreement with the report by Erhan et al. [7]. The underlying biological mechanisms for the association of obesity with periodontitis are not well-known; however, adipose-tissue-derived cytokines and hormones may play a key role, which in turn may modulate periodontitis [11] Furthermore, significant correlations between the clinical signs of gingivitis and periodontitis with leptin and CRP indicate a possible interaction between hormonal and metabolic phenotype in PCOS. On the other hand the clinical sign of dental caries, gingivitis and periodontitis correlate non-significantly with SFR and salivary fibrinogen. In persons with periodontitis, bacterial pathogens, endotoxins, and inflammatory cytokines may systemically trigger synthesis of acute-phase proteins (CRP), and enhanced lipid metabolism, along with increased serum cholesterol and triglyceride levels, which may contribute to the risk of systemic diseases such as CVD in agreement with Mattila et al.[12]. In women with PCOS, insulin stimulated leptin secretion is limited by the insulin resistance in adipocytes. An important feature of the obesity of PCOS is the accumulation of visceral fat (increased waist to hip ratio), which secretes more leptin in agreement with McConway et al. [13]. Leptin levels (serum and salivary) significantly increased with increasing metabolic syndrome score. Other associations were observed for CRP and fibrinogen levels in which it show significant correlations only in high scores of metabolic syndrome.This evidence in agreement with Feng [14]. The exact mechanism indicates that women with PCOS frequently have insulin resistance, meaning their body does not respond as quickly to insulin. The sluggish response will cause larger and larger amounts of insulin to be required before glucose is taken into the body tissues, and eventually a change in the way the body deals with sugar. Consistently high levels of glucose will be in the blood in agreement with Nicole and RN [15].

In Conclusion saliva was found to be a useful alternative to serum for the determination of leptin, CRP, and fibrinogen in PCOS. The susceptibility for dental carries, gingivitis, and periodontitis may significantly increase in PCOS that gingivitis and periodontitis are common finding in patients with PCOS, and that further supported by significant decrease in salivary flow rate. Salivary leptin and CRP may become important markers for inflammation related to health.

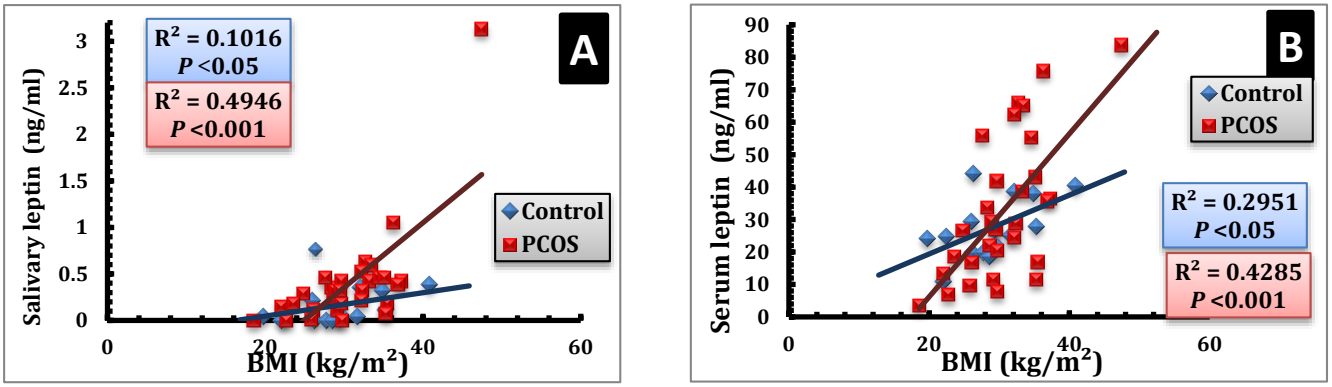


Figure 3. Regression line showing the strength of correlation between BMI and leptin concentration of both PCOS and controls in: A Saliva B Serum.

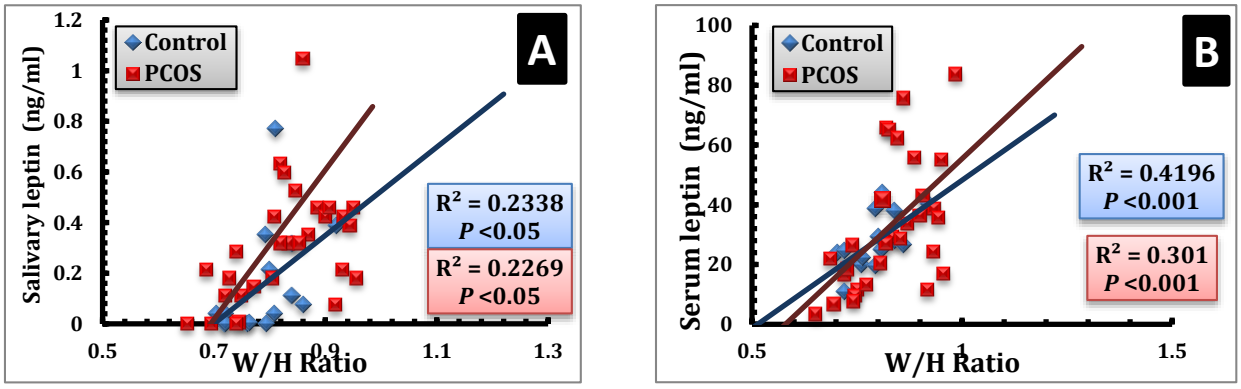


Figure 4. Regression line showing the strength of correlation between W/H Ratio and leptin concentration of both PCOS and controls in A Saliva B Serum.

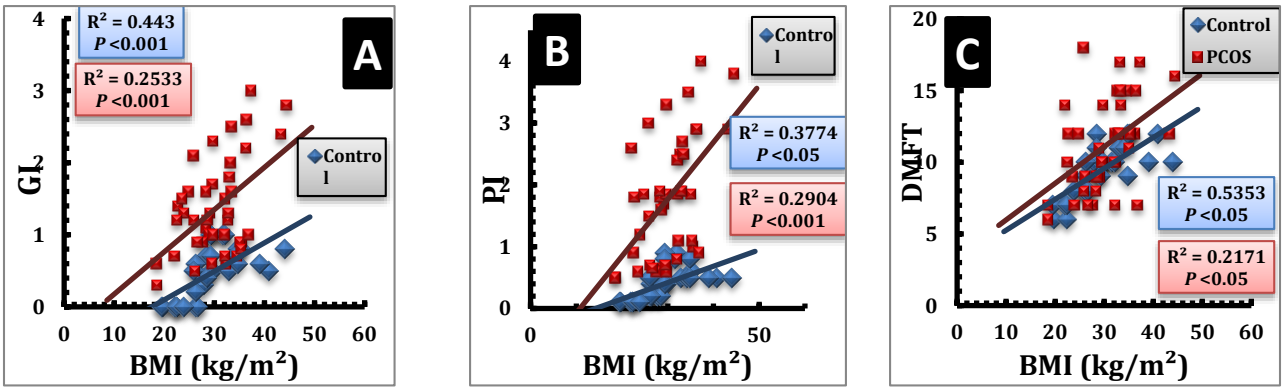


Figure 5. Regression line showing the strength of correlation between body mass index and oral health measures of both PCOS and controls that include A-Gingival index B- Periodontal index C- DMFT index.

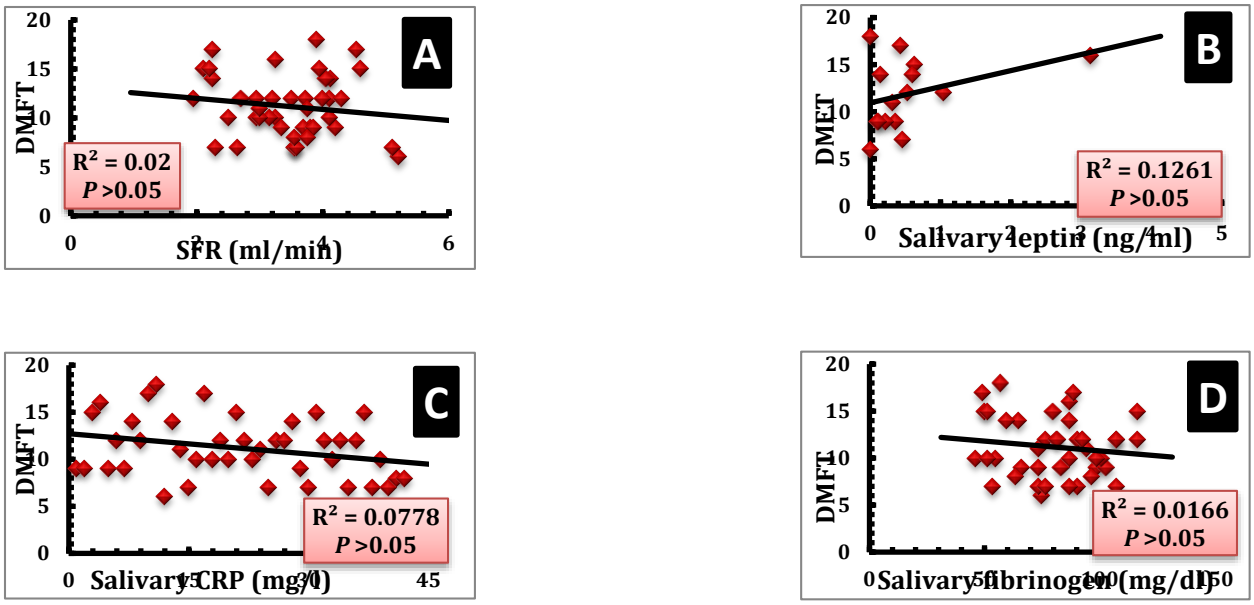


Figure 6. Regression line showing the strength of correlation between DMFT index score and A Salivary flow rate, B Salivary leptin, C Salivary CRP, D Salivary fibrinogen.

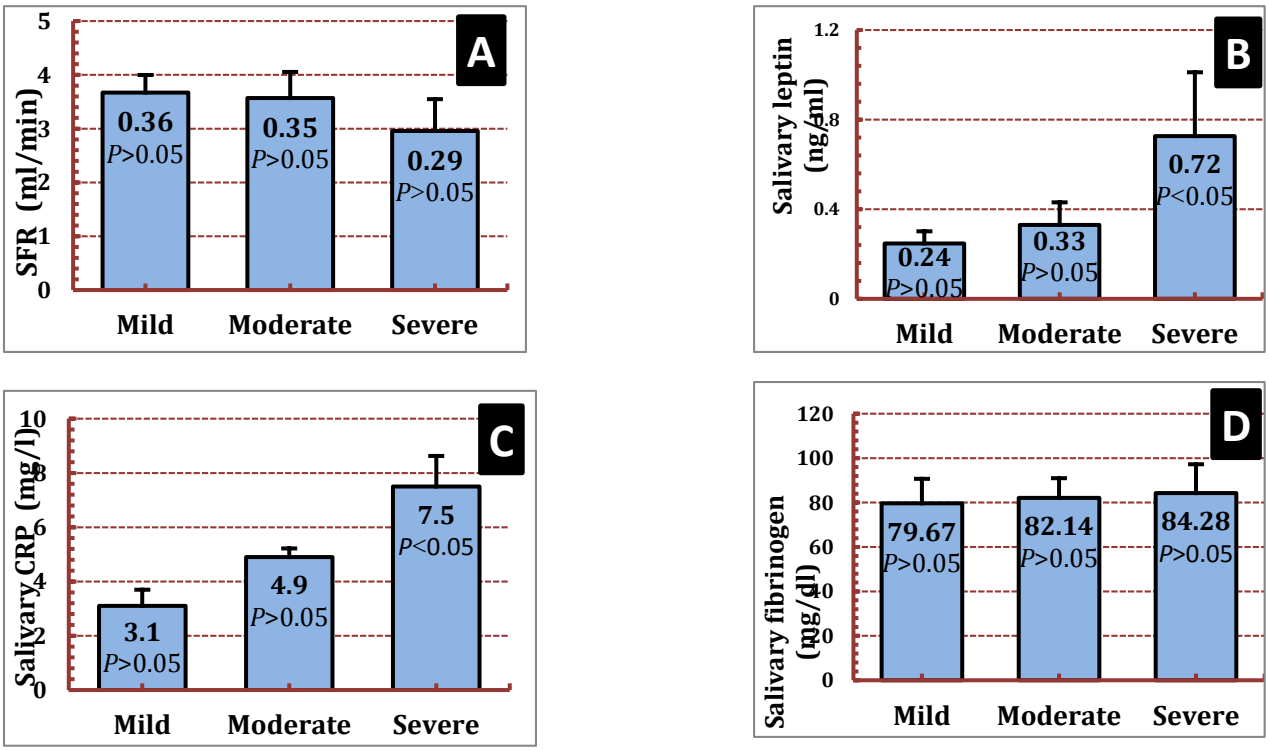


Figure 7. Comparison of the relationship between gingival inflammation grades in PCOS A Salivary flow rate, B Salivary leptin, C Salivary CRP, and D Salivary fibrinogen.

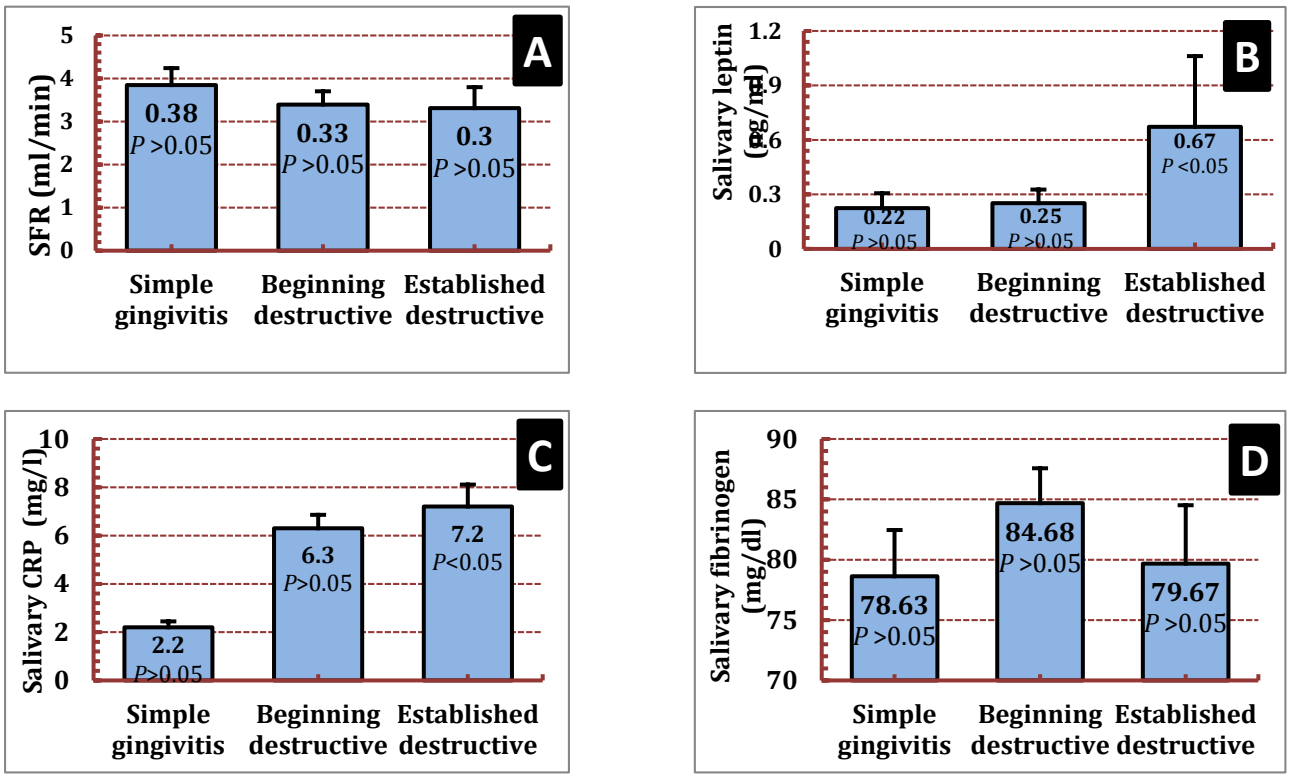


Figure 8. Comparison of the relationship between periodontal tissue inflammation grades in PCOS A SFR, B Salivary leptin, C Salivary CRP, and D Salivary fibrinogen.

Table 4. Oral health measures, inflammatory biomarkers and leptin correlation between the components

	No.	Salivary Leptin ng/ml	Serum Leptin ng/ml	Salivary CRP ≥ 6 (mg/l)	Serum CRP ≥ 6 (mg/l)	Salivary Fibrinogen (mg/dl)	Plasma Fibrinogen (mg/dl)	GI	PI	DMFT	SFR
		(Mean ±SD)		No.		(Mean ±SD)					
1Component	13	0.46 ± 0.08 *	52.33 ± 12.21 *	4 NS	2 NS	103.06 ± 9.21 NS	355.67 NS	1.7 ± 0.7 NS	1.9 ± 0.8 NS	11.3 ± 3 NS	0.36 ± 0.05 NS
2Components	7	0.42 ± 0.11 *	45.85 ± 13.69 *	1 NS	1 NS	94.48 ± 3.98 NS	304.15 346.88 NS	1.5 ± 0.75 NS	1.8 ± 0.99 NS	11.5 ± 3.62 NS	0.326 ± 0.093 NS
3 Components	4	1.04 ±0.31 *	55.74 ± 10.2 *	1 NS	1 NS	116.61 NS	359.17 NS	1.75 ± 0.59 NS	1.9 ± 0.93 NS	12.25 ± 2.06 NS	0.345 ± 0.036 NS
4 Components	3	2.13 ±0.42 *	73. 63 ±36.47 *	2 *	2 *	88.63 *	322 *	2.7 ± 0.14 *	3.56 ± 0.58 *	15 ± 1 NS	0.246 ± 0.05 *
5 Components	1	3.13 *	83.74 *	1 *	1 *	92.45 *	320.06 *	3 *	2.9 *	12 NS	0.195 *

The results are expressed as number and mean±SD. NS: Not significant, *: Significant, CRP: C-reactive protein, GI: Gingival index, PI: Periodontal index, DMFT: Decayed Missed Filled Teeth SFR: Salivary flow rate, no.: number, SD: standard deviation.

REFERENCES

1. 1.Diedrich, K, Bouchard P, Dominguez F, Matzuk M, Franks S, Hamamah S, Simon C (2011). Contemporary genetic technologies and female reproduction. Human Reproduction Update 17 (6): 829–847 Page 836.
2. 2.Balen AH, Laven JS, Tan SL (2003).Ultrasound assessment of the polycystic ovary:international consensus definitions. Hum Reprod Update.;9:505–14.
3. 3.Glueck CJ, Papanna R, Wang P (2003). Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. Metabolism.;52:908–15.
4. 4.Brennan AM, Mantzoros CS (June 2006). Drug Insight: the role of leptin in human physiology and pathophysiology--emerging clinical applications. Nat Clin Pract Endocrinol Metab 2 (6): 318–327.
5. 5.Hamann A, Matthaei S (1996). Regulation of energy balance by leptin. Exp. Clin. Endocrinol. Diabetes 104 (4): 293–300.
6. 6.Gilliam BE; Reed, Melinda R; Chauhan, Anil K; Dehlendorf, Amanda B; Moore, Terry L (2011). Evidence of Fibrinogen as a Target of Citrullination in IgM Rheumatoid Factor-Positive Polyarticular Juvenile Idiopathic Arthritis. Pediatric Rheumatology 9 (8).
7. 7.Erhan Dursun, Akalın FA, Güncü GN, Çınar N, Aksoy DY, Tözüm TF, Kılınc K, Yıldız BO (Jan 2011). Periodontal disease in polycystic ovary syndrome.; Volume 95, Issue 1 , Pages 320-323.
8. 8.Greenland P, Knoll MD, Stamler J, Neaton JD, Dyer AR, Garside DB, Wilson PW (2003). Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA, 290:891-897.
9. 9.Haffajee AD, Sokransky SS (2006). Introduction to microbial aspects of periodontal biofilm communities, development and treatment. Periodont, 42:7-12.
10. 10.Kershaw EE, Flier JS (2004). Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 89:2548–2556.
11. 11.Mattila KJ, Pussinen PJ, Paju S (2005). Dental infections and cardiovascular diseases: a review. J Periodontol 76(11 Suppl):2085–2088.
12. 12.McConway MG, Johnson D, Kelly A, Griffin D, Smith J, Wallace AM (2000). Differences in circulating concentrations of total, free and bound leptin relate to gender and body composition in adult humans. Ann Clin Biochem.; 37:717-723
13. 13.Feng-Hsiang Chiu, Chung Hsun Chuang, Wen-Cheng Li, Yi-Ming Weng, Wen-Chih Fann, Hsiang-Yun Lo, Cheng Sun, Shih-Hao Wang (2012). The Association of Leptin and C-reactive Protein With the Cardiovascular Risk Factors and Metabolic Syndrome Score in Taiwanese Adults;11(40)
14. 14.Nicole Galan, RN (July 22, 2009). PCOS and Insulin Resistance What You Need to Know About PCOS and Insulin Resistance.

Research Article

Non Palpable Breast Mass : Radiological & Pathological Evaluation

Wasan Ismail Al-Saadi , Basim Shehab Ahmed ,Qahtan Adnan Mahdi ,Israa Qader ,Yaarub Idrees

Date Submitted: 30/6/2015
Date Accepted:11/11/2015

Address for Correspondence:
Ass. Prof. Dr. Wasan I. Al-Saadi
Department of Radiology, College of Medicine, Al-Nahrain University
e-mail:drwasan72@gmail.com

Abstract

Background: Breast imaging modalities potentiates the detection of early, clinically occult breast masses thus allowing early treatment & reduces the mortality rate from breast cancer.
Objective: (i) To evaluate the diagnostic value of breast imaging modalities (mammography and / or ultrasonography) for the characterization of non-palpable breast masses in conjunction with cytology / histopathology. (ii)To assess the relation between the non-palpable breast mass & the breast density, Body mass index & the location of the mass within the breast.
Patients and method: The results of mammographic, sonographic & cytologic/ histopathologic examination performed in 50 patients with non-palpable breast mass were assessed to determine the diagnostic value of imaging in characterization of breast mass.The BMI of each patient was calculated & the breast density at mammography was assessed, 10 patients were <35 years old &thus had sonographic examination only,40 patients had both mammography and ultrasonography. All the patients underwent guided FNA & only 23 patients had excisional biopsy.
Results : Seven out of 40 mammographically detected masses were malignant (17.5%)and 33 cases (82.5%) were benign ,9 cases out of 50 sonographically detected masses were malignant (18%) and 41cases were benign (82%).These results were correlated with FNAC and /or histopathology. The sensitivity , specificity, positive predictive value negative predictive value & accuracy of mammography was 100%,91.6%,57.1%,100% & 92.5% respectively and for ultrasonography was 100% ,91.1% ,55.5% , 100% & 92% respectively .Most of mammographically detected lesions were in breast of category 2 breast density(72.5%) & the majority of patients were overweight or obese(72%).Most of the lesions were found at the upper outer quadrant of the breast.
Conclusions: Ultrasonography & mammography are both sensitive & specific in the diagnosis & characterization of benign and malignant non-palpable breast masses. Patients with high BMI are more prone to have non palpable breast masses particularly at the upper outer quadrants

Key words: *Non palpable breast mass , Mammography, Ultrasonography , Histopathology*

INTRODUCTION

Radiological examination of the breast is established as an essential part of the multidisciplinary approach to effectively manage breast diseases. With increasing use of screening mammography and ultrasound for various indications, a large number of **non-palpable breast lesions** are being detected. The more widespread use of these modalities has been accompanied by tremendous development of both non invasive and invasive radiological procedures that are implicated to establish the diagnosis of non-palpable breast lesions(1).

The standard practice when dealing with a palpable breast mass relies on the "triple test " concept which incorporates the clinical assessment , radiological evaluation & tissue sampling (2-5)

When the three assessments are performed adequately and produce concordant results, the triple test diagnostic accuracy approaches 100 % (3-5).However when dealing with non palpable breast lesion , one of the corner stones of the triple assessment is missed , that is the clinical evaluation .Here , comes the role of the radiologist & the pathologist to establish the diagnosis & to map the way for future management of the lesion . Several studies had dealt with non palpable breast lesions(6-7) , the major concern of these studies was the breast cancer incidence when the lesion is non palpable . In the present study , the authors aimed at evaluating the diagnostic value of breast imaging modalities(mammography and / or ultrasonography) for the characterization of non palpable breast masses with respect to cytology / histopathology & to find out the possible role of the location of the lesion within the breast & the Body mass index (BMI) in making the mass non palpable

PATIENTS AND METHODS

This was a prospective study which was conducting at the breast clinic at Al-Imamain Alkadhimain Medical City during the period From September 2013 to June 2014 . The patients presented to the breast clinic either for screening purpose or with sign and symptoms related to the breast. No palpable lesion was detected on the initial clinical breast examination performed by the same surgeon. Then these patients underwent mammography &/or bilateral breast ultrasonography. Those patients in whom a mass was found upon imaging were subjected to recall clinical breast examination by other surgeons unaware of the imaging findings to confirm the initial impression of no palpable abnormality , & these constituted the study sample which included a total of 50 women . Informed consent was obtained from the patients to participate in the study .Clinical data were collected from the patients & the body mass index was calculated . Ten of the patients were <35 years old & thus had sonographic examination only, 40 patients had both mammography and ultrasonography. All the patients underwent guided FNA & only 23 patients had exisional biopsy**.** Mammography was performed for 40 patients using GE health care mammo.senographe device.Mediolateral (MLO) & craniocaudal (CC) views were taken for both breasts & other view as magnifications were performed when indicated. The density of the breast was determined as 1-4 according to American college of radiologist (ACR) criteria(8) . Analysis of the mass detected by mammography was performed by the same radiologist & each mass was assessed regarding its shape , the ease of definition, the margin criteria and the presence of calcifications to determine the Breast Imaging Reporting and Data System (BIRADS) category according to ACR recommendation (8).The location of the mass with respect to breast quadrant was determined. The largest diameter of the mass was used to indicate the size of the lesion. Sonographic examinations were performed for all patients using a high-resolution unit (VERSA; Siemens, Erlangen, Germany 1996) with a linear array probe centered at 7.5 MHz. The ultrasonography was performed by another radiologist & BIRADS category for the lesion was set depending on the shape , the delineation , homogeneity and echogenicity, margin & irregularity , microlobulation & angulation , the posterior shadowing or enhancement and calcification. Both radiologists were not aware of the imaging finding at the other modality to avoid interobserver bias. Ultrasound guided fine needle aspiration cytology was performed for all patients using 18-23 G needles . The tissue samples obtained were placed in a 10%formalin fixative & routine H & E staining was done. The samples were assessed by the same experienced cytopathologist .Twenty three patients with equivocal or suspicious cytological findings underwent exisional biopsy. The results were interpreted by two experienced histopathologists .

Data Analysis

Probability tests, *t*-tests, or Wilcoxon's rank sum tests, as appropriate

Logistic regression techniques were used to obtain diagnostic Values of the two imaging techniques , assessing the major effect of tumor size and BMI in palpability of breast lesions. Then these were correlated with the FNAC/biopsy . The 95% confidence interval (CI), and *P* value were presented to identify the significant difference between the diagnostic ability of the two imaging modalities (mammography and ultrasonography), & between these modalities and FNAC/biopsy .

Data were analyzed to evaluate the sensitivity , specificity , positive predictive value , negative predictive value and over all accuracy for each imaging modality

RESULT

The study included 50 women with non palpable breast mass , the mean patients age 47 years (range 19-69 years). The size of the masses ranged from 3-16.9 mm (mean= 9.9 mm) at mammography & 2.5-16mm (mean= 7.9mm) at ultrasonography

Fifty patients had ultrasonography , 33 patients(66%) were considered benign **(figure 1 a)** and 8 patients(16%)were considered probably benign . Only

18 % were considered as probably malignant or malignant **(figure2 a).** On the other hand 40 patients had mammography , the majority of the lesions were considered benign or probably benign **(figure1 b)** & only 17.5 % were considered as probably malignant of malignant **(figure2 b)** . The distribution of the lesions according to BIRADS categories is shown in table 1

Table 1. BIRAD categories of the lesions on ultrasonography & mammography

BIRAD categories of the lesions on Ultrasonography			BIRAD categories of the lesions on Mammography		
MALIGNANT BIRAD	No. of cases	%	MALIGNANT BIRAD	No. of cases	%
IV	8	16	IV	7	17.5
V	1	2	V	0	0
BENIGN BIRAD	No. of cases	%	BENIGN BIRAD	No. of cases	%
II	33	66	II	24	60
III	8	16	III	9	22.5

The majority of patients (n=29 , 72.5%) were classified as ACR2 density (scattered fibroglandular density) .Table 2 shows the distribution of benign & malignant lesions according to the breast density

Table 2. distribution of Mammographically detected masses according to breast tissue density n=40

Density	No.of cases	Benign		Malignant	
		No.	%	No.	%
ACR I	3(7.5%)	3	7.5	0	0.0
ACR II	29(72.5%)	26	65.0	3	7.5
ACR III	6(15%)	6	15.0	0	0.0
ACR IV	2(5%)	1	2.5	1	2.5

All patients underwent FNAC and only 23 patients had biopsy . the cytological results were correlated with that of mammography and ultrasonography in BIRAD system .All the benign lesions at BIRAD (II,III) in ultrasound and /or mammography were found to be benign on cytology /biopsy comprise 41 & 33 patients respectively, while 4 out of 7 masses suggested to be malignant on mammography and 5 of 9 masses suggested to be malignant on ultrasonography (BIRAD IV,V5)were proved to be malignant at cytology and biopsy (table3,4).

The statistical characteristics of imaging modalities(mammography and ultrasonogrphy) & their role in diagnosis of non-palpable breast mass are shown in table 5:

Table 3. Mammography vs. cytology in differentiation between benign and malignant breast mass (n=40)

	Mammography		Cytology/Histopathology		P value
	No.	%	No.	%	
Benign	33	82.5	36	90.0	0.3301
Malignant	7	7.5	4	10.0	

Table 4. Ultrasound vs. cytology in differentiation between benign and malignant breast mass (n=50)

	Ultrasonography		Cytology/Histopathology		P value
	No.	%	No.	%	
Benign	41	82.0	45	90.0	0.249
Malignant	9	18.0	5	10.0	

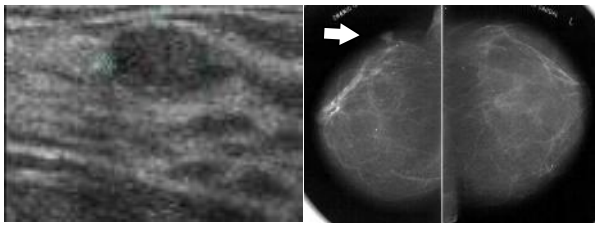
Table 5. Statistical analysis of Mammography & Ultrasonography

	Statistical potential for mammography in non-palpable breast masses	Statistical potential for Ultrasonography in non-palpable breast masses
Sensitivity	100%	100%
Specificity	91.6%	91.1%
accuracy	92.5 %	92 %
Positive predictive value	57.1%	55.5%
Negative predictive value	100%	100%

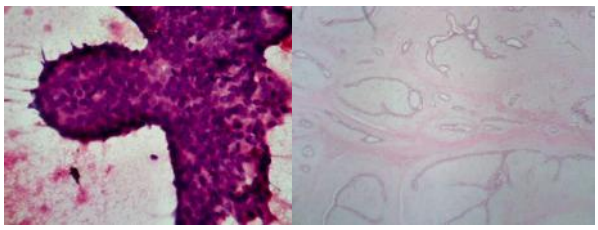
Most of patients with non-palpable breast mass were obese (BMI> 30) while those with BMI <18.5 are the least who had non-palpable breast mass.The distribution of patients with respect to the BMI is shown in table 6

Table 6. Distribution of non-palpable breast masses according to body mass index

BMI	No . of cases	Benign		Malignant	
		No.	%	No.	%
Underweight <18.5	2(4%)	2	4.0	0	0.0
Normal 18.5-24.9	12(24%)	11	22.0	1	2.0
Overweight 25-29.9	17(34%)	15	30.0	2	4.0
Obese >30	19(38%)	17	34.0	2	4.0

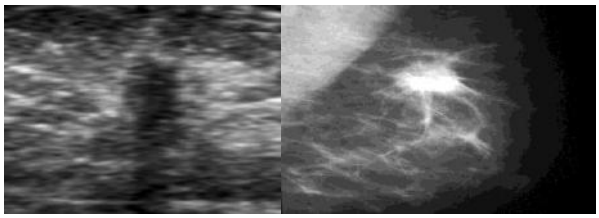


(a) **(b)**

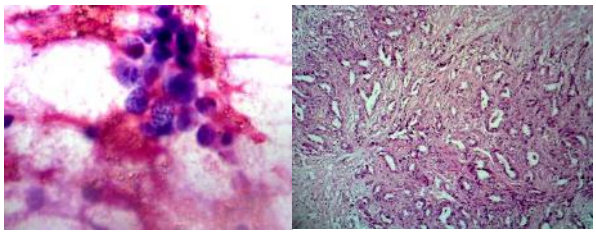


(c) **(d)**

Figure 1. A 55 year patient with mastalgia ,no palpable abnormality at clinical examination .(a)Rt. breast ultrasound revealed well defined oval mass lesion (9.4x5.4mm) with posterior enhancement (BIRAD II) (b) A crainocaudal view OF mammography shows well defined oval mass at the outer quadrant (arrow) (BIRAD II, (c) A cytology smear shows sheets of benign looking ductal epithelial & myoepithelial cells X40 ,(d)Histopathology: variable size slit like structures lined by benign ductal epithelial & myoepithelial cells with surrounding fibrous stroma(fibroadenoma) X40



(a) **(b)**



(c) **(d)**

Figure 2. : A 42 year old patient with Lt .breast nipple discharge.(a) ultrasound revealed hypoechoic mass (13 x10.6mm) with microlobulations & focal posterior shadowing (BIRADS IV) . (b)mammography MLO speculated mass lesion (BIRAD V).(c) A cytology smear shows discohesive malignant pleomorphic cells with variable size hyperchromatic nuclei X40 (d) Histopathology reveals infiltrative moderately differentiated ductal carcinoma X40

DISCUSSION

Screening mammography remains the primary imaging modality for the detection of clinically occult cancer at an earlier stage and thus permitting early treatment(9) , however at many times, other imaging modalities are needed to overcome some of limitations of mammography & to assist in making the diagnosis .Ultrasonography is a perfect adjunct to the mammography particularly in the evaluation of dense breasts which represent a challenging entity for mammography usually below 35 years of age. In the breasts where solid lesions and cysts are obscured at mammography due to dense fibroglandular tissue, ultrasonography help in diagnosis and to decrease the number of surgical biopsies .

In the present study mammography had 100% sensitivity with 91.6% specificity in differentiation of nonpalpable breast masses ,its positive predictive value was as high as 57.1% & the negative predictive value was 100% . These figures are consistent with those of Ohlinger et al (10) and Leconte et al.(11) , the higher sensitivity in our series can be attributed to the mammographic breast density as most of our patients (72.5%) had ACR 2 breast density which facilitate the lesion detection & thus increasing the mammographic sensitivity . Ultrasonography was very valuable in the differentiation of benign from malignant lesions with sensitivity and negative predictive value of 100%, specificity of 91.1% and overall accuracy of 91.9%. Both mammography & sonography have comparable high sensitivity & specificity in the evaluation of non palpable breast mass.

Concerning the tumor size , Its well known that the size is one of prognostic factors in patients with breast carcinoma (12) and its assessment in patient with breast cancer is based on radiologic assessment when pathological measurement are equivocal . In the current our study the average size of mammographically detected breast mass was 9.92 mm (range 3-16.9 mm) and the average tumors size detected by ultrasonography was 7.9mm (range 2.5 -16 mm) which indicate that the ultrasound imaging modality can predict the smallest size tumor . These figures agree with the results of Leconte *et al.* (11)Buchberger *et al.* (13), , Crystal *et al.* (14) and Kolb *et al.* (15), who were also able to show that it is feasible to use ultrasonography for the detection of early breast cancer <1 cm which were in fact mammographically occult in those studies.

Regarding the location of the breast mass the present study revealed that most of the non-palpable breast masses are located in the upper outer quadrant , this may be considered as one of the causes for the lesions being

non palpable because it is more mobile & thicker than the inner quadrants that are relatively fixed .

The role of the patients body mass index(BMI) had been observed as a contributing factor for the mass to be non-palpable. The present study identified that a high percentage of non-palpable breast mass were detected in obese (38%) and over weight women (34%) and the most detected malignant lesions affect over weight and obese patients , this fact confirmed by Vecchia et al (16).

Conclusions & recommendations

- Mammography and ultrasonography are highly sensitive and specific tool for the detection of breast masses which are not palpably by clinical breast examination . Ultrasonography and mammography cannot replace each other but to suggest single modality, ultrasonography is better in younger population with ACR breast density of 3 & 4 where as mammography is better & more sensitive in older population with ACR breast density of 1 & 2 . However, sono-mammographic correlation is best in both. ultrasonographic examination need to be considered in every patients who have a dense breast at mammography to detect occult or non palpable lesion

- Patients with high BMI are more prone to have non palpable breast mass particularly at the upper outer quadrants

REFERENCES

1. Gravelle Mammography 7th edition . In: Sutton D (ed.) A textbookof radiology and imagingDMRD,FCan, AR(hon)MD,FRCP,. Churchill Livingstone, New York, pp [1451-1486]2002, FIRST printed in india 2009,reprinted 2012
2. Morris KT, Vetto JT, Petty JK, Lum SS, Schmidt WA, Toth-Fejel S, et al. A new score for the evaluation of palpable breast masses in women under age 40. Am J Surg. 2002;184:346–7.
3. Steinberg JL, Trudeau ME, Ryder DE, Fishell E, Chapman JA, McCready DR, et al. Combined fine-needle aspiration, physical examination and mammography in the diagnosis of palpable breast masses: their relation to outcome for women with primary breast cancer . Can J Surg. 1996;39:302–11.
4. Kamphausen BH, Toellner T, Ruschenburg I. The value of ultrasound-guided fine-needle aspiration cytology of the breast: 354 cases with cytohistological correlation. Anticancer Res. 2003;23:3009–13.
5. Clarke D, Sudhakaran N, Gateley CA. Replace fine needle aspiration cytology with automated core biopsy in the triple assessment of breast cancer. Ann R Coll Surg Engl. 2001;83:110–2
6. Azavedo E ; Svane G; Ringertz H. The Role of the Radiologist in Screening for Nonpalpable Breast Tumors in Sweden. Investigative Radiology.1991-volume 26 –issue 2 , pp 174-178
7. Azavedo E ; Svane G , Auer G .Stereotactic Fine-Needle Biopsy In 2594 Mammographically Detected Non-Palpable Lesions. LANCET.1989. Volume 333 , no.8646, pp.1033-1036
8. ACR Practice Guideline For The Performance Of Screening And Diagnostic Mammography Preamble, Revised 2013
9. Eric L. Rosen1, Edward Sickles ,Delia Keating3 ,Ability of Mammography to Reveal Nonpalpable Breast Cancer in Women with Palpable Breast Masses AJR. 1999:172,
10. Ohlinger R, Heyer H, Thomas A, Paepke S, Warm H, Klug U,*et al*. Non-palpable Breast Lesions in Asymptomatic Women:Diagnostic Value of Initial Ultrasonography and Comparison with Mammography.Anticancer Research(2006); 26: 3943-3956
11. Leconte I.,Feger C, Galant C.,Berlière M., Berg B., Hoore W., et al. Mammography and Subsequent whole-Breast Sonography of Nonpalpable Breast Cancers, the Importance of Radiologic Breast Density. American Journal of Roentgenology. 2003;180: 1675-167
12. Smart CR, Hendrick RE, Rutledge JH III, Smith RA. Benefit of mammography screening in women aged 40 to 49 years. *Cancer* 1986;75:1619–1626.
13. Buchberger W, de Koekkoek-Doll P, Springer P, Obrist P and Dunser M: Incidental findings on sonography of the breast: clinical significance and diagnostic workup. AJR Am J Roentgenol *173*: 921-927, 1999
14. Crystal P, Strano SD, Shcharynski S and Koretz MJ: Using sonography to screen women with mammographically dense breasts. AJR Am J Roentgenol 2003;181: 177-182,.
15. Kolb T, Lichy J and Newhouse J: Comparison of the performance of screening mammography, physical examination of factors that influence them: an analysis of 27825 patient evaluations. Radiology 2002;225: 165-175,
16. Vecchia C, Giordano SH, Hortobagyi GN, Chabner B. Overweight, obesity, diabetes, and risk of breast cancer: interlocking pieces of the puzzle. Oncologist. 2011;16(6):726-9.

Research Article

Infection with herpes simplex virus type 2 (HSV-2) in pregnant women

Israa Hashim Saadoon, ***Ph.D;*** **Sumaya T. Saihood**, ***F.I.C.O.G.***

Date Submitted: 17/9/2015
Date Accepted:12/11/2015

Address for Correspondence:
Dr. Sumaya T. Saihood
Department of Obstetrics & Gynecology, College of Medicine, Tikrit University,Iraq.

Abstract

Background: During pregnancy, certain infections in the mother can be more severe than usual like hepatitis or latent viruses such as herpes simplex virus (HSV) and cytomegalovirus (CMV) can reactivate and infect the fetus. Disseminated herpes simplex (HSV) virus infection during pregnancy is rare, but important to recognize, as disseminated disease can have a serious outcome for both the mother and neonate. **Aim of the study:** The aim of this study was to identify seroprevalence of HSV-2 in pregnant women in Tikrit city and its relation with abortion.

Methods: The current study included 135 pregnant women who attended to Tikrit Teaching Hospital for the period from beginning of October/2013 to the end of April/2014. Blood samples were collected from each woman for laboratory diagnosis of HSV-2 IgG and HSV-2 IgM antibodies.

Results: The research revealed that HSV-2 IgG and HSV-2 IgM antibodies were found in 37 (27.4%) and 13(9.62%) respectively out of 135 pregnant women. There was significant correlation between HSV-2 IgG seropositive and age of pregnant women, while no such relation was found between HSV-2 IgM and age. There is no-significant relation between both HSV-2 IgG and HSV-2 IgM antibodies with gestational time of pregnancy. Antibodies of HSV-2/ IgG and IgM were detected in 40.54% and 53.85 % respectively of pregnant women with history of abortion and the result was significant at P< 0.05. There was non-significant relation between HSV-2 IgG antibodies and HSV-2 IgM antibodies and No. of abortions.

Conclusions: Herpes simplex virus type 2 (HSV-2) infection is one of the important infections in pregnant women. The current study revealed a significant role of HSV-2 in abortion and since the first time infection of the mother associate with risk of transmission of infection to the fetus or newborn and its sequelae it is necessary to screen all pregnant women for HSV-2 infection for proper management.

Key words: HSV-2, pregnant women, abortion.

INTRODUCTION

The HSV-2 virus is classified in the alphaherpes subfamily of the family *Herpesviridae.* All herpesviruses are morphologically similar, with an overall size of 180 to 200 nm. [1, 2]

In the infected person, there is an active and latent phase. After an incubation period of about a week, the active phase begins. During the active phase, the virus multiplies explosively between 50,000 and 200,000 new virions are produced from each infected cell. The primary infection and reactivation can occur without any symptoms and apparently healthy people can transmit HSV-2 to their sexual partners or their newborns.[3] The chance of acquiring infection increases with age.[4]

Herpes Simplex Virus Type 2 is usually acquired as a sexually transmitted disease, so antibodies to this virus are seldom found before puberty. Surveys using type-specific glycoprotein antigens recently determined that 17% of adults in the United States possess HSV-2

antibodies, with seroprevalence higher among women than men, higher among blacks than whites, and age related.[2]

Babies exposed to the initial genital herpes lesions as they pass through the birth canal can be infected. Herpesviruses can permanently disable or kill these young patients. Recurrent lesions, however, are not as harmful to newborns, presumably because maternal antibodies provide protection. Patients shedding herpesviruses from bursting blisters can infect their own or another person's esophagus, eyes, or skin. An estimated 4 billion people worldwide are infected with herpesviruses, and about 86 million have genital herpes lesions.[5] Seroprevalence of HSV-2 in developing Asian countries is comparable (10–30%) to that observed in North America and Northern Europe.[6]

Because of the high morbidity and mortality rates of neonatal infection, special attention must be paid to preventing transmission during delivery. Where active HSV lesions are present on maternal tissues, Cesarean section delivery may be used to minimize contact of the infant with infected maternal genital secretions, but Cesarean delivery may not be effective if rupture of the membranes precedes delivery by more than several hours. Avoiding the birth canal is particularly important if the mother has a primary HSV infection late during pregnancy.[7] The aim of this study was to identify seroprevalence of HSV-2 in pregnant women in Tikrit city and its relation with abortion.

PATIENTS AND METHODS

The current research included 135 pregnant women (aged from 17-49 years) who attended to Tikrit Teaching Hospital for the period from beginning of October/2013 to the end of April/2014. Pregnant women with HBV and/or HCV infection and those with other chronic diseases were excluded from this study.

Five ml of blood was collected from each woman by vein puncture using disposable syringes. The blood was placed in plastic disposable tubes; it was left to stand at room temperature (20- 25°C) to allow it to clot, then the sera were separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until the time of test.

Serological investigation included detection of HSV-2/ IgG antibodies and HSV-2/ IgM antibodies by using enzyme-linked immunosorbent assay (ELISA); from Biocheck, CA, USA.

Statistical Analysis

The statistical analysis was performed using chi-square test. P values less than 0.05 were considered statistically significant.

RESULT

The current study revealed that out of 135 pregnant women, HSV-2 IgG and HSV-2 IgM antibodies were detected in 37 (27.4%) and 13(9.62%) respectively… Table 1.

Table 1. Frequency of HSV-2/ IgG and IgM Antibodies in Pregnant Women.

No. of Women	HSV-2/ IgG		HSV-2/ IgM	
	No.	%	No.	%
135	37	27.4	13	9.62

The current study revealed significant correlation between HSV-2 IgG seropositive and age of pregnant women, while the relation between HSV-2 IgM and age was non-significant… Table 2.

Table 2. Frequency of HSV-2/ IgG and IgM Antibodies According to Age Groups.

Age Group (yr.)	No. of women	HSV-2/ IgG		HSV-2/ IgM	
		No.	%	No.	%
≤ 29	76	14	37.84	9	69.23
30-39	38	12	32.43	3	23.08
40-49	21	11	29.73	1	7.69
Total	135	37	100	13	100
X^2		10.0001		1.1307	
P		< 0.05		> 0.05	

The current research showed that the highest rate of HSV-2 IgG antibodies was found in women within the 3rd trimester of pregnancy, while the highest rate of HSV-2 IgM was found in women within the 1st trimester of pregnancy. However, the result was non-significant… Table 3.

Table 3. Frequency of HSV-2/ IgG and IgM Antibodies According to Gestational Age.

Gestational Age	No. of Women	HSV-2/ IgG		HSV-2/ IgM	
		No.	%	No.	%
1st trimester	43	9	24.32	7	53.85
2nd trimester	55	13	35.14	4	30.77
3rd trimester	37	15	40.54	2	15.38
Total	135	37	100	13	100
X^2		4.5074		3.2945	
P		> 0.05		> 0.05	

The present study revealed that 40.54% and 53.85 % of pregnant women with HSV-2/ IgG and IgM respectively had history of abortion. The result was significant at P< 0.05… Table 4.

Table 4. Association between HSV-2/ IgG and IgM Antibodies and History of Abortion.

History of Abortion	No. of Women	HSV-2/ IgG		HSV-2/ IgM	
		No.	%	No.	%
+ ve	38	15	40.54	7	53.85
- ve	97	22	59.46	6	46.15
Total	135	37	100	13	100
X^2		3.8702		4.6971	
P		< 0.05		< 0.05	

The present study showed that the highest rate of seropositive for HSV-2/ IgG was found in women with 2 abortions followed by those with three abortions or more. The relation was non- significant at P < 0.05. The highest rate of HSV-2/ IgM was recorded in women with 1 abortion followed by those with 2 and those with three abortions or more (the same rate for each group). The result was non- significant… Table 5.

Table 5. Association between HSV-2/ IgG and IgM Antibodies and No. of Abortions.

No. of Abortions	No. of Women	HSV-2/ IgG		HSV-2/ IgM	
		No.	%	No.	%
1	17	4	26.67	3	42.86
2	13	6	40	2	28.57
≥ 3	8	5	33.33	2	28.57
Total	38	15	100	7	100
X^2		3.827		0.317	
P		> 0.05		> 0.05	

DISCUSSION

Antibodies to HSV-2 rise during the age of adolescence and sexual activity. During primary infections, IgM antibodies appear transiently and are followed by IgG and IgA antibodies that persist for long periods. Antibodies appear in 4–7 days after infection and reach a peak in 2–4 weeks. They persist with minor fluctuations for the life of the host. Detection methods available include neutralization, immunofluorescence, and enzyme-linked immunosorbent assay.[2] Data obtained by the present research revealed that HSV-2 IgG and HSV-2 IgM antibodies were found in 27.4% and 9.62% of pregnant women. Another study concerning pregnant women in Babylon province reported that 28.9% and 22.2% of cases were positive for HSV-2 depending on IgM and IgG respectively.[8]

Concerning pregnant mothers visiting the antenatal clinic and delivery room in King Fahd Hospital of the University (KFHU) in Al-Khobar, Saudi Arabia…6.5% had detectable level of HSV-2 IgG antibodies, and 0.5% had detectable level of HSV-2 IgM antibodies.[9] In Turkey, high levels of HSV-2 (42%) were found amongst pregnant women in the city of Erzurum in Eastern Anatolia Region, Turkey.[10] In Istanbul however, lower HSV-2 seroprevalence was observed. Only 5% of pregnant women were infected with HSV-2.[11]

Comparing the developing countries, substantially higher rates of HSV-2 have been observed in sub-Saharan Africa, where prevalence in adults ranges from 30% to 80% in women. [12] In fact the strongest association with HSV-2 infection appears related to the number of sexual partners.

The present work revealed that the highest rate of HSV-2 IgG antibodies was found in women within the age group ≤ 29 years, followed by those within the age group 30-39 years. The result was significant (P< 0.05). Regarding HSV-2 IgM antibodies, the highest rate was detected in women who aged less than or equal to 29 years, followed by those within the age group 30-39 years. However, the result was non-significant (P > 0.05)… Table 2. Data obtained by the present work are in agreement with published data. In Babylon province , the distribution of age with type of infection according IgM antibodies to herpes showed that the main age of infection was from (25-40 years).[8] In Saudi Arabia, prevalence of HSV-2 IgG was statistically significantly associated with the age of the mother. Possible explanation for this finding is the increase in sexual activity with the increase in age.[9] In each country of Europe, HSV-2 seropositivity becomes more common from adolescence onwards and increases in the population with age, with a decline in the older age groups in some countries.[13]

The present work revealed that HSV-2 IgG seropositive was found at highest rate in of women within the 3rd trimester of pregnancy(40.54%), followed by those within the 2nd trimester (35.14%) and those within the 1st trimester of pregnancy (24.32%). Concerning HSV-2 IgM, the highest rate was found in women within the 1st trimester of pregnancy (53.85%) followed by those within the 2nd trimester and those within the 3rd trimester (30.77% and 15.38% respectively). However, the result was non-significant… Table 3.

Al- Marzoqi *et al* [8] reported that the highest rate of IgM antibodies to HSV-2 was detected in women within the 2nd trimester (63.5%) followed by those within the 3rd trimester and 1st trimester of pregnancy (19.2% and 17.3% respectively). On the other hand, HSV-2 IgG was detected in 27.1% of serum samples obtained during the first trimester of pregnancy from Saudi women attending Maternity and Children's Hospital in Makkah.[14]

Some maternal infections, especially during the early gestation, can result in fetal loss or malformations because the ability of the fetus to resist infectious organisms is limited and the fetal immune system is unable to prevent the dissemination of infectious organisms to various tissues. [15] The danger of intrauterine HSV transmission is highest during the first 20 weeks of gestation because it can lead to abortion, stillbirth and congenital anomalies. [16]

Recent findings revealed that first-time infection of the mother is the most important factor for the transmission of genital herpes from mother to foetus/newborn. In fact, the pregnant woman who acquires genital herpes as a primary infection in the latter half of pregnancy, rather than prior to pregnancy, is at greatest risk of transmitting these viruses to her newborn.[17] When primary HSV infection occurs during late pregnancy, there is not adequate time for antibodies to develop and suppress viral replication before labor.[18]

The current research revealed significant relation between HSV-2 IgG antibodies and HSV-2 IgM and history of abortion.…Table 4. Non-significant relation was found between both HSV-2 IgG antibodies and HSV-2 IgM antibodies and No. of abortions.…Table 5. Previous study concerning herpes simplex infections with incidence of abortion in pregnant women in Babylon province revealed that abortion according to herpes was 5.8%, first trimester was the highest ratio of infection than other two trimesters.[8] However, another study in Egypt confirmed a significant increase of HSV in patients with recurrent abortions compared with pregnant patients without recurrent abortions, which denotes that HSV may predispose to this condition.[19] For unknown reasons, the HSV-2 virus is associated with a higher-than-normal rate of cervical cancer and miscarriages.[3] Herpesvirus reactivation frequently occurs during the first months of pregnancy because of the progesterone-linked immunodepression.[20]

Kapranos and Kotronios[21] reported on the significant role of HSV in the first trimester pregnancy loss and its detection by sensitive and accurate nested PCR, which would permit prompt antiviral therapy for a successful future pregnancy: HSV was detected in 43.2% of early pregnancy loss and in 16.7% cases of elective pregnancy termination.

Herpes can be spread to an infant during vaginal delivery, leading to congenital (neonatal) herpes. Congenital herpes is one of the most life-threatening of all infections in newborns, affecting approximately 1,500 to 2,200 babies per year in the United States. It can result in neurological involvement as well as blindness. As a result any female who has had genital herpes should have a caesarean section instead of delivering vaginally.[3]

REFERENCES

1. Levinson W. Review of Medical Microbiology and Immunology. 13th ed. McGraw−Hill companies. Inc. United States of America. 2014
2. Brooks GF,Carroll KC, Butel JS, Morse SA, Mietzner TA. Jawetz, Melnick and Adelberg's Medical Microbiology. 26th ed. McGraw−Hill companies. Inc.United States of America. 2013: 467-91.
3. Willy JM, Serwood LM, Woolverton CJ. Prescott, Harley and Klein's Microbiology.7th ed. McGraw-Hill companies. Inc. United States of America. 2008: 933.
4. Fatahzadeh M, Schwartz RA. Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management. J Am Acad Dermatol 2007; 57 (5): 737–63.
5. Acheson NH. Fundamentals of Molecular Virology. 2nd ed. John Wiley & Sons, Inc.2011; 285- 301.
6. Weiss H. Epidemiology of herpes simplex virus type 2 infection in the developing world. Herpes 2004. 11 Suppl 1: 24A–35A.
7. Ryan KJ, Ray CG. Sherris Medical Microbiology. 6th ed. McGraw-Hill companies. Inc. United States of America. 2014; 245-69.
8. Al-Marzoqi AHM, Kadhim RA, Al-Janabi DKF, Hussein J, Hussein HJ, Al Taee ZM. Seroprevalence study of IgG and IgM antibodies to toxoplasma, rubella, cytomegalovirus, *Chlamydia trachomatis* and herpes simplex II in pregnancy women in Babylon province. Journal of Biology, Agriculture and Healthcare 2012; 2 (10):159-64.
9. Obeid O E. Prevalence of herpes simplex virus types 1 and 2 and associated sociodemographic variables in pregnant women attending King Fahd Hospital of the University. J Family Community Med. 2007 Jan-Apr; 14(1): 3–7.
10. Smith JS, Robinson NJ. Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. J Infect Dis 2002; 186 Suppl 1: S3–28.
11. Dolar N, Serdaroglu S, Yilmaz G, Ergin S. Seroprevalence of herpes simplex virus type 1 and type 2 in Turkey. J Eur Acad Dermatol Venereol 2006; 20 (10): 1232–6.
12. Weiss H. Epidemiology of herpes simplex virus type 2 infection in the developing world. Herpes 2004; 11:24A-35A.

.١٣

14. Pebody RG, Andrews N, Brown D, *et al*. The seroepidemiology of herpes simplex virus type 1 and 2 in Europe. Sex Transm Infect 2004; 80 (3): 185–91.
15. Ghazi HO, Telmesani AM, Mahomed MF. TORCH agents in pregnant Saudi women. Med Principles Pract 2002; 11:180–2
16. Levett, PN. Seroprevalence of HSV-1 and HSV-2 in Barbados. Medical Microbiology and Immunology 2005; 194: 105-7.
17. Sauerbrei A, Wutzler P. Herpes simplex and varicella-zoster virus infections during pregnancy: current concepts of prevention, diagnosis and therapy, Part 1:

herpes simplex virus infections. Med Microbiol Immunol 2007; 196(2): 89-94.

18. Anzivino E , Daniela Fioriti D, Mischitelli M, *et al*. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. Virology Journal 2009, 6:40-51.

19. Enright AM, Prober CG. Neonatal herpes infection: Diagnosis, treatment and prevention. Semin Neonatol 2002; 7:283-91.

20. Zaki M E, Goda H. Relevance of parvovirus B19, herpes simplex virus 2, and cytomegalovirus virologic markers in maternal serum for diagnosis of unexplained recurrent abortions. Arch Pathol Lab Med 2007; 131:956–60.

21. Burlingham WJ. A lesson in tolerance – maternal instruction to fetal cells. N Engl J Med 2009; 360:1355–7.

22. Kapranos NC, Kotronias DC. Detection of herpes simplex virus in first trimester pregnancy loss using molecular techniques. *In Vivo* 2009; 23:839- 42.

Research Article

The role of maternal serum placental growth factor level in predicting delivery within two weeks in pre eclamptic women

Henan Dh. Skheel Aljebory *MBChB FICOG* , **Handi Shaker** *MBChB*

Date Submitted:7/10/2015
Date Accepted:15/11/2015

Address for Correspondence:
Henan Aljebory, Assistant professor, Department of obstetrics and gynecology, College of Medicine, Al-Mustansiriya University, Baghdad, Iraq. E-mail: *hanandhali@yahoo.com*

Abstract

Background: Placental growth factor (PlGF) is an angiogenic factor, a secondary marker of placental dysfunction in preeclampsia, with known low plasma levels in the disease.
Objectives: to evaluate the diagnostic accuracy of plasma PlGF concentrations in women presenting with suspected preeclampsia before 35 weeks' gestation in determining the need for delivery.
Patients & Methods: A prospective study was done at Al Yarmouk Teaching hospital at the Department of Obstetrics and Gynecology, Between the 15th of March 2014 to 14th of January 2015 all pregnant women with gestational age of (20 weeks-37 weeks), who were admitted because of suspected preeclampsia were included in the study. Detailed history and examination was done. Two blood samples were obtained, first sample was for biochemical markers The other for Placental Growth Factor assay.
Results: The outcome was delivery of confirmed preeclampsia within 14 days. Of 100women, 47(47%) developed confirmed preeclampsia. PlGF <5th centile had high sensitivity (0.95; 95% confidence interval, 0.87–0.99) and negative predictive value (0.88; 0.83–0.97) for preeclampsia within 14 days; specificity was lower (0.59; 0.41–0.74). Area under the receiver operating characteristic curve for low PlGF (0.87, standard error 0.03) for predicting preeclampsia within 14 days was greater than all other commonly used tests in women presenting with suspected preeclampsia ($P<0.001$ for all comparisons).
Conclusion: Women presenting before 37 weeks' gestation with suspected preeclampsia, low PlGF has high sensitivity and negative predictive value for preeclampsia within 14 days.

Key words: Placental growth factor, Hypertension, Pregnancy

INTRODUCTION

Preeclampsia is a multisystem disorder affecting 8% of pregnant women [1, 2] .The pathophysiology of preeclampsia still unclear, despite intensive research. One of the favored hypotheses is that preeclampsia is generated by shallow invasion of the extra villous trophoblast followed by an incomplete remodeling of the maternal vascular structures which leads to uteroplacental insufficiency and fetal growth retardation [3–5] Insufficient invasion seems to lead to an altered placental angiogenesis, thus implicating causal importance in the origin of preeclampsia. An imbalance of angiogenic and growth factors at the maternal-fetal interface and a consecutive imbalance of these factors in maternal blood might lead to the clinical symptoms of hypertension and proteinuria [6–9]. An imbalance between the factors promoting angiogenesis such as vascular endothelial growth factor or placental growth factor (PlGF) and the factors antagonizing angiogenesis such as soluble fms-like tyrosine kinase 1 (sFLT1) plays a fundamental role in the pathogenesis of preeclampsia [10 , 11]. PlGF serum levels of patients with preeclampsia are significantly lower than the levels in non-preeclampsia pregnancies [11]. At present, the women at risk of preeclampsia are identified on the basis of epidemiological, clinical and anamnestic risk factors. Until now, there has been no clinically useful screening test to predict the development of preeclampsia in the early phases of pregnancy [12]. The purpose of all the screening tests for preeclampsia must be the detection of a high-risk group as early as possible in pregnancy and to offer a prophylactic treatment to the women at high risk. . Although the pathologic origins of preeclampsia likely occur during placentation, the clinical signs and symptoms typically do not emerge until after 20 weeks

of gestation and only 38% of women had both hypertension and proteinuria before the development of eclampsia [13, 14] A diagnosis of PE based on blood pressure and proteinuria has a positive predictive value of approximately 30% for predicting PE-related adverse outcomes[12]

Since women with suspected hypertensive disease are routinely monitored every 2 weeks, the test must be applicable for a subsequent 14-day window to impact management strategies. The primary aim of this study was to evaluate the diagnostic accuracy of plasma PlGF concentrations in women presenting with suspected preeclampsia before 35 weeks' gestation in determining the need for delivery.

PATIENTS AND METHODS

A prospective study was done at Al Yarmouk Teaching hospital at the Department of Obstetrics and Gynecology, the study was approved by the Iraqi Council of Medical specialization (Iraqi scientific committee of Obstetrics and Gynecology). Between the 15th of March 2014 to 14th of January 2015 all pregnant women with gestational age of (20 weeks-37 weeks), who were admitted because of suspected preeclampsia were included in the study. Criteria contributing to suspicion of clinical diagnosis of pre-eclampsia (PE) includes, *de-novo* elevated blood pressure, aggravation of pre-existing hypertension *de-novo* protein in urine

, aggravation of pre-existing proteinuria, epigastric pain, excessive edema /severe swelling (face, hands, feet) headache, Visual disturbances, Sudden weight gain (>1 kg/week in third trimester) PE-related findings, elevated liver enzymes, low platelets, (Suspected) intrauterine growth restriction, abnormal uterine artery Doppler (mean PI>95th centile in second trimester and/or bilateral notching). Those with confirmed preeclampsia were excluded.

Included patients were classified as preeclampsia, gestational hypertension, and chronic hypertension. Gestational hypertension is defined as blood pressure >= 140/90 mmHg on two occasions according to the classification of the International Society for the Study of Hypertension in Pregnancy at least 4 hours apart after 20 weeks of gestations without significant proteinuria at the time of evaluation.

Chronic hypertension is defined as the presence of hypertension prior to 20 weeks of gestation or known before pregnancy. Isolated proteinuria is defined as > = 300 mg/24h without hypertension at time of evolution. Low platelets are defined as < 150×10^5 /L and elevated liver enzymes as aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 2normal, eg, > 80 IU/mL.

Generalized edema is defined as de novo edema of the face, upper and lower limbs.

Preeclampsia defined as the association of hypertension (more than 140/90 mm Hg in seated position with cuff of sphygmomanometer at the level of the heart) and significant proteinuria (300 mg or more 24 hr urine sample). PlGF is classified as:

- Very low (<12 pg/ml)
- Low (< 5th centile) or<100pg/ml
- Normal (>=5th centile) ≥100pg/ml [15]

After taking verbal consent from the patient, explanation of the nature of the study was done. Detailed history and examination was done. Two blood samples were obtained, first sample was for biochemical markers (Blood urea, serum creatinine, liver enzymes, platelet count, PCV and urine for proteinuria. The other for Placental Growth Factor assay. Samples were obtained, centrifuging of them was done and they were stored at (-2C) till time of examination.

We followed the patient who later developed confirmed PE and those who remained without complete picture of PE. Collected data about time of delivery since admission and the outcome of babies were analyzed.

Placental growth factor assay was done by ELISA kit Cusabio. China CSB-EO470

Primary analysis was the diagnostic accuracy of low plasma PlGF (<5th centile for gestational age) to predict need to delivery for preeclampsia within 14 days of testing in women with suspected preeclampsia before 35 weeks' gestation. The secondary analyses included women presenting later (35–36+6; ≥37 weeks), or by using a lower threshold (<12 pg/mL)

Statistical analysis

Each questionnaire assigned a serial identification number. The data were reviewed, cleaned with double check entry into the computer using Statistical Package for Social Sciences (SPSS) version 20.

The Shapiro–Wilk test was used to test continues variables for their normality of distribution, the test revealed that plasma placental growth factor level and fetal birth-weights in the present study were not normally distributed as the test p-value for all variables was analyzed

The data presented as mean, median, standard deviation, inter quartile range, frequency and percentages tables and box-plot and clustered bar charts.

Receiver Operator (ROC) curve was used to assess the sensitivity and specificity of placental growth factor test in prediction of closeness of the birth after first inclusion in the resent study. Level of p – value less than 0.05 was significant.

RESULT

The present study revealed the following results; of the 100 participated patients 47 (47%) of them were diagnosed to have preeclampsia, while 29 (29%) of them had gestational hypertension, 16 (16%) had chronic hypertension and 8 (8%) had isolated proteinuria. As shown in figure 1 below.

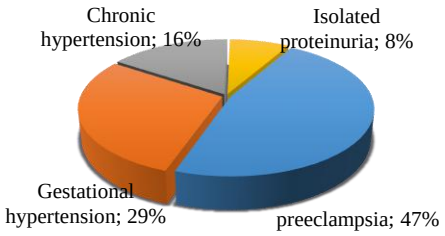


Figure 1. Categories of the participant patient according to their diagnosis, n=100.

The study also revealed that, 64 (64%) of them gave birth within two weeks and 36(36%) delivered after two weeks. As shown in figure 2 below.

Figure 2. Patient categories according to their time of delivery after inclusion in the study, (n=100).

Table 1 below illustrates the characteristics of the patients according to the time of their delivery.

The results revealed that neither ages of the participant nor their gestational ages were showing statistically significant differences (p=0.455, p=0.193) respectively.

Participants' systolic blood pressure at the time of their inclusion in the study also showed no significant differences between the two groups (p=0.087).

In the other hand; diastolic blood pressure of those delivered within two weeks of the inclusion was significantly higher than those delivered later (more than two weeks), (p=0.001).

As well; more than one half (57.8%) of the patients' group who delivered earlier were nulliparous, compared to about on thirds of those delivered after two weeks of the inclusion, this difference showed to be statistically significant (p=0.009).

Table 1. The patients with suspected NOHL classified according to the PTA in the speech frequencies 500-2000 Hz.

Characteristics of patients	Within 2 weeks (n=64) Mean±(SD)	>2 weeks (n=36) Mean±(SD)	p-value [a]
Maternal age (years)	29.9±(5.5)	29.0±(5.8)	0.455
Systolic bl. pressure mmHg	157.2±(16.0)	151.4±(16.1)	0.087
Diastolic bl. pressure mmHg	103.1±(10)	93.5±(11.1)	0.001*
Gestational age at inclusion (Weeks)	31.0±(4.9)	32.6±(6.3)	0.193
	No. (%)	No. (%)	p-value [b]
Nulliparous	37 (57.8)	11 (30.6)	0.009*
Nulliparous Singleton	34 (91.9)	10 (90.9)	0.918
Nulliparous Twins	3 (8.9)	1 (9.1)	

a=independent t-test (two tailed), b=Pearson's chi-square, SD=standard deviation, * significant at α<0.05.

Table 2 below illuminates the comparison of pregnancy's outcomes of the patients between those delivered within two weeks of the inclusion and those who delivered later on. The present study stated that; there was no significant difference neither in fetal numbers nor in modes of delivery between those delivered earlier and those who delivered later than two weeks of the inclusion, (p=0.781, p=0.31) respectively.

Regarding proteinuria; about two thirds 42 (65.6%) of the females delivered earlier than two weeks had positive proteinuria, in comparison to about one third 13 (36.1%) of those delivered beyond two weeks after their inclusion, this difference was shown to be statistically significant (p=0.004).

Significantly higher percentages of stillbirth had been appeared among those with early delivery as compared to those delivered after two weeks of inclusion in the present study, (p=0.022). Preeclampsia is prevalent in more than one half 38 (59.4%) of the patients who delivered prematurely (within 2 weeks), compared to only one fourths of those delivered after two weeks, this difference had shown to be statistically significant (p=0.001).

As for the gestational age at the delivery it had been revealed that; the majority of those delivered after two weeks of inclusion 32 (88.9%) their gestational age was 34 weeks or older, compared to 38 (59.4%) of those delivered within two weeks of inclusion in the present study, this difference was statistically significant (p=0.002).

Also it had been revealed that the median of the birth-weight of the newborn fetuses was higher for patients delivered beyond the two weeks (2.9 kilograms), compared to (2.45 kilograms) for patients delivered within two weeks since the inclusion in the recent study, this difference was statistically significant (p=0.006).

The levels of placental growth factor in the plasma [Median=31 pg/ml and Inter-quartile range (16-49 pg/ml)] for patients delivered within two weeks, which is low as compared to [Median=57 pg/ml and Inter-quartile range (21.6-180 pg/ml)] for patients delivered beyond the two weeks since the inclusion in the recent study, this difference was statistically significant (Mann-Whitney non-parametric test, p<0.001).

More than one thirds 22 (34.4%) of patients delivered within two weeks had very low level of placental growth factor, compared to only 3 (8.3%) of the patients delivered after two weeks since inclusion in the recent study. While, 28 (77.8%) of the patients delivered after two weeks had normal level of placental growth factor, compared to 29 (45.3%) of patients delivered within two weeks since inclusion in the recent study, this differences showed to be statistically significant (p=0.004).Table 2

It is also illustrated in figure 3 below as the area under the curve for placental growth factor was higher and more predictive with higher sensitivity than other parameters like uric acid, platelet count, systolic and diastolic blood pressure.

Table 2. Pregnancy outcomes of the participated patients according to their time of delivery after inclusion in the study, (n=100).

Pregnancy outcomes	Within 2 weeks (n=64) No. (%)	>2 weeks (n=36) No. (%)	p-value [a]
Pregnancy			
Singleton	**58 (90.6)**	**32 (88.9)**	**0.781**
Twins	**6 (9.4)**	**4 (11.1)**	
Positive Albumin in urine	**42 (65.6)**	**13 (36.1)**	**0.004***
Stillbirth	**15 (22.3)**	**2 (5.6)**	**0.022***
Mode of delivery			
Vaginal delivery	**27 (42.8)**	**19 (52.8)**	**0.31**
Caesarean section	**37 (57.2)**	**17 (47.2)**	
Intrauterine growth restriction (IUGR)	**32 (50.0)**	**10 (27.8)**	**0.031***
Preeclampsia	**38 (59.4)**	**9 (25.0)**	**0.001***
Gestational age at delivery			
≥ 34 weeks	**38 (59.4)**	**32 (88.9)**	**0.002***
<34 weeks	**26 (40.1)**	**4 (11.1)**	
Parameters	**Median (IQR)**	**Median (IQR)**	**p-value [b]**
Birth weight of the fetus (kg)	**2.45 (0.95-2.98)**	**2.90 (2.6-3.0)**	**0.006***
Placental growth factor in the plasma (pg/ml)	**31 (16-49)**	**57 (21.6-180)**	**<0.001***
Placental growth factor levels	**No. (%)**	**No. (%)**	**p-value [a]**
Very low PlGF <12 pg/ml	**22 (34.4)**	**3 (8.3)**	**0.004***
Low PlGF <5th centile	**13 (20.3)**	**5 (13.9)**	
Normal ≥ 5th centile	**29 (45.3)**	**28 (77.8)**	

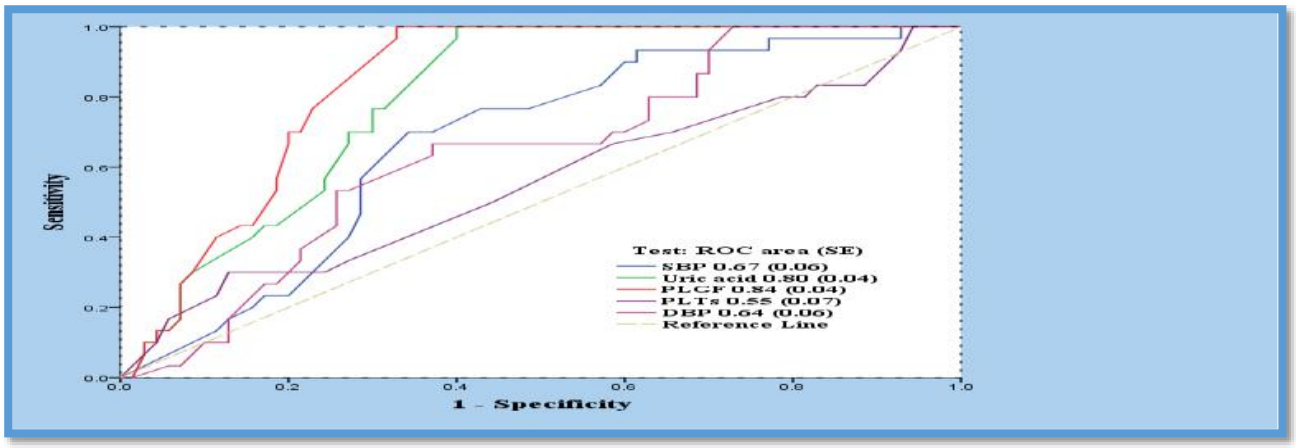


Figure 3. Receiver-operator curve to assess sensitivity of PlGF, SBP,DBP, platelets and uric acid in predicting patients with preeclampsia needed to deliver within 2 weeks after inclusion in the study, (n=100).

Table 3. Statistics test performance for Low PlGF in Predicting Adverse Outcomes, n=100.

Level of placental growth factor	Gestation at enrollment (weeks, days)		
	$<35^{+0}$ (n=55)	35^{+0} - 36^{+6} (n=17)	$\geq 37^{+0}$ (n=28)
$<5^{th}$ centile for	Preeclampsia requiring delivery within 14 days		
Sensitivity	0.95 (0.87–0.98)	0.76 (0.59–0.80)	0.61 (0.49–0.68)
Specificity	0.59 (0.41-0.74)	0.65 (0.54–0.77)	0.73 (0.65–0.81)
Positive predictive value	0.45 (0.36–0.55)	0.68 (0.55–0.79)	0.72 (0.63–0.83)
Negative predictive value	0.88 (0.83–0.97)	0.69 (0.57–0.84)	0.70 (0.66–0.78)
Positive likelihood ratio	2.0 (1.5–2.5)	2.0 (1.5–2.8)	2.3 (1.7–3.2)
Negative likelihood ratio	0.05 (0.02–0.18)	0.40 (0.31–0.70)	0.52 (0.41–0.73)
PlGF <12 pg/ml	Preeclampsia requiring delivery within 14 days		
Sensitivity	0.65 (0.55–0.78)	0.47 (0.38–0.59)	0.42 (0.36–0.58)
Specificity	0.94 (0.84-0.98)	0.85 (0.74–0.97)	0.83 (0.75–0.93)
Positive predictive value	0.72 (0.66–0.85)	0.75 (0.67–0.84)	0.72 (0.63–0.85)
Negative predictive value	0.85 (0.80–0.96)	0.59 (0.47–0.74)	0.60 (0.54–0.72)
Positive likelihood ratio	4.1 (3.2–7.5)	2.3 (1.6–3.1)	2.1 (1.5–2.9)
Negative likelihood ratio	0.4 (0. 2–0.6)	0.67 (0.47–0.87)	0.65 (0.51–0.78)

Table 4. Time for delivery (days) from day of inclusion according to the level of PlGF for patients with preeclampsia, n=47.

Level of placenta l growth factor	Gestation at enrollment (weeks, days)		
	$<35^{+0}$ (n=26)	35^{+0} - 36^{+6} (n=8)	$\geq 37^{+0}$ (n=13)
Very low PlGF <12 pg/ml	7 (9-19)	2 (5-8)	2 (7-9)
Low PlGF <5th centile	10 (19-27)	4 (7-13)	1 (3-5)
Normal ≥ 5th centile	33 (45-81)	5 (9-18)	1 (5-7)

DISCUSSION

In this prospective study of 100 pregnant women presenting with suspected preeclampsia, low plasma PlGF (lower than the fifth centile for gestation) or less than 100pg/ml had very high sensitivity and very low negative predictive value for pinpointing those women who actually had the disorder and would need delivery within 14 days. We found that the test was most accurate

in the earlier stages of pregnancy. Less than 35 weeks, the sensitivity of the assay in predicting the need for delivery within 14 days was 0.95 (95% CI 0.87–0.98) and its negative predictive value was 0.88 (95% CI 0.83–0.97),between 35 and 36 weeks' gestation, the sensitivity of low PlGF in predicting the need for delivery within 14 days was 0.76 (95% CI 0.59–0.80) and its negative predictive value was 0.69 (95% CI 0.57–0.84)and at 37 weeks or more, the test's sensitivity was lower at 0.61 (95% CI 0.49–0.68) and its negative predictive value was 0.70 (95% CI 0.66–0.78).And that's important for the woman, it's important for the doctor, and for the health service The real importance of the test is to flag the women who need greater surveillance, and on the other hand avoid unnecessary hospital admission for low risk. . Maternal plasma PlGF declines in the latter half of the third trimester, reducing test performance at >35 weeks' gestation; an ideal test would maintain separation between preeclampsia cases and other women, which is probably cannot be achieved by using a single biomarker at all gestations. More accurate determination of very low PlGF values (less than the current limit of detection of 12 pg./mL) could be useful; however, the high clinical sensitivity reported in this study relates to the prspecified threshold of <5th centile(low PlGF, or PlGF <100pg/m:) rather than very low PlGF .The PlGF test was significantly better than all other commonly used tests, such as diastolic and systolic blood pressure, alanine transaminase, uric acid and proteinuria, in determining preeclampsia requiring delivery within 14 days, when used alone or in combination (P<0.001 for all comparisons). Lucy C. *et al* in a prospective study of pregnant women between (20-35) weeks gestation who were suspected to have PE, they found that low PlGF (< 5th centile for gestation) had high sensitivity and negative predictive value for patients with PE who should be delivered within 14 days. PlGF test was better than other currently used tests and presented a great help in the management of such women [(15)]. Jeanne S. *et al* in a prospective study for 96 pregnant women who were suspected PE or IUGR, PlGF was measured, adverse outcome were identified (sever PE, SGA < 10th centile, elective delivery for maternal or fetal complication < 34 weeks). They observed that PIGF was lower for patients with PE and was markedly lower for patients with adverse outcome, so PlGF helps to identify women who will experience an adverse outcome and those who will not within time period of 15 days [(16)].

Attila M. *et al* observational study for women less than 35 weeks gestation, single maternal blood sample was taken and PlGF level was measured. Results were compared with last Doppler ultrasound measurement of fetal flow prior to delivery. Positive PlGF test was found with abnormal fetal flow which required delivery. In conclusion PIGF test provided useful information before 35 weeks gestation to identify fetuses requiring urgent delivery and those at risk of later adverse outcome not identified by fetal flow Doppler ultrasound [(17)].

In our study women with suspected (PE)and high diastolic blood pressure were significantly having lower level of PlGF .Patrcia G. *et al* studied the levels of angiogenic factors and their relation with PE in regard to increased diastolic blood pressure. Samples of pregnant women (33-35) weeks gestation were collected, blood pressure was measured and preeclampsia was define according to the National High Blood Pressure Education Program Working Group (NHB-PEPWG), that to say (systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg). PlGF was measured by ELISA, they observed that PlGF was reduced significantly in high diastolic blood pressure in PE patients compared to normotensive patients [(18)]

Nulliparous women with suspected preeclampsia were more significantly prone to have low level of PlGF than multiparous this is probably because preeclampsia is more common in nulliparous. Robert N. *et al in a* cross sectional study on nulliparous women, they observed that serum placental growth factor was reduced in abnormal pregnancy relative to control subjects as early as 15 – 19 weeks of gestation in preeclampsia with small for gestational age (SGA) neonates [(19)], also Francois A. *et al* who did prospective study on nulliparous women from 11 – 13 weeks gestation, combined the relation between uterine artery Doppler and serum placental biomarkers (PlGF). They observed that there was relation of clinical characteristics and first trimester maternal serum PlGF provided an accurate screening for early onset PE in nulliparous women [(20)]although these studies done at earlier gestational age than our study.

Regarding our result in delivery of small gestational age neonate with low PlGF (P value 0.006) which is agreed by Roberto Romero *et al* in a case control study included pregnant women grouped as Patient with uncomplicated pregnancy who delivered appropriate for gestational age (AGA) neonates, patient who delivered SGA but did not developed preeclampsia and Patient who developed preeclampsia. They observed that patients who destined to develop PE (term or preterm) and those who delivered small for gestational age (SGA) had lower plasma concentration of PIGF than those with normal pregnancy throughout gestation, also there were no significant differences in the plasma concentration of sVEGFR-1 between patients destined to deliver SGA and those with normal pregnancy.[(21)] Tjoa M. *et al* in a

prospective study of 72 pregnant patients they observed that between (17 – 21) weeks of pregnancy significant low level of PIGF was found in plasma of women with IUGR [22]. Samantha J. *et al* incase control study of 16 cases (9 placental IUGR, 7 constitutionally small).The PlGF positive when concentration was (< 5th centile for gestational age for normal pregnancy), was found in IUGR fetuses so PlGF identified placental IUGR from constitutionally small fetuses [23].

Some studies have demonstrated that PlGF concentrations begin to decrease from 9-11weeks before the onset of preeclampsia, with greatest reductions during the 5 weeks before the onset of hypertension or proteinuria ([24-26] Sohrabi N. *et al* collected samples from pregnant mothers (8-12) week gestation. PlGF was measured, the result were compared between the patients who later developed PE and those with normal pregnancy. They observed that there was significant difference in PlGF between those who would develop PE from those who would not. So serum level of PlGF in 1st trimester of pregnancy can be used to predict the occurrence of PE [27].

These alterations in the PlGF level are more pronounced in women with early-onset preeclampsia, especially before the 26th week of pregnancy [28, 29]. The main problem of all these screening tests is that the strategies to develop prophylaxis are limited. Beyond 20 weeks of pregnancy, the only option is to improve pregnancy care by allowing closer and carful prenatal monitoring, recognition of preeclampsia earlier in the disease course and administration of steroids for fetal lung maturation [30]. The diagnostic and predictive value of the soluble fms-like tyrosine kinase-1), a trophoblast derived antiangiogenic factor sFlt1/PlGF ratio in patients at risk of placenta-related disorders, i.e. preeclampsia (PE), HELLP syndrome, IUGR and stillbirth, has been show in the recent literature and estimation of the sFlt-1/PlGF ratio has become an additional tool in the management of these disorders, primarily PE. [15]

In this study we measured PlGF once at time of admission and we observe the correlation with the clinical features, repeat measurements of the PlGF or sFlt-1/PlGF ratio are suggested by some studies to improve individual risk assessment in these patients, but this has to be proven by further studies. To date, the use of sFlt-1, PlGF or the sFlt-1/PlGF ratio has not been incorporated into official guidelines. In this study, we have aimed to give good clinical practice guidance for implementation of this method into the management of pregnant women. Use of the sFlt-1/PlGF ratio may help to optimize care by improving management of women with suspected preeclampsia.

Conclusion

Evidence is building in support of the utility of PlGF as an accurate and specific marker identifying the underlying cause of disease, placental dysfunction. A proposal has been made for the definition of pre-eclampsia to include PlGF as a marker of placental dysfunction.

.

REFERENCES

1. Sibai BM, Dekker G, Kupferminc M: Preeclampsia. Lancet 2005; 3 65: 7 85–799.
2. Redman CW, Sargent IL: Latest advances in understanding preeclampsia. Science 2005; 3 08: 1592–1594.
3. Dietl J: The pathogenesis of pre-eclampsia: new aspects. J Perinat Med 2000; 2 8: 464– 471.
4. Oudejans CBM, Tjoa ML, Westerman BA, Mulders MAM, Van Wijk IJ, Van Vugt JMG: Circulating trophoblast in maternal blood. Prenat Diagn 2003; 23: 1 11–116.
5. Fisher SJ: The placental problem: linking abnormal cytotrophoblast differentiation to the maternal symptoms of preeclampsia. Reprod Biol Endocrinol 2004; 2 : 5 3–56.
6. Sane DC, Anton L, Brosnihan KB: Angiogenic growth factors and hypertension. Angiogenesis 2004; 7 : 1 93–201.
7. Lam C, Lim KH, Karamunanchi SA: Circulating angiogenic factors in the pathogenesis and prediction of preeclampsia. Hypertension 2005; 4 6: 1077–1085.
8. Gellhaus A, Schmidt M, Dunk C, Lye STJ, Kimmig R, Winterhager E: Decreased expression of angiogenic regulators CYR61 (CCN1) and NOV (CCN3) in human placenta is associated with pre-eclampsia. Mol Hum Reprod 2006; 12: 3 89–399.
9. Schmidt M, Gellhaus A, Kasimir-Bauer S, Winterhager E, Kimmig R: Angiogenic factors during pregnancy: indicators of preeclampsia. Geburtsh Frauenheilkd 2007; 6 7: 2 28–235.
10. Stepan H, Faber R, Dornhöfer N, Huppertz B, Robitzki A, Walther T: New insights into the biology of preeclampsia. Biol Reprod 2006; 74: 772–776.
11. Schmidt M, Dogan C, Birdir C, Callies R, Kuhn U, Gellhaus A, Janetzko A, Kimmig R, Kasimir-Bauer S: Altered angiogenesis in preeclampsia: evaluation of a new test system for measuring placental growth factor. Clin Chem Lab Med 2007; 45: 1 504–1510
12. Conde-Agudelo A, Villar J, Lindheimer M: World Health Organization systematic review of screening tests for preeclampsia. Obstet Gynecol 2004; 104: 1 367–1391
13. Redman CW, Sargent IL. Placental debris, oxidative stress and pre-eclampsia. Placenta. 2000 Sep;21(7):597-602. PubMed PMID: 10985960. Epub 2000/09/14.
14. Ogge G, Chaiworapongsa T, Romero R, et al. Placental lesions associated with maternal underperfusion are more frequent in early-onset than in late-onset preeclampsia. Journal of perinatal medicine. 2011 Nov;39(6):641-52. PubMed PMID: 21848483. Pubmed Central PMCID: PMC3213694. Epub 2011/08/19.
15. Lucy C. Chappell, PhD; Suzy Duckworth, MBBS; Paul T. Seed, CStat; Melanie Griffin, MBChB; Jenny Myers,

PhD; Lucy Mackillop, MA; Nigel Simpson, MBBS.et al.. Diagnostic Accuracy of Placental Growth Factor in women with suspected Preeclampsia. A Prospective Multicenter Study, 2013; 128:2097-2098.

16. Jeanne Sibiude ; Jean Guibourdenche ;Marie-Danielle Dionne; Camille Le Ray; Olivia Anselem; Raphaël Serreau; François Goffinet;Vassilis Tsatsaris. Placental Growth Factor for the Prediction of Adverse Outcomes in Patients with Suspected Preeclampsia or Intrauterine Growth Restriction. Published: November 28, 2012, DOI: 10.1371/journal.
17. Attila Molvarec; Nóra Gullai; Balázs Stenczer; Gergely Fügedi; Bálint Nagy and János Rigó Jr. Comparison of placental growth factor and fetal flow Doppler ultrasonography to identify fetal adverse outcomes in women with hypertensive disorders of pregnancy: an observational study. BMC Pregnancy and Childbirth 2013,
18. Patrícia Gonçalves Teixeira, Antônio Carlos Vieira Cabral, Silvia Passos Andrade, Zilma Silveira Nogueira Reis, Lívia Pieroni Barroso da Cruz, Jacqueline Braga Pereira, Breno Oliveira de Barcelos Martins, and Cezar Alencar de. Placental Growth Factor (PlGF) Is a Surrogate Marker in Preeclamptic Hypertension. Hypertension in Pregnancy2008; 27:65–73, DOI: 10.1080/10641950701825937.
19. Robert N. Taylor, MD, PhDa, Jane Grimwood, PhDa, Rennae S. Taylor, BScN, MHScc, Michael T. McMaster, PhDb, Susan J. Fisher, PhDa,b, Robyn A. North, MD, PhDc. Longitudinal serum concentrations of placental growth factor: Evidence for abnormal placental angiogenesis in pathologic pregnancies. American Journal of Obstetrics and Gynecology2003; 188: 177– 182.
20. François Audibert, MD, MSc; Isabelle Boucoiran, MD, MSc; Na An, MD, MSc; Nikolai Aleksandrov, MD; Edgard Delvin, MD, PhD; Emmanuel Bujold, MD, MSc; Evelyne Rey, MD, MSc. Screening for preeclampsia using first trimester serum markers and uterine artery Doppler in nulliparous women. Am J Obstet Gynecol 2010;203:383.
21. Roberto Romero, Jyh Kae Nien, Jimmy Espinoza, David Todem, Wenjiang Fu, Hwan Chung,A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neonatal Med 2008; 21: 9–23.
22. Tjoa ML, van Vugt JM, Mulders MA, Schutgens RB, Oudejans CB, van Wijk IJ. Plasma placenta growth factor levels in midtrimester pregnancies. Obstet Gynecol. 2001 Oct;98(4):600-7.
23. Samantha J. Benton, BSc, Yuxiang Hu, MD, Fang Xie, MD, PhD, Kenneth Kupfer, PhD, Seok-Won Lee, PhD, Laura A. Magee, MD, MSc, Peter von Dadelszen, MBChB, DPhil. Can placental growth factor in maternal circulation identify fetuses with placental intrauterine growth restriction? Presented at the International Federation of Placenta Associations Meeting 2011, Geil, Norway, Sept. 14-17, 2011.
24. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA: Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004; 350: 672–683.
25. Polliotti BM, Fry AG, Saller DN, Mooney RA, Cox C, Miller RK: Second-trimester maternal serum placental growth factor and vascular endothelial growth factor for predicting severe, early-onset preeclampsia. Obstet Gynecol 2003; 1 01: 1 266–1274.
26. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA: Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. Am J Obstet Gynecol 2003; 1 88: 1 77–182.
27. Sohrabi Nahid, Yazdan Mehr Khadije, The Study of Diagnostic Value of Placental Growth Factor for Predicting Pre-eclampsia in the First Trimester of Pregnancy, journal of Surgery, 2014 2 (1), pp 12-15.
28. Ohkuchi A, Hirashima C, Matsubara S, Suzuki H, Takahashi K, Arai F, Watanabe T, Kario K, Suzuki M: Alterations in placental growth factor levels before and after the onset of preeclampsia are more pronounced in women with early onset severe preeclampsia. Hypertens Res 2007; 3 0: 1 51–159.
29. Crispi F, Llurba E, Domínguez C, MartínGallán P, Cabero L, Gratacós E: Predictive value of angiogenic factors and uterine artery Doppler for early- versus late-onset preeclampsia and intrauterine growth restriction. Ultrasound Obstet Gynecol 2008; 3 1: 3 01–309.
30. Staff AC, Benton SJ, von Dadelszen P, Roberts JM, Taylor RN, Powers RW, Charnock-Jones DS, Redman CW. Redefining preeclampsia using placenta derived biomarkers. Hypertension. 2013;61:932–942.

Research Article

Validation of proximal isovelocity surface area in Mitral valve stenosis

Raed S.Abed , Ghazi F. Haji , Ghassan M.Mahmood

Date Submitted: 3/5/2015
Date Accepted:15/11/2015

Address for Correspondence:
Dr . Ghazi F. Haji, College of Medicine - Baghdad University

Abstract

Background: Mitral stenosis (MS) is a disabling and eventually lethal disease unless treated with valvotomy or valve replacement .It has been proposed recently that measuring the flow convergence region proximal to an orifice by Doppler flow mapping can provide a means of calculating regurgitant flow rate. However, this method also can be used to derive cardiac output or flow rate proximal to stenotic orifices and therefore to calculate their areas by the continuityEquation (area=flow rate/velocity) .Applying this method in mitral stenosis would provide a important way to elicited the underlying concept because the predicted areas could be compared with those measured directly by planimetry.

Objectives: To validate the proximal isovelocity surface area (PISA) method in measuring mitral valve area (MVA) in patients with mitral valve stenosis by transthoracic echocardiography.

Methods: This is a cross sectional study conducted in Ibn-Al Bitar center for cardiac surgery for a duration from March 2010 to March 2011 We studied patients with mitral stenosis using 2D imaging and Doppler echocardiography. Doppler color flow recordings of mitral inflow were obtained from the apex, and the radius of the proximal flow convergence region was measured at its peak diastolic value from the orifice to the first color alias along the axis of flow. Flow rate was calculated assuming uniform radial flow convergence toward the orifice, modified by a factor that accounted for the inflow funnel angle formed by the mitral leaflets. Mitral valve area was then calculated as peak flow rate divided by peak velocity by continuous-wave Doppler. A=(6.28r2 X aliasing velocity /peak MS velocity)Xα 1/180°

Results: In the 60 patients studied, mitral valve area by planimetry ranged from 0.5 to 2.2 cm2 with a mean of (1.15±43) . Mitral valve area by the proximal isovelocity surface area (PISA) method agreed well with direct planimetry with no significant p value (p 0.30) with no significant effect of mitral regurgitation (P 0.6) or atrial fibrillation (P 0.12) on the relation between calculated and plan metered Area -Proximal isovelocity surface area (PISA) method also agreed well with mitral valve area calculated from the Doppler-derived pressure half-time in sinus rhythm (p 0.7) and in aterial fibrillation (p 0.9),but not correlate well in presence of mitral valve regurgitation with significant p value (p 0.05).

CONCLUSION: that PISA is a useful method in the measurement of MVA and can be used as third echocardiographic method in patients with unreliable results with classical echocardiographic methods.

Key words: :Transthoracic echocardiography, Proximal isovilocity surface area, Mitral valve stenosis

INTRODUCTION

Mitral stenosis (MS) is a disabling and eventually lethal disease unless treated with valvotomy or valve replacement. Almost all cases are due to rheumatic heart disease with symptoms usually appearing 16 to 40 years after the episode of acute rheumatic fever..[1] Mitral stenosis is, in most patients, a progressive disease. Progression is slow in asymptomatic patients, but becomes more rapid after the onset of symptoms.[2].

The normal mitral valve orifice has a cross sectional area of 4 to 6 cm^2. When the orifice is reduced to 2 cm^2, mitral stenosis is mild and there is a small pressure gradient between the left atrium and ventricle. [3]. The mean rate of progressive valve narrowing is approximately 0.1 cm2/year, [4,5].The mitral valve was the first structure to be identified by echocardiography[6.7,8]. A standard echocardiographic examination of the mitral valve consists of an M-mode tracing, multiple two dimensional views, and Doppler flow evaluation[9] .

M-mode echocardiography — The early diastolic closure slope, the E-F slope, produces an easily recognized pattern ,Although this method is the least reliable means of quantitating the severity of obstruction, a slope of less than 10 mm/sec (normal is >60 mm/sec) from a valve recording made during suspended respiration is evidence for severe mitral stenosis[10]

Two dimensional echocardiography — The elevated gradient initiates the opening motion in an abrupt manner, generating the opening snap and a characteristic "knee bend" appearance on the precordial long axis view.[10,11] ·. The peak gradient can be calculated from the modified Bernoulli equation: $P = 4V2$, where V is the peak velocity as measured by CW Doppler. The mean gradient is calculated from the time velocity integral across the MV as measured by CW Doppler. :*Mean gradient* 0–5 mmHg (mild stenosis) 5–10 mmHg (moderate stenosis) >10 mmHg (severe stenosis) .using pressure gradients alone to estimate the severity of stenosis can be problematic.[12] direct measurement of the orifice area via planimetry is possible .

Doppler echocardiography — The most frequently used measurement for the determination of the transmitral gradient during diastole is the pressure half-time[10] . The P1/2 is the time it takes for the pressure gradient across the MV to decrease by half . The valve area is calculated using the equation, MVA = 220/P1/2 ,where MVA = Mitral valve area and P1/2 = pressure half-time. It has been proposed recently that measuring the flow convergence region proximal to an orifice, as imaged by Doppler color flow mapping, can provide a means of calculating regurgitant flow rate. [13.14.15].

The proximal isovelocity surface area (PISA) method for estimating valve area is a new technique based on the continuity principle that may circumvent the limitations of the traditional methods[16,17] The MV orifice area can be determined by the continuity principle . MVA = Area LVOT x VTI LVOT/VTI MV This method correlates well with the invasive assessment of MVA

An extension of the continuity principle is to use the proximal isovelocity surface area to calculate the MVA Here, the point of "comparison" is changed from the aortic or pulmonic outflow tracts.[12].

Aim of the study

To validate the proximal isovilocity surface area (PISA) method in measuring mitral valve area (MVA) in patients with mitral valve stenosis by echocardiography

PATIENTS AND METHODS

This is a comparative cross-sectional study conducted in Ibn-Al Bitar center for cardiac surgery for a duration from March 2010 to March 2011including 60 patients randomly selected from patients who attended the center, all the patients with typical rheumatic mitral stenosis selected for image quality suitable for quantification. All patients were sent for ECG to document atrial fibrillation. Echocardiography was done by at least two well-trained operators. Echocardiography:Two-dimensional echocardiography, Doppler ultrasound, and color flow mapping were performed using a (En Visor C) ultrasound imager equipped with a (S 4-2) transducer and a standard velocity map. Color flow mapping of the mitral inflow was obtained from the apical window, with color gain adjusted to eliminate random color in areas without flow. From this window, the four-chamber view provided the most consistent image of the largest proximal convergence radius and allowed the proximal flow to be viewed most nearly parallel to the ultrasound beam. This view was scanned to image the largest proximal flow convergence region, and the aliasing velocity was reduced by shifting the color baseline to maximize this area on the image. Mitral inflow velocities were measured from the apex by continuous-wave Doppler, and the mitral valve was scanned in the parasternal short axis view to image the smallest orifice area for planimetry. The collected data had been tabulated in term of frequency distribution tables that showed the frequency, the mean and standard deviation of different parameters of mitral valve measurements.

Statistical analysis had been done using student's t-test and P value of less than 0.05 had been selected as the level of statistical significance

RESULT

This study had enrolled 60 patients with mitral valve stenosis ,There were 18(30%) men and 42(70%) women(Fig1) ; mean age was (40.5±11.3)Years as shown in table 2. 25(42%) patients were in sinus rhythm and 35(58%) were in atrial fibrillation(Fig2). 17(28.3%) patients had associated mitral regurgitation (3 mild, 7 moderate, and 7 severe by Doppler color flow assessment of jet extent into the left atrium)(Fig3). Eleven patients had aortic insufficiency, which was generally mild.

In the 60 patients studied, mitral valve area by planimetry ranged from 0.5 to 2.2 cm2 with a mean of (1.15+0.43)The radius of the proximal convergence region ranged from 0.6 to 1.9 cm (mean, 1.2±0.29 cm), with most of the aliasing velocities in the range of 19 to 43 cm/s. The angle formed by the mitral leaflets ranged from 80° to 158° (mean, 119±140), and peak velocity by continuous wave range from 1.30 to 3.00 m/s with mean.(2.03±0.49) Mitral valve area by the proximal isovelocity surface area method agreed well with direct planimetry (Table 6) with no significant p value (p0.30) with no significant effect of mitral regurgitation (P 0.6) or atrial fibrillation (P 0.12) on the relation between calculated and planimetered areas. Mitral valve area by proximal isovelocity surface area also agreed well with mitral valve area calculated from the Doppler-derived pressure half-time in sinus rhythm (p 0.7)(Table8) and in atrial fibrillation (p 0.9)(Table7),but not correlate well in presence of mitral valve regurgitation with significant p value (p 0.05)(Table 9).

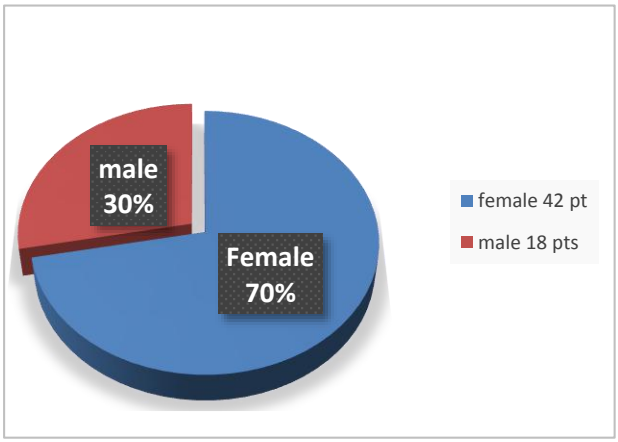


Figure1. Distribution according to gender in patients with mitral stenosis

Table 1. Descriptive Statistics

Variables	No.	Minimum Area	Maximum Area	Mean ±SD
Area by pressure half time cm^2	60	0.50	2.20	1.1592±0.4360
Area by planimetry cm^2	60	0.5	2.2	1.158±0.436
Area by PISA cm^2	60	0.36	2.60	1.1322±0.5106
Peak velocity m/s	60	1.30	3.00	2.0528±0.4975
Angle	60	80^0	158^0	119.95±14.17
Radius cm	60	0.60	1.91	1.2058±0.2941
Aliasing velocity m/s	60	18	79	37.92±13.29

Table 2: distribution of patients according to age

Age years	No.	%
10-20	**2**	**3.4**
21-30	**22**	**36.65**
31-40	**23**	**38.3**
41-50	**10**	**16.65**
51-60	**3**	**5**
Total	**60**	**100**
Mean ±SD	**40.5+11.3**	

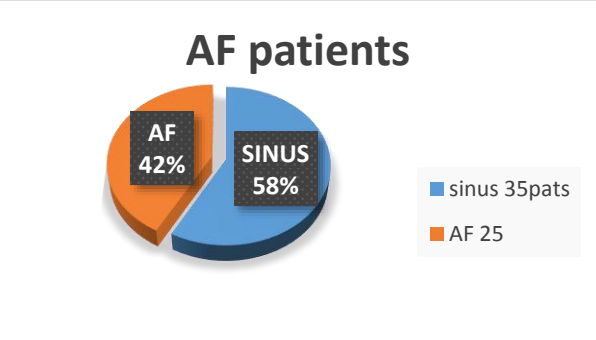


Figure 2. Percent of sinus and AF in mitral stenosis patient

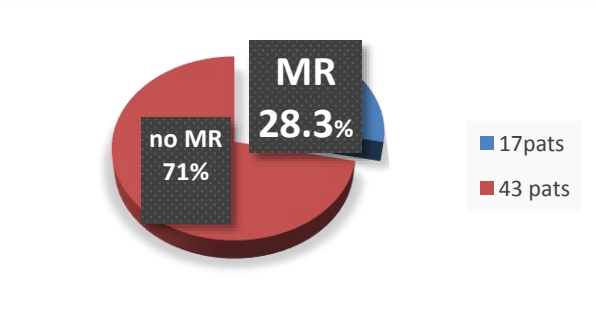


Figure 3. Percent of association of mitral regurgitation in mitral stenosis patient

Table 3. PISA vs planimatry

p=0.30			
Area cm^2	**No**	**Mean±SD**	**Std. Error Mean**
planimetry	60	1.158±0.436	5.635E-02
PISA	60	1.1322±0.5106	6.592E-02

Table4. PISA vs pressure half time

P=0.29			
Area cm^2	**No.**	**Mean± SD**	**Std. Error Mean**
Pressure half time	60	1.159±0.4360	5.629E-02
PISA	60	1.132±0.5106	6.592E-02

Tabe 5. MVA by PISA, pressure half time and planimetry in AF patients

Group Statistics					
Area cm^2	**sinus**		**AF**		
	No.	**Mean± SD**	**No.**	**Mean±**	**P**
Pressure1/2time	35	1.086±0.388	25	1.261±0.484	0.12
Planimetry	35	1.085±0.389	25	1.261±0.485	o.12
PISA	35	1.063±0.470	25	1.228±0.557	0.21

P value significant if < 0.05

Tabe 6. MVA by PISA, pressure half time and planimetry in MR patients

Group Statistics						
Area cm^2						
	N0 MR			**MR**		
	No.	**Mean ±**	**SD**	**No.**	**Mean±SD**	**P**
Pressure1/2time	43	1.173±0.427		17	1.33±0.33	**0.05**
planimetry	43	1.173±0.428		17	1.12±0.46	0.69
PISA	43	1.152±0.516		17	1.08±0.50	0.63

Table 7. MVA by PISA vs pressure half time and planimetry in AF patients

AF			
Area cm^2		**No.**	**Mean ±SD**
	Area.plan	25	1.261±0.485
	PISA	25	1.228±0.557
	Pressure1/2time	25	1.261±0.484
	PISA	25	1.228±0.557

Table 8. MVA by PISA vs pressure half time and planimetry in sinus rythem

sinus			
Area		**N0**	**Mean±SD**
	Pressure1/2time	35	1.0863±0.3886
	PISA	35	1.0631±0.4702
	Area.plan	35	1.085±0.389
	PISA	35	1.0631±0.4702

Table 9. MVA by PISA vs pressure half time and planimetry in MR

MR group				
Area cm^2		**No.**	**Mean±Std. Deviation**	**P**
	Pressure1/2time	17	1.33±0.33	**0.05**
	PISA	17	1.08±0.50	
	planimetry	17	1.12±0.46	0.30
	PISA	17	1.08±0.50	

Table 10. MVA by PISA vs pressure half time and planimetry in no MR

No MR		
Area cm^2	**No**	**Mean±SD**
Pressure1/2time	43	1.1737±0.4276
PISA	43	1.1523±0.5160
planimetry	43	1.173±0.428
PISA	43	1.1523±0.5160

DISCUSSION

The advent of Doppler flow mapping has led to attempts to extract quantitative physiological information from the displayed flow fields in accordance with fluid mechanical principles. One such approach is based on the observed flow accelerate on proximal to regurgitant and shunt lesions[20-21].

It has been proposed that these laminar flows could provide a measure of regurgitant flow rate unaffected by the complexities of turbulent flow beyond the orifice. The ability to visualize such flows with existing systems has indeed correlated with moderate to severe regurgitation[13],. On the other hand, flow convergence regions are seen proximal to stenotic lesions as well, where they are especially prominent because they represent the entire forward output across such valves. In particular, observations of such regions in mitral stenosis have suggested the possibility of testing these principles in the context of that disease.

The results of this study validate the flow convergence concept by demonstrating that it can be used to calculate mitral orifice area by continuity in patients with mitral stenosis. The results agree well with two independent measurements of orifice area: direct planimetry and the Doppler pressure half-time method,. The results also point out the need to account for geometry proximal to the orifice in applying the flow convergence method. Without correcting for the angle of the inflow funnel, the area would have been overestimated by up to 100%. The effect of inflow angle is also likely to be important in assessing flows through regurgitant orifices within a nonplanar leaflet geometry surrounding the orifice. And the correcting factor measured by dividing α/180 which is the angle formed by the inflow funnel of both mitral leaflets was one of the most important factors deciding the final results of MVA by PISA method, in addition to the radius which measured from mitral valve leaflets tip to first colour aliasing, unfortunately the correcting factor was one of limiting factors in applying PISA method in MVA measurement because measurement of the α angle not present in echocardiography device software built-in and needs off-line analysis and measurement by protractor .

In addition to validating the flow convergence concept, this technique may provide a simple and useful alternative to calculate orifice area in mitral stenosis when the pressure half-time is affected by altered chamber compliance or aortic insufficiency . and direct planimetry from the parasternal window is technically limited. This calculation may be not dependable if mitral stenosis is associated with mitral regurgitation or atrial fibrillation.

The small size of the proximal convergence region on the video image can limit measurement precision. Such errors can be minimized by lowering the aliasing velocity to increase the measured radius. This also provides

the most hemispheric isovelocity contours for which the proximal convergence calculation is most accurate, because in the immediate vicinity of an orifice, isovelocity contours tend to flatten out, leading to flow underestimation.[13,22] (In the current study, the measured radii, which represented the entire forward flow through a narrowed inlet, were relatively long [1.2+0.3 cm] compared with the visualized orifice dimension, predisposing to accurate flow calculations.) Shifting the baseline to reduce the aliasing velocity has potential limitations; for example, if the aliasing velocity is very low relative to the Nyquist limit[23,24] , selective suppression of low velocities by the color wall filter will cause overestimation of the mean velocity output displayed by color Doppler and therefore overestimation of the proximal flow convergence radius[25].

Despite this potential, moderate degrees of baseline shifting have been used in practice with accurate results. The limited temporal resolution of Doppler color flow mapping can cause variability in measuring peak flow rate [26].

In mitral stenosis, however, flow rate varies relatively slowly after its early diastolic peak, minimizing this variability. Potential variations in leaflet geometry proximal to the orifice may not always be accounted for completely by the simple angle correction used. In this regard, it is worth noting that the continuity equation, in principle, predicts effective orifice area at the vena contracta, where velocity is highest, as opposed to the anatomic orifice area, which is generally somewhat larger [27].

In this study, however, the continuity and planimetered (anatomic) values coincided well. A potential explanation for this is that milder restriction of the converging flow by the leaflets in planes perpendicular to the measured funnel angle was not taken into account, causing a mild overestimation of flow rate and predicted area. In the end, however, the empirical modification used provides good agreement with planimetered values in the clinical application studied.

The current study results are nearly comparable with results of Robert D. et al study in which the accuracy of PISA area estimates in mitralstenosis is at least comparable to those of planimetry and pressure half.time and reasonable accuracy of the PISA method ispossible in irregular rhythms [28] .

Also current study results are comparable with result of Dilek Ural et al study in which MVAs measured by PISA are closely correlated to classical echocardiographic methods, especially to planimetry , However, in cases with inadequate image qualities, planimetry lost its reliability, and measurements of PISA was not affected . its results were closer to Doppler pressure half-time.[29].

Also the current study results were comparable with result of L Rodriguez et al study in which Mitral valve area by the proximal flow convergence method agreed well with direct planimetry and showed no significant effect of mitral regurgitation or atrial fibrillation on the relation between calculated and planimetered areas. Correlation was also good with mitral valve area calculated from the Doppler-derived pressure half-time[30] .But the current study results were not comparable to the study by Messika-Zeitoun et al which was showing that using a fixed angle value of 100^0

provides an accurate estimation of the mitral valve area (MVA) by the proximal isovelocity surface area (PISA) method in patients with mitral stenosis (MS) in attempt to make PISA method more popular in assessing the MVA. The method proposed by Messika- Zeitoun et al. is subject to some difference with our study. In their study the angle range was 90^0- 115^0. This means that there is 11% overestimation and 11.5% underestimation at most with the use of 100^0 as the fixed angle. the angle range is quite narrow In a previous study by Messika-Zeitoun et al.while in our study the range of the angle was very wide with range of (80^0-158^0) which explain clearly the non-similarity between the current study results and the study by Messika- Zeitoun et al[31].

Conclusion :

The proximal isovilocity surface area (PISA) method by echocardiography allows accurate estimation of mitral valve area in mitral stenosis. This application provides a unique opportunity for comparing predictions based on flow convergence with directly measured values-in this case, planimetered orifice areas and area by pressure half time . Therefore, these results validate the proximal isovilocity surface area (PISA) method by echocardiography in measuring mitral valve area MVA in patient with mitral valve stenosis .

REFERENCES

1. Rowe, JC, Bland, EF, Sprague, HB, White, PD. The course of mitral stenosis without surgery: ten- and twenty-year perspectives. Ann Intern Med 1990; 52:741.
2. Horstkotte, D, Niehues, R, Strauer, BE. Pathomorphological aspects, aetiology and natural history of acquired mitral valve stenosis. Eur Heart J 1991; 12 Suppl B:55.
3. Hugenholtz, PG, Ryan, TJ, Stein, SW, Abelmann, WH. The spectrum of pure mitral stenosis. Hemodynamic studies in relation to clinical disability. Am J Cardiol 1992; 10:773.
4. Sagie, A, Freita, N, Padial, LR, et al. Doppler echocardiographic assessment of long-term progression of mitral stenosis in 103 patients: Valve area and right heart disease. J Am Coll Cardiol 2001; 28:472.
5. Gordon, SP, Douglas, PS, Come, PC, Manning, WJ. Two-dimensional and Doppler echocardiographic determinants of the natural history of mitral valve narrowing in patients with rheumatic mitral stenosis: implications for follow-up. J Am Coll Cardiol 2002; 19:968.
6. Feigenbaum, Harvey; Armstrong, William F.; Ryan, Thomas Title: Feigenbaum's Echocardiography,lippincot and william, 6th Edition;2011;11,p320
7. Edler, I. Ultrasound cardiogram in mitral valve disease. Acta Chir Scand 2006; 111:230.
8. Edler, I. Ultrasoundcardiography in mitral valve stenosis. Am J Cardiol 1997; 19:18.
9. Schiller, NB, Foster, E, Redberg, RF. Transesophageal echocardiography in the evaluation of mitral regurgitation. The twenty-four signs of severe mitral regurgitation. Cardiol Clin 1993; 11:399.
10. Nichol, PM, Gilbert, BW, Kisslo, JA. Two-dimensional echocardiographic assessment of mitral stenosis. Circulation 2007; 55:120.
11. Wann, LS, Weyman, AE, Feigenbaum, H, et al. Determination of mitral valve area by cross-sectional echocardiography. Ann Intern Med 2011; 88:337.
12. Robert J. Ostfeld; Essential Echocardiography; A Practical Handbook; Mitral Stenosis; © 2007 Humana Press Inc;Ch.13,239-244
13. Recusani F, Bargiggia GS, Yoganathan AP, Raisaro A, Valdes-Cruz L, Sung HW, Bertucci C, Gallati M, Moises VA, Simpson IA,Tronconi L, Sahn DJ. A new method for quantification of regurgitant flow rate using color flow imaging of the flow convergence region proximal to a discrete orifice: an in vitro study. Circulation.2001;83:594-604.
14. Utsunomiya T. Ogawa T. Tang HA, Henry WL, Gardin JM. Dopplercolor flow mapping of the "proximal isovelocity surface area":a new method for measuring volume blood flow across an orifice.JAm Coll Cardiol. 1999;13:225A. Abstract.
15. Utsunomiya T, Ogawa T, Doshi R, Patel D, Quan M, Henry WL,Gardin JM. Doppler color flow "proximal isovelocity surface area"method for estimating volume flow rate: effects of orifice shape and machine factors. JAm Coll Cardiol. 2001;17:1103-1115.
16. Loyd D, Wranne B, Pressure half time dose not always predict mitral valve area correctly, J Am Soc Echocardiog; 1998;1:313-12.
17. Karp k, Teien D, Bjerele p,Eriksson P. Reassessment of valve area determination in mitral stenosis by the pressure half time method ;impact of left ventricular stiffness and peak diastolic pressure difference. Jam Coll Cardiol 1999;13;594-9.tiv
18. Smith MD,Handshoe R,Handshoe S,Kwan, DeMariaAN.Comparative accuracy of two dimensional echocardiography and Doppler pressure half time method in assessing the severity of mitral stenosis in patients with and without prior commisurotomy.Circulation 1989;73;100-7.
19. Nishimura R, Rihal C, Tajik J,Holms D.Accurate measurement of the trasmitral gradient in patient with mitral stenosis; asimultaneous catheterization and Doppler echocardiography study.J Am Coll Cardiol 1994;24:152-8.
20. Okamoto M, Tsubokura T, Nakagawa H, Morichika N, Amioka H,Yamagata T, Hashimoto M, Tsuchioka Y, Matsuura H, Kajiyama G. The suction signal detected by color Doppler echocardiographyin patients with mitral regurgitation: its clinical significance. Am J Cardiol. 1988;18:739-746.
21. Sahn DJ, Simpson IA, Murillo A, Valdes-Cruz L. Observations ofacceleration proximal to restrictive orifices in congenital heartdisease: important clues for the interpretation of Doppler colorflow maps. Circulation. 1988;78(suppl II):II-649. Abstract.
22. Rodriguez L, Flachskampf FA, Abascal VM, Levine RA, Harrigan P, Thomas JD. Regurgitant flow rate calculated by proximal isovelocitysurface area is independent of orifice shape. Circulation.1989;80(suppl II):II-570. Abstract.
23. Rodriguez L, Anconina J, Flachskampf FA, Weyman AE, LevineRA, Thomas JD. Impact of finite orifice size on proximal flowconvergence: implications for Doppler quantification of valvular regurgitation. Circ Res. 1992;70:923-930.
24. Shandas R, Gharib M, Liepmann D, Shiota T, Sahn DJ. Experimentalstudies to define the geometry of the flow convergenceregion: laser Doppler particle tracking and color Doppler imaging.Echocardiography. 1992;9:43-

50.

25. Vandervoort PM, Thoreau DT, Weyman AE, Thomas JD. Wallfiltering significantly increases Doppler velocity in proximal flowconvergence. Circulation. 1991;84(suppl II):II-104. Abstract.
26. Cape EG, Levine RA, Muralidharan E, Heinrich R, YoganathanAP. Increased heart rate can cause underestimation of regurgitantflow by proximal isovelocity surface area (PISA). Circulation. 1992;86(suppl I):I-804. Abstract
27. Yoganathan AP, Cape EG, Sung H-W, Williams FP, Jimoh A.Review of hydrodynamic principles for the cardiologist: applicationsto the study of blood flow and jets by imaging techniques. JAm Coll Cardiol. 2008;12:1344-1353.
28. Robert D.Rifkin, Kathleen Harper, Comparison of Proximal Isoveiocity Surface Area Method With Pressure Half-Time and Planimetry in Evaluation of Mitral Stenosis JACC Vol. 26, No. 2;August 1995:458-65,
29. Dr. Dilek Ural, Prof. Dr. Barış Ilerigelen; Value of Proximal Isovelocity Surface Area Method in Calculation of Mitral Valve Area in Patients with Mitral Stenosis, Türk Kardiyol Dern Arş Volume: 25 Issue: 8 November 1997; 25:471-476
30. L Rodriguez, JD Thomas, V Monterroso, AE Weyman, P Harrigan, LN Mueller ; Validation of the proximal flow convergence method. Calculation of orifice area in patients with mitral stenosis ,*Circulation* 1993;88;1157-1165;
31. David Messika-Zeitoun et al, Evaluation of mitral valve area by the proximal isovelocity surface area method in mitral stenosis: Could it be simplified? Eur J Echocardiography (2007) 8, 116-121

Research Article

Comparison between short and long segment posterior spinal fixation in thoracolumbar burst fractures

Hayder Qatran Raheem, ***F.I.C.M.S***

Date Submitted: 26/ /2015
Date Accepted:24/11/2015

Address for Correspondence:
Dr. Hayder Qatran Raheem, Lecturer in Neurosurgery Department, College of Medicine, Al-Mustansiryia University,
Email: aljubourihayder@yahoo.com

Abstract

Background: thoracolumbar area of the spine is the most common site for injuries and the surgical treatment of fixation for treatment of unstable burst fracture remain controversy.
Objectives: to compare the effect of long and short segment posterior pedicular fixation on vertebral burst fracture without neurological deficit since the subject is controversy.
Methods: 14 patients had been taken and divided into two categories A and B, each category consists of 7 patient (5 males and 2 females) whom undergo burst fracture at thoracolumbar region (D12, L1 or L2) which is type B according to "Dennis classification" and all the patient were intact neurologically but had local kyphosis angle more than 15 degree according to Cobb method of measuring. Category A was operated by short segment posterior fixation while category B was operated by long segment posterior fixation. The result was based on the correction of kyphosis angle (immediately and at 18-24 months period follow up), operation's time, the amount of blood loss and instrumental failure.
Result: the long segment fixation was significantly ($p<0.05$) better than short segment in correction of the angle of kyphosis in long follow up period (more than 18 months) rather than immediate post-operative period. The short segment fixation had instrumental complications (fracture of screws and dislodgement) while there was no instrumental failure in long segment fixation. According to the blood loss and operation time there was also significant differences.
Conclusion: the long segment fixation had better result in long term follow up on the angle of kyphosis; there was no instrumental complication (fractures of screws or dislodgment) due to distribution of the weight load on more levels in spite of increase in the time of operation and blood loss which are significant.

Key words: Burst fracture, Kyphosis angle, Short segment and long segment fixation

INTRODUCTION

Thoracolumbar area is highly susceptible to high energy trauma like road traffic accident or fall from height.[11] fracture in this area is the most common type, accounting more than 50%.[15] The aim of treatment of burst fracture in thoracolumbar area of the spine is to restore the angle of kyphosis and remove the compression of spinal canal; nevertheless, there were controversy in using short segment or long segment posterior fixation. Since the introduction of "fixateur interne" of short segment pedicular screw by "Dick et al"[1] till now the controversy still exist.[16] surgical method of treatment provide early mobilization and correction of kyphotic angle.[18]The aim of this research is to know whether the short segment (SS) pedicular fixation is the best choice in treating burst fracture of thoracolumbar spine by comparing it with long segment (LS) pedicular fixation.

In this study we depend on Dennis classification[2] of Burst fracture in which the type of fracture depends on the morphology by X-ray (figure 1)

- **Type A**: Fracture of both end-plates. *The bone is retropulsed into the canal.*

- **Type B**: Fracture of the superior end-plate. It is common and occurs due to a combination of axial load with flexion.
- **Type C**: Fracture of the inferior end-plate.
- **Type D**: Burst rotation. This fracture could be misdiagnosed as a fracture-dislocation. The he mechanism of this injury is a combination of axial load and rotation.
- **Type E**: Burst lateral flexion. This type of fracture differs from the lateral compression fracture in that it presents an increase of the interpediculate distance on anteroposterior roentgenogram.

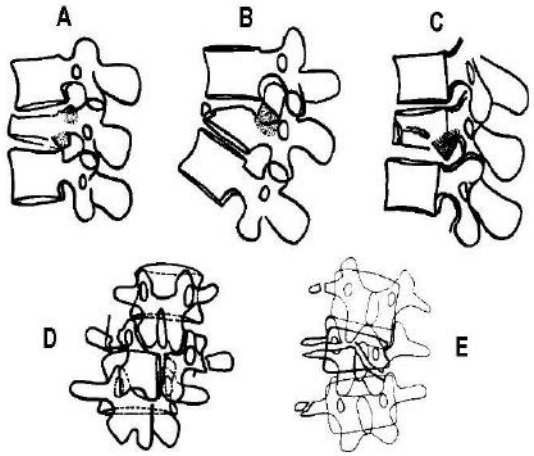


Figure 1. Dennis classification of Burst fracture

There are many classification methods for thoraco-lumbar burst fracture. We depend on Denis classification (taking in consideration to include only neurologically intact patient). Denis

Also The kyphosis angle was measured by using lateral thoracolumbar plain x ray and Cobb method by drawing line parallel to the upper border of the normal vertebra above the burst fracture and line parallel to the lower border of the normal vertebra below the burst fracture from these lines a perpendicular two lines were crossed to make the angle.[3] (figure 2A,B)

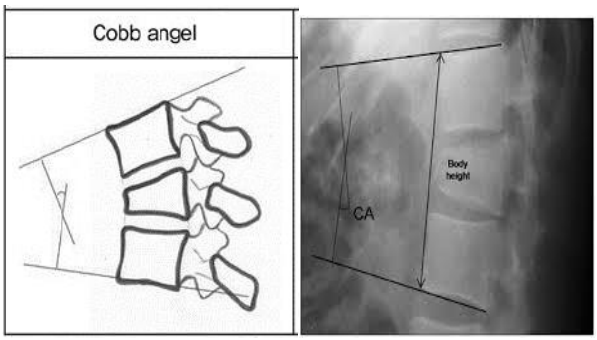


Figure 2 (A) Cobb method of measuring the kyphosis angle. (B) x-ray pre-op. Cobbs angle-

PATIENTS AND METHODS

Fourteen patients were included in this study; all of them had thoracolumbar burst fracture. The inclusion criteria were limited to neurologically intact patients; the angle of kyphosis more than 15 degrees; the fracture vertebra either D12 or L1 or L2 and the burst fracture type B according to Dennis classification.[2]The study included 10 males and 4 female, the mean of age 34.2 years (16-60). The levels of facture were L1 in 8 patients, D12 in 4 patients and L2 in 2 patients. The causes of fractures were road traffic accidents in 8 patients and fall from height in 6 patientsThe patients were divided into two categories according to the way of treatments. Category A (7patients) were treated by short segments fixation and category B (7patients) were treated by long segment fixation.

All of the patients were operated in Red Crescent Hospital in Baghdad, after 1-2 months of injury.

In category A the fixation was one level above and one level below the fracture site while in category B two levels above and two levels below the fracture sites. The instruments used were titanium pedicular screws and titanium rod to connect between the screws above and below vertically in each side.

The kyphosis angle was measured by using lateral thoracolumbar plain x ray and Cobb method.

The angle of kyphosis was measured pre-operatively, immediate post-operatively and after 18 months follow up. The failure in correction of kyphosis angle was determined by its increment equal or more than 10 degrees. The time of operation and the blood loss were measured.

Statistical comparison between 2 categories was done by MedCalc software (*t* and *U* test). The significant P value was < 0.05.

RESULT

The time of operation in short segment (SS) fixation was (120-180) minutes while in long segment (LS) fixation (160-250) minutes, the amount of blood loss in SS fixation was (200-500) milliliters while in LS fixation was (400-750) milliliters. In both categories comparison of (operation time and blood loss) was statistically significant.

The pre-operative, immediate post-operative and follow up post-operative (>18 months) of the kyphosis angle was measured and there was significant statistical

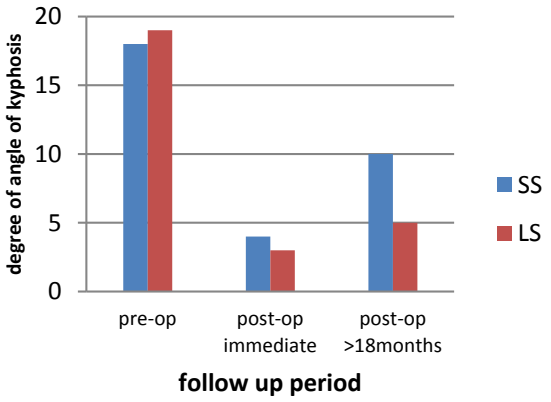
difference between two categories in follow up post-operative only (as shown in table 1).

Table 1. the results of comparison between short and long segment fixations

	Category A (SS)	Category B (LS)	P value
Age	32 +/- 11(16-55)	35+/-12 (18-60)	0.80
Time of follow up	22-27 months	24-26	0.76
Angle of kyphosis pre-op	18 +/- 2	19 +/- 1	0.20
Angle of kyphosis post-op immediately	4 +/- 1	3 +/- 1	0.25
Angle of kyphosis post-op >18 months	10 +/- 1	5 +/- 1	<0.05
Operation time	120-180 minutes	160-250 minutes	<0.05
Blood loss	200-500 ml	400-750 ml	<0.05

Four out of seven patients from (SS) category had correction loss of angle of kyphosis > 10 degrees (all in long post-operative period >18 months) with a failures rate 57%, while there was no correction loss in (LS) category. One patient had fracture and dislodgment in (SS) category. The difference between two categories was statistically significant. "Figure 3"

Figure 3: angle of kyphosis measurement showing no significant differences according to pre and immediate post-operative periods in both categories, whereas there was significant difference according to pre and long post-operative periods between two categories.



DISCUSSION

Depending on their experience, the surgeon choose their surgical approaches.[19] some doctors recommended conservative treatment like bed rest, physical therapy and braces ; however, this will not treat the kyphotic angulation and restore the original shape.[20]

Before "Dick et al"[1] whom invented what was called "fixateur interne" in 1985, there were two great surgeons who used pedicular screws, Boucher [7] at 1959 and Roy-Camille et al [8] at 1963. At that time (SS) method was considered the best way for the treatment of burst fracture.[1, 4-6] Some studies showed that (LS) fixations were superior to (SS) one.[7-9] Yu et al [10] founded that (SS) fixations had a failure rate in thoracolumbar region. Moon et al[12] founded that (LS) fixation was more effective than (SS) method. Korovessis et al[13] reported that claw figuration on two levels above and below the site of fracture was the best way in creating hard fixation. Katonis et al[15] reported that fixation two levels above the burst fracture associated with low instrumental complications. The advantage that makes (SS) method reliable are preserving the spinal motion segments, less blood loss and operative time is shorter.[17]

In this study with the comparison of (SS) and (LS) the failure in correction of kyphosis angle occurred in 4 patients all of them in (SS) category in a long period follow up result (statistically significant) demonstrated that it was not the best way in treatment of burst fracture in thoracolumbar region in spite of the significant long time and significant blood loss between the two categories. The failure rate in (SS) category was 57 % and there were also complication of screw fracture and dislodgment in one of (SS) category patient.

Conclusion

Long segment (LS) fixation occurred to be more successful than short segment (SS) one in treatment of thoracolumbar burst fractures.

REFERENCES

1. Dick et al. A new device for internal fixation of thoracolumbar and lumbar spine fractures: the fixateur interne. Paraplegia.1985; 23:225–232.
2. Denis F. The three column spine and its significance in the classification of acute thoracolumbar spine injuries. Spine. 1983; 8:817–831.
3. Farcy JPC, Weidenbaum M, Glasmann SD. Sagittal index in management of thoracolumbar burst fractures. Spine. 1990; 9:958–965.
4. Oner FC. Posterior instrumentation in spinal fractures. Presented at the 6th International Congress on Spinal Surgery, Ankara, 2002.
5. Sanderson PL, Fraser RD, Hall DJ, et al. Short segment fixation of thoracolumbar burst fractures without fusion. Eur Spine J. 1999; 8:495–500.
6. Rommens PM, Weyns F, Van Calenbergh F, et al. Mechanical performance of the Dick internal fixator. A clinical study of 75 patients. Euro Spine J. 1995; 4:104–109.
7. Muller U, Berlemann U, Sledge J, et al. Treatment of thoracolumbar burst fractures without neurologic deficit by indirect reduction and posterior instrumentation: bi segmental stabilization with mono segmental fusion. Euro Spine J. 1999; 8:284–289.
8. Mc Namara MJ, Stephens GC, Spengler DM. Trans-pedicular short segment fusions for treatment of

lumbar burst fractures. J Spinal Disord.1992; 5:183–187.
9. Rommens PM, Weyns F, Van Calenberg F, et al. Mechanical performance of the Dick internal fixator. A clinical study of 75 patients. Euro Spine J.1995; 4:104–109.
10. Yu SW, Fang KF, Tseng IC, et al. Surgical outcomes of short-segment fixation for thoracolumbar fracture dislocation. Chang Gung Med J.2002; 25. Abstract.
11. Bensch FV, Koivikko MP, Kiuru MJ, Koskinen SK. The incidence and distribution of burst fractures.Emerg Radiol. 2006;12:124–9.
12. Moon MS, Moon YW, and Kim YS, et al. Stabilization of fractured thoracic and lumbar spine with Cotrel–Dubousset instrument. J Orthop Surg.2003; 11:59–66.
13. Korovessis PG, Baikousis A, Stamatakis M. Use of the Texas Scottish Rite Hospital instrumentation in the treatment of thoracolumbar injuries. Spine. 1997; 22:882–888.
14. Katonis PG, Kontakis GM, Loupasis GA. Treatment of thoracolumbar and lumbar spine injuries using Cotrel–Dubousset instrumentation. Spine.1999; 24:2352–2357.
15. Dai LY, Jiang LS, Jiang SD. Posterior short-segment fixation with or without fusion for thoracolumbar burst fractures. J Bone Joint Surg Am. 2009;91:1033–1041. [PubMed]
16. M. B. Harris, "Commentary: thoracolumbar spine fractures: is more knowledge better?" The Spine Journal, vol. 13, no. 3, pp. 222–223, 2013.
17. X. Li, Y. Ma, J. Dong, X.-G. Zhou, and J. Li, "Retrospective analysis of treatment of thoracolumbar burst fracture using mono-segment pedicle instrumentation compared with short-segment pedicle instrumentation," European Spine Journal, vol. 21, no. 10, pp. 2034–2042, 2012.
18. H. Y. Kim, H. S. Kim, S. W. Kim, et al., "Short segment screw fixation without fusion for unstable thoracolumbar and lumbar burst fracture : a prospective study on selective consecutive patients," Journal of Korean Neurosurgical Society, vol. 51, no. 4, pp. 203–207, 2012.
19. R. Schmid, R. A. Lindtner, M. Lill, M. Blauth, D. Krappinger, and C. Kammerlander, "Combined posteroanterior fusion versus transforaminal lumbar interbody fusion (TLIF) in thoracolumbar burst fractures," Injury, vol. 43, no. 4, pp. 475–479, 2012.
20. Rajasekaran S. Thoracolumbar burst fractures without neurological deficit : the role for conservative treatment. Eur Spine J 2010 ; 19 : S40-47.

Research Article

Effects of metformin on omentin-1 serum levels in a newly diagnosed type 2 diabetes mellitus: Randomized, placebo controlled study

Hayder M. Al-Kuraishy, Miqat T. Hamada ,Abdilkarim Y. Al-Samerraie

Date Submitted: 16/11/2015
Date Accepted:28/12/2015

Address for Correspondence:
Dr. Hayder M. Al-kuraishy, Department Of Pharmacology, College of Medicine ,Almustansiriya University
Email:Hayderm36@Yahoo.Com

Abstract

Metformin is one of the most common used anti-diabetes drugs for treatment of T2DM. Metformin is considered as anti-hyperglycemic drug, it lowers blood glucose levels in T2DM without producing significant hypoglycemia. Plasma omentin-1 concentrations and expression of its mRNA in human omental adipose tissue were significantly lower in patients with impaired glucose tolerance and T2DM. The objectives of the present study were to establishing and elucidation of omentin-1 serum levels in metformin treated T2DM patients. The selected subjects patients were divided into two groups: Group A: includes thirty of a newly diagnosed T2DM patients with (mean age 49.32± 11.18 years), were initially treatment with metformin at time diagnosis according to ADA criteria. Group B: includes thirty healthy volunteers. The duration of treatment was three consecutive months. After a period of 12 hrs fasting, the blood samples (10ml) were withdrawn from all subjects by vein puncture before starting the study (before starting metformin) and after three months of metformin treatment. There was a significant difference in the baseline serum omentin-1 levels between metformin and control groups (p=0.023) and significant rising in serum omentin-1 levels after three month s duration of treatment with metformin. Conclusions: In newly diagnosed T2DM patients, omentin-1 levels were lower compared to control subjects. Three months of treatment with metformin lead to in a significant elevation in omentin-1 serum levels compared with baseline values.

Key words: *Metformin, Omentin-1, T2DM*

INTRODUCTION

Type 2 diabetes mellitus represents 90 to 95% of the overall diabetes types worldwide [1]. The incidence of T2DM is increasing worldwide, primarily due to increases in the prevalence of consumption of high-calorie diets, obesity and sedentary lifestyle [2]. Obesity alone has been founded to be a contributing factor to around 55% of T2DM [3]. Most patients with T2DM exhibited abdominal obesity, which contributes for development of insulin resistance [4]. T2DM usually affects individuals who are obese and most of the cases are diagnosed at age more than forty years. However, the demographic profile of T2DM is changing nowadays, where the prevalence of T2DM is increasing among young adults and even among children, pathological abnormalities in T2DM which are impairment of insulin secretion from a dysfunctional pancreatic β-cell and/or insulin action due to development of insulin resistance [5]. Insulin resistance is the most important risk factor for T2DM and is characterized by the inability of the target tissues to respond for insulin action [6].

In response, the pancreatic β-cells needed to secrete an extra amount of insulin for maintenance of euglycemic state, however; with time, the defects in the secretion of insulin will prevent the pancreatic β-cells from preserving a high rates of insulin secretion. Consequently, this will result in impairment of glucose tolerance and eventually development of T2DM [7]. Insulin resistance may be exists for several years before diagnosis of DM and continue to progress during the course of the disease [8].

The chronic elevation in glucose and lipid levels will causes gluco-lipotoxicity, which in turn contributes to pancreatic β-cell failure by causing activation of the stress response, enhanced apoptosis with reduced proliferation and exacerbates insulin resistance.

Metformin is one of the most common used anti-diabetes drugs for treatment of T2DM [9]. Metformin is considered as anti-hyperglycemic drug, it lowers blood glucose levels in T2DM without producing significant hypoglycemia [10]. The glucose-lowering properties of metformin are inhibition of gluconeogenesis , glycogenolysis and enhancing insulin-stimulated glucose uptake by skeletal muscle and adipocytes [11].However, the main effect of metformin appears to be through decreasing hepatic glucose output due to inhibition of respiratory-chain complex 1 in the mitochondria, causing transient reduction in the status of cell energy which promotes stimulation of adenosine monophosphate -activated protein kinase (AMPK), that plays an important role in regulating of energy balance, moreover, within skeletal muscle, stimulation of AMPK increases glucose uptake and lipid oxidation while; in liver stimulation of AMPK decreases gluconeogenesis and synthesis of the lipid[12].

Omentin-1 was initially found in intestinal cell (called intelectin), it is mainly expressed at visceral adipocity and low levels of omentin expression have been establish in human muscle, kidney, intestine, endothelial cells, and cardiac tissues, but it is highly expressed in placenta and ovary [13].

Abnormal omentin-1 secretion is thought to have a role in the pathophysiology of insulin resistance, inflammatory processes, endothelial dysfunction and cardiovascular diseases [14].

Plasma omentin-1 concentrations and expression of its mRNA in human omental adipose tissue were significantly lower in patients with impaired glucose tolerance and T2DM [15]. In T2DM patients, fasting serum omentin-1 levels were found to be linked negatively with insulin level and HOMA-IR [16]. A newly diagnosed T2DM female patients had been found to have considerably low serum levels of omentin-1 than age-matched control females, also both diabetic and control groups with insulin resistant have appreciably lower serum omentin-1 levels than patients without insulin resistance, as insulin resistance worsened, omentin-1 levels will decline [17]. In addition, lower circulating omentin-1 levels were founded within T2DM patients [18].

Thus, the objectives of the present study were to establishing and elucidation of omentin-1 serum levels in metformin treated T2DM patients.

PATIENTS AND METHODS

This study was carried out on thirty healthy control subjects (mean age 47.8± 9.3 years), and thirty T2DM patients, who attended the Endocrinology and Diabetes specialized center, Al-Mustansiriya University – Baghdad/Iraq. The control subjects did not have any medical disorders and were not receiving any medications. The enrolled patients attended the Endocrinology and Diabetes Center from December 2014 till June 2015. The selected subjects patients were divided into two groups:

Group A: includes thirty of a newly diagnosed T2DM patients with (mean age 49.32± 11.18 years), were initially treatment with metformin at time diagnosis according to ADA criteria.**Group B**: includes thirty healthy volunteers.The duration of treatment was three consecutive months.

The following exclusion criteria were used for all patients:

1-Having type 1 diabetes
2-Having any chronic illnesses of heart, kidney, thyroid, lung or liver.
3-Taking corticosteroids.
4-Female subjects taking hormonal replacement therapy
5-Patients with acute renal failure.
6-Patients with malignancy.

Anthropometric measurements: Blood pressure, height (cm), weight (kg) and body mass index BMI were determined for all patients at the initiation of the study as well as 3 months later. BMI=weight (kg)/height (cm) ^{2}[19].

Biochemical measurements: After a period of 12 hrs fasting, the blood samples (10ml) were withdrawn from all subjects by vein puncture before starting the study (before starting metformin) and after three months of metformin treatment. 9 mls of blood were placed in plane tubes, and 1 ml was placed in EDTA -tubes and stored at (2-8 ^{o}C) for HbA_{1c} measurement within one week. The remaining blood sample was centrifuged (at 3000 rpm for 10 minutes) to get the serum, which is used for the determination of fasting glucose and postprandial blood glucose. The remaining serum was frozen at (–20^{o}C) for estimation of serum insulin and omentin-1 levels.

Determination of blood glucose (FBG) Levels: Fasting blood glucose level was determined by using a ready-made kit (Biolabo, Glucose GOD-PAP, France) on the KENZA 240 TX Automatic Biochemistry Analyzer, based on method of Borham and Trindoer.

Determination of Serum Insulin Levels: Serum insulin level was determined using a ready-made kit (accubind,

ELISA Microwells, Monobind Inc., USA), the insulin ELISA kit is a solid phase ELISA depending on the sandwich principle. The absorbance is spectrophotometrically measured at 450 nm.

Determination of Insulin Resistance (IR): Insulin resistance was determined from the HOMA-IR (homeostasis model assessment of insulin resistance) depending on the following formula [20]:

$$HOMA-IR = \frac{\text{fasting serum insulin concentration } \left(\frac{\mu U}{ml}\right) \text{ x FBG } \left(\frac{mg}{dl}\right)}{405}$$

Determination of HbA_{1c}:HbA_{1c} level was determined using a ready-made kit (Clover A1c, Infopia Inc., Anyang, Korea) based on boronate affinity method [21].

Determining Serum Omentin-1 Levels: Serum omentin-1 concentrations were determined using a commercially available ELISA kit (Biovendor, Czech). All kit measurements were according to the kit constructions.

Analysis of the current data was carried out using of Statistical Packages for Social Sciences- version 22(SPSS-22). Data were presented in simple measures of mean, percentage, frequency, range and standard deviation.The significance of difference of different percentages (qualitative data) was tested by using Pearson Chi-square test (χ^2-test). The significance of difference was tested by using paired-t-test for difference of paired observations regarding p value <0.05 as significance.

RESULT

Sixty patients were included in this study, thirty newly diagnosed T2DM patients started with metformin treatment and thirty healthy volunteers were regarded as control group. Demographic data and baseline characteristics were not significantly different among the two groups, table (1). There was a non-significant difference in the baseline BMI values between metformin group and control group (p=0.476). Within the same group, there was a non-significant difference in BMI values after three months of treatment compared with baseline values and metformin group. There was a significant difference in the baseline fasting blood glucose, postprandial blood glucose and HbA_{1c} levels between metformin and control groups (p=0.0001). Within the same group, there was a significant decrease in these parameters after three months of treatment compared with baseline values in metformin group.

There was a non significant difference in the baseline serum insulin levels between metformin and control groups (p=0.136). Within the same group, also there was significant difference in serum insulin levels after three months of treatment compared with baseline values in metformin group p<0.01. There was a significant difference in the baseline insulin resistance values between metformin and control groups (p=0.0001). However, within the same group, there was a non significant difference in insulin resistance values after three months of treatment compared with baseline values and metformin group. There was a significant difference in the baseline serum omentin-1 levels between metformin and control groups (p=0.023); and significant raising in serum omentin-1 levels after three month s duration of treatment with metformin, table (2).

Table 1. Demographic data and baseline characteristics of the patients and control groups.

Variables		Control group (n=30)	Metformin group (n=30)	*P* value
Age (*yr*)		47.80±9.31	49.32±11.17	0.104
Gender (no.)	Male	20	24	0.126
	Female	10	6	
BMI (kg/m^2)		28.52±4.02	29.28±4.18	0.454
Smoker (no.)		7	5	0.519

Data presented as mean ± SD; and numbers.

Table 2. effects of metformin on anthropometric and biochemical measures on type 2 DM before and after three months of treatment.

	Control		Metformin	
Variables	Before (Mean ±SD)	After (Mean ±SD)	Before (Mean ±SD)	After (Mean ±SD)
BMI (Kg/m^2)	28.52±4.02	28.342±3.02	29.28±4.18	28.25±5.56
F BG (mg/dl)	93.60±8.73	90.60±8.73	228.56±57.57¶	206.44±71.64**
PP G level mg /dl)	116.87±11.22	117.67±18.22	228.56±57.57¶	148.56±11.47**
HbA1C (%)	5.03±0.32	5.03±0.31	10.20±1.76¶	7.74±1.76**
Serum Omentin-1 (mIU/dl)	10.25±7.20	10.15±4.25	6.59±4.84¶	8.84±7.27*
Serum insulin(µIU/ml)	10.46±6.30	10.48±5.30	14.11±9.40¶	10.32±8.10**
Insulin Resistance	2.38±1.41	2.25±1.22	6.40±4.95¶	4.11±3.43*

P*<0.05;p*<0.01(metformin versus control after three months duration); ¶*p*< 0.05 (baseline metformin versus control (before)**

The correlations of serum omentin-1, serum insulin and insulin resistance with other parameters (pre- and post-treatment) in metformin group. Regarding serum omentin-1 in the metformin group, the results showed non-significant correlations between serum omentin-1 level and all of the other parameters both at baseline and after three months of treatment with metformin. With respect to insulin resistance in metformin group, the results showed that insulin resistance value at baseline was positively correlated with fasting blood glucose, HbA_{1C}, and fasting serum insulin. After three months of treatment with metformin, insulin resistance value was significantly positively correlated with fasting blood glucose and fasting insulin levels. Fasting insulin levels were significantly correlated with insulin resistance values, both at baseline and after three months of treatment with metformin.Moreover, in the present study, serum omentin-1 is highly correlated with serum insulin at baseline status r=0.95, figure (1).After three months of treatment with metformin, serum omentin-1 is highly correlated with serum insulin at post-treatment with metformin r=0.74, figure (2).At pretreatment stage, serum omentin-1 was highly correlated with insulin resistance, r=0.93, figure (3), but this correlation becomes more significant after three months of treatment with metformin r=0.97 figure (4).

Table 3. Correlations of serum omentin-1, serum insulin and insulin resistance in metformin group with other parameters pre- and post-treatment.

Parameters		Omentin 1 (MIU/dl)		IR level		Fasting Plasma Insulin (MIU/dl)	
		Before	After	Before	After	Before	After
Fasting blood glucose (mg/dl) Before	r	-0.129	0.102	0.444*	0.562**	0.017	0.569**
	P	0.491	0.586	0.012	0.001	0.927	0.001
Fasting blood glucose (mg/dl) After	r	-0.055	0.034	0.516**	0.547**	0.335	0.236
	P	0.768	0.855	0.003	0.001	0.065	0.201
HbA_{1C} (%) Before	r	-0.222	0.226	0.486**	0.490**	0.223	0.404*
	P	0.229	0.221	0.006	0.005	0.229	0.024
HbA_{1C} (%) After	r	-0.041	0.054	0.424*	0.294	0.293	0.136
	P	0.827	0.774	0.018	0.108	0.110	0.465
Omentin- 1 (MIU/dl) Before	r	-	0.132	-0.271	-0.200	-0.300	-0.232
	P		0.480	0.141	0.280	0.100	0.210
Omentin 1 (MIU/dl) After	r	0.132	-	0.211	-0.059	0.122	-0.171
	P	0.480		0.254	0.751	0.512	0.358
Fasting Plasma Insulin (MIU/dl) Before	r	-0.300	0.122	0.874**	0.056	-	-0.047
	P	0.100	0.512	0.0001	0.763		0.800
Fasting Plasma Insulin (MIU/dl) After	r	-0.232	-0.171	0.163	0.924**	-0.047	-
	P	0.210	0.358	0.382	0.0001	0.800	
Post prandial glucose level (mg/dl) Before	r	0.039	0.074	0.077	0.044	-0.121	0.107
	P	0.835	0.692	0.679	0.812	0.516	0.567
Post prandial glucose level (mg/dl) After	r	-0.049	0.030	0.234	0.252	0.180	0.206
	P	0.794	0.873	0.206	0.171	0.331	0.267
IR level Before	r	-0.271	0.211	-	0.290	0.874**	0.163
	P	0.141	0.254		0.113	0.0001	0.382
IR level After	r	-0.200	-0.059	0.290	-	0.056	0.924**
	P	0.280	0.751	0.113		0.763	0.0001
BMI (Kg/m^2) Before	r	0.136	0.142	-0.031	0.182	-0.101	0.184
	P	0.465	0.444	0.869	0.327	0.590	0.322
BMI (Kg/m^2) After	r	0.202	0.108	0.046	0.207	-0.027	0.210
	P	0.276	0.563	0.805	0.263	0.887	0.258

***P<0.05**

****p<0.01**

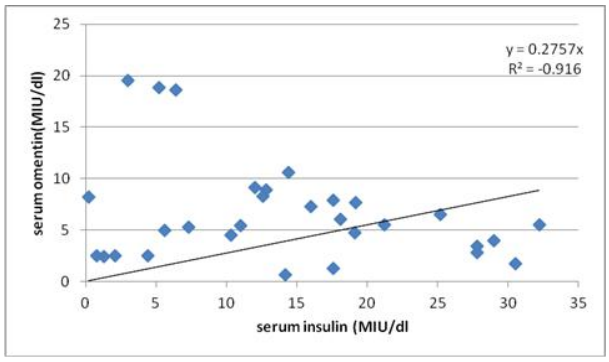


Figure 1. Correlations between serum omentin-1 and serum insulin in metformin group (pre-treatment).

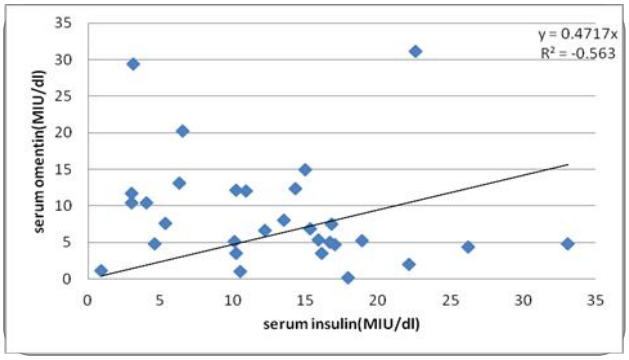


Figure 2 Correlations between serum omentin-1 and serum insulin in metformin group after 3 months (post-treatment).

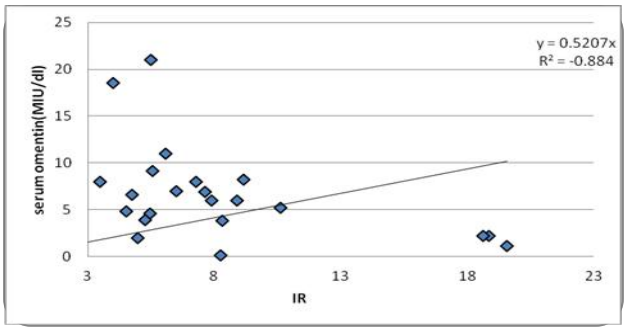


Figure 3 Correlations between serum omentin-1 and insulin resistance (IR) in metformin group (pre-treatment).

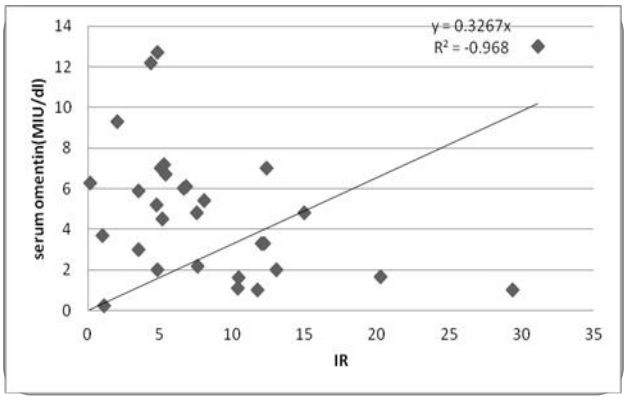


Figure 4. Correlations between serum omentin-1 and insulin resistance (IR) in metformin group after three months of treatment (post-treatment).

DISCUSSION

Abdominal fat was found to be more pathogenic compared to subcutaneous fat in producing IR, T2DM, and cardiovascular events. Adipose tissue has been found to secrete different active molecules, called adipokines that can significantly affect glucose and lipid metabolism. These adipokines include visfatin, resistin, adiponectin, interleukin-6, leptin and TNF-α. Omentin-1 was founded to be preferentially expressed in visceral fat more than subcutaneous fat. Omentin-1 may be a novel hormone that may act as a modulator of systemic metabolism, including insulin action in subcutaneous adipose tissue [22]. Interestingly, it was found that individuals with impaired glucose homeostasis, as well as T2DM, have a reduced serum omentin-1 levels.

Concerning the effects of three months therapy with metformin alone on serum glycemic indices (FBG, PBG, HbA_{1c}, serum insulin and IR), significant reduction was reported in FBG, PBG and HbA_{1c} levels.

The mean value of BMI for the newly diagnosed T2DM was (29.28±4.18 kg/m^2) which occurs at near the upper limit of overweight classification 25–29.9 kg/m^2) [23]. There is a close links between the pathophysiology of obesity and T2DM. Previous studies showed that risk of T2DM was increased directly with body weight and obesity, especially the central obesity which increased the risk of T2DM by 10-11 folds [24]. a non significant difference was showed in BMI values after three months of treatment compared with baseline values in metformin group which was agreed that metformin usually produce weight loss and the result of the current study was inconsistent with observations made by several authors where significant decreased in body weight were associated with metformin treatment [25].

Metformin therapy decreased fasting serum glucose concentrations significantly, results that are matched with other studies [26]. The reduction in FBG reported with metformin are mainly as consequence of reduced hepatic glucose output (mainly by inhibiting gluconeogenesis and to a lesser degree, glycogenolysis) and increased glucose uptake by skeletal muscle as well as by adipocytes [27]. The glucose production by liver was reported to increase at least twofold in T2DM [28]. The exact mechanism by which metformin reduces this production still unclear, but its major site of action appears to be the mitochondria of hepatocytes, producing an inhibition of cellular respiration leading to decrease gluconeogenesis [29].

In addition, metformin has been found to reduce FFA oxidation by 10-30% [30]. Elevated FFA levels are commonly found in diabetes, and they contribute also to

increased hepatic glucose output and insulin resistance [31] .

Metformin also enhances insulin-induced inhibition of gluconeogenesis from several substances, including glycerol, lactate, pyruvate, and amino acids, and antagonizes glucagon-induced gluconeogenesis [32].

Postprandial blood glucose is reduced by metformin therapy either by increasing splanchnic utilization of glucose or through enhancing the peripheral uptake of glucose since; at gastrointestinal tract, there is no significant effect of metformin on glucose absorption [33]. However, metformin was found to accumulates in the gastrointestinal wall where favors glucose metabolism to produce lactate [34]. The combination of these effects will lowers postprandial glucose level. Moreover, metformin was found to increases uptake of glucose by the muscle under conditions of elevated glucose level [35]. In addition, metformin lower plasma glucagon concentrations and antagonize glucagon actions [36]. Finally, an increased post-meal hepatic blood flow by metformin may increases hepatic glucose uptake [37].

The finding of increased insulin resistance in newly diagnosed T2DM enrolled in the current study was in agreement with a well established finding that insulin resistance is a characteristic pathological abnormality of T2DM and consider an essential contributor for the development of T2DM [38]. However, significant changes were revealed in insulin levels and insulin resistance after three months of metformin treatment, findings that were compatible with findings of other studies in which metformin, an insulin-sensitizing agent, decreases insulin resistance in T2DM patients, with subsequent decrease in baseline as well as glucose-stimulated insulin secretions [39]. Concerning the newly diagnosed T2DM patients who started treatment with metformin, the results of this study showed that newly diagnosed T2DM patients had significantly lower serum omentin-1level. Omentin-1 is relatively a newly identified adipokine. The previous studies showed negative correlations between omentin-1 serum levels and gene expression with T2DM [40]. In vitro study had shown that omentin-1 increases glucose uptake by human adipocytes through enhancing protein kinase B (AKT) phosphorylation as well as transduction of insulin signal [41]. The findings of the current study confirm that T2DM patients demonstrated decreased levels of omentin-1. The reduced levels of omentin-1 reported in T2DM patients may lead to a reduction in insulin-mediated uptake of glucose in both visceral and subcutaneous fats as well as other insulin-sensitive tissue [42]. However, it may be difficult to determine whether a high glucose level is the cause or the result of the observed low serum omentin-1 levels and by which mechanisms glucose affects omentin-1 level. Tan, *et al.*, found that both glucose as well as insulin can significantly decrease the omentin-1 production in adipose tissue explants in a dose-dependent manner and that a high insulin level can significantly reduced the levels of serum omentin-1in healthy individuals [43]. Thus, omentin-1 synthesis is regulated directly or indirectly by plasma glucose and insulin levels [44]. In addition, other possible may contributs for decreasing serum omentin-1 levels were reported for the newly diagnosed T2DM could be excessive body weight since; mean of BMI for the newly diagnosed T2DM involved in the current study was 29.28±4.18 kg/m^2,according to BMI Classification, those patients are considered overweight (BMI between 25–29.9 kg / m^2) [45].

This possibility is matched with the finding of increased level of omentin-1 in patients with anorexia nervosa associated with significant reduced body fat stores [149]. It was observed that lean individuals had significantly increased serum omentin-1levels compared to those who are obese or overweight [46].

After three months treatment of the newly diagnosed T2DM with metformin, there was no significant change in the level of omentin-1 compared to baseline level. Serum omentin-1 levels were reported to increases following metformin treatment in women with PCOS , and following treatment of metformin plus liraglutide in T2DM Chinese patients [47]. There was significant effect of metformin therapy on omentin-1 level in the current study, which be attributed to the differences among the patients characteristics between the current study the Chinese study such as gender distribution, ethnicity, as well as the lack of a 'metformin monotherapy' group in the Chinese study [48].

Conclusions

In newly diagnosed T2DM patients, omentin-1 levels were lower compared to control subjects. Three months of treatment with metformin lead to in a significant elevation in omentin-1 serum levels compared with baseline values.

Recommendations for Future study

Further studies are needed to investigate the effects of metformin combined with SGLT2 inhibitors or sitagliptin on serum omentin-1 level.

REFERENCES

1. American Diabetes Association. (2015) Classification and Diagnosis of Diabetes. Diabetes Care.38 (Suppl. 1), S8–S16.
2. Jennifer D. diabetes mellitus. (2013) In: Leon S, Alan H, Paul F, Larry N. Comprehensive pharmacy review for

NAPLEX. 8th ed. Lippincott Williams & Wilkins; pages: 930-954.
3. Centers for Disease Control and Prevention. (2004) Prevalence of overweight and obesity among adults with diagnosed Diabetes United States, 1988-1994 and 1999-2002. Morbidity and Mortality Weekly Report. 53(45), 1066-1068.
4. Triplitt C, Reasner C. Diabetes mellitus. In: DiPiro JT, Talbert RL, Yee GC, *et al*, eds. (2011) Pharmacotherapy: A Pathophysiologic Approach. 8th Ed. New York: McGraw-Hill; pages: 1255–1302.
5. Holt R. (2004) Diagnosis, epidemiology and pathogenesis of DM: an update for psychiatrics. British J Psychiatry.184, S55-S63.
6. Kangduk C, Young B. (2010) Molecular Mechanism of Insulin Resistance in Obesity and Type 2 Diabetes. Korean J Intern Med. 25, 119-129.
7. Goldstein B. (2002) Insulin resistance as the core defect in type 2 diabetes mellitus. Am J Cardiol. 90(5), 3-10.
8. Molina PE. (2010) Endocrine pancreas. In: Endocrine Physiology. 3rd ed. New York: McGraw-Hill. Pages: 167–188.
9. American Diabetes Association. (2009) Standards of medical care in diabetes. Diabetes Care 32 Suppl 1: S13-61.
10. Benoit V, Bruno G, Nieves S, Jocelyne L, Marcn F, Fabrizio A. (2012) Cellular and molecular mechanisms of metformin: an overview. Clinical Science. 122 (6), 253-70.
11. Dmitri K, Samy I, James R. (2002) Metformin: An Update. Ann Intern Med.137, 25-33.
12. James G, Gerard A, Miles F. (2010) Drugs for diabetes: part 1 metformin. Br J Cardiol.17, 231–4.
13. Yang R, Lee M, Hu H, Pray J, Wu H, Hansen B *et al*. (2006) Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol Endocrinol Metab. 290, E1253–E1261.
14. Anh V, Maha S, Brooke C, Lisa A, Christina L. (2014) Evaluation of the relationship between circulating omentin-1 concentrations and components of the metabolic syndrome in adults without type 2 diabetes or cardiovascular disease . Diabetology & Metabolic Syndrome. 6, 1-9.
15. Tan B, Adya R, Randeva H. (2010) Omentin: a novel link between inflammation, diabesity, and cardiovascular disease. Trends Cardiovasc Med. 20, 43-8.
16. Yan P, Liu D, Long M, Ren Y, Pang J, Li R. (2011) Changes of serum omentin levels and relationship between omentin and adiponectin concentrations in type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes.119, 257-63.
17. Gürsoy G, Kırnap N, bah O, Acar Y, Demirba B, Akçayöz S, *et al* . (2010) The relationship between plasma omentin-1 levels and insulin resistance in newly diagnosed type 2 diabetic women. Clinical Reviews and Opinions. 2(4), 49-54.
18. Tan B, Pua S, Syed F, Lewandowski K, O'Hare J, Randeva H. (2008) Decreased plasma omentin-1 levels in Type 1 diabetes mellitus. Diabet Med, 25, 1254–1255.
19. Friedewald W, Levy R, Fredrickson D. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18, 499–502.
20. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 28 ,412-9.
21. Flückiger R, Woodtli T, Berger W. (1984) Quantitation of glycosylated hemoglobin by boronate affinity chromatography. Diabetes.33, 73-6.
22. El-Mesallamy H, El-Derany M, Hamdy N. (2011) Serum omentin-1and chemerin levels are interrelated in patients with type 2 diabetes mellitus with or without ischaemic heart disease. Diabet Med. 28, 1194-200.
23. Julie S, Susan Cl, Kayce S, Tommy J. Diabetes mellitus. In: Marie A, Patrick M, .Barbara G, Jill M., Terry L, *et al*. (2013) Pharmacotherapy Principles & Practice. 3rd edition. Pages: 757-786
24. Field AE, Coakley EH, Must A, Spadano JL, Laird N, *et al*. (2001) Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med.161, 1581–1586.
25. Garber A, Duncan T, Goodman A, Mills D, Rohlf J.(1997) Efficacy of metformin in type 2 diabetes: results of a double-blind, placebo-controlled, dose-response trial. Am J Med. 103, 491–497.
26. Cusi K, DeFronzo R. (1998) Metformin: a review of its metabolic effects. Diabetes Review. 6, 89-131.
27. Wiernsperger N, Bailey C. (1999) The antihyperglycaemic effect of metformin: therapeutic and cellular mechanisms. Drugs. 58 (l), 31-9.
28. Jeng C, Shen W, Fuh M, Chen Y, Reaven G. (1994) Relationship between hepatic glucose production and fasting glucose concentration in patients with NIDDM. Diabetes. 43, 440–1444.
29. Dominguez L, Davidoff A, Srinivas P, Standley P, Walsh M, *et al*. (1996) Effects of metformin on tyrosine kinase activity, glucose transport, and intracellular calcium in rat vascular smooth muscle. Endocrinology. 137, 113-21.
30. Hundal R, Krssak M, Dufour S, Laurent D, Lebon V, *et al*. (2000) Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes. 49. 2063-9.
31. Wilcock C, Bailey C. (1991) Reconsideration of inhibitory effect of metformin on intestinal glucose absorption. J Pharm Pharmacol. 43, 120-1.
32. Dominguez L, Davidoff A, Srinivas P, Standley P, Walsh M, *et al*. (1996) Effects of metformin on tyrosine kinase activity, glucose transport, and intracellular calcium in rat vascular smooth muscle. Endocrinology. 137, 113-21.
33. Cuber J, Bosshard A, Vidal H, Vega F, Wiernsperger N, *et al.* (1994) Metabolic and drug distribution studies do not support direct inhibitory effects of metformin on intestinal glucose absorption. Diabete Metab. 20, 532-9.
34. Wiernsperger N, Bailey C. (1999) The antihyperglycaemic effect of metformin: therapeutic and cellular mechanisms. Drugs. 58 (l), 31-9.
35. Li G, Srijib G, Kathleen M, Russ B, Teri E. (2012) Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics. 22(11),820–827.
36. Kühl C, Lindkaer Jensen S, Vagn Nielsen O, Pedersen J. (1976) The effect of dimethylbiguanide (DMB) on plasma insulin and glucagon concentrations in portal and peripheral venous blood. Diabetologia. 12, 155.
37. Ohnhaus E, Berger W, Duckert F, Oesch F. (1983) The influence of dimethylbiguanide on phenprocoumon elimination and its mode of action. A drug interaction study. Klin Wochenschr. 61:851-7.
38. DeFronzo R. (1988) The triumvirate: β-cell, muscle, liver: a collusion responsible for NIDDM. Diabetes 1988;37:667–687.

39. Wilcock C, Bailey C. (1991) Reconsideration of inhibitory effect of metformin on intestinal glucose absorption. J Pharm Pharmacol. 43, 120-1.
40. Yang R, Lee M, Hu H, Pray J, Wu H, Hansen B *et al*. (2006) Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol Endocrinol Metab. 290, E1253–E1261.
41. Tan B, Adya R, Randeva H. (2010) Omentin: a novel link between inflammation, diabesity, and cardiovascular disease. Trends Cardiovasc Med. 20, 43-8.
42. Pan HY, Guo L, Li Q. (2010) Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes. Diabetes Res Clin Pract .88, 29–33.
43. Bee K , Raghu A, Farhatullah S, Kris C, Paul O, Hendrik L , *et al* .(2008) Omentin-1, a Novel Adipokine, Is Decreased in Overweight Insulin-Resistant Women With Polycystic Ovary Syndrome. Diabetes.57, 801-808. (28)
44. Yang R, Lee M, Hu H, Pray J, Wu H, Hansen B *et al*. (2006) Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol Endocrinol Metab. 290, E1253–E1261.
45. In Koda-Kimble and Young's. (2013) Applied Therapeutics: The clinical use of drugs, 10th ed. by Lippincott Williams & Wilkins. Pages: 1223-1300.
46. De Souza Batista C, Yang R, Lee M, Glynn N, Yu D, *et al*. (2007) Omentin plasma levels and gene expression are decreased in obesity. Diabetes. 56,1655–1661.
47. Tan BK, Adya R, Farhatullah S, Chen J, Lehnert H, *et al.* (2010) Metformin treatment may increase omentin-1 levels in women with polycystic ovary syndrome. Diabetes. 59, 3023–3031.
48. Yan P, Li L, Yang M, Liu D, Liu H, Boden G, *et al*. (2011) Effects of the long-acting human glucagon-like peptide-1 analog liraglutide on plasma omentin-1 levels in patients with type 2 diabetes mellitus. Diabetes Res Clin Pract. 92, 368-74.

Research Article

Measurement of Serum Omentin-1 in Patients with Acute Coronary Syndrome

Walaa Ahmed AL-Jedda, Abbas Naji H.Al-Sharifi, Mushtaq Abed H. AL-Taie

Date Submitted: 29/4/2015
Date Accepted:10/1/2016

Address for Correspondence:
Dr. Walaa Ahmed AL-Jedda, Department of Biochemistry, College of Medicine ,Almustansiriya University

Abstract

Objective: Acute coronary syndrome (ACS) is an insufficient supply of the myocardium with oxygenated blood in which atherosclerosis is an essential cause of myocardial ischemia. Adipokine biomarkers plays an essential role in the atherosclerosis and hence coronary artery disease. Serum Omentin-1 is the biomarker that believed to be independently associated with acute coronary syndrome. The present study was carried out to evaluate: serum Omentin-1 in patients with acute coronary syndrome [ST- Elevation Myocardial Infarction (STEMI), Non-ST-segment Elevation Myocardial Infarction (NSTEMI), and Unstable Angina pectoris (UA)], that may help in guiding the diagnosis and treatment.

Methods: The study included a total (100) individuals; (75) patients with acute coronary syndrome (ACS) and (25) healthy control. A total number of 75 patients with acute coronary syndrome (ACS) (50 male ,25 female) as: 25 patients with STEMI (22 male and 3 female) 25 patients NSTEMI(16 male and 9 female) and 25 patients with unstable angina UA(12 male and13 female)were enrolled in this study all taken from Coronary Care Unit (CCU)at AL-Yarmouk Teaching Hospital periods of study . Each patient was clinically examined by the consultant cardiologist and the diagnosis was achieved by electrocardiograph (ECG), and cardiac enzyme levels.

Venous blood used for measuring serum omentin-1, total cholesterol, triglyceride, High density lipoprotein cholesterol and low- density lipoprotein cholesterol, fasting blood sugar, serum creatinine and. Body mass index was calculated according to the following equation: BMI=weight/height2, also blood pressure was measured.

Results: Serum omentin-1 levels were significantly lower in patients groups in comparison with control group, while total cholesterol, and low- density lipoprotein cholesterol levels were significantly higher in patients with coronary artery disease than controls. Negatively significant correlation was found between serum omentin-1 with (BMI and total cholesterol).

Conclusion: Serum Omentin-1 levels were lower in acute coronary syndrome patients in comparison with control group , serum omentin-1 showed significant changes with the development and progression of acute coronary syndrome and would be valuable in the assessment of patients with acute coronary syndrome.

Omentin-1 may be used as a predictor of ACS.

Key words: *Acute coronary syndrome, Omentin-1, Lipid profile.*

INTRODUCTION

Acute coronary syndrome is a term an acute encompasses both unstable angina and myocardial infarction (MI). It is characterized by new-onset or rapidly worsening angina. MI occurs when symptoms occur at rest and there is evidence of myocardial necrosis, as demonstrated by an elevation in cardiac troponin or creatine kinase-MB isoenzyme. (1) An acute coronary syndrome may present as a new phenomenon or against a background of chronic stable angina. The culprit lesion is usually a complex ulcerated or fissured athermanous plaque with adherent platelet-rich

thrombus and local coronary artery spasm (2). Lipid Research Clinics-Coronary Primary Prevention Trial revealed that lowering total and LDL or bad cholesterol levels significantly reduced CAD. More series of clinical trials using statin drugs have provided conclusive evidence that lowering LDL cholesterol reduces the rate of myocardial infarction (MI), the need for percutaneous coronary intervention and the mortality associated with CAD-related causes.(3)

Omentin/intelectin, discovered in 2005, is a novel adipokine, a secretory protein of 313 amino acids. It is a protein expressed and secreted from visceral but not subcutaneous adipose tissue that increases insulin sensitivity in human adipocytes. (4)

It is codified by two genes (1 and 2) with omentin-1 predominating as the circulating form of the adipokine (5). It stimulates insulin-mediated glucose transport in human adipocytes and triggers Akt signaling. The expression of Omentin is greater in visceral than subcutaneous adipose tissue (6).

Omentin, like other periadventitial epicardial adipokines, could play an important role in cardiovascular disease CVD pathogenesis, particularly in coronary atherosclerosis, as the absence of a fibrous fascial layer allows for more diffusion of adipokines and free fatty acids between epicardial adipose tissue and the underlying vessel wall in addition to the myocardium. Thus, omentin-1 appears to be a 'protective adipokine' with respect to CVD, given that it induces vasodilatation and inhibits endothelial cell EC migration, vascular inflammation and angiogenesis. As well as reducing endothelial dysfunction, it is also anti-inflammatory. This is a relatively novel adipokine and so one may expect much more of its role in CVD to be illuminated in the near future. (7)

The metabolic syndrome is a clustering of metabolic, proinflammatory, and prothrombotic factors that increases the risk of cardiovascular disease and type 2 diabetes (8). Adipose tissue dysregulation and altered secretion of numerous adipokines are present in the metabolic syndrome. However, in regards to omentin-1, few studies have evaluated the relationship between omentin-1 and the metabolic syndrome in patients without concomitant type 2 diabetes and/or cardiovascular disease (9).

PATIENTS AND METHODS

This study included (100) individuals: (75) patients and (25) control healthy , the criteria of inclusion were: un stable angina pectoris and STEMI and NSTEMI patients with recent acute myocardial infarction admitted into the coronary care unit (CCU) from February to July 2014 at AL-Yarmouk Teaching hospital .

A total number of 75 patients (50 male, 25 female) consisting of: 25 patients with unstable angina pectoris (12 male and13 female) and 25 patients with STEMI (22 male and 3 female) and 25 patients NSTEMI (16 male and 9 female), were enrolled in this study. Each patient was clinically examined by the consultant and the diagnosis was achieved by electrocardiograph ECG, cardiac enzymes and cardiac Troponin.

Several modalities of therapeutic agents: anti- angina, anticoagulants, and lipid lowering agents are the cornerstones in managements of ACS patients

The criteria of exclusion were patients with valvular heart disease, malignant disease, and infectious diseases, inflammatory diseases such as collagen disease, neoplasm, hematological disorders, advanced renal disease, liver disease and diabetes mellitus.

Control group consisted of 25 healthy persons (14males and 11 females) participants matched by age and BMI .Clinical examination of patients and controls with was done for BP, weight (Kg) and height (m), the litter two were measured for BMI. The BMI was calculated using Quetelet's equation: BMI (kg/m^2) = $weight/(height)^2$ taking the cut off $\geq$ 25 kg/m^2 as indication of overweight-obese

Ten milliliter (10mls) of blood were obtained by venepuncture after (10-12) hours overnight fasting, using a 10 ml disposable syringe between 9.00 and 11.00 A.M. Then centrifuged at 3000 rpm for 10 minutes to collect serum. Serum was divided into aliquots (250µl) in Eppendorff tubes and stored in a freezer (-20ºC) until use.

Human Omentin-1: Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for Omentin-1.

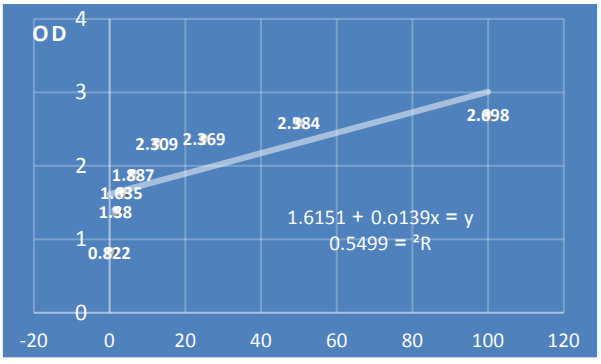


Figure 1. Omentin Concentration (pg/ml)

Assessment of Lipid Profile

Total Serum Cholesterol (enzymatic colorimetric assay),Serum Triglyceride :(enzymatic colorimetric assay),High Density Lipoprotein (HDL) Cholesterol (colorimetric method),Low Density Lipoprotein (LDL) cholesterol and Very Low Density Lipoprotein cholesterol (VLDL) were determined by using Friedewald's formula

VLDL-C calculated in mg/dl = TG/5

Calculated LDL-C in mg/dl = Total Cholesterol – (HDL + VLDL)

Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences- version 22). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means or Paired-t-test for difference of paired observations (or two dependent means), or ANOVA test for difference among more than two independent means. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (χ^2-test) with application of Yate's correction or Fisher Exact test whenever applicable

RESULT

Table (1) shows the mean age and gender of seventy – five (75) patients divided in to three groups: 25 patients with ST segment Elevation Myocardial Infarction (STEMI) with amen age of 55.1±10. 3(range 39-70) years and consisted22 males and 3 females, 25 Non ST segment Elevation Myocardial Infarction (NSTEMI) with amen age of 58.0±9.5 (range 36-70) years and consisted16 males and 9 females, 25 unstable Angina (UA) with amen age of 55.88±11.2 (range 27-70) years and consisted 12 males and 13 females .This study included also 25 healthy control subjects with amen age of 51.2±8.3 (range 37-65) years and consisted 14 males and 11 females. The mean age of control was not significantly different from that of the STEMI, UA, and NSTEMI Sequentially.In table-(2) body mass index (BMI) for all studied groups showed no significant difference between STEMI ,NSTEMI ,and UA patients and control group(28.2±5.6 kg/m^2)(range 19.0-42.6),(28.8±3.8 kg/m^2)(range20.3-34.2),(31.5±7.0 kg/m^2)(range20.8-46.0)and(27.3±4.6 kg/m^2)(range20.5-37.5) respectively. Regarding blood pressure measurements (systolic and diastolic blood pressure) there were also no significant differences between the same groups. The patients were treated with several modalities of therapeutic agents: anti- angina, anticoagulants, and lipid lowering agents were the cornerstones in management of ACS patients.

Table (3): shows that there were significant differences between STEMI,NSTEMI, UA patients and controls regarding the fasting serum lipid profile and shows the number of low ,normal or a high parameter in all of the STEMI,NSTEMI ,Unstable angina and controls

Serum Omentin-1 Study*:* Table (3) shows levels of serum omentin-1 in patient with acute coronary syndrome (ACS) compared with controls .These results a significant difference between STEMI, NSTEMI, UA, patients and control. Regarding the serum negative levels ,the higher level of serum omintin-1 in control: mean ±SD(636.32±191.44) and was reduced in ACS patients at the time of admission in to was the hospital ,and the level were lower about 5 times in unstable angina mean± SD (128.32±38.81) ,and the level were lower by about 8 times in NSTEMI mean ±SD(83.20±7.89),and lower by about 26 times in STEMI mean ±SD (24.76±7.6)

DISCUSSION

Coronary artery disease is a widely spread disease and is associated with significant mortality. Atherosclerosis is the most common reason of myocardial ischemia, which is a gradual inflammatory disorder of arterial wall that results in occlusion of the coronary artery(s) resulting in acute coronary syndromes (ACSs); the UA, NSTEMI, and STEMI. (10) Multiple factors such as hypertension, smoking, dyslipidemia, obesity, and family history of coronary artery disease play a crucial role in the pathogenesis of CAD (11)

Obesity is an independent modifiable risk factor for coronary heart disease (CHD) in both genders, it is associated with increased insulin resistance, hyperinsulinaemia, elevated levels of triglycerides and cholesterol, and increased activity of sympathetic nervous system. BMI it considered as a clinical tool for evaluating obesity, increasing body mass index (BMI) result in increase the risk of CVD events. And this results agreements with Yusuf S.et .al . (12)

Results of this study revealed that coronary artery disease had significant positive correlation with the BMI. This result agreed with Wolk R. study who found that, body mass index (BMI) as an independent risk factor of acute coronary syndrome, in patients with established coronary atherosclerosis, it was associated with unstable angina and myocardial infarction (13)

while Chua S. et al. and Iqbal M. studies found that obesity was the strongest risk factor for STEMI (14)

Body mass index (BMI) versus in this study were classified in to three groups: normal, overweight and obese and the patients became more risky for the acute coronary syndrome (ACS) for each of ST – segmented elevation myocardial (STEMI),non ST-segmented elevation myocardial (NSTEMI),and unstable angina (UA) when there was increased BMI, that agreed with (Bhaskaran K et. al.2015).(15) Our study showed that blood pressure measurements (systolic and diastolic blood pressure) had no significant differences between the same groups probably the patients were treated with several modalities of therapeutic agents: anti- angina , anticoagulants, and lipid lowering agents (16).

Some lipid profile components significant play a role in cardiovascular risk assessment. The oxidation of LDL cholesterol is considered as one of the important risk factors for atheroma formation, which is the underlying cause of coronary artery disease (17).

Current data revealed that there were significant elevation in serum total cholesterol concentration and serum LDL-C concentration in ACS patients as compared to the control group, these results are supported by other studies which assumed that elevated levels of total and low density lipoprotein cholesterol (TC, LDL-C) were considered as important risk factors for CAD (18).

The present study showed that serum omentin-1 levels were lower in patients with ACS than in control and previous study the study found that serum omentin-1 levels were independently associated with ACS prevalence. (19)

The study results revealed a significant difference between STEMI, NSTEMI, UA, patients and control. Regarding the serum omentin-1 level, so the higher level of serum omentin-1 in control group mean ±SD(636.32±191.44) and reduced in ACS patients at the time of admission to the hospital : Lower levels by about 5 times in unstable angina patients mean± SD (128.32±38.81) ,and by about 8 times in NSTEMI patients mean ±SD(83.20±7.89),and was lowers by patients compared with control group , in regarded to the following study paramaters:

-Age in the STEMI - Weight (Kg) in the STEMI, NSTEMI, UA. - BMI (Kg/m2) in the STEMI, NSTEMI, UA.

-S.B.P. In the UA.

- D.B.P. in the UA.

- Triglycerides (mg/dl) in the STEMI

- VLDL-C (mg/dl) in the STEMI about 26 times in STEMI patients mean ±SD (24.76±7.6) . This study found that was is a significant association between serums Omentin-1 in levels ACS

However, there was no significant association between serum omentin-1 level and lipid profile components , The majority of patients in this study had already received statin therapy; thus, the relationship between serum concentrations of Omentin-1 and lipid variables may best be observed in newly diagnosed and untreated patients with ACS .(20)

Levels of serum omentin-1 in all the STEMI, NSTEMI, UA and control group showed an association with BMI. A negative association between serum OMENTIN-1 levels and BMI in patients with ACS and control group was recorded in some studies. (21)

Levels of serum omentin-1in all the STEMI, NSTEMI, UA and controls correlation with total cholesterol. There was a positive correlation between serum omentin-1 levels and total cholesterol in patients with NSTEMI, UA when compared with control group and negative correlation in STEMI. (22) There was a positive correlation between serum omentin-1 levels and HDL in patients with ACS and control (23)

Conclusion:

Serum Omentin-1 level is significantly decreased in patients with acute coronary syndrome. These findings may help in the diagnosis and treatment of patients at high risk for CAD

REFERENCES

1. Brian R.Walker,Nicki R,Colledge, Stuart H.Ralston ,IanD. Penman: atherosclerosis in Cardiovascular disease , D.E.New- N.R. Grubb-A.Bradbury, Davidson's Principles and practice of Medicine,22th edition,part 2 ,practice of medicine section 18 2014,p 589.
2. Grech ED, Ramsdale DR (June 2003). "Acute coronary syndrome: unstable angina and non-ST segment elevation myocardial infarction". BMJ 326 (7401): 1259–61.
3. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med. Apr 7 2005;352(14):1425-35.
4. Saremi A, Asghari M & Ghorbani A 2010 Effects of aerobic training on serum omentin-1 and cardiometabolic risk factors in overweight and obese men. Journal of Sports Sciences 28 993–998.
5. De Souza Batista C.M., Yang R.Z., Lee M.J., Glynn N.M., Yu D.Z., Pray J., Ndubuizu K., Patil S., Schwartz A. Omentin plasma levels and gene expression are decreased in obesity. Diabetes. 2007

6. Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, Shuldiner AR, Fried SK, McLenithan JC & Gong DW 2006 Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. American Journal of Physiology. Endocrinology and Metabolism 290 E1253–E1261. (doi:10.1152/ ajpendo.00572.2004)
7. Celia M de Souza Batista, Rong-Ze Yang, Mi-Jeong Lee, Nicole M Glynn, Dao-Zhan Yu, Jessica Pray, Kelechi Ndubuizu, Susheel Patil, Alan Schwartz, Mark Kligman, Susan K Fried, Da-Wei Gong, Alan R Shuldiner, Toni I Pollin, John C McLenithan Omentin plasma levels and gene expression are decreased in obesity , 2007 Feb, American diabetes association
8. Raj S, Kaur H, Adams-Huet B, Bremer AA. Increased chemerin and decreased omentin-1 in both adipose tissue and plasma in nascent metabolic syndrome. J Clin Endocrinol Metab. 2013;6:E514–517. doi: 10.1210/jc.2012-3673.
9. Ang JP, Liu XT, Zheng QS, Xue YS, Wang B, Zhao LY. Serum omentin-1 levels are inversely associated with the presence and severity of coronary artery disease in patients with metabolic syndrome. Biomarkers. 2011;6:657–662. doi: 10.3109/1354750X.2011.622789
10. Thygesen K., Alpert J., Jaffe A., Simoons M., Chaitman B., White H., et al.: Third universal definition of myocardial infarction: European Heart Journal, 2012; 33: 2551-67
11. Aggarwa A., Aggarwa S., Guha Sarkar P., and Sharma V.: Predisposing Factors to Premature Coronary Artery Disease in Young (Age . 45 Years) Smokers: A Single Center Retrospective Case Control Study from India, Journal of Cardiovascular and Thoracic Research, 2014; 6(1): 15-9.
12. Yusuf S., Hawken S., Ounpuu S., Dans T., Avezum A., Lanas F., et al.: Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study, Lancet, 2004;364(9438):937-52.
13. BMI Classification". Global Database on Body Mass Index. World Health Organization. 2006. Retrieved July 27, 2012.
14. Chua S., Hung H., Shyu K., Cheng J., Chiu C., Chang M., et al.: Acute ST-elevation Myocardial Infarction in Young Patients:15 Years of Experience in a Single Center, Clinical Cardiology, 2010; 33(3):140-8.
15. Bhaskaran K1, Douglas I2, Forbes H2, dos-Santos-Silva I2, Leon DA2, Smeeth L3. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5·24 million UK adults. Lancet. 2014 Aug 30;384(9945):755-65. doi: 10.1016/S0140-6736(14)60892-8. Epub 2014 Aug 13.
16. Gadi Shlomai, MD, Eran Kopel, MD, MPH, Ilan Goldenberg, MD, Ehud Grossman, MD The association between elevated admission systolic blood pressure in patients with acute coronary syndrome and favorable early and late outcomes , December 01, 2014
17. Prabodh V., Chowdary N., Reddy R., Shekhar R., and Vidya D.: Lipid Profile Levels On The Second Day Of Acute Myocardial Infarction, International Journal of Pharmacological and Biological Sciences, 2012; 3(3): 245-50.
18. Srilakshmi P., Gopinath M., Bhaskar M., Rambabu K., and Reddy G.: Non-fasting samples for estimation of serum lipid levels in patients with coronary artery disease, Journal of Clinical Science Research, 2014; 3:206-9.
19. Mehmet Ali Kobata, e, Ahmet Celika, Mehmet Balina, Yakup Altasa, Adil Baydasb, Musa Bulutb, Suleyman Aydinc, Necati Daglib, Mustafa Ferzeyn Yavuzkirb, Selcuk Ilhand . The Investigation of Serum Vaspin Level in Atherosclerotic Coronary Artery Disease J Clin Med Res • 2012;4(2):110-113
20. Mehmet Ali Kobata, e, Ahmet Celika, Mehmet Balina, Yakup Altasa, Adil Baydasb, Musa Bulutb, Suleyman Aydinc, Necati Daglib, Mustafa Ferzeyn Yavuzkirb, Selcuk Ilhand . The Investigation of Serum Vaspin Level in Atherosclerotic Coronary Artery Disease. J Clin Med Res • 2012;4(2):110-113.
21. Shan-Shan Wu, Qiu-Hua Liang, Yuan Liu, Rong-Rong Cui, Ling-Qing Yuan, and Er-Yuan Liao, Omentin-1 Stimulates Human Osteoblast Proliferation through PI3K/Akt Signal Pathway, International Journal of Endocrinology Volume 2013 (2013), Article ID 368970, 6 pages
22. Xia ZHONG1, #, Hai-yang ZHANG1, #, Hui TAN1, Yi ZHOU1, Fu-li LIU1, Fu-qin CHEN2, *, De-ya SHANG1, *Association of serum omentin-1 levels with coronary artery disease 1department of Emergency, Provincial Hospital Affiliated to Shandong University, Ji-nan 250021, China; 2Department of Endocrinology, Qilu Hospital, Shandong University, Ji-nan 250012, China. 2011.
23. H.-Y. Pan, L. Guo, and Q. Li, “Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes,” Diabetes Research and Clinical Practice, vol. 88, no. 1, pp. 29–33, 2010

Table 1. Age and gender of Study groups.

		STEMI		Non-STEMI		Unstable Angina		Control		P value
		No	%	No	%	No	%	No	%	
Age (years)	<40	1	4.0	1	4.0	2	8.0	2	8.0	0.364
	40---49	9	36.0	4	16.0	4	16.0	9	36.0	
	50---59	4	16.0	8	32.0	9	36.0	9	36.0	
	=>60years	11	44.0	12	48.0	10	40.0	5	20.0	
	Mean ±SD (Range)	55.1±10.3 (39-70)		58.0±9.5 (36-70)		55.8±11.2 (27-70)		51.2±8.3 (37-65)		
Gender	Male	22	88.0	16	64.0	12	48.0	14	56.0	0.021*
	Female	3	12.0	9	36.0	13	52.0	11	44.0	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.										

Table 2. Clinical Examination (BMI and BP) of Study Groups

	STEMI		Non-STEMI		Unstable Angina		Control		P value
	No	%	No	%	No	%	No	%	
Normal (18.5-24.9)	7	28.0	4	16.0	5	20.0	7	28.0	0.052
Overweight (25-29.9)	7	28.0	9	36.0	5	20.0	14	56.0	
Obese (=>30)	11	44.0	12	48.0	15	60.0	4	16.0	
BMI(Kg/m2) Mean ±SD (Range)	28.2±5.6 (19.0-42.6)		28.8±3.8 (20.3-34.2)		31.5±7.0 (20.8-46.0)		27.3±4.6 (20.5-37.5)		
Weight (Kg) Mean ±SD (Range)	77.9±18.9 (50.0-132.0)		78.9±17.3 (56.0-121.0)		80.8±15.7 (58.0-112.0)		77.8±13.3 (53.0-110.0)		
Height (cm) Mean ±SD (Range)	165.6±7.3 (150-180)		164.7±10.5 (147-188)		160.9±8.9 (148-178)		168.9±9.8 (148-182)		
SBP (mmHg) Mean ±SD (Range)	127.5±24.7 (80-180)		127.6±25.1 (95-200)		122.4±25.4 (80-180)		118.6±7.0 (110-130)		0.410
DBP (mmHg) Mean ±SD (Range)	77.7±12.3 (60-110)		74.4±11.6 (55-100)		72.9±16.7 (50-115)		77.0±5.2 (70-90)		0.473
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.									

Table 3. Fasting Serum Lipid Profiles: in all Studied Groups. Results are expressed as Number (%) and p Value

		STEMI		Non-STEMI		Unstable Angina		Control	
		No	%	No	%	No	%	No	%
Total cholesterol (mg/dl)	Low	-	-	-	-	-	-	-	-
	Normal (100-199)	19	76.0	16	64.0	23	92.0	25	100
	High	6	24.0	9	36.0	2	8.0	-	-
	P value			0.003*					
Triglycerides (mg/dl)	Low	1	4.0	-	-	3	12.0	3	12.0
	Normal (65-149)	12	48.0	11	44.0	14	56.0	21	84.0
	High	12	48.0	14	56.0	8	32.0	1	4.0
	P value			0.004*					
HDL-C (mg/dl)	Low	13	52.0	17	68.0	12	48.0	12	48.0
	Normal (40-60)	11	44.0	8	32.0	11	44.0	6	24.0
	High	1	4.0	-	-	2	8.0	7	28.0
	P value			0.021*					
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.									

Table 4. Serum Levels of Omentin-1 in Patient with Acute Coronary Syndrome Compared to Controls.

Serum Omentin-1 (pg/ml)		STEMI	Non-STEMI	Unstable Angina	Control
	Number	25	25	25	25
	Mean ±SD	24.76±7.60	83.20±7.89	128.32±38.81	636.32±191.44
	Standard Error of Mean	1.521	1.578	7.762	38.289
	Range	(12.1-42)	(70-103)	(70-210)	(217-915)
	Percentile 05th	15	71	75	240
	25th	19	79	103	540
	50th (Median)	23	82	120	672
	75th	29	89	147	775
	95th	39	97	193	890
	99th	42	103	210	915
P value compare to Control		0.0001*	0.0001*	0.0001*	-
P value compared to Unstable Angina		0.0001*	0.0001*	-	-
P value compared to Non-STEMI		0.0001*	-	-	-
P value comparing all		0.0001#	-	-	-
*Significant difference between two independent means using Students-t-test at 0.05 level					
#Significant difference among three independent means using ANOVA test at 0.05 level					

Table 5. Significance of difference of serum Omentin-1 versus all study parameters composed among study groups and control.

Omentin-1	STEMI	Non-STEMI	Unstable Angina	Control
Age	0.004*	0.146	0.755	0.374
Gender	0.893	0.601	0.024*	0.300
BMI	0.0001*	0.003*	0.0001*	0.0001*
Family history	0.566	0.650	0.206	-
Smoking	0.161	0.578	0.082	0.722
Hb	0.056	0.025*	0.111	0.663
FBS	0.175	0.960	0.587	-
Creatinine	0.370	0.023*	0.462	-
Cholesterol	0.414	0.927	0.266	-
TG	0.038*	0.774	0.289	0.078
HDL	0.906	0.140	0.577	0.182
*Significant difference between two independent means using Students-t-test or ANOVA test for difference among three independent means at 0.05 level				

Table 6. Serum Lipid Profile components in Patient with Acute Coronary Syndrome Compared

	STEMI	Non-STEMI	Unstable Angina	Control	p-value
Total cholesterol (mg/dl)	181.9±45.9 (115-306)	185.2±38.6 (110-258)	161.8±28.5 (111-228)	161.3±19.9 (123-196)	0.021#
Triglycerides (mg/dl)	143.6±58.9 (56.0-326.0)	168.7±52.0 (79.0-303.0)	129.7±52.1 (49.0-244.0)	99.5±29.7 (51.0-152.0)	0.0001#
HDL-C (mg/dl)	40.5±11.3 (24.0-61.0)	36.4±8.7 (24.0-53.0)	41.8±13.5 (24.0-76.0)	47.0±13.5 (30.0-72.0)	0.021#
VLDL-C (mg/dl)	28.7±11.8 (11.2-65.2)	33.7±10.4 (15.8-60.6)	25.9±10.4 (9.8-48.8)	20.0±6.0 (10.2-30.4)	0.0001#
LDL-C (mg/dl)	112.6±43.8 (42.4-205.8)	115.0±35.5 (53.6-186.6)	94.0±29.4 (37.6-154.2)	94.4±20.0 (65.0-130.8)	0.039#
#Significant difference among three independent means using ANOVA test at 0.05 level					

Table 7. Serum levels of omentin-1 expressed as mean ±SD versus all study parameters: the number and type of patients (low, normal, high) in each group are shown and their means composed with that of controls.

		Serum Omintin-1 (pg/ml)							
		STEMI		Non-STEMI		Unstable Angina		Control	
		No	Mean ±SD	No	Mean ±SD	No	Mean ±SD	No	Mean ±SD
Age (years)	<40	1	19.00±	1	71.00±	2	156.50±75.66	2	845.00±63.64
	40---49	9	19.46±4.04	4	79.75±4.19	4	123.50±42.70	9	639.11±90.16
	50---59	4	23.00±4.69	8	81.75±5.20	9	123.00±38.90	9	631.22±246.28
	=>60years	11	30.27±7.47	12	86.33±9.15	10	129.40±34.80	5	557.00±227.17
Gender	Male	22	24.69±7.95	16	82.56±9.03	12	146.08±30.00	14	672.21±177.82
	Female	3	25.33±5.51	9	84.33±5.61	13	111.92±39.76	11	590.64±206.77
BMI (Kg/m2)	Normal (18.5-24.9)	7	32.71±6.65	4	92.75±9.46	5	174.20±36.02	7	818.43±78.59
	Overweight (25-29.9)	7	24.57±4.96	9	84.67±5.20	5	144.80±20.64	14	633.21±106.30
	Obese (=>30)	11	19.83±5.14	12	78.92±6.07	15	107.53±27.89	4	328.50±167.70
Family History	Positive	3	22.33±3.06	8	82.13±6.10	11	117.09±36.06	-	-
	Negative	22	25.10±8.02	17	83.71±8.73	14	137.14±39.87	25	636.32±191.44
Smoking	Smoker	15	23.01±7.26	11	82.18±8.32	6	152.33±28.56	5	608.20±234.82
	Non-smoker	10	27.40±7.71	14	84.00±7.76	19	120.74±39.10	20	643.35±185.54
Hemoglobin (g/dl)	Anemia	10	28.30±8.94	6	89.33±7.63	12	141.25±41.86	4	675.75±79.70
	Normal	15	22.41±5.73	19	81.26±7.09	13	116.38±32.93	21	628.81±206.54
FBS (mg/dl)	Low	-	-	-	-	-	-	-	-
	Normal (65-110)	13	26.77±8.46	10	83.10±4.43	15	131.87±40.28	25	636.32±191.44
Serum Creatinine (mg/dl)	High	12	22.59±6.19	15	83.27±9.70	10	123.00±37.95	-	-
	Low	-	-	-	-	-	-	-	-
	Normal (0.30-1.25)	23	24.35±7.79	22	81.91±6.86	21	125.76±40.40	25	636.32±191.44
Total cholesterol (mg/dl)	High	2	29.50±2.12	3	92.67±10.02	4	141.75±29.66	-	-
	Low	-	-	-	-	-	-	-	-
	Normal (100-199)	19	25.48±8.35	16	83.31±8.22	23	130.91±39.31	25	636.32±191.44
Triglycerides (mg/dl)	High	6	22.50±4.32	9	83.00±7.75	2	98.50±14.85	-	-
	Low	1	17.00±	-	-	3	157.67±76.40	3	726.67±89.54
	Normal (65-149)	12	28.58±7.95	11	83.73±9.87	14	129.21±33.62	21	642.29±184.56
HDL-C (mg/dl)	High	12	21.59±5.54	14	82.79±6.29	8	115.75±29.08	1	240.00±
	Low	13	24.85±8.56	17	81.59±6.61	12	119.67±34.40	12	563.17±236.84
	Normal (40-60)	11	24.36±7.05	8	86.63±9.68	11	135.64±43.16	6	688.33±130.42
	High	1	28.00±	-	-	2	140.00±52.33	7	717.14±92.50

Research Article

Diagnostic Sharpness of Ultrasound guided needle True -Cut biopsy in diagnosis of breast lesions

Sahar Basim Ahmed ,Theer Jwad Kadhim

Date Submitted: 30/11/2015
Date Accepted:10/1/2016

Address for Correspondence:
Dr. Sahar Basim Ahmed, Department of Radiology, Al-Yarmuk Teaching Hospital

Abstract

Objective : The objective of our study was to determine the diagnostic accuracy of sonographically guided core needle biopsy for breast masses **Materials and methods :** In this retrospective study , 65 patients undergo Ultrasound guided needle core biopsy for breast lesions performed between March 2013 and April 2015 at dedicated breast clinic in AL-Yarmook teaching hospital .The histological agreement between the core pathology and subsequent excision pathology , and 2 years follow up for cases of benign cases were studied .

Results: A sixty -five tru-cut biopsies were performed during the study period , histopathology show 55 (84.6) malignant lesions , 9 (13.8 %) benign lesions and 1 (1.5%) case inconclusive (suspicious) . Repeat True -cut biopsies were done on 2 of the malignant cases which confirmed to be malignant , 1 of benign cases also proved to be benign , and 1 inconclusive specimen was found to be malignant on repeat TCB . Trucut biopsy had a sensitivity of 98.2 % , specificity of 100% , positive predictive value of 100% ,negative predictive value of 90% , and diagnostic accuracy of 98.4 % .

Conclusion : Ultrasound guidance should be used to perform core biopsies in evaluating all breast abnormalities visible on ultrasound . Adherence to principles of triple assessment following biopsy allows for early recognition of the majority of false negative cases .

Key words: Ultrasound , True -Cut biopsy , breast lesions

INTRODUCTION

Breast cancer is the most common type of cancer in Iraqi women , breast screening is a method of detecting breast cancer at a very early stage . The first step is a mammogram that can detect small changes in breast tissue that may indicate cancers that are too small to be felt either by the women herself or by a doctor , most of the lesions detected by screening are not malignant . the suspicious abnormalities detected on mammography require further evaluation with biopsies . [1,2,3]

Nowadays ,percutaneous imaging breast biopsy is a reliable alternative to surgical biopsy for histological diagnosis . Percutaneous biopsy is less invasive than surgery , can be performed quickly , does not deform the breast , causes minimal scaring , complications (hematoma and infection) are infrequently found (less than one case in 1000) fewer surgeries are needed for patients who undergo percutaneous biopsies and therefore the cost of diagnosis is lower [4,5,6,7,8] .

Virtually any breast lesion that is clearly seen on Ultrasound can be sampled under Ultrasound guidance [9] .All lesions classified as BI-RADS 4 and 5 ,clearly visible on Ultrasound ar e amenable to Ultrasound CNB , this technique can also be used for some BI-RADS 3 lesions under certain circumstances : genetic or family risk , medical or social difficulties for follow up

,pregnancy ,extreme anxiety and others ,including the patient decision .[10.11]

The process of p of significant breast lesions involve the correlation of clinical imaging and the histological findings . This is best achieved with a multidisciplinary open forum with the clinician , radiologist and pathologist reaching a consensus on the management of each case using predefined protocols . The highest levels of diagnostics accuracy are achieved if such triple approach of imaging , clinical diagnosis and biopsy is used . In cases where cancer is reported such an approach allows for preoperative counselling of the women regarding treatment options and may assist in the planning of single stage surgery [12,13,14] .In cases where a benign diagnosis is reported or confirmed the need for excision biopsy is eliminated , the women can be reassured and appropriate management options discussed [15] .

There are many advantages of Ultrasound guidance include :

Ultrasound involve non ionizing radiation .

full control of the needle position in real time .

Ultrasound equipment is widely available and cost effective technique [16].

So Ultrasound -guided True-cut needle biopsy is a well tolerated and reliable procedure for providing a tissue diagnosis of malignancy before definitive treatment and obviating the need for formal excision biopsy of lesions for which there is a low index of suspicion [17] .As well as diagnostic objevtives , ultrasound guidance allow us to perform other intersting theraputics procedures such as evacaution of liquid or semi-solid collectins and placement of markers or coils for neoadjuvent chemotherapy . More recently , ultrasound guidance has been usefull for tumor ablation using radiofrequncy, cryoablation , laser therapy or focused ultrasound [18] . The complications of Ultrasound CNB are infrequent and not significant .Both hematomas and infections are very rare accounting for less than 1 /1000 biopsies [8] .The possibility of pneumothorax exists but it is very rare using the free -hand technique and a horizontal approach . Patients should be informed about a possible complications of the technique.

Aim of this research is to study the diagnostic value effectiveness of the true cut needle biopsy under Ultrasound guidance for suspicious breast lesion .

PATIENTS AND METHODS

This is retrospective study looking at Ultrasound guided automated true cut needles biopsies (TCNB) of breast lesions performed between March 2013 and April 2015 . sixty five patients attend a dedicated breast clinic at Al-Yarmook Teaching Hospital .Clinical findings were recorded as normal , benign , suspicious or malignant . Breast imaging either by mammography or Ultrasound was usually performed on the same day of patient attendance , with the TCNB performed within a few days of patients diagnosis . Radiological findings were also classified as benign , suspicious or malignant . True cut needle biopsy was performed under local anesthesia using a needle mounted in a automated firing device employing a spring loaded mechanism (Bard products) , this can be operated with one hand , facilitating simultaneous Ultrasound scanning performed using a 7.5 MHz linear array real time probe . The biopsy device automatically advances the cutting needle into the biopsy site , the needle tip appear as a bright echo and its track during biopsy is demonstrated to confirm that the correct area has been sampled . Usually three passes were made in each patient .

The approach to the lesion should be as parallel to the chest wall as possible to avoid pneumothorax . Moreover the transducer should be oriented parallel to the needle in order to facilitate the needle visualization .

The biopsy were fixed in 10% formalin and embedded in paraffin wax , and stained with haematoxylin and eosin for histological examination . Additional immunohistochemistry was performed on selected samples if felt to be appropriate .

The histology obtained on TCNB was compared with a final diagnosis obtained from subsequent excision biopsy, wide local excision or mastectomy .

Each true cut biopsy (TCB) diagnosis was matched with the histopathology results and labeled as follows : true positive (TP) when positive TCB result for malignancy is confirmed in the histological study of the post surgical specimen , false positive (FP) when positive TCB result for malignancy is not confirmed in the histological study of the post -surgical specimen , true negative (TN) when negative TCB result for malignancy is obtained and no carcinoma in the histological study of the post-surgical specimen is found ,and false negative (FN) when negative TCB result for malignancy is obtained but carcinoma is detected in the histological study of the post-surgical specimen .

sensitivity (SN) was measured as the proportion of patients with an associated carcinoma and a positive TCB result for malignancy . The formula used for sensitivity was ; SN = TP / (TP + FN) . While specificity (SP) was based on the proportion of patients without associated carcinoma and a negative TCB result for malignancy . the formula used for specificity was SP= TN/(TN +FP) . Positive predictive value (PPV) was

considered as the proportion of patients with a positive TCB result and histopathological confirmation of malignancy of the post surgical specimen , the formula used for positive predictive value was PPV = TP / (TP + FP) .Negative predictive value (NPV) was defined as the proportion of patients with negative TCB results and without carcinoma in the histological study of the post surgical specimen . the formula is NPV = TN /(TN + FN). Diagnostic accuracy (DA) was based on the proportion of patients diagnosed correctly using the diagnostic test .The formula used for diagnostic accuracy was DA = (TP+TN) / (FP +FN+TP+TN).

RESULTS

Sixty five TCB procedures were performed between June 2013 and July 2015 at AL-Yarmook teaching hospital in Baghdad . The median age of patients was 52 years old range (28 to 72 years) (table 1) . The histopathological diagnosis of the TCB specimen showed 55 cases (84.6 %) to be malignant lesions , 9 cases (13.8 %) as benign lesions and 1 case (1.5%) as inconclusive . A repeat TCB was done on 2 of the malignant cases , 1 of the benign cases also on the 1 inconclusive TCB specimens .Of the two malignant cases ,in which repeat TCB was performed , were found to be malignant on the second TCB . the result of the one benign case in which repeat TCB was performed , turned out to be benign also . The one inconclusive specimen was found to be malignant on repeat TCB . The indication for those repeat TCBs was inadequate samples or for confirmation of a diagnosis rendered on a scanty or hypocellualr specimen . Final diagnosis of TCB specimen after repeat TCB showed a total of 56 (86.1 %) malignant cases and 9 (13.9 %) benign cases .Comparison of core biopsy and final diagnosis on excision biopsy was made (Table 2) , there were 55 true-positive cases , 9 true negative cases , 1 false negative case and no false positive cases , TCB reveal a sensitivity of 98.2% , 100 % specificity , PPV of 100% , NPV of 90% , and DA of 98.4% , it also provided a definitive histological type and grade that correlate with the final histopathology report in 33 (60%) of the 55 malignant cases .

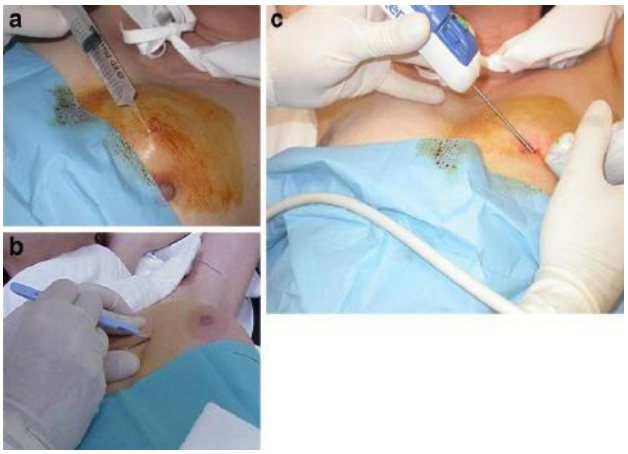
Table 1. Age distribution of the patients

Age	Number	Percentage
20-29	2	3 %
30-39	6	9.2 %
40-49	16	24.6 %
50-59	29	44.6 %
60-69	11	16.9 %
≥70	1	1.5 %

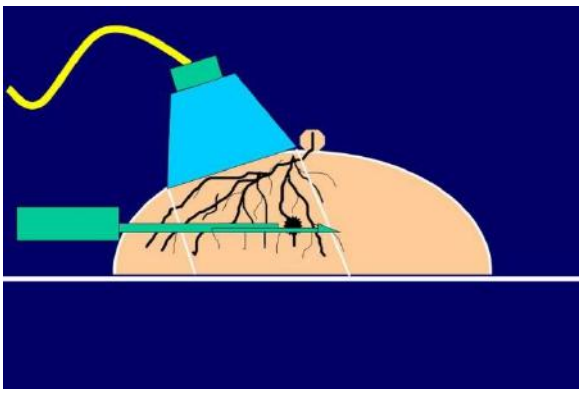
Table 2. Tru-Cut biopsy VS final histology

Findings	Core biopsy		Final diagnosis	
	Number	%	Number	%
Benign	9	13.8	9	13.9
Malignant	55	84.6	56	86.1
Inconclusive	1	1.5	-	-
Total	65	100.0	65	100.0

Types of true cut biopsy needles



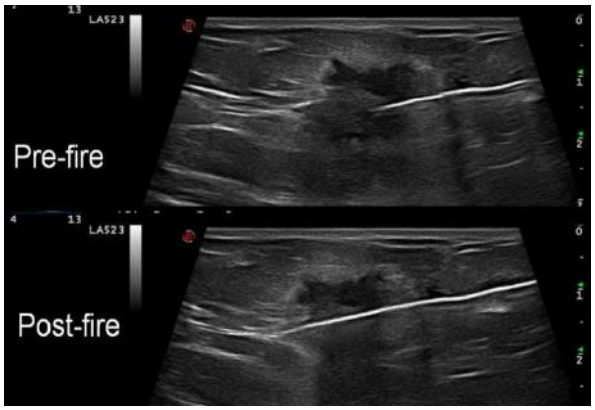
The few steps in performing Ultrasound guided needle guided biopsy after local anasthesia .



The needle passhorizontal to skin surface , nearly perpendicular to Ultrasound beam .

Table 3. Comparison of studies conducted to determine the usefulness of TCB in the diagnosis of breast mass.

Authors	Year	sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
Basim et al	**2015**	**98.2%**	**100 %**	**100%**	**90 %**	98.4 %
Husain and Rikabi [20]	**2013**	**98.1 %**	**100 %**	**100 %**	**98.9 %**	99.3 %
Lacambra et al [22]	**2011**	**96%**	**99%**	**99%**	**94 %**	-
Ahmed et al [24]	**2010**	**94.6%**	**91.3%**	-	-	94.8 %
Bdour et al [21]	**2009**	**97%**	**100 %**	-	-	-
et al [23] **Brunner**	**2009**	**95 %**	**100%**	**100%**	**90%**	-
Homesh et al [30]	**2005**	**92.3 %**	**94.8 %**	**100%**	**100%**	93.4 %
Gukas et al [31]	**2000**	**88.9 %**	**96.8 %**	-	-	93.5%



Inpre-fire :needle tip put in proximity to lesion margin to be sure that at post fire the needle cut from the margin & cenral core of the mass also .

DISCUSSION

Accurate preoperative assessment of breast cancer is crucial for treatment planning , including operative procedures as well as neoadjuvant preoperative therapies . Core needle biopsy has been shown to be an excellent tool , working with true tissue specimen using an automated firing gun under ultrasound control in conjunction with clinical and radiological assessment provides an accurate , reliable and safe of establishing the diagnosis of patients with breast lesions [19] .Our result yielded a high sensitivity of 98.2% with 100 % specificity and a PPV , NPV , and DA of 100% , 90 % , 98.4% respectively .In fact there were no false positive results .This means that TCB provides a breast cancer diagnosis with a high degree of confidence . Any patient with TCB results that are consistent with breast carcinoma should be referred to surgery and oncology for immediate management and treatment , compared to several studies that were conducted on the diagnostic usefulness of TCB in the diagnosis of breast masses [20,21,22,23] (table 3) . our specimens contained a single FN case (1.5%) of inconclusive result in compare to 1.7 % inadequacy rate has been reported [19] , 2.2 % [20] & 6.2 % [24] in other studies. After repeat TCB reveal a malignant specimen , which lower the diagnostic accuracy to 98.4 % , the most probable explanation for the false negative in the current study could be sampling error ,slide misinterpretation or small size of needle used , many studies used a 14 g needle size for biopsy and number of samplings per patient shown that a minimum of 4-5 cores are necessary to obtain definitive diagnosis and decrease the false negatives cores [25,26] .

however false negative diagnosis are unavoidable and may delay the diagnosis and treatment of breast cancer .For reducing them , the imaging -histological correlation is of critical importance in percutanous imaging -guided breast biopsy to confirm that tissue was retrieved from the target lesion [27,28,29]) .

The main disadvantage of Ultrasound CNB is the limitation of performing biopsy for lesions not seen on Ultrasound . Most clustered micro calcifications ,especially if they are not inside a mass , cannot be identified on Ultrasound . However high resolution transducer can demonstrate some clustered microcalcifications even in the absence of mass .

Although most Ultrasound CNB procedures are easy to perform , in some special situations (deeply located lesions , patients with implants , axillary lesions , etc) a high level of experience is needed to get reliable results

In conclusion, this study has shown that Ultrasound -guided biopsy is highly accurate in obtaining a histological diagnosis in breast cancer. We would recommend that this modality is used in patients who have breast lesions visible on Ultrasound. All BI-RADS 4 and BI-RADS 5 , and some BI-RADS 3 ones , should be percutaneously punctured ,and in cases with

benign results ,surgery can be avoided if there a good radiological - pathological correlation and no borderline result is obtained .Careful adherence to the principles of triple assessment following biopsy and the support of multidisciplinary review is essential to avoid delay in diagnosis of breast cancer in patients with false negative core biopsies .

REFERENCES

1. Thompson WR , Bowen JR , Dorman BA , et al. Mammographic localization and biopsy of nonpalpable breast lesions . Arch Surg 1991 ; 126 : 730-4 .
2. Fajardo LL , Pisano ED , Caudry DJ , Gatsonis CA , Berg WA , Connolly J , et al . Stereotactic and sonographic large - core biopsy of nonpalpable breast lesions . Acad. Radio. 2004 ; 11:293-308 .
3. Thurfjell E. Mammographically-guided fine needle aspiration in differential diagnosis of cystic versus solid rounded masses smaller than 2 cm detected at mammographic screening . Breast Cancer Res Treat 2002; 75:221-6 .
4. Liberman L. percutaneous imaging -guided core breast biopsy : state of the art at the mellennium. AJR Am J Roentgenol . 2000 ; 174 : 1191-1199.[PubMed].
5. Parker SH , Lovin JD , Jobe WE Luethke JM , Hopper KD , Yakes WF et al. steriotactic breast biopsy with biopsy gun.Radiology.1990;176:741- 747 .[PubMed] .
6. Liberman L. Percutaneous image -guided core breast biopsy , Radiol Clin North Am. 2002 ; 40-483 -500 .doi:10.1016/S0033- 8389(01)00011-2.9 [PubMed] .
7. Parker SH . Percutaneous large core biopsy . Cancer . 1994; 74 :256- 262. doi : 10.1002/cncr.2820741309.[PubMed] .
8. Parker SH, Burbank JRJ , et al. Percataneous large core breast biopsy : a multi-institutional study . Radiology . 1994 ; 193 : 359-364 .
9. Schueller G, Schueller - Weidekamm C , Helbich TH. Accuracy of Ultrasound -guided large -core needle breast biopsy . Eur Radiol. 2008 ; 18:1761-1773. doi: 10.1007/s00330-008-0955-4. [Pub Med].
10. Smith DN, Rosenfield Darling ML , Meyer JE , et al . The utility of Ultrasonographically guided large -core needle biopsies . J Ultrasound Med. 2001; 20: 43 -49 .[Pub Med] .
11. Schoonjians JM, Brem RF. Fourteen-gauge ultrasonographically guided large core needle biopsy of breast masses . J Ultrasound Med.2001; 20: 967 - 972 .[Pub Med] .
12. Apestegu , a L , Pina L, Inchusta M , Mellado M , et al . Non palpable well defined probably benign breast nodule : management by fine needle aspiration biopsy and long interval follow up mammographically . Eur Radiol 1997 ; 7:1235-9 .
13. Collacol M, Delima RS , Werner B , Torres IFB . Value of fine needle aspiration in the diag of breast lesions .Acta Cyto 1999; 43:587-92 .
14. Howat AJ ,Hoda RS , Correspondence . Why pathologists should take needle aspiration specimens . Cytopathology 1995; 6:419-20.
15. 15. Rimm DL , Stastny J , Rimm EB , Ayer S , et al . Comparison of the costs of fine needle aspiration and open surgical biopsy as methods for obtaining a pathological diagnosis . Cancer 1997; 81:51-6.
16. 16. Liberman L , Feng TL , Dershaw DD , Morris EA , et al . US - Guided core breast biopsy : use and cost - effectiveness .Radiology .1998 ; 208: 717 -723. [Pub Med] .
17. 17. Woodcock NP , Glaves I , Morgan DR , et al .Ultrasound -guided True cut biopsy of the breast . Ann R Coll Surg Engl. 1998 July 80 (4):253 - 256.
18. 18. Esser S, Bosch MA , Diest PJ , Mali WJ, et al . Minimally invasive ablative therapies for invasive breast carcinomas : an over veiw of current literature .World J Surg . 2007 ; 31:2284-2292. doi:10.1007/s00268-007-9278 .
19. 19. Mary F . Dillon , Arnold D. K. Hill , Cecily M. Quinn ,etal . The Accuracy of Ultrasound , Stereotactic , and Clinical Core Biopsies in the Diagnosis of Breast Cancer, with an Analysis of False - Negative cases .Ann Surg. 2005 Nov; 242(5):701-707 .
20. 20. Ammar Rikabi and Sufia Hussain . Diagnostic Usefullness of True-C ut biopsy in the diagnosis of breast lesions . Oman Med J. 2013;28(2):125-127.
21. Bdour M ,Hourani S , Mefleh W ,Shabatat A, et al . Comparison between fine needle aspiration cytology and true cut biopsy in the diagnosis of breast cancer . J Surg Pak 2008 ; 13(1):19-21 .
22. Lacambra MD , Lam CC ,Mendoza P , Chan SK , et al .Biopsy sampling of breast lesions : Comparison of core-needle and vacuum -assisted breast biopsies . Breast Cancer Res Treat 2012 April; 132(3):917-923.
23. Brunner AH , Sagmeister T , Kremer J , Riss P , et al . The accuracy of frozen section analysis in ultrasound - guided core needle biopsy of breast lesions . BMC Cancer 2009; 24:9:341 .
24. Ahmed ME, Ahmed I , Akhtar S. Ultrasound guided fine needle aspiration cytology versus core biopsy in the preoperative assessment of non-palpable breast lesions . J Ayub Med Coll Abbottabad 2010 Apr 22(2):138-142 .
25. Dennison G , Anand R , Maker SH, et al . A Prospective study of the use of fine needle aspiration and core biopsy in the diagnosis of breast cancer .Breast J 2003; 9:491-493 .
26. Fishman JE ,Milikowski C , Ramsinghani R , et al . US-guided core needle biopsy of the breast : how many specimens are necessary ? Radiology .2003 ; 226 : 779 -782 .
27. Mainiero MB, Koelliker SL ,Lazarus E , et al.Ultrasound -guided large core needle biopsy of the breast : frequency and result of repeat biopsy . J Women ,s imaging 2002 ; 4:52-57 .
28. Schoonjans JM , Brem RF. Fourteen -gauge ultrasonographically guided large core needle biopsy of breast masses .J Ultrasound Med 2001 ; 20:967 -972 .
29. Crystal P , Koretz M , Shcharynsky S , Makarov V , et al .Accuracy of sonographically guided 14-gauge core needle biopsy : results of 715 consecutive breast biopsies with at least two-year follow up of benign lesions . J Clin Ultrsound 2005 ; 33 :47-52 .
30. Homesh NA, Issa MA , El-Sofiani HA. The diagnostic accuracy of fine needle aspiration cytology versus core needle biopsy for palpable breast lump . Saudi Med J 2005 Jan;26(1):42-46.
31. Gukas ID, Nwana EJ , Ihezue CH , Momoh JT , etal .Tru-cut biopsy of palabaple breast lesions :a practical option for pre-operative diagnosis in developing countries .Cent Afri J Med 2000 May ;46(5):127-130 .

Research Article

The effect of maternal age on the outcomes of in vitro fertilization Sulaimani region

Shilan Hussein Karim

Date Submitted: 13/9/2015
Date Accepted:12/1/2016

Address for Correspondence:
Department of Anatomy and Embryology, School of Medicine, University of Sulaimani.
shilanmed@yahoo.com
shilan.karim@univsul.edu.iq

Abstract

Background In Vitro fertilization IVF can be a very effective fertility treatment. However, we need to have sufficient eggs of sufficient quality in order to make it successful. Both egg quantity and egg quality are very much related to female age A woman's ability to conceive a child reduces with age, on average, the younger the women have the higher chances of success.

Objectives This work is intended to study the impact of maternal age on the outcome of IVF resulting in biochemical pregnancy in Sulaimani region.

Methods This retrospective study has being done in Dwarozh IVF private center in Sulaimani, between December 2008 and January 2011 on 125 women. All of these women had undergone thorough IVF treatment according to established protocols. According to maternal age the women were divided in to group 1 below 35 years and group 2 from 36 years and above.

For each cycle number of retrieved oocyte, injected oocyte, fertilization, number of embryo transfer, quality and grading of the embryo with the result of biochemical pregnancy has been studied.

Then comparison between the two maternal age groups has been done to show the significance of maternal age on the outcome of IVF.

Results For the Group 1(G1), the effect of maternal age on Fertilizes oocytes is significant, as the T-value is 37.035577. The P-Value is < .00001. The result is significant at p < .05.and the percentage of positive biochemical pregnancies was **45.83%.**

While for Group 2 (G2) the effect of maternal age on fertilized oocyte is significant, as the T-value is 58.997473. The P-Value is < .00001. The result is significant at p < .05 and the percentage of biochemical pregnancies were **33.96%**

Conclusion Maternal age has significant effect on the number of fertilized oocytes and percentage of biochemical pregnancy for the IVF process. The percentage of biochemical pregnancy is more in age group less than 35 years.

ABBREVIATIONS: IVF: In vitro fertilization, Oocyte R: oocyte retrieval, MI: Meiosis I, MII: Meiosis II, GV: Germinal vesicle, ET: embryo transfer, Bhcg: Beta human chorionic gonadotropin.

Key words: In vitro fertilization, Maternal age, Fertilization, Biochemical pregnancy.

INTRODUCTION

Ovarian function declines as women approach their later reproductive years until menopause, and increasing age is associated with lowered fecundity and infertility. Women experience a decline in natural fertility that begins in the mid-30s, and they will often reach sterility many years before the complete cessation of menses. Although IVF may aid some couples who present with fertility issues, it

will not compensate for the decline in natural fertility that occurs with delayed child-bearing.IVF is also invasive, expensive, and not covered by most provincial health plans for this indication. In addition,

complications of pregnancy increase for both the mother and the offspring with advanced maternal age (1). The age at which women should be advised against proceeding with initial or further infertility treatment is one of the many unresolved questions in this area of women's health and was the subject of investigation in this study (2).

There is no universal definition of advanced reproductive age in women, in part because the effects of increasing age occur as a continuum, rather than as a threshold effect. Fertility clearly declines with advancing age, especially after the mid-30s, and women who conceive are at greater risk of pregnancy complications (3).Advanced maternal age, in a broad sense, is the instance of a woman being of an older age at a stage of reproduction, although there are various definitions of specific age and stage of reproduction. The variability in definitions regarding age is in part explained by the effects of increasing age occurring as a continuum rather than as a threshold effect. The most commonly used definition of advanced maternal age is 35 years or more at the time of childbirth. Assisted reproductive technologies have helped many families to have their healthy offspring during the past decades. *In vitro* fertilization and embryos transfer (IVF-ET) has become increasingly popular and the pregnancy rate was improved by the development of the technology (3, 4). Many women in advanced age want to have babies through IVF. However, it has been reported that one of the main limiting factors to fertility and reproductive outcomes was female's age (5, 6). Women over 38 years old had poor IVF outcomes, especially in women over 40 years old (7). Then which age period is the best period for pregnancy? Are IVF outcomes worse in women between 30 and 35 years old than those in women under 30 years old? Not many papers mentioned this.

In this study, we examined the difference of IVF outcomes between two maternal age groups, 35 and below (G1) with 36 years and above (G2). There are a lot of studies conducted on the effect of maternal age on the outcome of IVF, as a study done by YAN JunHao (2012) in China concluded that patients with higher maternal age had worse IVF outcomes. In women of fertile age, patients between 20 and 30 years old have the best IVF outcomes. Patients over 40 years old have poor IVF outcome and high miscarriage rate, which suggested the necessity of preimplantation genetic screening (PGS) (8).

In other study the results indicated that maternal age adversely affects both clinical pregnancy rates and rates of spontaneous abortion, when summed across treatments and stimulation protocols. (9).

Other study conclude that To determine true efficacy, success rates have to be related to patient characteristics, such as age and cause of infertility (10).

PATIENTS AND METHODS

All women undergoing IVF in Dwarozh IVF private center in Sulaimani, between December 2008 and January 2011 were included in the retrospective study. All of these women had undergone thorough IVF treatment according to established protocols.The protocol includes:

1. **Ovarian stimulation**: done by gynaecologist starting from the second day of cycle by transvaginal ultrasound and hormonal assay (FSH,LH).Induction by GnRH (Gonadotropin), then follow up by ultrasound, until it show more than 4 follicles with diameter of 16-17mm then administration of HCG (Human chorionic gonadotropin) that assists maturation of ova.
2. **Oocyte retrieval**: Done after 34-36 hours after HCG administration by transvaginal ultrasound ,the oocyte cumulus complex collected under stereo microscope, and washed with flushing media then placed in incubator 37 C and 6% CO2 for 1-2 hours.
3. **Denudation of the oocyte**: Removal of surrounding cumulus cells is accomplished by combined enzymatic exposure to hyaluronidase enzyme in buffered media and mechanical treatment under a stereo microscope. Each oocyte then examined under microscope to assess maturation stage and its integrity. Metaphase MII was assessed according to the absence the germinal vesicle (GV) and the presence of an extruded polar body . Metaphase MI was assessed according to the absence of both GV and extruded polar body and prophase PI when GV was obvious in the absences of extruded polar body.
4. **Sperm preparation:** the collected semen allowed to be liquefy for 15-30 minutes, followed by centrifugation and incubation.
5. **ICSI procedure (intracytoplasmic sperm injection):** was carried out on heated stage (37C) of an inverted microscope 40 hours after HCG trigger for MII stage retrieved oocytes and 64 hours after HCG trigger for immature oocytes that had undergone nuclear maturation.After injection ,the oocyte were washed and stored in culture media and kept in incubator at 37Cfor 16-18 hours.

6. **Assessment of fertilization, embryo quality and embryotransfer:** At 16-18 hours after microinjection, the oocytes were checked for survival and fertilization. The criteria for normal fertilization are the presence of two clearly visible pronuclei, embryo cleavage and quality are evaluated 2 days after ICSI. Embryo transfer was performed on day 2 or 3 .For each couple ,one to three embryos are transferred for female age below 35 years old and more than three embryos for females more than 35 years old. Then cycles followed by serum B-HCG and ultrasound for confirmation of Pregnancy. Biochemical pregnancy was verified by plasma -HCG>20 IU L 14 days after embryo transfer.

Patients

A total of 125 IVF-ET cycles were included in this study. The IVF outcomes were analyzed in two different groups sorted by the age period. The groups were G1 that include 35 years old and below (72 cycles), and G2 were 36 years and above (53 cycles).

IVF outcomes

The means of age, retrieved oocyte, MII, MI, injected and fertilized oocyte, and number of embryo transfer and rate of biochemical pregnancy by BHCG were compared between the two age groups.

Ethics

This study was a retrospective analysis on the clinical practice outcomes and the data of our study were approved by scientific and ethics committee of school of medicine in university of Sulaimani.

Statistical analysis

For G1, the T-value is 37.035577. The P-Value is < .00001. The result is significant at p < .05, between maternal age (less than 35) and the fertilized oocytes. Also the percentage of positive biochemical pregnancies (that are detected by BHCG) was 45.83%.For G2 the T-value is 58.997473. The P-Value is < .00001.

The result is significant at p < .05. between maternal age (more than 35) and the fertilized oocytes. Also the percentage of positive biochemical pregnancies (that are detected by BHCG) was 33.96%.

RESULT

A total of 125 IVF-ET cycles were included in this study. The IVF outcomes were analyzed in two age

Groups, G1: 35 years old and below (72 cycles), and G2 were 36 years and above (53 cycles).There is a significant effect of maternal age on numbers of fertilized oocytes which leads to high percentage of positive biochemical pregnancy in IVF procedure.

Table 1.Showing the results for Group 1 and Group 2

	Group 1 Mean +SD	Group 2 Mean +SD
Age	28.98+3.75	39.16+2.41
Oocyte Retrival	11.26+5.45	8.07+5.76
MII	8.37+4.81	5.94+4.8
MI	1.83+1.28	1.57+0.79
GV	2.09+1.42	1.77+1.26
Injected oocyte	8.37+4.81	5.94+4.8
Fertilized	5.77+3.77	4.2+3.56
No. of ET	3.08+1.12	2.73+1.3
Grade A	1.26+1.18	0.88+0.89
P-value	< .00001	< .00001
Bhcg	45.83%	33.96%

Table 2. Showing the sum and percentages of data for 35 years and below G1

N=72	Oocyte Retrived	MII	MI	GV	Injected Ovum	Fertilized	Bhcg
Sum	811	603	57	65	603	416	33
%		74.35	7.02	8.01		68.98	45.83

Table (3) Showing the Sum and percentage of data of 36 years and above

N= 53	Oocyte Retrived	MII	MI	GV	Injected Ovum	Fertilized	Bhcg
Sum	428	315	44	32	315	228	18
%		73.59	10.28	7.47		72.38	33.96

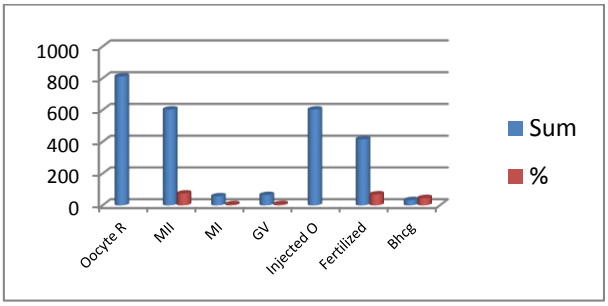


Figure 1. Showing the sum and percentages of the parameters for Group 1

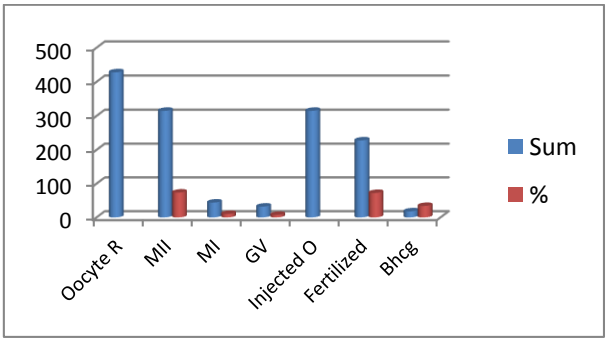


Figure 2. Showing the sum and percentages of the parameters for Group 2

DISCUSSION

Ovarian aging will have begun before women notice any clinical changes to their menstrual cycles; therefore, they are often unaware that they may be at greater risk of infertility.Ovarian reserve testing has been explored as a means to determine a woman's fertility potential and provide an assessment of ovarian aging. Although chronological age alone serves as a good marker of ovarian reserve, some women will experience a decline in their natural fertility sooner than average, while some older women may

maintain above average ovarian function. Identification of these two groups, in which ovarian reserve is inconsistent with chronological age, may be useful for counselling and planning treatment.

There are increased rates of obstetrical and maternal complications with increasing maternal age, including maternal death, hypertension, prematurity, fetal and neonatal death, and operative delivery (1).

It is recognized by many reporters that with the increasing of maternal age, the IVF outcomes become increasingly worse (4). Some IVF centers even limited the last age for IVF as 43 years old (8), Women with advanced maternal age will have poor ovary response during controlled ovary hyperstimulation (COH), low retrieved oocyte number, low oocyte fertilization rate, low good quality embryo rate,

low embryo implantation rate, low pregnancy rate, high miscarriage rate, high preterm delivery rate and high birth defect rate (9,12,13,14).

We found that first of all, in current study, the fertility significantly dropped in women over 35 years old, as shown in table 2 and 3.There is a slow decline in pregnancy rates in the early 30's, The decline is more substantial in the late 30's and early 40's, Very few women over 44 are still fertile. Miscarriage rates also increase as the mother ages. Although there are a high percentage of fertilized oocyte in G2 (72.38%) but the positive biochemical pregnancy percentage are lower than G1 (33.96%) as shown in the Fig.(1 and 2) .This result is going with other study done in China by Liu K, Case A (15).

Advanced maternal age may cause the aging of oocyte which will result in abnormal fertilization and development, such as polyspermy, division arrest, implantation failure and miscarriage.

In addition, It is reported that genetic abnormalities, uterine anatomical defects, antiphospholipid antibodies, environmental factors, advanced maternal age, obesity, endometriosis, etc. may be the possible causes of miscarriage in natural and assisted conceptions (16).

To draw the conclusion, patients with higher maternal age had worse IVF outcomes. Patients between 20 and 30 years old have the best IVF outcomes in women of fertile age. Patients over 40 years old have really poor IVF outcomes and high miscarriage rate, which suggested the necessity of preimplantation genetic screening (PGS) (9).

Conclusion

Maternal age has significant effect on the number of retrieved oocyte, and the outcome of Biochemical pregnancy in IVF process.

REFERENCES

1. Kimberly Liu, MD, Toronto ON ,Allison Case, MD, Saskatoon SK ,Advanced Reproductive Age and Fertility, No. 269, SOGC Clinical Practice Guidelines, November 2011.
2. Kenny DT, In vitro fertilisation and gamete intrafallopian transfer: an integrative analysis of research, 1987-1992. Br J Obstet Gynaecol. 1995 Apr; 102(4):317-25.
3. Salihu HM, Shumpert MN, Slay M, et al. Childbearing beyond maternal age 50 and fetal outcomes in the United States. Obstet Gynecol 2003; 102:1006.
4. Gnoth C, Maxrath B, Skonieczny T, *et al*. Final ART success rates: a 10 years survey. Hum Reprod, 2011, 26: 2239–2246
5. De Mouzon J, Goossens V, Bhattacharya S, *et al*. Assisted reproductive technology in Europe, 2006: results generated from European registers by ESHRE. Hum Reprod, 2010, 25: 1851–1862
6. Balasch J. Ageing and infertility: an overview. Gynecol Endocrinol,2010, 26: 855–860.
7. Tatone C. Oocyte senescence: a firm link to age-related female subfertility. Gynecol Endocrinol, 2008, 24: 59–63
8. Hassold T, Chen N, Funkhouser J, *et al*. A cytogenetic study of 1000 spontaneous abortions. Ann Hum Genet, 1980, 44: 151–178
9. YAN JunHao†, WU KeLiang†, TANG Rong, DING LingLing & CHEN Zi-Jiang, Effect of maternal age on the outcomes of in vitro fertilization and embryo transfer (IVF-ET), SCIENCE CHINA, Life SciencesAugust , 2012 Vol.55 No.8: 694–698
10. Dianna T. Kenny PhD, The Impact of Maternal Age on Clinical Pregnancy an Spontaneous Abortion in Women Undergoing In Vitro Fertilization and Gamete Intra-Fallopian Transfer, Australian and New Zealand Journal of Obstetrics and Gynaecology, Vol 34 Issue 4.
11. Feichtinger W Results and complications of IVF therapy. Curr Opin Obstet Gynecol. 1994 Apr;6(2):190 - 7
12. Hourvitz A, Machtinger R, Maman E, et al. Assisted reproduction in women over 40 years of age: how old is too old? Reprod Biomed Online, 2009, 19: 599–603
13. Thum M Y, Abdalla H I, Taylor D. Relationship between women's age and basal follicle- stimulating hormone levels with aneuploidy risk in in vitro fertilization treatment. Fertil Steril, 2008, 90: 315–321
14. Griffiths A, Dyer S M, Lord S J, et al. A cost-effectiveness analysis of in-vitro fertilization by maternal age and

number of treatment attempts. Hum Reprod, 2010, 25: 924–931

15. Liu K, Case A. Advanced reproductive age and fertility. J Obstet Gynaecol Can, 2011, 33: 1165–1175.

16. Nybo Andersen A M, Wohlfahrt J, Christens P, et al. Maternal age and fetal loss: population based register linkage study. BMJ, 2000, 320: 1708–1712

Research Article

Apolipoproteins and lipid profile in patients with oral diseases and systemic arterial hypertension

Huda Shakir Ahmed *B.D.S, M.Sc.*, **Noor Thair Tahir** *Ph.D*, **Hind Shakir Ahmed** *Ph.D*

Date Submitted: 10/6/2015
Date Accepted:13/1/2016

Address for Correspondence:
Department of Oral Surgery, College of Dentistry/ University of Baghdad,

Abstract

Background: Systemic arterial hypertension and dyslipidemia are major risk factors for cardiovascular disease, and have been shown to be associated with oral diseases.
Objective: The objective of this study was to estimate apolipoproteins and lipid profile in patients with oral diseases and systemic arterial hypertension.
Methods: This study was conducted at Medical City Hospital during the period from October 2014 until the end of April 2015. Sixty arterial hypertensive patients were enrolled in this study; their age range was (38-50) years and they were compared with 30 healthy subjects as control group. Apolipoproteins A1, B, and lipid profile were estimated for each subject.
Results: The serum levels of apolipoprotein B, total cholesterol, triacylglycerol, and low density lipoprotein cholesterol were higher while apolipoprotein A1 and high density lipoprotein cholesterol levels were lower in hypertensive patients with oral diseases as compared to the control, which was statistically significant ($P < 0.05$).
Conclusion: Hypertensive patients with oral diseases had dyslipidemia and need measurement of blood pressure and lipid profile at regular intervals to prevent comorbidities.

Key words: Oral diseases, Hypertension, Dyslipidemia.

INTRODUCTION

Arterial hypertension (HTN) was defined as a blood pressure (BP) ≥140 mmHg and/or ≥90 mmHg, and/or use of antihypertensive medication [1]. Hypertension occurrence varies by age, race, education, and so forth [2, 3].Patients with arterial HTN are at increased risk of developing adverse effects with dental diseases [4]. Periodontal disease is often modified by systemic diseases [5]. The periodontal diseases are a group of chronic inflammatory diseases, involving the soft tissue and bone surrounding the teeth in the jaws. Periodontal diseases are characterized by inflammation of tooth-supporting tissues caused by bacterial infection [6]. Gingivitis is a very frequent reversible condition, which may progress into periodontitis with further destruction of periodontal tissues ligament [7]. This process is attributed to the release of toxic products from the pathogenic bacteria plaque in addition to the inflammation of gingival tissues elicited by the host response. Hypertension increases the risk of a range of adverse cardiovascular (CV) events such as atherosclerosis, stroke, and coronary heart disease [8].

It is well identified that arterial hypertension and periodontitis share common risk factors, namely, smoking, stress, increased age, and socioeconomic factors. However, according to the scientific statement issued by the American Heart Association (AHA) published in circulation, observational studies support an association between periodontal disease and cardiovascular disease (CVD), independent of shared risk factors [9]. There are also some studies that have been focused on antihypertensive treatment and oral diseases stating that patients under antihypertensive treatment show positive effects on periodontitis i.e. they have increased number of pockets and there is a linear trend between periodontal disease severity and antihypertensive treatment [10]. Recent studies have shown that the inflammatory property of periodontal disease help to promote blood clot formation in arteries [11]. Moreover, periodontitis patients also were found to

have higher levels of plasma oxidized low density lipoprotein (LDL) levels, which means higher risk of developing atheroma plaque [12].Apolipoprotein B (apo B) is the major proteins of LDL, and apo A1 is the major protein of high density lipoprotein (HDL). Because of their associations with the respective lipoproteins, apo B is positively and apo A1 is inversely associated with CV risk [13].The aim of the present study was to estimate apolipoproteins and lipid profile in patients with oral diseases and systemic arterial HTN.

PATIENTS AND METHODS

This study was conducted at the Medical City Hospital during the period from October 2014 until the end of April 2015. Sixty hypertensive patients were enrolled in this study; their age range was (38-50) years and they were compared with 30 healthy subjects as control group.

Oral Examination:Oral manifestations mainly observed were gingivitis, periodontitis, lichenoid reactions, hyposalivation, and facial nerve paralysis. Gingivitis and periodontitis were confirmed from patients Russell's periodontal index, and hyposalivation was noted by asking questions to the hypertensive patients regarding symptoms [14].

Measurements: Body mass index (BMI) was calculated as weight in kilograms, divided by height in meters squared (kg/m^2) and waist circumference (WC) was measured from every individual. Blood pressures were recorded according to the guidelines adopted by WHO [15]. Hypertension was defined as an average systolic blood pressure (SBP) ≥140 mmHg and diastolic blood pressure (DBP) ≥90 mmHg without antihypertensive medication according to the 7th report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-7) [16].

Biochemical Analysis: About 5 milliliters of blood sample was obtained from every patient and control. The separated serum was used for measurements of apolipoproteins and lipids. Apo A1 and apo B were measured by turbidimetry while lipids were estimated by enzymatic colorimetric methods.

Statistical Analysis: Statistical analysis was done by using an excel program version 16. T-test was used to estimate P value. P value less than 0.05 was set to indicate significant difference.

RESULT

Oral manifestations in hypertensive patients are shown in table (1) and figure (1). Around 85.5% of patients presented with gingival bleeding on probing and were characterized by redness of the marginal gingiva. 15.3% of patients presented with hyposalivation. 3% of the total patients presented with lichenoid reactions and they were characterized by linear striations occurring on the buccal mucosa. 1.5% of patients presented with facial nerve paralysis and 15% patients presented with gingival enlargement characterized by firm nodular gingival overgrowth seen on both buccal and facial and lingual or palatal aspects of the marginal gingival. 80.5% of the patients under study presented with Russell's periodontal index score ranging from 2-4.9.

Distribution of demographic and medical variables in hypertensive group is shown in table (2). Hypertensive patients had high levels of apo B, TC, TAG, and LDL-C ($P< 0.05$) while had a low levels of apo A1 and HDL-C ($P= 0.05$), table (3).

Table 1. Oral manifestations in hypertensive patients

Parameter	Hypertensive
Number	60
Gingival Bleeding	85.5 (51%)
Hyposalivation	9 (15.30%)
Lichen Planus	2 (3.0%)
Facial Paralysis	1 (1.50%)
Gingival Enlargement	9 (15.0%)
Peridontitis	48 (80.50%)

Table 2. Distribution of demographic and medical variables in hypertensive group

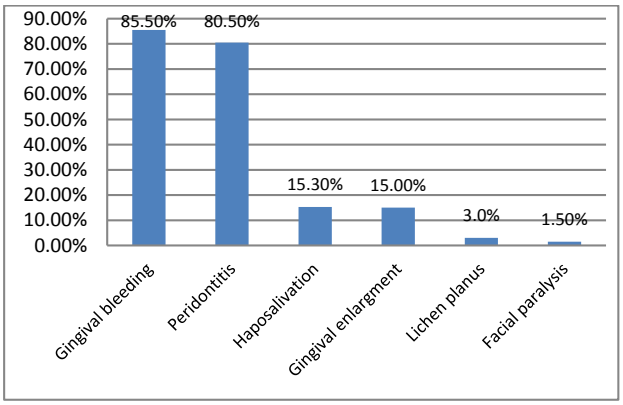
Parameter	Frequency (%)
Demographic Variables:	
- Age	
38-50	40 (67%)
50-56	20 (33%)
- Gender	
Male n. (%)	30 (50%)
Female n. (%)	30 (50%)
- Duration of Hypertension	
2-5 years	45 (75%)
5-10 years	15 (25%)
- Stage of Hypertension	
Stage 1	20 (33%)
Stage 2	25 (42%)
Stage 3	15 (25%)
Medical Variables:	
- BMI	
< 30 kg/m^2	40 (67%)
> 30 kg/m^2	20 (33%)
- WC	
≤ 85 cm	42 (70%)
≥ 90 cm	18 (30%)
- Drugs Uses	
Diuretics	9 (15%)
β-blockers	11 (18%)
Vasodilators	40 (66%)

Table 3. Biochemical characteristic of hypertensive and control group

Clinical Data	Hypertensive	Control	P Value
Age (Years)	47.0 ± 9.15	42.38 ± 3.60	0.001
SBP (mmHg)	147.12 ± 8.20	118.21 ± 5.30	0.001
DBP (mmHg)	98.0 ±7.39	75.0 ±5.22	0.012
BMI (kg/m^2)	28.20± 3.90	26.70± 4.30	0.15
WC (cm)	87.01 ± 3.89	75.20 ± 5.14	0.001
Apo A1 (mg/dl)	138.0± 2.50	174.0± 2.30	0.001
Apo B (mg/dl)	145.20 ± 3.80	68.50 ± 3.45	0.001
TC (mg/dl)	194.20 ± 6.42	169.70 ± 19.20	0.01
TAG (mg/dl)	194.20 ± 9.49	128.50 ± 14.0	0.001
HDL-C (mg/dl)	40.80 ± 7.20	55.0 ± 4.60	0.05
LDL-C (mg/dl)	115.70 ± 5.96	89.1 ± 18.50	0.003
VLDL (mg/dl)	38.0±19.0	26.50 ± 5.30	0.05
Duration of Hypertension (Years)	6.0±4.5	-	-

*** P < 0.05**

Figure 1. Oral manifestations observed in hypertensive patients



DISCUSSION

Hypertension or arterial HTN is a chronic medical state in which the BP in the arteries is elevated [17]. Gingival bleeding was one of the frequent clinical features seen in hypertensive patients with 80.5% of the hypertensive patients presented with the symptoms of periodontitis, which was similar to the results of Maiborodin et al., study [18].

One of the main risk factors for hyposalivation is the use of certain medications. In addition, polypharmacy has been revealed to significantly influence patients' saliva flow [19]. Hyposalivation was related to elevating BP and in patients who were under antihypertensive medication especially with diuretics such as thiazides and calcium channel blockers [20].

The results of this study showed that 15.3% of hypertensive patients who were under medication with diuretics presented with hyposalivation. These results were similar to the study of Glick et al., [21].

Gingival enlargement is also one of the most frequent clinical finding in patients with HTN taking antihypertensive medication particularly calcium channel blockers which is in agreement with the study of Andrew et al., [22]. Gingival enlargements appear clinically as an excessive growth of the gingiva which may occur as a side-effect of systemic medications [23].

Lichen planus are white lesions characterized by linear striations occurring on the buccal mucosa. These are sometimes seen in hypertensive patients as a manifestation secondary to the use of the drug or medication [24]. The results of this study showed 3% of hypertensive patients with lichen planus and as their investigation, it is supported the hypothesis of relationship between lichen planus and HTN. The results were parallel to the study of Harting and Hsu [25].

Facial nerve paralysis in HTN is because of edema or hemorrhage in the facial canal, but the exact etiology is unknown. Usually facial nerve paralysis is seen in patients with malignant hypertension. It is characterized by the sustained increase in SBP ≥ 200 mmHg and/or sustained increase in DBP ≥ 120 mmHg. The results of the present study were similar to the study results of Margabanthu et al., [26].

There is growing evidence that suggests a relationship between periodontitis and the risk factors for CVD, including dyslipidemia and HTN [27].

Additional prospective mechanisms have become clear: continuous and lasting exposure to bacteria of the oral cavity or bacterial toxins may initiate pathological changes in blood vessel walls and therefore act as a precursor of atherosclerosis in susceptible hosts. In this way, periodontal pathogens can penetrate the epithelial barrier of periodontal tissues and achieve systemic spread through the bloodstream [28]. By this dynamic mechanism, periodontal pathogens can infect the vascular epithelium and atherosclerotic plaques, causing inflammation and plaque instability followed by acute myocardial ischemia. Furthermore, periodontal pathogens produce a variety of virulence factors that have deleterious effects on the vascular system, resulting in platelet aggregation and adhesion and formation of lipid laden foam cells and deposits of cholesterol that contribute to the formation of atheromas [29].

The role of apo A1 which generally has anti-inflammatory and atheroprotective properties has been examined in the development of pre HTN and HTN [30].

The means values of serum apo B, TC, TG, and LDL-C were significantly higher and statistically significant between the hypertensive patients compared to the control. The means values of serum apo A1 and HDL-C

levels were lower in the hypertensive patients compared to the control and were statistically significant.

Present findings and those of others discussed indicate that any relevant degree of pro-inflammatory state offsetting the balance of anti-inflammatory processes may be prominently involved in the development of HTN, be it low levels or dysfunctional high levels of apoA1, HDL particles or elevated concentrations of apo B. These findings are similar to the findings of some other studies [31, 32, 33].

In conclusions, the results of this study suggest that hypertensive patients with oral diseases had a disturbance in lipoprotein metabolism. High levels of pro-inflammatory apo A1 may thereby be accounted for a prospective association with HTN.

Recommendations: Hypertensive patients with oral diseases need measurement of BP and lipid profile at regular intervals throughout their primary health care to prevent CVD and stroke. Oral health programme should be emphasized in the preventive measure.

REFERENCES

1. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003; 26(11): 3160-3167.
2. Hogan J and Radhakrishnan J. The assessment and importance of hypertension in the dental setting, Dental Clinics of North America, 2012; 56:731-745.
3. Dorobantu M., Darabont RO, Badila E, and Ghiorghe S. Prevalence, awareness, treatment, and control of hypertension in Romania: results of the SEPHAR study, International Journal of Hypertension, 2010; 2010:1-6.
4. Popescu SM, Scrieciu M, Mercuu V, tuculina M, and Dascslu I. Hypertensive patients and their management in dentistry. Hindawi Publishing Corporation. ISRN Hypertension. 2013; 2013:1-8.
5. Glascoe AL, Brown RS, Marshall KL and Smith DR. Periodontal & Oral-Systemic Relationships: Reproductive Health. Austin J Dent. 2015; 2(3):1-5.
6. Jin LJ, Chiu GK, and Corbet EF. Are periodontal diseases risk factors for certain systemic disorders-what matters to medical practitioners? Hong Kong Medical Journal, 2003; 9(1):31-37.
7. Tonetti MS and VanDyke TE. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases, Journal of Periodontology, 2013; 84(4):S24-S29.
8. Fisher MA, Borgnakke WS, and Taylor GW. Periodontal disease as a risk marker in coronary heart disease and chronic kidney disease, Current Opinion in Nephrology and Hypertension, 2010; 19(6):519-526.
9. Lockhart PB, Bolger AF, Papapanou PN et al. Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association? A scientific statement from the american heart association, Circulation, 2012; 125(20):2520-2544.
10. 10- Morita T, Yamazaki Y, Mita A, Takada K, Seto M, Nishinoue N, Sasai Y, Motohashi M and Maeno M. A cohort study on the association between periodontal disease and the development of metabolic syndrome. J. Periodontol. 2010; 81: 512-519.
11. Kumar P, Mastan KMK, Chowdhary R, Shanmugam K. Oral manifestations in hypertensive patients: a clinical study. J Oral Maxillofac Pathol, 2012; 16(2):215-221.
12. Tamaki N, Tomofuji T, Ekuni D, Yamanaka R, Morita M. Periodontal treatment decreases plasma oxidized LDL level and oxidative stress. Clin Oral Investig, 2011; 15(6): 953-958.
13. Walldius G, Jungner I, Aastveit A, Holme I, Furberg C, SnidermAN a. The apo B/apo A1 ratios to estimate the balance between plasma proatherogenic and antiatherogenic lipoproteins and to predict coronary risk. Clin Chem Lab Med. 2004; 42(12):1355-1363.
14. Ellis J, Syemour RA, Steela JG, Robertson P, Butler TJ, Thomason JM. Prevalence of gingival overgrowth induced by calcium channel blockers a community based study. J Periodontol 1999; 70(1):63-67.
15. World Health Organization. International Society of Hypertension: guideline for management of hypertension. Guideline subcommittee. Journal of Hypertension. 1999; 17:151-183.
16. Krousel-Wood M, Muntner P, Carson A, et al. Hypertension control among newly treated patients before and after publication of the main ALLHAT results and JNC 7 guidelines. J Clin Hypertens (Greenwich). 2012; 14(5):277-283.
17. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, Lackland DT, Lefevre ML, et al. 2014 Evidence-based guideline for the management of high blood pressure in adults: Report From the Panel Members Appointed to the Eighth Joint National Committee (JNC8). JAMA 2013; 311 (5): 507-520.
18. Maiborodin IV, Kolmakova IA, Pritchina IA, Chupina VV. Changes in gum in cases of arterial hypertension combination with periodontitis. Stomatologiia (Mosk) 2005; 84(6):15-19.
19. Singh ML, Papas A. Oral Implications of Polypharmacy in the Elderly. Dent Clin North Am. 2014; 58(4):783-796.
20. 20- Smidt D, Torpet LA, Nauntofte B, Heegaard KM, Pedersen AM. Associations between labial and whole salivary flow rates, systemic diseases and medications in a sample of older people. Community Dent Oral Epidemiol. 2010; 38(5):422-435.
21. Glick M. New guidelines for prevention, detection, evaluation and treatment of high blood pressure. J Am Dent Assoc 1998; 129(11):1588-1594.
22. Andrew W, Evelyn W, Francis M, Mark J, Mark C. Pattern of gingival overgrowth among patients on antihypertensive pharmacotherapy at a Nairobi hospital in Kenya. Open Journal of Stomatology, 2014; 4(4):169-173.
23. Seymour, RA, Ellis, JS, and Thomason JM. Risk factors for drug-induced gingival overgrowth. Journal of Clinical Periodontology, 2000; 27(4):217-223.
24. Christensen E, Holmstrup P, Wiberg-Jorgensen F, Neumann- Jensen B, Pindborg JJ. Arterial blood pressure in patients with oral lichen planus. J Oral Pathol 1977; 6(3):139-142.
25. Harting MS and Hsu S. Lichen planus pemphigoides: a case report and review of the literature Dermatol Online J. 2006; 12(4):1-10.

26. Margabanthu G, Brooks J, Barron D, Miller P. Facial palsy as a presenting feature of coarctation of aorta. Interact Cardiovasc Thorac Surg 2003; 2(1):91-93.
27. Barmasheva A, Orekhova L, and Musaeva R. Requirement for prevention of periodontitis in patients with metabolic syndrome. Barmasheva et al. EPMA Journal 2014; 5(1):A113.
28. Tonetti MS and Van Dyke TE. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Periodontol. 2013; 40(14): S24-29.
29. Friedewald VE, Kornman KS, Beck JD, Genco R, Goldfine A, Libby P, Offenbacher S, Ridker PM, Van Dyke TE and Roberts WC. The American Journal of Cardiology and Journal of Periodontology Editors' Consensus: periodontitis and atherosclerotic cardiovascular disease. Am J Cardiol. 2009; 80(7):1021-1032.
30. Onat A, Hergenc G, Bulur S, Uğur M, Kucukdurmaz Z, Can G. The paradox of high apolipoprotein A-I levels independently predicting incident type-2 diabetes among Turks. Int J Cardiol 2010; 142(1):72-79.
31. Anjum R, Zahra N, Rehman K, et al. Comparative analysis of serum lipid profile between normotensive and hypertensive Pakistani pregnant women. J Mol Genet Med. 2013; 7(2):1-5.
32. Bambara R, Mittal Y, Mathur, A. Evaluation of lipid profile of north Indian hypertensive subjects. Asian Journal of Biomedical and Pharmaceutical Sciences. 2013; 3:38-41.
33. Onat A, Can G, Ornek E, Cicek G, Ayhan E, Doğan Y. Serum γ-glutamyltransferase: independent predictor of risk of diabetes, hypertension, metabolic syndrome and coronary disease. Obesity (Silver Spring) 2012; 20(4): 842-848.

Research Article

High –sensitivity c-reactive protein in hypertensive patients living in United Arab Emirates

Abbas AL-Sharifi ***FICMS***

Date Submitted:7/10/2014
Date Accepted:15/1/2016

Address for Correspondence:
Abbas AL-Sharifi, Prof. of Cardiology and Internal Medicine, Department of Internal Medicine, College of Medicine, Al-Mustansiriya University, Baghdad, Iraq.
E-mail: ***abbasalsharifi@yahoo.com***

Abstract

Background: ; Inflammation has been found to correlate with endothelial dysfunction and relate to renin-angiotensin system, as a result it has hypothesized that hypertension may be in part an inflammatory disorder.High-sensitivity C- reactive protein(Hs-CRP) is an independent predictor of future cardiovascular events and it predicts risk of incident hypertension.
Objectives: of this study is to determine the relationship of the Hs-CRP, a marker of systemic inflammation , with hypertension in hypertensive patients living in United Arabic Emirates.
Patients & Methods: This study was conducted in,Dubai between May 2008 and April 2009. 86 patients with hypertension and 84 healthy individuals were included in this study. Hs-CRP and lipid profile;Total cholesterol(TC).Triglyceride(TG),Low density lipoprotein(LDL),and High density lipoprotein (HDL) were measured for both groups.
Results: Hs-CRP levels were higher in hypertensive patients than in control, but this difference was not statistically significant (p=0.217).Frequency of low and high risk levels was significantly higher in hypertensive patients than in control (p=0.003),while at average risk levels frequency was higher in control individuals than in patients (p=0.003).Overall there was positive association between increasing levels of Hs-CRP and risk of developing hypertension. There was no significant correlation between Hs-CRP and BMI, (r=0.6,p=0.447).SBP(r=0.0.197;p=0.139) and DBP(r=0.112,p=0.455).
ConclusionIn: High s-CRP is an independent risk factor for hypertension ,so it is recommended to be done as part of strategies of global risk assessment.

Key words: Hypertension, High-sensitivity C-reactive protein, prevalence, risk levels

INTRODUCTION

There are a large number of studies done allover the world suggested that inflammation is important in atherosclerosis .Inflammation has also been hypothesized to play a role in the development of hypertension ,and cross sectional evidence demonstrates higher hs-CRP levels among those individuals with elevated blood pressure (BP)[1-6]

Large number of prospective trials have shown that inflammatory biomarker, highs-CRP, is an independent predictor of future cardiovascular events and it predicts risk of incident hypertension and diabetes [7-8] . Inflammation has been shown to correlate with endothelial dysfunction and relate to renin-angiotensin system[9], as a result it has hypothesized that hypertension may be in part an inflammatory disorder. Scientific studies have found that the higher the hs-CRP , the higher the risk of having heart attack.

Studies done in United states and Europe have also found that elevated levels of highs-CRP among apparently healthy men and women are a strong predictor of future cardiovascular events, and showed that there is association between sudden cardiac death ,peripheral arterial disease and highs-CRP.[10-13]

Higher level of hs-CRP may increase BP by reducing nitric oxide production in endothelial cells[14].

The aim of this study was to see whether hs-CRP ,a marker of systemic inflammation, is associated with incident hypertension.

PATIENTS AND METHODS

This study was done in Dubai, UAE, during the period between May 2008-April 2009 .86 hypertensive patients and 84 controlled people were included in this study.

A clinical and demographic data of all patients and control individuals were recorded. .Only patients with history of high BP are included in the study .The individual with a history of diabetes mellitus ,CAD and chronic kidney disease were excluded .

Inclusion criteria included adult patient living in UAE whether local people or expatriate .Hypertensive patients is defined as either a new physician diagnosis, the initiation of antihypertensive treatment or self-reported systolic BP of at least 140 mmHg or diastolic BP of at least 90 mmHg .

Fasting venous blood samples were taken from each patient and control people, which

was analyzes for hs-CRP and lipid profile including total, cholesterol(TC),Triglyceride(TG),High density lipoprotein(HDL),and Low density lipoprotein (LDL), Hs-CRP was measured by immunoturbidimetric method. lipid profile were analyzed by enzymatic colorimetric method. All the above tests were done by Olympus AV400fully automated chemistry analser.

Statistical analysis was performed using(SPSS-17) computer Software Program Statistical Package for Social Science.Data were presented as mean, SD,frequency and percentage ,.The t-test and chi-square test were applied for testing significance using 0.05 as level of significance. .A p-value of <0.05 was considered as statically significant. Spearman's correlation coefficient was determined for correlation and regression between hs-CRP and other parameters in hypertensive patients.

RESULT

Baseline characteristic of the study population, lipid profile and hs-CRP are shown in **Table I**. There was significant differences in age ,BMI, and BP between patients and control subjects(P=0.0001).

TC and LDL were significantly higher in patients with hypertension (P=0.028,0,002) respectively ,while TG and HDL levels dose not show significant difference between two groups (P=0.466,0.814) respectively .Hs-CRP levels were higher in hypertensive patients than control (6.38 V 4.32) ,but this mean level was not statically significant.

Frequency of low and high risk levels was significantly higher in hypertensive patients than control people (19.3%V 10.5% ;62.75 V 42.1%) respectively with(P=0.003).

At moderate risk levels frequency was significantly higher in controls than hypertensive patients (18.1% V 47.4%, P=0.003) as shown in(**Table II**).

Overall, there was a positive association between increasing levels of hs-CRP and risk of developing hypertension (**Table II**).This study showed that patients with systolic hypertension have moderate to severe levels of high s-CRP,30.8% and 69.2% respectively. Patients with diastolic hypertension have high s-CRP levels ranged between slight 20% to high level 80%, while those patients with both systolic and diastolic hypertension have high s-CRP ranged from mild, moderate to severe levels as shown in **Figure I**

There was no significant correlation between hs-CRP and BMI, (r=0.6 p=0.447).SBP(r=0.0.197;p=0.139) and DBP(r=0.112,p=0.455) as shown in Figure 2 and **3** respectively.

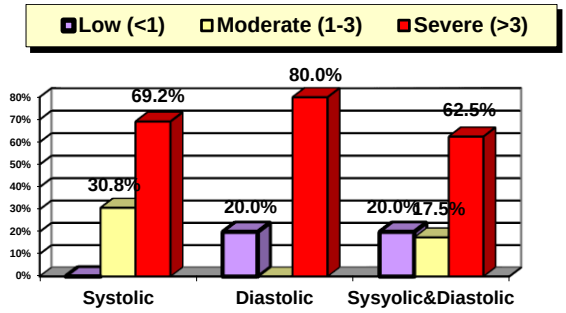


Figure 1 The high S-CRP and type of hypertension..

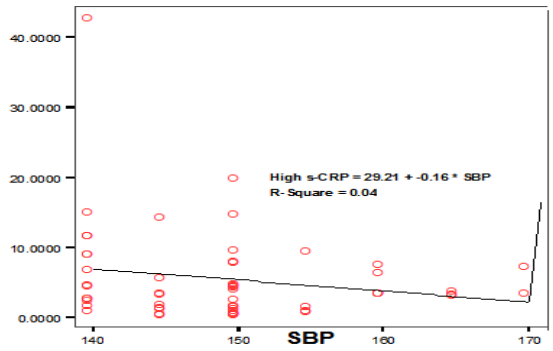


Figure 2. The correlation between high sensitive C-Reactive protein and systolic hypertension (r= -0.197, P=0.139).

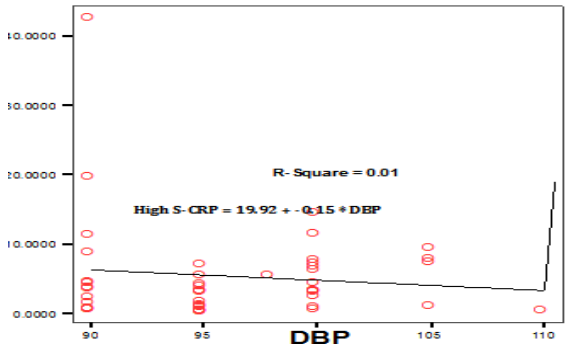


Figure 3. The correlation between high sensitive C-Reactive protein and systolic hypertension (r= -0.197, P=0.139).

Table 1. The Clinical Characteristic and Demographic data of Control and Patients with Hypertension.

	Patients No (%)	Control No(%)	P value
Age (years)	**47.6±9.0**	**37.40±6.54**	**0.0001***
Males	**59 (68.6%)**	**70 (83.3%)**	**0.076**
Females	**27 (31.4%)**	**14 (16.7%)**	
BMI	**31.82±6.21**	**29.68±4.65**	**0.050***
SBP	**142.63±12.40**	**123.86±9.66**	**0.0001***
DBP	**89.42±8.92**	**79.29±5.69**	**0.0001***
Total Cholesterol	**218.16±33.59**	**201.98±38.60**	**0.028***
Triglycerides	**167.83±97.61**	**155.74±81.33**	**0.508**
HDL	**45.96±9.75**	**46.47±13.38**	**0.814**
LDL	**146.24±29.18**	**123.84±37.73**	**0.002***
High sCRP	**6.38±9.77**	**4.32±4.29**	**0.217**

Table 2. The percentage distribution of different risk categories of control and hypertensive patients.

High s-CRP	**Patients No (%)**	**Control No(%)**	**P value**
Low (<1)	17 (19.3%)	9(10.7%)	0.003*
Moderates (1-3)	16 (18.1%)	38 (45.2%)	
Severe (>3)	53 (62.6%)	37 (44.1%)	

DISCUSSION

Despite the fact that up to 50 millions US individuals are affected ,the etiology of hypertension often remained unclear[15]. Although hypertension awareness, treatment, and control rates have increased during the past three decades, the identification of individuals at risk for hypertension remains a high priority[15].

The study data showed the prevalence of undesirable risk levels of hs-CRP was higher in both low and sever ranges, and the difference was significant. Moreover the hs-CRP levels was significantly higher in healthy individual in moderate risk range. A possible explanation is that, those control individuals in moderate risk group, could have BMI higher than hypertensive patients (42.9%) of those people are overweight in comparison with(34%) in hypertensive patients ,or could have another risk factors such as undiscovered diabetes or renal impairment, which could cause such a level in hs-CRP.The study provides evidence that levels of hs-CRP is independently increased in patients who are without baseline cardiovascular factors .Diabetes mellitus ,renal disease ,coronary artery disease and stroke which could be a cause of hs-CRP. This result is consistent to what mentioned in JNC7.[15] .High s-CRP has been reported to decrease production of nitric oxide by endothelial cells and thus might indirectly promote vasoconstriction and up regulating angiotensin type-I receptor expression, affecting the renin angiotensin system and contributing to the pathogenesis of hypertension,[16,17,18]

The data showed that hs-CRP has been associated more with DBP than SBP, this result is inconsistent with study done by Sesso,et all ,who showed that CRP has been more strongly associated with systolic BP than DBP[19].

The current data provide evidence that hs-CRP is independent risk factor for high BP, but we cannot confirm that ,because the study showed also high highs-CRP in overweight and obese control individual, so more studies are required to clarify this issue, similar finding have been reported by Haider et al.[20]

Conclusion and recommendations;

1-Hs-CRP is an independent factor and can be useful parameter to differentiate hypertensive patient from normal individual especially those who are not overweight or obese.2-Hs-CRP test should be recommended to be routinely done in hypertensive patient as part of strategies of global risk assessment.

REFERENCES

1. Ford ES, Giles WH, Serum C-reactive protein and fibrinogen concentrations and self-reported angina pectoris and myocardial infarction: findings from National Health and Nutrition Examination Survey III. J Clin Epidemiol. 2000;53:95-102.
2. Rhode LE, Hennekens CH, Ridker PM. Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. Am J Cardiol. 1999;84:1018-1022
3. Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. Arterioscler Thromb VascBiol. 2002;22:1668-1673
4. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. Hypertension . 2001;38:399-403
5. Yamada S, Gotoh T , Nakashima Y, et al. Distribution of serum C-reactive protein and its association with atherosclerotic risk factors in a Japanese population: Jichi Medical School Cohort Study. Am J Epidemiol. 2001; 153:1183-1190
6. Bautista LE, Lopez-Jaramillo P, Vera LM, et al. Is C-reactie protein an independent risk factor for essential hypertension? J Hypertens . 2001;19:857-861.
7. Libby P, Ridker PM, Maseri A. Inflammation and Atherosclerosis. Circulation. 2002; 105:1135-43.
8. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. J Am Coll Cardiol. 2007; 49(21):2129-38.
9. Braiser AR, Recinos A, 3rd, Eledrisi MS. Vascular inflammation and the renin-angiotensin system. Arterioscler Thromb Vasc Biol. 2002;22:1257-1266.
10. Ridker PM, Hennekens CH, Buring JE, Rifai N. C reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342:836-43.
11. Ridker PM, Buring JE, Shih J , Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation. 1998;98:731-3.
12. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 2000;321:199-204.
13. Mendall MA, Strachan DP, Butland BK, Ballam L, Morris J, Sweetnam PM, et al. C-reactive protein: relation to total factors in men. Eur Heart J 2000;21:1584-90.
14. Verma S, Wang CH, Li SH, et al. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation. 2002;106:913-919.
15. Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA. 2003;289:2560-2571.
16. Wang CH,LiSH,Weisel rd,et al. C-reactive protein upregulates angiotensin type I receptors in vascular smooth muscle. Circulation.2003;107;398-404
17. Verma S,Anderson TJ.The ten most commonly questions about endothelial function in cardiology.Cardio Rev.2001;9;250-252
18. Ross . Atherosclerosis;an inflammatory disease. N Engl J Med. 1999;340;115-126
19. Sesso HD, Stampfer MJ, Rosner B, et al. Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in men. Hypertension. 2000;36:801-807
20. Haidari,M,Javadi E ,Sadeghi B,Hajilooi ,M ,Ghanbili J.Evaluation of C-reactive protein, a sensitive marker of inflammation, as a risk factor for stable coronary artery disease. Clin Biochem.2001;34(4);309-15

Research Article

Evaluation of Serum Interleukin-6, Body Mass Index and bone mineral density in Patients with Nodal Osteoarthritis

Abbas Toma Joda *F.I.B.M.C* , **Adnan F. Al-Najar** *MBChB, MSC,* **Mustafa Saleam Khalaf**

Date Submitted: 26/12 /2015
Date Accepted:24/3/2016

Address for Correspondence:
Dr. Abbas Toma Joda, *Lecturer in Department of Internal Medicine/, College of Medicine, Al-Mustansiryia University*

Abstract

Background: Nodal osteoarthritis is a type of osteoarthritis and its common worldwide, the etiology is unknown but there are a biochemical markers recognized. Many studies have explain that adiponectin have an important role in the pathogenesis of osteoarthritis, but little is studied about the relation between serum Interleukin-6 , body mass index and bone minerals density with nodal osteoarthritis.

Objectives: To evaluate serum level of Interleukin-6 , body mass index and bone minerals density in nodal osteoarthritis patients.

Methods: Sixty patients with nodal osteoarthritis and sixty controls were included in the study; serum Interleukin-6, body mass index and bone mineral density were measured in all subjects. Student t-test was applied to find out the significant difference between two means.

Result: This study shows a significant increase in body mass index (BMI) in those patients with nodal osteoarthritis (mean $\pm$ SD) (31.6 $\pm$ 8.7) , P-value < 0.05. the mean of serum IL-6 is significantly increased in patients (mean $\pm$ SD) (3.2 $\pm$ 2.2) P-value < 0.05 . but not in control group and there's an obvious decrease in the bone mineral density between nodal osteoarthritis patients and control group(mean $\pm$ SD)(-2.2$\pm$ -1.1) P-value < 0.05.

Conclusion: This study shows the role of Interleukin-6 as inflammatory mediator in nodal osteoarthritis and osteoporosis. The BMI has a role in Interleukin-6 level and it's increased in nodal osteoarthritis patients.

Key words: body mass index, Nodal osteoarthritis, serum Interleukin-6.

INTRODUCTION

Osteoarthritis (OA) is a heterogeneous group of disease containing a different spectrum of clinical symptoms and signs. In generalised OA the hand is commonly involved, and polyarticular interphalangeal OA is taken as the marker for predisposition to OA at multiple sites(1).

Nodal OA (NOA), a type of OA, which is a degenerative disease of cartilage that covers the bone surfaces at the joints which begins to wear out, it characterized by polyarticular interphalangeal and thumb base of hand with Heberden's and Bouchard's nodes formation ,it is more common in women, and there is a clear genetic predisposition(1).

The incidence of hand osteoarthritis in people over 55 years of age was 13.4% for men and 26.2% for women, and these levels are increased with age(2).

The pathogenesis of OA appears to be the result of interaction between mechanical, cellular, and biochemical forces ,cytokines like tumor necrosis factor-α and interleukin-6, and even fragments of the cartilage itself induce chondrocytes to be differentiated (3).

As a result, these cells increase their synthesis of matrix metalloproteinases (MMPs) that cause the loss of

proteoglycans, at the same time, a decrease in tissue Inhibitors of Matrix Metalloproteinases (TIMPs) occurs (4).

Interleukin-6 (IL-6) is a substance produced by blood cells T-cells, as well as macrophages and endothelial cells (5). However, IL6 is a cytokine, and it is involved in relaying information between cells as both a signaling molecule and a signaling protein. IL-6 may behave as both an anti-inflammatory agent and a pro-inflammatory mediator, depending on certain conditions (6).

IL-6 plays an important role in regulating cell growth as well as immune function. In fact, its release is triggered by tissue damage or infection. Receptor sites are found on the surface of numerous cells throughout the body. From these sites, interleukin-6 transports a variety of proteins through the three major signal transduction pathways: protein kinase C, cAMP/protein kinase A, and calcium release also each IL-6 molecule performs a specific action, depending on the cell that initiated its release (6).

IL-6 is also known as a myokine, a type of cytokine triggered by muscle contraction and then discharged into the blood stream, this exchange promotes a variety of biologic actions. For one thing, it increases the breakdown of fats. It also improves insulin resistance, resulting in better uptake and utilization of glucose. Therefore, IL-6 therapy may have an application in treating certain conditions, such as obesity and diabetes type II (7).

IL-6 is considerd as the best marker for function of Immune system, impaired or uncontrolled IL-6 gene expression can produce unwanted immune responses and lead to a variety of diseases, including autoimmune disorders. Patients with rheumatoid arthritis, for example, typically have elevated levels of IL-6 in their synovial tissue. To combat this dysfunction, researchers continue to investigate different ways to inhibit binding of interleukin-6. This includes development of an anti-IL-6 receptor antibody (8).

This study is aimed to evaluate the serum IL-6, body mass index and bone mineral density in patients with nodal osteoarthritis

PATIENTS AND METHODS

One hundred twenty (60 patients with NOA & 60 healthy controls) were enrolled in this study. The patients studied in this case-control study have been selected from patients attended Rheumatology and Rehabilitation Out-Patient Clinic, In Al-Yarmouk Teaching Hospital during the period from November 2014 to February 2015.

They were randomly selected, diagnosed clinically and radiologically. Many laboratory tests have been done for each patients to exclude other possible causes of arthritis, these tests were include: ESR, C-reactive protein (CRP), Rheumatoid factor (RF) and serum uric acid. A pre-tested questionnaire was designed to obtain information from both patients and control group about past medical and drug history.

About five milliliters of venous blood was aspirated using disposable syringes and needles. Samples were collected between 09.00-12.00 Am. The blood was allowed to clot in plain tubes for 15 minutes, serum was obtained by centrifugation at 3000 rpm for 10 minutes and transferred into plain plastic tubes and kept frozen at (-20) C° until the time of assay

Measurement of IL-6 in serum: The IL-6 Enzyme immunoassay kit provides materials for the quantitative determination of IL-6 in serum and plasma. This assay is intended for in vitro diagnostic use only. The IL-6 was measured using a solid phase enzyme-linked immune-sorbent assay (ELISA) based on the sandwich principle (Figure 1).

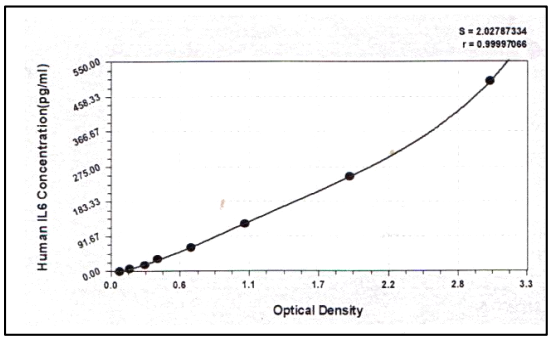


Figure 1. Standard Curve of IL-6 (pg/ml)

Cusabio - China Human IL-6 ELISA Kit was used to determine serum IL-6 level in this study. Normal values: 0.4-2.1 Pg/ml

The BMI was measured in this study according to world health organization (WHO) equation (weight / (hight)²)

The bone minerals density (BMD) was measured by DXA scan in Al-Yarmouk Teaching Hospital (Dexxum3-Korea Company) for both cases and controls.

The results were presented as sample size (n),mean ± standard deviation (SD) .the statistical significance of difference in mean between two groups was analyzed by student t-test. P-value < 0.05 was considered statistically significant.

All statistical analyses were done using IBMSPSS version 21 computer software (Statistical Packages for Social Sciences).

RESULT

Study showed significant increase of BMI in patients (mean ± SD) (31.6 ± 8.7) when compared to controls (mean ± SD) (26.2 ± 3.6) , P-value < 0.05. (Table-1) (Figure-2).Also this study showed significant increase of IL-6 level in patients mean (± SD) (3.2 ± 2.2) when compared to controls (mean ± SD) (1.6 ± 0.9) P-value < 0.05 . (Table 1) (Figure-3).Also this study showed significant decrease of BMD level in patients (mean ± SD) (-2.2 ± -1.1) when compared to controls (mean ± SD) (-0.7 ± -0.6), P-value < 0.05 . (Table-1) (Figure-4).

Table 1. Comparison of BMI and serum IL-6 in NOA patients with controls

Variable	NOA Patients	Controls	P- value
Number	60	60	
BMI (Kg/m2)			<0.05*
(Mean ± SD)	31.6 ± 8.7	26.2 ± 3.6	
IL-6 (Pg/ml)			<0.05*
(Mean ±SD)	3.2 ± 2.2	1.6 ± 0.9	
BMD(t-score)			<0.05*
(Mean ±SD)	-2.2 ± -1.1	-0.7 ± -0.6	

*** Significant differences**

Figure 3: Comparison of BMI (mean ± SD) Between Controls and NOA Patients.

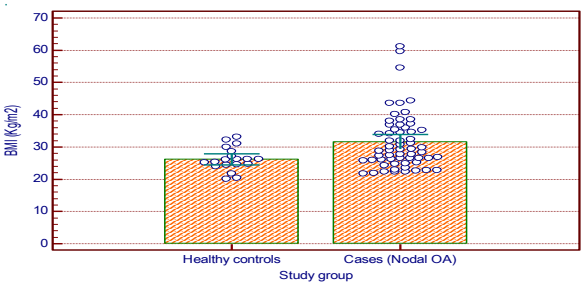
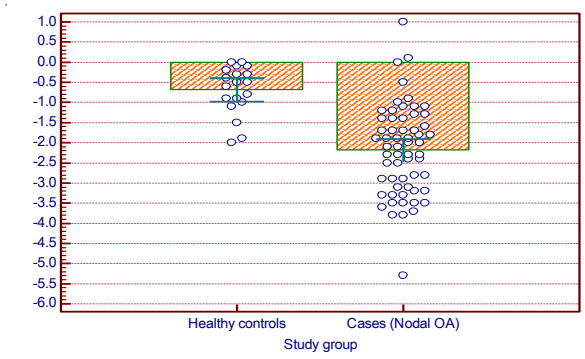


Figure 4: Comparison of Bone Mineral Density (BMD) (bone t-score) (mean ± SD) between controls and Nodal Osteoarthritis(NOA) patients



DISCUSSION

This study shows a significant increase of body mass index(BMI) between patients and controls this study agree with (Lauren , et al. 2013) who found that the patho-physiology of obesity-related OA is likely to be multi-factorial (9).

Structural joint damage is thought to result from both mechanical factors, including increased forces about the joint, decreased muscle strength and altered biomechanics during every day activities and metabolic factors, as being obese also increases the risk of OA in non weight-bearing joints such as the hands because the NOA consider as later status for OA (10).

But the study was disagree with (Raid , et al .2009) who found no associated changes between BMI in the cases and controls, but they proved the association between BMI and inflammatory hormones (like adipokinse as adiponectin and leptin)(11).

There are two methods show the relationship between bone mineral density(BMD) and OA generally and NOA specially, these are genetic and metabolic , because the two diseases occur in old age(12,13).

The genetic method shows relationship between OA and BMD by known gene responsible for OA and decrease BMD and bone turnover. (Tim , et al .2004) have proved this correlation (13). This proved when found urinary collagen cross links (markers of bone resorption) is due to share genes for two diseases (12).

The metabolic method shows the relationship between BMD and NOA as there is a correlation of sex hormones and turnover of bone. there are various studies about this subject, (Burger , et al .1996) shows that BMD increase for patients with general OA or NOA but after 60 years age the BMD decreases (13), (Valentina Živković , et al .2010) shows that at postmenopausal period the BMD decrease (14), (Abir Naguib, et al 2011) also shows that with age the bone turnover increase due to increase of urinary deoxypyridinoline (DPD) (16). This three studies supported the results of this study because our cases have age 60 years and more, also all the females in this study are postmenopausal (13, 14, 15).

This study shows significant increase of serum IL-6 in NOA patients when compared with controls. This difference is due to that all the NOA cases are postmenopausal, estrogen hormone decrease physiologically at this condition lead to error at bone formation because Osteoclast apoptosis is regulated by estrogens (16).

With estrogen deficiency, the osteoclasts live longer and are therefore able to absorb more bone and this will promote increase IL-6 level because interleukin 6 is a potent stimulator of bone resorption, and estrogen blocks the osteoblasts synthesis of interleukin- 6(16).

Estrogen may also antagonize the interleukin- 6 receptors, this lead to decrease osteocalcin then a decrease of BMD and the appearance of urinary deoxypyridinoline (DPD) (17).

This results agree with (Abir Naguib, et al 2011) who proved that the relationship between NOA ,bone turnover and urinary DPD (15). IL- 6 can be an important mediator in increased bone resorption of NOA patient because it mediated the inflammation in joints this agree with (Dequeker , et al .2003)(17).

Conclusion

This study proved the role IL-6 as inflammatory mediator in NOA patients, and BMI have a role in status of increased Interleukin-6 in NOA patients, as well as a relationship between NOA disease and decrease of BMD in elderly women.

REFERENCES

1. Jung-Yoon Choe ,Jisuk Bae ,Hyun-Young Jung and et al ." Serum resistin level is associated with radiographic changes in hand osteoarthritis" .Joint Bone Spine. 2012.2. 16-165 .
2. Zhang Y, Niu J and Kelly-Hayes M." Prevalence of symptomatic handosteoarthritis and its impact on functional status among elderly". Am J Epidemiol . 2002. 156.1021–1027.
3. Raid D.Hashim, Kismat M.Turki and Mohammed H. Alosami ."Estimation of serum leptin in female patients with nodal osteoarthritis " .J Fac Med Baghdad . 2009.51 .4.
4. Qishan Chen, Min Jin , Feng Yang and et al. " Matrix Metalloproteinases: Inflammatory Regulators of Cell Behaviors in Vascular Formation and Remodeling" . PMC Mediators Inflamm. 2013 . 288-315.
5. Cem Gabay." Interleukin-6 and chronic inflammation". Arthritis Research & Therapy. 2006.2. 3.
6. Simon A. Jones, Sankichi Horiuchi ,Nicholas Topley and et al. "The soluble interleukin 6 receptor: mechanisms of production and implications in disease". FASEB Journal. 2001. 15. 143-58.
7. Pura Muñoz-Cánoves ,Camilla Scheele ,Bente K. Pedersen and et al ."Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword ". FEBS J. 2013.17. 4131-48.
8. Lam J, Takeshita S, Barker JE and et al . "TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand" . J Clin Invest. 2000 .106 .1481 – 88 .
9. Lauren K King, Lyn March, and Ananthila Anandacoomarasamy ." Obesity & osteoarthritis". Indian journal of medical research . 2013 . 2 . 185-193.
10. Hashim, Raid D ,Kismat M. Turki and et al ." Estimation of serum leptin in female patients with nodal Osteoarthritis". Fac Med Baghdad. 2009.5. 4.
11. Tim D. Spector MD , Alex J and MacGregor MD. " Risk factors for osteoarthritis: genetics1 " . OsteoArthritis and Cartilage . 2004.12. 39–44.
12. Valkenburg HA, Hofman A and Grobbee DE, " Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age: the Rotterdam Study". Arthritis Rheum; 1996.39:81-6.
13. Bojana Stamenković, Jovan Nedović, Aleksandar Dimić and et al." Bone Mineral Density in Osteoarthritis" . Scientific Journal of the Faculty of Medicine in Niš. 2010.27.135-141.
14. Abir Naguib , Nermin Hossam , Mohamed Samy and et al ." The relationship between osteoarthritis of the hands,bone mineral density, and bone turnover markers" : Alexandria Journal of Medicine . 2011.47. 149–155.
15. Bharadwaj S, Naidu AG, Betageri GV and et al . "Milk ribonuclease-enriched lactoferrin induces positive effects on bone turnover markers in postmenopausal women". Osteoporos Int .2009.20 . 1603–11 .
16. Majkić-Singh N, Ilić M, Ignjatović S and et al ." Assessment of four biochemical markers of bone metabolism in postmenopausal osteoporosis" . Institute of Medical Biochemistry . 2002.48:407-413.
17. Luyten FP. "Osteoarhritis and osteoporosis: clinical and research evidence of inverse relationship". Aging Clin Exp Res. 2003.15.426-39..

MMJ

مجلة المستنصرية الطبية

المجلد ١٥العدد ١ نيسان ٢٠١٦

pISSN: 2070-1128

eISSN:2227-4081

MMJ

مجلة المستنصرية الطبية

المجلد ١٥ العدد ١ نيسان ٢٠١٦

مجلة علمية محكمة تصدر عن كلية الطب ـ الجامعة المستنصرية