Volume 11 (2) 2013 ISSN 1681-6579

IRAQI JOURNAL OF MEDICAL SCIENCES

Editorial Board Members

Iraqi Journal of Medical Sciences

Aims and Scope

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

Iraqi Journal of Medical Sciences publishes original articles, case reports, and letters to the editor, editorials, investigative medicine, and review articles. They include forensic medicine, history of medicine, medical ethics, and religious aspects of medicine, and other selected topics.

All correspondence and subscription information requests should be addressed to:

The Editor of **Iraqi Journal of Medical Sciences**
College of Medicine
Baghdad, Iraq
Tel and Fax: + 964-1-5224368
P. O. Box 14222, Baghdad, Iraq.
E-mail: iraqijms@colmed-alnahrain.edu.iq

Iraqi JMS FORMAT

INSTRUCTION TO AUTHORS

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

Types of Contributions: Original articles, review articles, case studies, editorials, medical education, history of medicine, ethics, practical points, medical quiz, conferences, meetings and letters to the Editor.

Manuscripts:

- Submission of a manuscript implies that is not being considered for publication anywhere.
- The author should provide the following:

A. A document officially state that the current work was carried out at the site which provides the certification. The document should be signed by the highest authorized member at that location.

B. Document stated clearly that his current work is in agreement with the medical ethics provided either from the local ethical committee in the place where he did his work or from the Ministry of Health - Depart. Of Training & Improving skill - Research & Educational facilities, the approval has to be stated separetly in the method section.

C. Publication fees are 60,000 Iraqi dinars and extra fees will be taken for extended paper (6000 dinars for each additional page (more than six pages) and up to 24000 dinars only).

- Manuscripts submitted to IJMS are subject to editorial evaluation and revision by three referees.
- The format of IJMS complies with the uniform requirements for manuscripts submitted to Biomedical Journals, published by the International Committee of Medical Journals Editors (ICMJE) (Vancouver, British Colombia, 1979) and its last update in October 2001, available on the web site www.icmje.org.
- Manuscript should be typewritten double spaced on size A4 (29.5x21 cm) paper with wide margins. Page should be numbered consecutively. One original and three photocopies including figures, tables, and photographs should be submitted. Begin each of following sections on separate page in the following sequence: Title page, abstract and keywords, text, acknowledgments, references, tables, and legends for illustration.
- Manuscript and figures will not be returned to the authors whether the editorial decision is to accept, revise or reject.
- Manuscripts must be accompanied by a covering paper signed by all authors that the paper has not been published in and will not be submitted to any other journal if accepted in IJMS.
- The page should contain (a) title of the manuscript, (b) names of each author (first name, middle initial and family name) including highest academic degree, (c) official academic and/or clinical title and affiliation (d) name and address of the institution where

the work was done (e) name and address (E-mail if available) of the author to whom correspondence should be sent.

Abstract: manuscript should include an abstract of not more than 150 words. Structured abstract typed on a separate sheet and consist of background, objective, method, results, and conclusion.

- **Keywords**: three to ten keywords should be provided on the same page as the abstract in English. As far as possible, be selected from the National Library of Medicine Medical Subject Headings.
- **Manuscript format:** It should be divided into the following parts: introduction, materials and methods, results and discussion.
- **References**: All references should be listed in consecutive numerical order by English numerical, in the order of citation in the text. Once a reference is cited all subsequent citations should be to the original number.

Examples

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

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

2. Books: Mann JI, Pyorala K, and Teuscher A. Diabetes in epidemiological perspective. London: Churchill Livingstone. 1983. p. 1-5.

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

- **Tables**: Each table should be typed on a separate page double-spaced, including all headings, number all tables with English numerals and include a short title. Vertical lines between columns are to be avoided.
- **Figures**: All figures must be suitable for reproduction without being retouched or redrawn. Figure number, name of senior author, and title of the work should be written lightly on the back with red pencil. Photographs must be supplied as glossy black and white prints. The top of the figures should be indicated clearly.
- **Legends**: Captions for figures must be typed; double spaced, and must not appear on the figure.

Proof Reading will be done by the secretarial office of the journal. The principal author will receive a copy of the journal. The authors are responsible for accuracy of all statements, data, and references included in the manuscript.

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

Iraqi Journal of Medical Sciences

A Medical Journal Encompassing All Medical Specializations

Issued Quarterly

CONTENTS

Iraqi Journal of Medical Sciences

A Medical Journal Encompassing All Medical Specializations

Issued Quarterly

CONTENTS

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Tips for Using YouTube in Medical Education

Akram A Jaffar *MBChB PhD*

Assistant Professor of Anatomy, Dept. of Basic Medical Sciences, College of Medicine, University of Sharjah, United Arab Emirates

Abstract

The role of online social networks in our everyday life has become crucial and undeniable. YouTube is a mainstay in online social networks. Created in 2005, YouTube is the third most visited Web site on the internet. Its educational value has been exemplified by the establishment of YouTube Education. Given the relative easiness of producing and uploading videos on YouTube and its free content, it has become a pool of a huge quantity of educational videos in different specialties uploaded by students and teachers. This paper aims to provide hints on effective usage of YouTube in medical education by evaluating an already existing video in order to recommend it to your students and by highlighting how to create and optimize your educational videos.

Introduction

Videos play an important role in supporting classroom teaching, self-directed learning, revision, and continuous medical education. The role of multimedia including videos in medical education has long been documented and has been reported to be more efficient in reducing classroom time and improving examination performance in many instances [1-5]. Students show interest in the subject and their learning is increased when instruction is integrated with multimedia tools [6-7].

Internet resources have become an integral part of everyday life, and undoubtedly, the media influences the way students learn and teachers teach [8]. The future generation of medical professionals who grew up in an environment sophisticated with information technology has been called 'digital natives' [9].

This 'Net Generation' has aptitudes, attitudes, expectations, and learning styles reflecting the environment in which they were raised [10-11]. This environment is decidedly different from that which existed when faculty were growing up. Thus, medical educators must find ways of engaging students more critically and creatively.

The more recent use of web applications facilitates sharing through social networks like YouTube (YouTube, LLC., San Bruno, CA), Facebook© (Facebook. Palo Alto, CA), and Twitter™ (Twitter, Inc., San Francisco, CA).

The use of social networks in an educationally relevant context can change the way students learn and communicate, as well as improve interactions between faculty and students [12-13].

The growth of such online resources and the advances in Web 2.0 technology are changing the information landscape and impacting teaching and learning.

It has been shown that social networking creates a blended, social constructive learning environment that encourages collaboration, conversation and sharing. Many social networks offer platforms that can be tailored by the individual learner [14-17].

YouTube popularity and educational potential

YouTube is a mainstay in online social networks. Created in 2005, now owned by Google, YouTube is the third most visited Web site on the internet behind Google and Facebook [18]. YouTube is a video-sharing website on which users can upload, share, and view videos. It provides an easy to use interface and the largest collection of a wide range of user-generated video content including videos with educational value. Each month, there are more than 1 billion unique users who visit YouTube and over 4 billion hours watched. Every minute, 72 hours of video are uploaded to YouTube with 70% of YouTube traffic coming from outside the United States. With the boom in the usage of intelligent hand held devices in the past couple of years, users, including medical professionals and students have instantaneous access to the growing collection of video content; thus, traffic from mobile devices tripled in 2011. Today, 25% of global YouTube views come from mobile devices [19].

In the Arab world, according to a survey published in June 2012, YouTube playbacks doubled in one year putting the region the number two spot in the world behind the United States. Saudi Arabia has scored the highest number of YouTube views in the world per Internet user. In the Arab region, Saudi Arabia is followed by Egypt, Morocco and United Arab Emirates. Whereas no statistics were provided on YouTube for Iraq, its Facebook and Twitter statistics are not among the leading in the region [20].

Technologies that were designed for purposes other than education, such as YouTube, are now frequently used in education. The educational value of YouTube has been exemplified by the establishment in 2009 of YouTube EDU, which became a home to high quality educational content from around the world. It aims to provide a global platform where anyone, anywhere can learn or teach. In the higher education category, channels from top educational institutions, including colleges and universities around the world have already subscribed. Several video categories are broadcasted including medical videos [21].

The use of YouTube in education is a present day topic. Little research exists in the literature about the recent use of YouTube in educating medical students and health professionals [22-28]. Considering its popularity and ease of access, the author considered YouTube an important platform for anatomy education. For that purpose, Human Anatomy Education channel was established on YouTube in early 2011 [29]. A survey in May 2012 on second year MBBS students at the University of Sharjah showed that among ten other social networks, YouTube channel ownership (52%) is the second after Facebook account ownership (86%) (unpublished observations). However the percentage of YouTube channel owners is almost double the percentage of an earlier survey in May 2011 (29%). In the latter survey, 98% of students indicated that they use YouTube for acquiring medical knowledge although with different frequencies [23].

Aims

This article aims to provide hints on effective usage of YouTube in medical education by evaluating an already existing video in order to recommend it to your students and by highlighting how to create and optimize your educational videos.

How to evaluate an educational YouTube video?

Given the relative easiness of producing and uploading videos on YouTube and its free content, it has become a pool of a huge quantity of educational videos in different specialties uploaded by students and teachers. However, the quality of such videos is not scrutinized. In addition the unregulated nature of the information contained within user generated wiki sites is potentially dangerous to those seeking online information; YouTube is not an exception in this respect. This mixture of variable content quality videos creates a burden on

students who search for an authentic source of information.

The ways search engines and/or Web 2.0 applications exploit social signals are usually not disclosed; however, social features are promising to improve the retrieval performance [30]. YouTube is the world's second largest search engine; however, unlike many professionally oriented databases, such as those used for peer-reviewed publications, the search engine utilized by YouTube allows for only a limited degree of search control since it is not best calibrated for searching educational content. Thus, some videos with high-standard educational quality content might not appear on the top of the list [26,31].

Given the lack of regulation of such videos, it is likely that a proportion of online resources are still of poor quality with substantial educational flaws. The student should be observant to quality and not be misled by low-standard educational content videos. Educators can help the student by recommending a short list of relevant videos to the topic under discussion. However, this process, while time-saving to the student is cumbersome to the teacher [23].

Educators should set criteria for rapid selection of a short list of relevant videos by using objective and subjective parameters. For objectivity, you may use video information metrics which include the page number on which the video was located in the search results (each page contains 20 videos), duration, date the video was uploaded, number of views, video category, and engagement parameters extracted from video statistics including likes, dislikes, comments and favourites.

Other objective information includes those related to the channel on which the video is uploaded. Channel information includes number of subscribers, channel views, number of uploaded videos on the channel, and whether the channel is dedicated for educational videos or not.

The page number indicates the relevance of the video to the search keywords and thus those found on the first page are more likely to be seen than those on lower pages. Number of video views provides an indication of the popularity of the video. The date the video was uploaded can give an idea about the number of views/day because recently uploaded videos will have lower total views than older videos and this might not serve as a comparative criterion to reflect popularity and usefulness. The engagement criteria are an indication of the concern of the viewer who might reflect on the video by liking, commenting, or adding to his favourites. It has been shown that for every 'dislike,' we get 10 'likes'- people like to tell other people about the stuff they love [19]. Camm et al., 2013 used a "like/dislike" ratio of 0.9 as a cut off for rating videos. This ratio is calculated by the number of likes divided by the total number of likes/dislikes [26]. The number of comments per se might not be a reliable indicator of a positive feedback because some comments might reflect a negative impact.

A brief analysis of the channel to which the video belongs provides a better insight on the video owner. Institutions and some educators may have established dedicated channels for teaching. On the other hand, some students have established such channels for their colleagues. Others might have mixed educational and non-educational content, this reflects non-dedication to education.

The number of uploaded videos, channel views, and subscribers are a good indicator of the owner's work in general. Some channel owners are sporadic producers who have produced the videos once and never added to them. The number of channel views is a collective figure of the views of all videos on the channel. It can be considered as a good reflection of the quality of the channel videos even though the particular video has not by itself attained high level indicators. The number of channel views is another reliable indicator of the quality of the owner's videos in general. However, in non-dedicated channels the above-mentioned channel indicators are not reliable in reflecting video or channel quality since the number of channel views or subscribers might have been

skyrocketed for the reason of presence of videos of another category like music or social videos.

In addition to YouTube metrics, the educator can set own subjective quality assessment criteria including appropriateness of content to the educational level of your students, authenticity, fulfilment learning objectives, communication skills of the presenter, and audio-visual quality of the produced video. This is in view that some authors have indicated that YouTube indices of preference (views, likes, dislikes, or search page) are not crucial in determining the value of educational video content [26,32]. Therefore, teaching institutions or professional societies should endeavour to identify and highlight good online teaching resources.

Reports of dissociation of YouTube provided video usage statistics and community engagement statistics with the relevance of the retrieved videos were published before YouTube launched "watch time". In October 2012, YouTube updated its suggested videos algorithm in order to focus on 'watch time." Thus the new algorithm for suggesting videos includes prioritizing videos that lead to a longer overall viewing session over those that receive more clicks [33]. By this way it can better surface the videos that viewers actually watch, over those that they click on and then abandon [34]. Previous to this, smart video producers had noticed that YouTube would reward clicks more than actual views. Watch Time should thus become an important metric to promote videos on YouTube because it is a sensitive metric of those people who are watching well beyond the first click. However, this new metric has not yet been researched in relation to educational videos.

How to create your educational video?

In every minute, 72 hours of video are uploaded to YouTube worldwide [19]; however, in the Arab region, which is number two spot in the world in YouTube video viewing, only one hour of YouTube video is uploaded per minute [20]. This is an indicator of the consumer tendency in the Arab region. It is at the same time an appeal to begin producing videos from own teaching material.

YouTube's slogan "Broadcast Yourself" can be easily met by consulting free self-learning resources. The creator hub on YouTube site provides a lot of information about how to get started and create a video. More refined information about creating videos with educational content are provided in YouTube EDU Playbook Guide which can be downloaded for free. It also provides information about how to apply for and become part of YouTube EDU [35].

Video capturing and editing methods that are suitable for producing educational videos are in many instances ubiquitous and user-friendly. Educational videos can fail if they go too high tech and if they go too low tech. Videos can be captured by video cameras or by using Camtasia Studio software (Techsmith Corporation, Michigan, USA), which is a computer screen capturing program in which it is also possible to record audio. In this way on-screen PowerPoint presentations can be captured as video. If a drawing pad with a stylus is also used, then writing, drawing illustrations or tracing certain features can be captured at the same time. As for mobile devices, Reflector (Squirrels LLC, USA) can airplay mirror the iPad, iphone screen on a computer which can then be captured and produced using Camtasia Studio.

The captured videos can be edited by using simple software such as Windows Live Movie Maker (Microsoft Corporation, Redmond, WA), Camtasia Studio, and the editing tool of YouTube itself.

These simple methods of capturing and editing are in many places comparable to those that are used by Khan Academy in producing educational videos. Khan Academy is a not-for-profit educational organization started by Salman Khan in 2008. Its mission is to provide a free, world-class education to anyone, anywhere. It all started when, in 2004, Khan began remotely tutoring his cousin who had difficulties in math. Eventually, word got around and his videos were hosted on YouTube. Now, with a library of over

4,100 videos on everything from arithmetic to physics, finance, medicine and history, the organization is receiving significant donations and grants [36].

How to optimize your educational video?

In a traditional classroom setting, no instructor would like to find his lecture hall empty or his students dozing in their chairs. In the virtual world, methods to engage the viewers could be different from face-to-face instruction since the instructor is not accomplishing visual contact with the attendants.

Education content video creators need to employ certain strategies to optimize their videos for the education category and build up more audience. The educator can use supplemental or full lessons. The contents could be single or in series. In anatomy education, for example, a course-based channel is recommended in which it is important to utilize annotations, metadata, and playlists to help the viewer navigate through the videos in a system oriented or region oriented sequence.

Because anatomy is a morphological subject then it is of the utmost essential to use three dimensional materials such as cadaver, prosections, and models to support learning. The use of multiple approaches within the same video or in a series of videos is important for understanding the multiple aspects of anatomy including gross, cross sectional, applied and surface anatomy.

The metadata (and description in particular) is a great way to communicate and outline your channel content for the viewer. It will help your audience find the right videos as they search. It is also important to give an outline of the objectives of each video session so viewers know what to expect. If the videos are course-based, it should be clear as to how this video session fits into the series or playlist. If such an outline is not provided within the video itself, it can be given in the "about" information tab. Also include links to related videos and the sequence they must be watched with both in the "about" information tab and as annotations on the video itself. For the latter purpose, you may use the annotation tab from the edit screen. Hints to the sequence can be included in the video title. These tips can help not only create but also repurpose existing educational materials for YouTube.

The use of supplemental subtitles is recommended to engage the viewer. It can also serve to learn difficult medical terminology. Subtitles can be also used to pose questions during the video session which may effectively engage the viewer.

Lecture-based videos can often be long. For these videos to become more consumable and accessible for the audience they need to be shortened. If not possible, then an abridged version of the video may guide interested viewers who can then commit to the full length version. However, viewers often find shorter videos more intelligible. As an example, Stanford University uploaded two versions of the 2005 Steve Jobs commencement speech - a full version included an introduction by the president, and the other video was edited to just feature the speech. The edited version has over 16.5 million views, whereas the full version has 1.5 million [35]. Long videos can be cut into shorter ones each addressing an objective or a group of objectives then the whole series is included in a playlist. Education channels can use the 'start and end time' features, allowing you to create playlists that feature specific time frames from videos within the playlist. Let's take the heart anatomy as an example; if you create a video on the gross anatomy of the heart and another on the surface anatomy of the thorax then you can select the part related to surface anatomy of the heart instead of the whole video to include it in a playlist of heart anatomy together with the gross anatomy video.

Annotations can be extremely useful to build an interactive, curriculum experience for your viewers and to help them navigate to the previous and next videos in the lesson.

Create an interactive lesson for your viewers by annotating to specific time codes of video so that users can jump to a particular section. Annotations can be used for another way of

interactivity by posing self-assessment questions for which answers and explanations are provided by hovering the pointer over a highlighted area using the spotlight annotation.

Evaluation and feedback

The use of YouTube can encourage learners to reflect on the material in an environment in which they are accustomed to. YouTube Analytics lets the channel owner monitor the performance of the channel and videos with up-to-date metrics and reports. It is a powerful evaluation tool that provides continuous feedback in which the channel owner can filter the reports by content, geography and date.

Concluding remarks

YouTube popularity should encourage educators to use it as a platform in teaching the Net Generation. The design and creation of videos requires extra time and effort by the faculty; however, capturing and editing tools are cheap and user-friendly. Educators need not worry that they might be superseded by YouTube videos with their interactive potentials because technological tools cannot completely replace classroom interactive teaching. Videos can release educators by reducing lecturing load and improving the quality of instruction. Establishing a YouTube Channel can offer a new forum for scholars to communicate and additional opportunities for educational research.

References

1. Leonard W. A comparison of student performance following instruction by interactive videodisc versus conventional laboratory. J Res Sci Teach. 1992; 29: 93-102.
2. Romanov K, Nevgi A. Do medical students watch video clips in eLearning and do these facilitate learning? Med Teach. 2007; 29: 490-94.
3. Saxena V, Natarajan P, O'Sullivan PS, et al. Effect of the use of instructional anatomy videos on student performance. Anat Sci Educ. 2008; 1: 159-65.
4. Bridge PD, Jackson M, Robinson L. The effectiveness of streaming video on medical student learning: A case study. Med Educ Online 2009;14:11. doi:10.3885/meo.2009.Res00311. Available from http://www.med-ed-online.org
5. McNulty JA, Hoyt A, Gruener G, et al. An analysis of lecture video utilization in undergraduate medical education: associations with performance in the courses. BMC Med Edu. 2009; 9:6. doi:10.1186/1472-6920-9-6
6. Schacter J. The Impact of Education Technology on Student Achievement: What the Most Current Research Has to Say. 1st Ed; 1999. Santa Monika, CA: The Milken Family Foundation. 12 p. URL: https://bookert-dev.ito.lacoe.edu/funding_coordination/docs/impact_of_et.pdf [accessed 25, April 2013].
7. Ruiz JG, Mintzer MJ, Leipzig RM. The impact of e-learning in medical education. Acad Med 2006; 81: 207-12.
8. Lowerison G, Sclater J, Schmid RF, et al. Are we using technology for learning? J Educ Tech Syst. 2005-2006; 34: 401-25.
9. Prensky M. Digital natives, digital immigrants, part II: Do they really think differently? On the Horizon. 2001; 9(6): 1-9. URL: http://www.marcprensky.com/writing/Prensky%20%20Digital%20Natives,%20Digital%20Immigrants%20-%20Part2.pdf [accessed 25 August, 2011].
10. Roberts G. Technology and learning expectations of the net generation. In: Oblinger DG, Oblinger JL (Editors). Educating the Net Generation. 1st Ed. 2005. Boulder, CO: EDUCAUSE. p 3.1-3.7. [Accessed 25 August, 2011].
11. DiLullo C, McGee P, Kriebel RM. Demystifying the Millennial student: A reassessment in measures of character and engagement in professional education. Anat Sci Edu. 2011; 4: 214-26.
12. Boulos MN, Maramba I, Wheeler S. Wikis, blogs and podcasts: A new generation of web-based tools for virtual collaborative clinical practice and education. BMC Med Educ. 2006; 6: 41-48.
13. Wilen-Daugenti T. 21st Century Trends for Higher Education. 1st Ed. 2007. San Jose, CA: Cisco systems Inc. 12 p. URL: http://ftp.ucv.ve/Documentos/Evento_Cisco/Top_trends_in_Education_White_Paper.pdf. [accessed 25 August, 2011].
14. Stanciu A, Mihai F, Aleca O. Social networking as an alternative environment for education. Accounting and Management Information Systems. 2012; 11: 56-75.
15. Lei C, Krilavicius T, Zhang N, et al. Using Web 2.0 tools to enhance learning in higher education. A case study in technological education. Proceedings of the International Multi-Conference of Engineers and Computer Scientists (IMECS) Vol II. 2012. URL: http://www.iaeng.org/publication/IMECS2012/IMECS2012_pp1153-1156.pdf. [accessed 28 April, 2013]
16. Nicole A. Buzzetto-More Social Networking in Undergraduate Education. Special Section on Social Networking, Teaching, and Learning. Interdiscip J Inform Knowl Manag. 2012; 7: 63-90.

17. Forgie S, Duff J, Ross S. Twelve tips for using Twitter as a learning tool in medical education. Med Teach. 2013; 35: 8-14.
18. Alexa. The Web Information Company. Global top sites. 2013. URL: http://www.alexa.com/topsites [accessed 25 April, 2013]
19. YouTube. About YouTube. 2013. YouTube, LLC., San Bruno, CA. URL: http://www.youtube.com/t/about_youtube [accessed 25 April, 2013].
20. Salem F, Mourtada R. Social Media in the Arab World: Influencing Societal and Cultural Change? Arab Social Media Report. 2012; 2(1). URL: http://www.arabsocialmediareport.com/home/ [accessed 20 April, 2013].
21. YouTube. YouTube EDU. YouTube, LLC., San Bruno, CA. URL: www.youtube.com/education . [Accessed 23 March, 2013].
22. Azer SA. Can ''YouTube'' help students in learning surface anatomy. Surg Radiol Anat. 2012; 34: 465-8.
23. Jaffar A. YouTube: An emerging tool in anatomy education. Anat Sci Ed. 2012; 5: 158-64.
24. Koya K, Bhatia K, Hsu J, et al. A. YouTube and the Expanding Role of Videos in Dermatologic Surgery Education. Seminars Cut Med Surg. 2012; 31: 163-7.
25. Logan R. Using YouTube in perioperative nursing education. AORN J. 2012; 95: 474-81.
26. Camm CF, Sunderlan N, Camm A. A Quality Assessment of Cardiac Auscultation Material on YouTube. Clin Cardiol. 2013; 36: 77-81.
27. Duncan I, Yarwood-Ross L, Haigh C. YouTube as a source of clinical skills education. Nurse Education Today. 2013. Published online. http://dx.doi.org/10.1016/j.nedt.2012.12.013 .
28. Raikos A, Waidyasekara P. How useful is YouTube in learning heart anatomy? Anat Sci Ed. 2013; Published online. doi: 10.1002/ase.1361
29. Human Anatomy Education Channel. 2011. Akram Abood Jaffar, University of Sharjah, Sharjah, United Arab Emirates. URL: www.youtube.com/user/akramjfr [accessed 25 April, 2013].
30. Chelaru SV, Rodriguez C, Altingovde I. Can social features help learning to rank YouTube videos? Web Information Systems Engineering – WISE. 2012; pp. 552-66.
31. Murugiah K, Vallakati A, Rajput K, et al. YouTube as a source of information on cardiopulmonary resuscitation. Resuscitation. 2011; 82: 332-4.
32. Tao D, Adsul P, Wray R, et al. Search strategy effectiveness and relevance of YouTube videos. Proc Am Soc Info Sci Tech. 2012; 49: 1-4.
33. Lardinois F. YouTube changes its search ranking algorithm to focus on engagement, not just clicks. Teckcrunch. 2012. http://techcrunch.com/2012/10/12/youtube-changes-its-search-ranking-algorithm-to-focus-on-engagement-not-just-clicks/. [accessed January, 2013].
34. YouTube. YouTube Support. YouTube, LLC., San Bruno, CA. URL: http://support.google.com/youtube/. [accessed April, 2013].
35. YouTube. Playbook Guide: Education. 2012. YouTube, LLC., San Bruno, CA. URL: http://storage.googleapis.com/support-kms-prod/SNP_2799330_en_v1. [Accessed January, 2013]
36. Khan Academy. Khan Academy, Inc. Mountain View, CA. URL: www.khanacademy.org. [Accessed 27 April, 2013].

Correspondence to: Dr Akram A Jaffar
E-mail: akramjfr@gmail.com
PO Box 45521, Sharjah, United Arab Emirates
Office: (+971 6) 5057225; Fax: (+971 6) 5585879

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

The Outcome of 810 nm Surgical Diode Laser in the Management of Oral Soft Tissue Lesions

Ammar S Al-Alawi *BDS MSc*

Dept. of Maxillofacial Surgery, Al-Kadhimyia Teaching Hospital, Ministry of Health, Baghdad, Iraq

Abstract

Background Surgical diode lasers have been used in oral surgical procedures with beneficial effects as compared to the conventional techniques.

Objective To evaluate the efficacy and safety of 810 nm surgical diode laser in the field of oral and maxillofacial surgery.

Methods Forty patients who had different oral lesions were attending consultation clinic of the Maxillofacial Surgery Department in the Al-Kadhimyia Teaching Hospital. The patients have been treated by 810 nm diode laser. The power of the diode laser was 2-5 Watts in continuous mode. Excisional biopsies were sent for histopathological examination. Intraoperative and postoperative clinical examinations were done.

Results The clinical observations revealed no bleeding intraoperatively and postoperatively, no infection and minimal swelling was seen postoperatively.

Conclusion The benefits of 810 nm diode laser application in oral and maxillofacial surgery have been justified based on its efficacy and safety. There is a good acceptance for this new modality of treatment by the patients.

Keywords Surgical diode laser, soft tissue lesion, oral surgery

Introduction

The use of laser in dentistry has increased over the past few years. The first laser was introduced into the fields of medicine and dentistry during the 1960s [1,2].

Unlike other light sources, the laser emits a coherent, collimated and monochromatic radiation. These characteristics render laser radiation with unique applications in the field of the medicine and surgery especially in the field of the oral and maxillofacial surgery. Different lasers have many advantages in oral surgery like haemostatic property, postoperative comfort to the patient and incision quality [3].

In laser-tissue interaction, the factors that determine the initial tissue effect include laser wavelength, laser power, laser mode (continuous, pulsed, and chopped beam), tissue optical properties, and tissue thermal properties [4]. The degree of absorption of the laser radiation inside the tissue components determines the type of interaction mechanism obtained by laser on soft tissue [5]. In the oral cavity, the pyogenic granuloma clinically presents as a sessile or pedunculated vascular mass with ulcerated surface, purplish-red in color, painless and soft lesion.

Fibrous epulis are most common soft tissue swellings of mouth. It presents near at the anterior part of the mouth and arises from interdental papilla on the gingiva between two teeth and it can also form on the buccal mucosa.

It is a hyperplastic response to the chronic irritation or trauma to the gingival margin by sharp edges of the carious cavity or by calculus.
Giant cell epulis is probably hyperplastic is usually and found on the gingival margin between teeth anterior to the premolar. The swelling is rounded, soft and typically red or purplish in color [6,7].
The most surgical lasers conducted in oral surgery are excimer laser which emits laser light between 200-400 nm, argon laser emit laser between 488 -514.5 nm, Nd: YAG is located in an invisible spectrum 1064 nm, Er: YAG laser which has wavelength 2940 nm, CO_2 laser has 10600 nm wave lengths. Diode laser emits wavelength of 790-980 nm and it can be used in the continuous as well as pulsed mode. According to the clinical application, contact handpiece is used for tissue cutting and non contact handpiece is used for tissue coagulation. The diode laser offers special effects in the oral cavity like bactericidal effect and inflammation reduction in periodontal pockets [8]. Due to the conservative nature of treatment accomplished with diode laser, it can be utilized in both aesthetic enhancement purposes and treatment of soft tissue lesions [9].
The aim of the current study is to evaluate the efficacy and safety of surgical diode laser in the field of oral and maxillofacial surgery.

Methods

This research dealt with 40 patients who presented with oral lesions and were attending the consultation clinic of the Maxillofacial Surgery Department at the Al-Kadhimyia Teaching Hospital and requiring surgical intervention for various oral lesions. The period of study was from Nov. 2008 to Nov. 2011.
The work-up of treatment included a clinical examination to validate the preoperative diagnosis depending on shape and site of the lesions.
Photographs were taken for all lesions before and immediately after treatment. Fig. 1 and 2 shows two types of clinical lesions preoperatively.

Fig. 1. Pyogenic granuloma of palatal mucosa.

Fig. 2. Fibroma on right side of buccal mucosa.

Diode laser (Diomed 15 laser) was used for oral surgical procedures. It is an integrated GaAlAs semiconductor laser. Its maximum output power is 15 W and it works in continuous, single, and repeated pulsed modes. Its wavelength is 810 nm, and the pulse duration is 0.1-1.0 second.
The laser soft tissue surgical operations were done by choosing an appropriate power ranging (2-5 W) with a contact and continuous mode. The exposure time was varied according to the tissue response. All the oral surgical procedures were done with local anesthesia (Lidocaine 2%, infiltration anesthesia).
Clinical evaluation (including presence of intra and postoperative hemorrhage, pain, swelling, infection and scar formation) was done immediately, three days later, and then one week postoperatively. In all surgical procedures, an excisional biopsy was taken and sent for

histopathological analysis to confirm clinical diagnosis of nature of the lesion.

Results

The locations of the oral lesions were at different sites in oral cavity. The sites of pyogenic granuloma were on palatal and buccal alveolar mucosa between teeth. The sites of fibroma were at the left and right buccal mucosa. The sites of giant cell granuloma were at interdental papillae of the anterior teeth. A pie graph showing the percentage of oral lesion types is illustrated in Fig. 3.

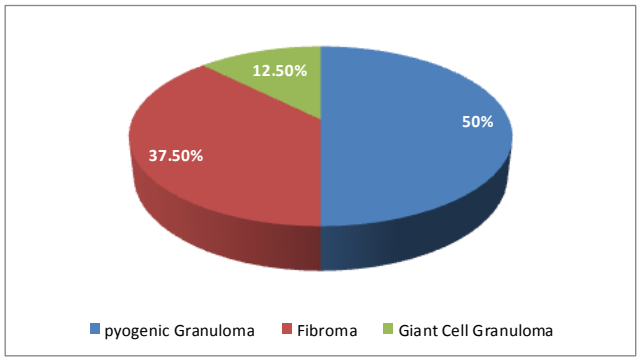


Figure 3. Pie chart showing the percentage of each type of oral lesions.

The intra and postoperative complications are shown in Table 1; the bleeding was not observed during the treatment or in postoperative period. Mild pain was observed in 5 patients on 3rd day postoperatively and it was managed by ponstan capsules 20 mg three times daily. In 4 cases minimal postoperative swelling was recorded on 3rd day. Postoperative infection was not recorded and there was no need for sutures. After completion of one week, no scar tissue formation was seen.

All patients tolerated well this modality of the oral soft tissue surgery (Fig. 4 and 5).

Table 1. Intra and postoperative complications of the oral lesions

Complication	IO	Day 0 PO	Day 3 PO
Bleeding	0	0	0
Pain	0	0	5
Swelling	0	0	4
infection	0	0	0

IO = intraoperative, PO = postoperative

Fig. 4. Complete fibroma excision by 3 W, continuous & contact mode surgical diode laser.

Fig. 5. Complete pyogenic granuloma excision by 4 W, continuous & contact mode surgical diode laser.

Discussion

Since the invention of laser in 1960, surgeons have been interested in applying this technology to improve outcomes in a variety of diseases and surgical procedures [10]. The instruments of choice for soft tissue surgery are the scalpel and the conventional electrosurgery unit. The scalpels have been used for many years because of their ease of use, accuracy, and minimal damage to the surrounding tissue; on the other hand, they cannot provide hemostasis especially in vascular tissue [11]. In our clinical work, intraoperatively and postoperatively bleeding was minimal or not observed and the

postoperative edema was greatly diminished. These results are in agreement with repo previous rese repasts revealing that the advantages of laser application in soft tissue surgery include a relatively bloodless surgical and postsurgical course, minimal swelling, coagulation and cutting minimal or no suturing [(12)].

In this clinical trial, uncontrolled postoperative pain was detected in few cases and postoperative infection was not reported and there was no scar formation. These results agreed with previous reports which proved the advantages of laser surgery including : sterilization of the surgical site while cutting tissue and a dry surgical field, reduced postoperative pain, less oedema, limited scarring and no-touch technique [(13,14)]. Clinically, the use of diode laser in the treatment of oral and maxillofacial diseases has found an application in the removal of premalignant lesions of the oral mucosa [(15)].

In clinical studies, the remarkable cutting ability and tolerable damage zone show clearly that diode laser system is effective and useful in soft tissue surgery of oral cavity because of its excellent coagulation ability, the smooth heal of wounds, and its simple use allows good modeling of the gingiva [(16)] and more precise incision margin is seen compared to other systems [(8)]. The evaluation of safety and efficacy of the diode laser system is already done for the treatment of facial pigments and vascular lesions and in oral surgery in fibroma, epulis fissuratum, and gingival hyperplasia [(17)].

Finally, it was concluded that the diode laser applications in the field of oral and maxillofacial surgery was found to be justified on the basis of its safety and efficacy in this study.

References

1. Goldman L, Hornby P, Meyer R, et al. Impact of the laser on dental caries. Nature. 1964; 203: 417.
2. Hussein A. Applications of Lasers in Dentistry: A Review. Arch Orofacial Sci. 2006; 1: 1-4.
3. Deppe H, Horch G. Laser applications in oral surgery and implant dentistry. Laser Med Sci. 2007; 2: 217-21.
4. Douglas N, Dederich NH. Laser/tissue interaction. Algha Omegan. 1991; 84: 33-6.
5. Walsh LJ. The current status of laser applications in dentistry. Australian Dent J. 2003; 48: 146-55.
6. Cawson RA, Odell EW. Cawsons essentials of oral pathology and oral medicine. 8th ed. UK: Churchill Livingstone; 2008. p. 314-7.
7. Stward GR, Harris M, McGowan DA. An outline of oral surgery. part I, 2nd ed. UK, WRITE; 1998. p. 298-304.
8. Romanos G, Nentwig G. Diode laser (980 nm) in oral and maxillofacial surgical procedure: clinical observation based on clinical applications. J Clin Laser Med Surg. 1999; 17: 193-9.
9. Reichwage DP, Barjenbruch T, Lemberg K, et al. Esthetic contemporary dentistry and soft tissue recontouring with the diode laser. J Indiana Dent Assoc. 2004; 83(1): 13-5.
10. Papadaki M, Doukas A, William A, et al. Vertical ramus osteotomy with Er:YAG laser: a feasibility study. Int J Oral Maxillofac Surg. 2007; 36: 1193-7.
11. Liboon J, Funkhouser W, Terris D. Comparison of mucosal incisions made by scalpel, CO_2 laser, electrocautery and costant-electrocautery. Otolaryngol Head Neck Surg. 1997; 116: 379-85.
12. Pick RM, Colvard DM. Current status of lasers in soft tissue dental surgery. J Periodontol. 1993; 64: 589-602.
13. Kafas P, Kalfas S. Carbonization of a radicular cyst using fiberoptic diode laser: A case report. Cases J 2008; 1: 113.
14. Frentzen M, Gotz W, Ivanenko M, et al. Osteotomywith 80-micros CO_2 laser pulses-histological results. Lasers Med Sci. 2003; 18: 119-24.
15. Eliades A, Stavrianos C, Kokkas A, et al. 808 nm diode laser in oral surgery: a case report of laser removal of fibroma. Res J Med Sci. 2010; 4: 175-8.
16. Goharkhay K, Mortiz A, Wilder-Smith P, et al. Effects on oral soft tissue produced by a diode laser in vitro. Laser Surg Med. 1999; 25: 401-6.
17. Desiate A, Cantore S, Tullo D, et al. 980 nm diode lasers in oral and facial practice: current state of the scienceand art. Int J Med Sci. 2009; 6: 358-63.

Correspondence to Dr. Ammar S Al-Alawi
E-mail: amasan2010@yahoo.com
Cell phone: + 964 7906635864
Received 3rd Oct. 2012: Accepted 17th Mar. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Hepatic Tissues under the Effect of Dexamethasone: Histological Study, Dose and Duration Related Changes

Khalida I Noel *MBChB MSc*

Dept. of Anatomy, Histology and Embryology, College of Medicine, Al-Mustansiyria University, Baghdad.

Abstract

Background Dexamethasone is a highly potent glucocorticoid. Treatment with dexamethasone results in several metabolic perturbations on nearly all organs of the body including the liver.

Objective This study had been carried out in order to investigate the effects of dexamethasone sodium phosphate as synthetic form of glucocorticoids on the rabbit liver as a model for human liver, by a light microscope, using two extreme of doses and two durations to show the dose and duration dependency.

Methods Liver specimens were obtained from rabbits treated with dexamethasone sodium phosphate and from control groups 1 and 2, the specimens were fixed and processed to evaluate the histological and histochemical changes**.**

Results Vacuolation and ballooning of hepatic cells were observed in the liver of the treated groups associated with degenerative changes of these cells, dilatation and congestion of central hepatic vein and sinusoidal capillaries were observed, positive periodic acid schiff's stain (PAS) reactions were noticed in the treated groups. All these changes were dose and duration related.

Conclusion Morphological changes induced in the liver by dexamethasone sodium phosphate could be accepted as side effects of these drugs.

Key words Liver, dexamethasone, histology, glycogen.

Introduction

The liver is a vital organ for processing nutrients absorbed from the gastrointestinal tract and for transforming them into materials needed by other tissues of the body [(1)]. It is under the influence of many hormonal actions such as insulin [(2)], glucagon [(3)] and the adrenal steroids [(4)]. The synthetic glucocorticoids are administered for a variety of disorders and illnesses, but their administration may be associated with development of multitude of complications involving almost all organ systems. The degree of complications depends on a number of factors, including length of treatment, time of day of administration, glucocorticoid preparation chosen, route of administration, dose administered and dosing intervals [(5)].

Dexamethasone is a potent glucocorticoid that is indicated for a wide range of diseases such as endocrine and non-endocrine diseases including rheumatoid arthritis, osteoarthritis and other connective tissue diseases and also indicated for inflammatory diseases such as respiratory disease, dermatological diseases etc. However, dexamethasone has wide spectrum of side effects on nearly all the body systems [(6)].

Serum corticosteroid-binding protein, transcortin, has been considered to be synthesized and secreted by liver cells [(7)]. Transcortin is involved in the selective transfer

of glucocorticoid across the plasma membrane and influences the intracellular entrance and transport to the nucleus of steroid bound to protein [8].

Glucocorticoids inhibit glucose and amino acid uptake in many instances and enhance lipolysis in adipose tissue [9]. In the liver, these steroids stimulate a number of enzymes and increase protein and glycogen content. There is an enhanced hepatic capacity for gluconeogenesis; which, with substrate from catabolism elsewhere, results in increased glucose production.

The integrated effects of glucocorticoids thus result in hyperglycemia, negative nitrogen balance and fat loss [9]. The general stimulatory glucocorticoid effect on the liver is in pattern of hypertrophy of hepatocytes, since the total protein content in the liver cell is increased [10].

The objective of this study was to explore the effects of dexamethasone sodium phosphate on the rabbit liver as a model for human liver, by a light microscope, using two extreme of doses and two durations to confirm the dose and duration dependency.

Methods

Healthy white New Zealand female rabbits weighing between 1000-1250 grams were kept in separate plastic cages, fed *ad-libitum* and used for scientific research from January to march 2012 in Al-Mustansiriyia College of Medicine Laboratories.

The animals were divided into six groups, seven animals in each. The first group was treated daily for 10 days with (0.5 mg/kg of body weight (b.w.) equal to 0.1 ml/kg b.w.) intramuscular injection of dexamethasone sodium phosphate as single injection every 24 hours (ZMC import-export GmbH Germany as 8 mg/2 ml ampoules) in the thigh muscle. The second group was treated with (1.5 mg/kg b.w. equal to 0.4 ml/kg b.w.) of the same reagent for 10 days. The third group was received (0.5 mg/kg b.w.) of dexamthasone for 15 days. The fourth one was treated with (1.5 mg/kg b.w.) of dexamethasone sodium phosphate for 15 days. The fifth group was considered as a control (1) animals, they received equal amounts of 0.9% saline solution as intramuscular injections for 10 days. The sixth group was received also 0.9% saline solution for 15 days and considered as the control (2) group [11].

Twenty- four hours after the last injection, the animals were anaesthetized with chloroform. After dissection of the abdomen, the liver were removed and they were fixed in 10% formaline solution for 24 hrs., dehydrated, cleared, and embedded in paraffin and the blocks obtained were sectioned and stained by:

1. Haematoxyllin and Eosin stain (H&E): routine slandered stains for general structure of liver [12].
2. Alcoholic periodic acid- Schiff's stain (PAS): for carbohydrates including glycogen, mucin, and most basement membranes [13]. Staining methods and techniques were done on the basis of Humason and Luna [12, 13].

Results

H&E sections show vacuolation and ballooning of hepatic cells, which started to appear more clearly in the second and fourth groups (as the dose and duration of treatment were increased) as seen in fig. 1.

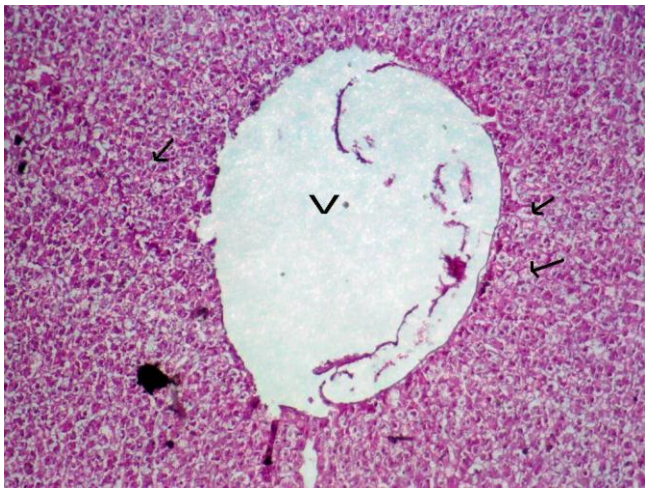


Fig. 1: Photomicrograph of liver cells of treated groups showing dilated and congested central vein (V), hepatic cells (arrows) H&E X100.

Degenerative changes of liver cells were noticed including; distortion of nuclei with distortion of

hepatic cell cytoplasm started to appear from the second group and further on till the fourth group where pyknotic nuclei were demonstrated (Fig. 2).

In some sections of first group, we noticed the appearance of hepatic cells with nuclei containing prominent nucleoli (Fig. 3). Dilatation and congestion of central hepatic vein were evident in all treated groups (Fig. 3). Sinusoidal dilatation and congestion also were demonstrated specially in the second and fourth groups (Fig. 3).

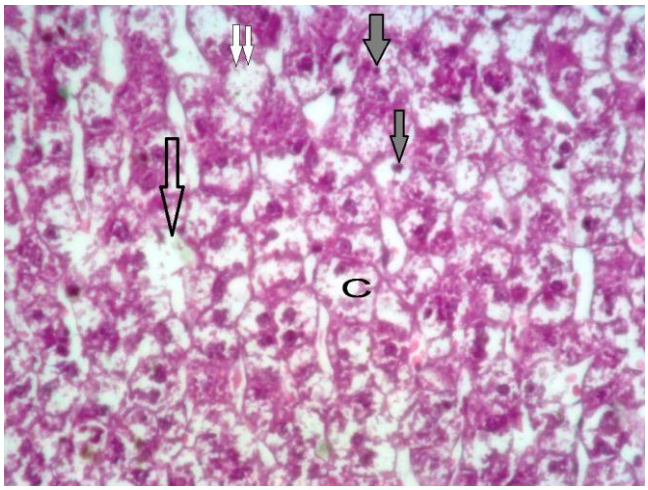


Fig. 2: Photomicrograph of treated liver cells (C) showing vacuolation (double white filled arrows), ballooning (single not filled arrow) and degenerative changes with pyknotic nuclei (gray filled single arrow) H&E X400.

Fig. 3: Photomicrograph of treated liver cells (C) showing prominent nucleoli in some cells (arrows), dilated and congested sinusoids (S), dilated and congested central vein (V) H&E X400.

All the above changes were dose and duration related when compared with the control animals (Fig.4).

With PAS stain, the strength of PAS reaction depends on the pattern of distribution of the dye appeared in the tissue. We noticed that there were positive (+ve) PAS reactions in all of the treated groups, but with differences in the strength of positivity, with presence of dispersed staining that indicated a +ve reaction to PAS and gradually this staining became heterogenic and then appeared as clumped masses (Fig. 5, 6, 7 and 8) and summarized in table 1.

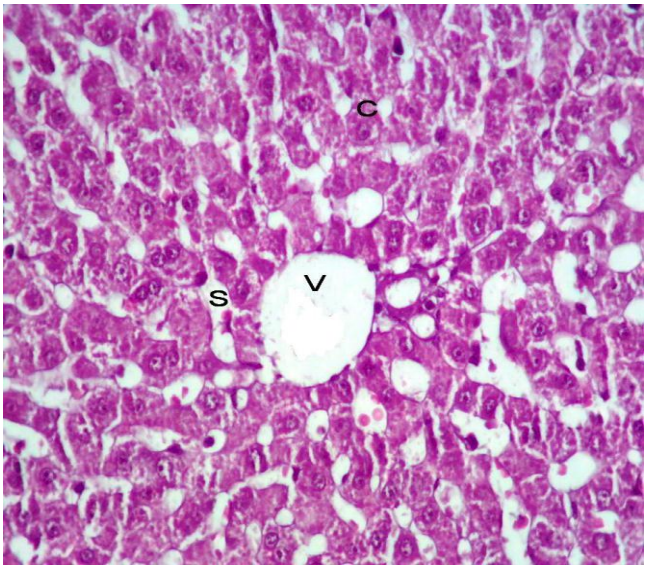


Fig. 4: Photomicrograph of control rabbit liver showing hepatic cells (C), central vein (V) and sinusoids (S) H&E X400.

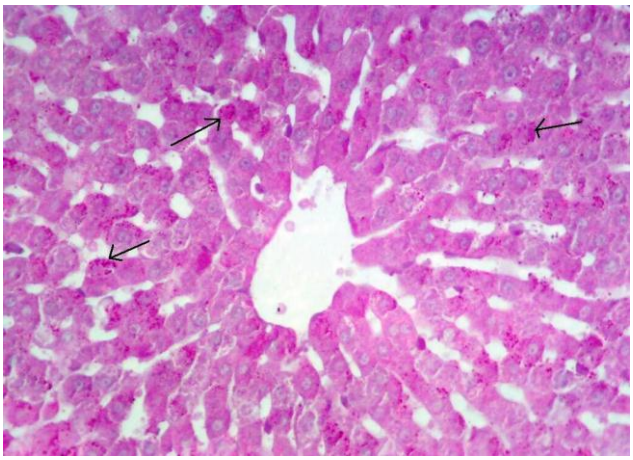


Fig.5: Photomicrograph of control rabbit liver with weak +ve PAS reaction (arrows) PAS X400.

Discussion

Drugs are an important cause of liver injury. Large number of drugs has been reported to cause liver injury. Drug-induced hepatic injury is the most common reason cited for withdrawal of approved drugs. Physicians must be vigilant in identifying drug-related liver injury because early detection can decrease the severity of hepatotoxicity if the drug is discontinued [6].

In the present study, the histological sections of treated rabbits with dexamethasone showed areas of ballooning and vacuolation of hepatocytes, which were directly proportional to the duration and dose of treatment.

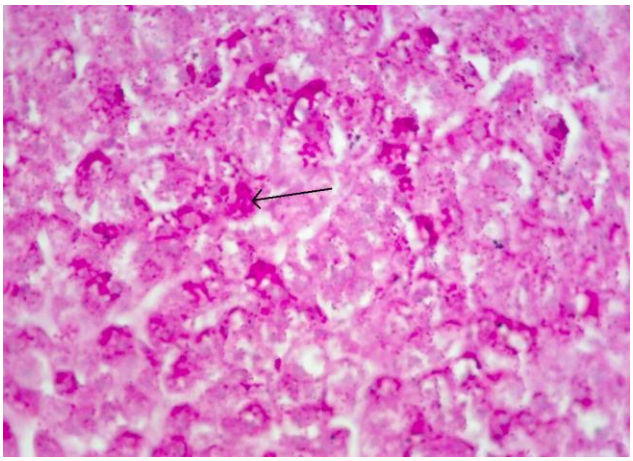


Fig. 6: Photomicrograph of treated rabbit liver cells with strong +ve PAS reaction (arrows) PAS X400.

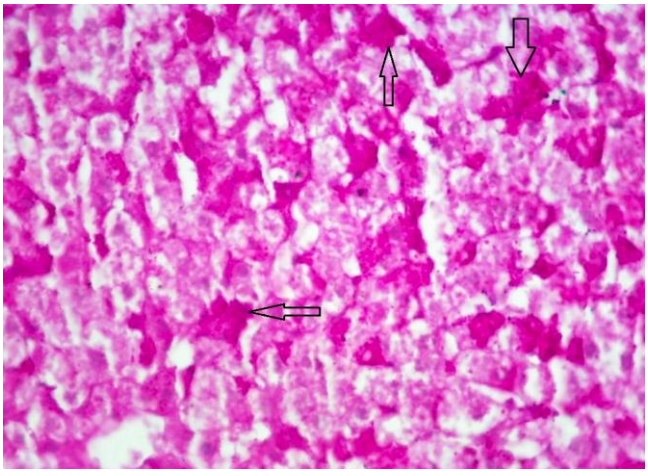


Fig. 7. Photomicrograph of treated rabbit liver cells with ++ve PAS reaction (arrows) PAS X400.

The histological sections stained with PAS showed that the vacuoles inside the hepatocytes contain glycogen inside their cytoplasm, there were positive (+ve) PAS reactions in all of the treated groups, but with differences in the strength of positivity, with presence of dispread staining that indicated a +ve reaction to PAS.

Gradually, this staining became heterogenic and then appeared as clumped masses which indicate strongest reaction of glycogen to PAS, that differ in the different doses and duration used in this study. Some researchers [14] stated that glycogen deposition was time and dose dependant and they indicated that repeated administration of dexamethasone increase liver weight and glycogen content and these changes were reduced by cessation of treatment.

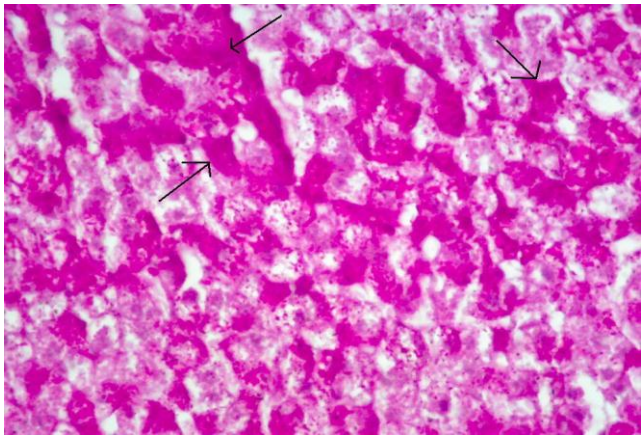


Fig. 8: Photomicrograph of treated rabbit liver cells with +++ve PAS reaction (arrows) PAS X400.

In the periphery (body organs outside the liver), glucocorticoids stimulate lipolysis and protein breakdown, releasing glycerol, fatty acids and amino acids, respectively, that act as substrates for gluconeogenesis[9].

Table 1: Periodic Acid Schiff's stain (PAS) reaction of liver cells in the study groups.

Study groups	PAS reaction
First group	Strong +ve
Second group	++ve
Third group	Strong +ve
Fourth group	+++ve
Fifth group (control (1))	Weak +ve
Sixth group (control (2))	Weak +ve

In the liver, glucocorticoids stimulate hepatic gluconeogenesis and increase the hepatic synthesis and storage of glycogen. Glucocorticoids also decrease glucose uptake in peripheral tissues, including adipose tissue, further contributing to increases in blood glucose. In response to elevated blood glucose, there is a compensatory increase in insulin secretion [6]. However, glucocorticoids inhibit the suppression of gluconeogenesis by insulin and cause insulin resistance in peripheral tissues, further contributing to hyperglycemia [9]. Treatment with dexamethasone causes time dependant changes in glucose and insulin levels, and increases the secretion of insulin, which makes the glycogen to be deposited, so that as the time increases, more glycogen deposited in the cytoplasm of hepatocytes [15]. Also dexamethasone causes up-regulation in insulin receptors in time dependant way due to stimulation of insulin receptors synthesis [16].

A number of biochemical processes in the liver, such as protein synthesis, glycogenesis, lipogenesis, certain mitochondrial functions, and the release of hydrolytic enzymes, are known to be affected by cortisone treatment. Many of these processes can be related to specific ultrastructural elements of the cytoplasm [17].

Some researchers [18] indicated that dexamethasone causes enhancement of smooth endoplasmic reticulum (SER), which is functionally associated with the increase in glycogen. The hepatocytes show increase in the amount of SER preceding glycogen deposition. In addition to that dexamethasone increases the activity of glycogen synthase which increases the glycogen accumulation [19] and inhibits activation of glycogen phosphorylase [20].

Degenerated hepatocytes ballooning is not due to glycogen deposition only, but also due to lipid accumulation. This is because glucocorticoids cause an increase in hepatic synthesis and secretion of VLDL [21]. Glucocorticoids cause time dependant accumulation in triglyceride within the cytoplasm of hepatocytes due to decrease in its secretion or due to increase synthesis and /or esterification of fatty acids [22].

There was evidence that glucocorticoids cause progressive increase in fragility of intracellular organelles, such as lysosomes with alteration in the plasma membrane properties [23] and decrease in the number with marked changes in the ultrastructure of mitochondria [17]. It is also known that dexamethasone reduces the number of mitochondria in treated hepatocytes and decreases the oxidative phosphorylation and their active respiration [24], this may lead to a disturbance in electrolyte balance through the "Sodium-Potassium pump". As this mechanism is energy dependent, the efflux of potassium ions may happen with the influx of sodium ions, increasing osmotic pressure in the cytoplasm beside the alteration in plasma membrane function; which attract water molecules. As a result, swelling of the cells occurs; also the leakage of hydrolytic enzymes may cause macromolecular crowding [25].

Dexamethasone, which is a synthetic form of glucocorticoid, inhibits the synthesis of arachidonic acid and prostaglandin which normally act as antiaggregant agents [26]. This, together with hypertension and polycythemia [27], caused the sinusoidal dilatation and congestion, which have been noticed in the treated groups of this study with the marked differences between them which indicate dose and duration dependency.

From our results, we concluded that the morphological changes induced in the liver by dexamethasone sodium phosphate were accepted to be side effects of these drugs.

References

1. Mescher AL. Liver. In: Junqueira's Basic Histology, 12th ed. McGraw Hill Company. 2010; 287-96.
2. Garfield AS, Mohamed SA, Cardell RR. The effects of insulin replacement and withdrawal on hepatic ultrastructure and biochemistry. Am J Anat. 1984; 170: 127-42.
3. Striffler JS, Cardell EL, Cardell RR. Effects of glucagon on hepatic glycogen and smooth endoplasmic reticulum. Am J Anat. 1981; 160: 363-79.
4. Cardell RR. Action of metabolic hormones on the fine structure of rat liver cells. III Effects of adrenalectomy and administration of cortisone. Anat Record. 1974; 180(2): 309-30.

5. Halfer EL, Rose LJ. Corticosteroid and adrenal suppression. Drug. 1989; 38(5): 838-45.

6. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. Endocr Rev. 2000; 21: 55-89.

7. Kraujelis K, Desrius A. Transcortin biosynthesis and intracellular distribution of rat liver polyribosomes synthesizing transcortin. J Steroid Biochem. 1989; 32(1A): 121-5.

8. Kraujelis K, Ulinskaite A, Meilus V. Transcortin in rat kidney: subcellular distribution of transcortin synthesizing polyribosomes. J Steroid Biochem Mol Biol. 1991; 38(1): 43-7.

9. Macajova M, Lamosova D, Zeman M. Physiological effects of Leptin, Insulin and Triamcinolon on adult male Japanese Quail. Acta Vet Brno. 2003; 72: 515-22.

10. Baxter JD, Forsham PH. Tissue effects of glucocorticoids. Am J Med. 1972; 53: 573-86.

11. Shaya KIN. The effect of dexamethasone on the histology of pancreas in female rabbits, MSc thesis, University of Mosul, 2008.

12. Humason GL. Animal tissue technique. 3rd ed. San Francisco: WH. Freman and Company; 1972.

13. Luna LG. Manual of histological methods. 3rd ed. New York: McGraw-Hill Company; 1968. P. 9-166.

14. Kim YH. Effect of corticosteroids on the hepatic cell and bile secretion. Korean Med Database 1967; 10(12): 29-42.

15. Kori´G, Isenovi´ E, Stojanovi´c-Šušuli´ V, et al. Time Dependent Effects of Dexamethasone on Serum Insulin, Level and Insulin Receptors in Rat Liver and Erythrocytes. Gen Physiol Biophys. 2006; 25: 11-24.

16. Salhanick AI, Krupp MN, Amatruda JM. Dexamethasone stimulates insulin receptor synthesis in cultured rat hepatocytes. J Biolo Chem. 1983; 258: 14130-5.

17. Wiener J, Alden VL, Daniel VK, et al. Quantitative description of cortisone-induced alteration in the ultrastructure of rat liver parenchymal cells. J Cell Biol. 1968; 37: 47-61.

18. Jerome WG, Cardell RR. Observations on the role of smooth endoplasmic reticulum in glucocorticoid-induced hepatic glycogen deposition. Tissue Cell 1983; 15(5): 711-27.

19. Margolis RN, Curnow RT. Effects of dexamethasone administration on hepatic glycogen synthesis and accumulation in adrenalectomized fasted rats. Endocrinology 1984; 115(2): 625-9.

20. Stalmans W, Laloux M. Glucocorticoids and hepatic glycogen metabolism. Monogr Endocrinol. 1979; 12: 517-33.

21. Garfield SA, Scott AC, Cardell RR. Alterations in hepatic fine structure after chronic exposure of rats to dexamethasone. Anat Rec. 1978; 192(1): 73-87.

22. Mendoza-Figueroa, Hernandez A, De Lourdes Lopez M, et al. Intracytoplasmic triglyceride accumulation produced by dexamethasone in adult rat hepatocytes cultivated on 3T3 cells. Toxicology 1988; 52(3): 273-86.

23. Rutgers HC, Batt RM, Vaillant C, et al. Subcellular pathologic features of glucocorticoid-induced hepatopathy in dogs. Am J Vet Res. 1995; 56(7): 898-907.

24. Roussel D, Dumas J-F, Simard G. et al. Kinetics and control of oxidative phosphorylation in rat liver mitochondria after dexamethasone treatment. Biochem J. 2004; 382: 491-9.

25. Abdelhalim MA, Jarrar BM. Gold nanoparticles induced cloudy swelling to hydropic degeneration, cytoplasmic hyaline vacuolation, polymorphism, binucleation, karyopyknosis, karyolysis, karyorrhexis and necrosis in the liver. Lipids Health Dis. 2011; 22(10): 166-70.

26. Galliani I, Santi P, Falcieri E. Morphological aspects of steroid hormone action on rat hepatocyte. Boll Soc Biol Sper. 1993; LXIX (3): 145-51.

27. Rogers WA, Rueoner BH. A retrospective study of probable glucocorticoid-induced hepatopathy in dogs. JAVMA. 1977; 170(6):603-606.

Correspondence to: Dr. Khalida I Noel
E-mail: kanarsawa@yahoo.com
Received 10th Sept. 2012: Accepted 5th Mar. 2013

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Serum Testosterone and Postprandial Lipids in Relation to Sexual Dysfunction in Males with Cardiovascular Disease

Zainab AA Al-Shamma[1] *BSc MSc*, **Yahya YZ Fareed**[1] *BSc PhD*, **Ali MH AL-Yassin**[2] *CABN FRCP/GFRCP*

[1]Dept. of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, [2]The Scientific Council of the Arab Internal Medicine, Baghdad, Iraq

Abstract

Background Earlier studies have suggested that total testosterone (Testo) concentrations influence lipid metabolism. Whether these concentrations are prospectively associated with an adverse lipid profile and an increased risk of incident dyslipidemia has not yet been investigated.

Objective Test the hypothesis that increased levels of postprandial triglycerides (TG) are associated with hypogonodism in male patients with cardiovascular disease (CVD).

Methods Forty male patients with CVD aged 30-60 years and 46 normal healthy controls were studied. Postprandial blood glucose, lipid profile, urea and creatinine were measured. In addition, Total testosterone, sex hormone binding globulin (SHBG), luteinizing hormone and follicle stimulating hormone were done by Enzyme-Linked Immuno-Sorbent Assay. Body mass index was calculated.

Results Negative correlation between Testo, and postprandial TG in both CVD and control groups was found with significant differences in Testo between these two groups, while SHBG correlated negatively with postprandial TG, in control group.

Conclusion Postprandial triglyceride levels were associated with risk of CVD. These findings are particularly interesting and may contribute to an explanation for the higher cardiovascular disease risk in men with lower total testosterone concentrations.

Key words CVD, Dyslipidemia, postprandial TG, Testosterone.

Introduction:

Causes of cardiovascular disease (CVD) are diverse but atherosclerosis and/or hypertension are the most common. Although cardiovascular disease usually affects elderly, the antecedents of cardiovascular disease, notably atherosclerosis begins in early life, making primary prevention efforts necessary from childhood [(1)].

There is therefore increased emphasis on preventing atherosclerosis by modifying risk factors, such as healthy eating, exercise, and avoidance of smoking. Almost all CVD in a population can be explained in terms of a limited number of risk factors. Dyslipidemia may come at the top of the list [(2,3)]. Dyslipidemia is a disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency. It may be manifested by elevation of serum total cholesterol, the low density lipoprotein cholesterol (LDL-c) and the triglyceride (TG), and a decrease in serum high density lipoprotein cholesterol (HDL- c). Definitions of dyslipidemia are based on guidelines from the World Health Organization: HDL < 0.9 mmol/l or TG ≥ 1.7 mmol/l [(4)].

The role of triglycerides as a risk factor of ischemic stroke remains controversial, however some studies reported a strong association between elevated levels of postprandial triglycerides and increased risk of myocardial infarction, ischemic heart disease, and ischemic stroke [5-7]. The atherosclerosis has long been hypothesized to be a disorder influenced by postprandial effects of TG as early as 1950, when Moreton, suggested a linkage between chylo-micronemia, fat tolerance, and atherosclerosis by affecting endothelial function and producing the atherogenic small LDL particles [8].

Thus measurement of postprandial triglycerides, particularly because they peak 3-4 h after ingestion of a fat-rich meal, might provide more relevant information on vascular risk than measurements based on fasting concentrations [9]. Recent studies found a strong association between elevated levels of postprandial triglycerides, and increased risk of ischemic heart disease [10].

Low serum testosterone levels have been associated with several components of metabolic syndrome, including CVD, hypertension, abdominal obesity, insulin resistance, and inflammatory markers in male individuals independent of age [11,12].

Also, it has been shown that low endogenous testosterone levels are associated with increased risk for both all-cause and cardiovascular mortality [13,14]. Testosterone is a muscle-building hormone, and there are many testosterone-receptor sites in the heart. The weakening of the heart muscle can sometimes be attributed to testosterone deficiency [15].

Testosterone is not only responsible for maintaining heart muscle protein synthesis; it is also a promoter of coronary artery dilation and helps to maintain healthy cholesterol levels [16-18].

The aim of the present study was to stress on the importance of postprandial lipids, in general, and triglycerides, in particular, and its relation to serum testosterone level in evaluating the risk of CVD in males.

Methods

This study included 40 male patients with CVD of age range between 30-60 years and disease duration of 2-15 months, who were attending the Coronary Care Unit (CCU) at Baghdad medical city during the period from December 2011 to June 2012 between 9.00 and 12.00 am. Patients with diabetes mellitus and thyroid disease were excluded from the study. The study also included 46 normal male volunteers of matching age and BMI, Who were non-smokers; non alcoholics and none, had dyslipidemia as revealed from previous laboratory tests.

Ten milliliters (10 ml) of venous blood were withdrawn from both patients and controls, collected in plain tube and centrifuged for 15 minutes at 3000rpm after being allowed to clot at room temperature for 30 minutes. The separated sera were divided into aliquots and stored frozen at -20 ^{o}C to be used for hormonal assays. Postprandial blood glucose, lipid profile, urea and creatinine were measured immediately after separation of the serum. In this study the determination of patients' androgen sex hormones; luteinizing hormone (LH) [19,20], follicle stimulating hormone (FSH) [21], testosterone [22], sex hormone binding globulin (SHBG) [23] were measured by enzyme linked Enzyme-Linked Immune Sorbent Assay (ELISA, Sandwich Assay). Body mass index was calculated as body weight (in Kg)/Sq. height (in meter).

Statistical study

All values were expressed as mean ± standard deviation (mean ± SD). All Statistical analysis was performed using Social process statistical system (SPSS version 15.0). Independent student t-test was performed to assess differences between two means. Pearson correlation coefficient was used to determine the correlation between quantitative data. P value < 0.05 was considered significant.

Results

As shown in table 1, there is a significant difference in total testosterone between the two

groups (P < 0.0001), while no significant differences in SHBG, LH, and FSH (P > 0.05) can be noted. All other biochemical parameters measured were significantly different (including serum lipids, glucose urea and creatinine).

Table 1. Demographic features of cardiovascular disease patients and control subjects

Parameter	CVD Patients N = 40	Control group N = 46
Age (yr)	46.43 ± 9.8	44.17 ± 8.1
BMI (kg/m^2)	30.65 ± 5.53	30.09 ± 5.27
glucose (mmol/l)	6.07 ± 1.64	5.39 ± 1.26*
TG (mmol/l)	2.5 ± 0.73	1.76 ± 0.54‡
Cholesterol (mmol/l)	5.74 ± 0.73	4.42 ± 0.73‡
HDLc (mmol/l)	0.91 ± 0.22	1.18 ± 0.21‡
LDLc (mmol/l)	3.7 ± 0.71	2.38 ± 0.7‡
VLDL c (mmol/l)	1.14 ± 0.33	0.8 ± 0.25‡
Atherogenic index	4.38 ± 1.45	2.15 ± 0.87‡
Urea (mmol/l)	7.42 ± 1.95	5.94 ± 0.71‡
Creatinine (µmol/l)	88.6 ± 25.67	74.18 ± 7.47†
FSH (IU/l)	7.3 ± 5.15	7.4 ± 5.52
LH (IU/l)	6.96 ± 2.72	5.61 ± 2.08*
TT (nmol/l)	9.34 ± 3.51	15.14 ± 5.16‡
SHBG (nmol/L)	32.43 ± 16.9	33.39 ± 15.39

TT = total testosterone, * = P < 0.05, † P = < 0.005, ‡ P = < 0000.1

In the control group, there was a significant negative correlation of BMI with each of total testosterone, and SHBG (r = - 0.263, P = 0.04) (r = - 0.259, P = 0.049) respectively, and significant negative correlations of the postprandial TG with the total testosterone, and SHBG as shown in the fig. 1 and 2, respectively.

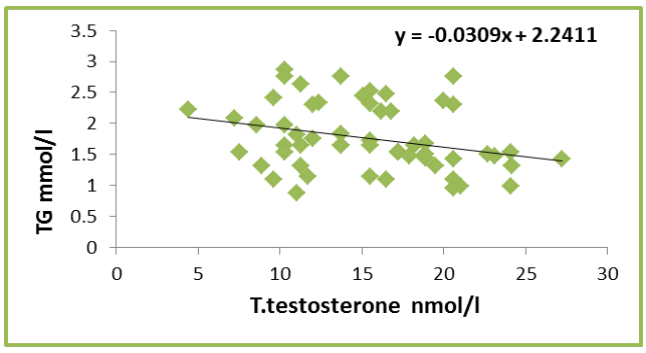


Fig. 1. Correlation between serum total testosterone and postprandial triglycerides

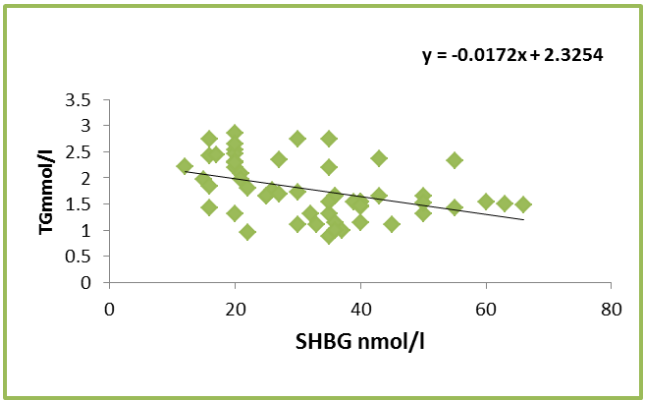


Fig. 2. Correlation between serum sex hormone and postprandial triglycerides

In the CVD patients' group serum total testosterone was negatively correlated with BMI (r = - 0.348, P = 0.028). There was also a negative correlation between total testosterone and postprandial TG as shown in fig. 3.

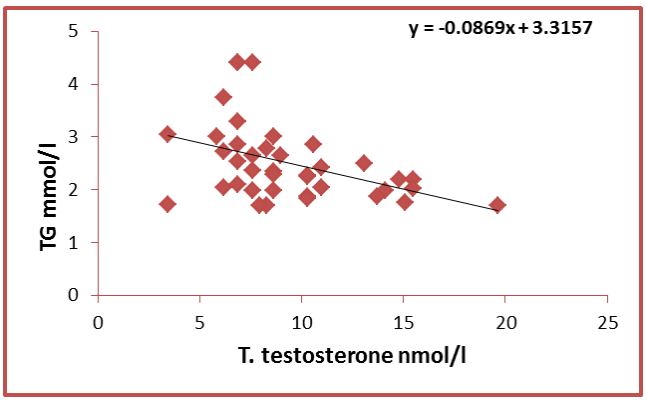


Fig. 3. Correlation between serum total testosterone and postprandial Triglyceride

Discussion

The present results show clearly low serum testosterone in the CVD patients relative to the normal controls, and this reduction in serum testosterone was associated with higher rise in the serum postprandial triglycerides. On the other hand the low serum testosterone negatively correlated with the BMI. Previous report had suggested a role for testosterone in visceral obesity [24]. Visceral fat contains a good number of androgen receptors, and these appear to inhibit the action of lipoprotein lipase and fatty acid/triglyceride uptake; the androgen receptors thus limit fat accumulation [25].

Natural decline of testosterone in the middle aged men and hypogonadism have been reported to associate visceral obesity [26]. Few prospective studies have demonstrated a protective link between endogenous testosterone and CV events [27]. Earlier cohort studies have documented the association of high serum TG with the risk and mortality from ischemic heart disease and stroke [10,28,29].

Increased levels of postprandial TG indicate the presence of increased levels of remnants from chylomicrons and VLDL [28]. These cholesterol-containing, triglyceride-rich lipoproteins penetrate the arterial endothelium, and may get trapped within the subendothelial space potentially leading to the development of atherosclerosis [30,31]. The majority of cross-sectional studies have found a positive correlation of endogenous testosterone with HDL and a negative correlation with total cholesterol, LDLc and triglycerides. Thus normal men with low testosterone appear to have adverse lipid profiles, and hypogonadal men have a potentially atherogenic dyslipidaemia prior to treatment [32,33]. These findings, together with previous reports on the importance of post prandial serum lipids in the prediction of atherosclerosis risk and consequent CVD [34,35], may lead to the speculation that serum testosterone and postprandial TG levels (or either of them) are better predictors than fasting serum lipids for assessment of the CVD risk in normal men or those with classical dyslipidemia. This would, also, necessitate an early testing and early start of preventive measures.

References

1. Antonio M, Gotto Jr. Cholesterol, Inflammation and Atherosclerotic Cardiovascular Disease: Is It All LDL? Trans Am Clin Climatol Assoc. 2011; 122: 256-89.
2. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries. Lancet. 2004; 364(9438): 937-52.
3. Fuster V, Kelly BB, Vedanthan R. Promoting global cardiovascular health: moving forward. Circulation 2011; 123: 1671-78.
4. WHO Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of Diabetes mellitus. Geneva: World Health Organisation, 1999.
5. Bowman TS, Sesso HD, Ma J, et al. Cholesterol and the risk of ischemic stroke. Stroke. 2003; 34: 2930-34.
6. Shahar E, Chambless LE, Rosamond WD, et al. Plasma lipid profile and incident ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) study. Stroke. 2003; 34: 623-6.
7. Patel A, Barzi F, Jamrozik K, et al. Serum triglycerides as a risk factor for cardiovascular diseases in the Asia-Pacific region. Circulation. 2004; 110: 2678-86.
8. Moreton JR. Chylomicronemia, fat tolerance and atherosclerosis. J Lab Clin Med. 1950; 35: 373-84.
9. Zilversmit DB. Atherogenesis: a post-prandial phenomenon. Circulation 1979; 60:473-85.
10. Freiberg JJ, Hansen AT, Jensen JS, et al. Non fasting Triglycerides and Risk of Ischemic Stroke in the General Population. JAMA. 2008; 300: 2142-52.
11. Yeap BB. Testosterone and ill-health in aging men. Endocrinol Metab. 2009; 5: 113-21.
12. Yeap BB. Are declining testosterone levels a major risk factor for ill-health in aging men? Int J Impot Res. 2009; 21: 24-36.
13. Eckardstein A, Wu FC. Testosterone and atherosclerosis. Growth Horm IGF Res. 2003; 13: S72-S84.
14. Khaw KT, Dowsett M, Folkerd E, et al. Endogenous testosterone and mortality due to all-causes, cardiovascular disease and cancer in men: European prospective investigation into cancer in Norfolk (EPIC-Norfolk) Prospective Population Study. Circulation. 2007; 116: 2694-701.
15. Hayward CS, Webb CM, Collins P. Effect of sex hormones on cardiac mass. Lancet. 2001; 357: 1354-56.
16. Rosano GM, Leonardo F, Pagnotta P, et al. Acute anti-ischemic effect of testosterone in men with coronary artery disease. Circulation. 1999; 99: 1666-70.
17. Jankowska EA, Biel B, Majda J, et al. Anabolic deficiency in men with chronic heart failure: prevalence and detrimental impact on survival. Circulation. 2006; 114: 1829-37.
18. Jackson G. Testosterone deficiency syndrome (TDS) and the heart. Eur Heart J. 2010; 31: 1436-7.
19. Danzer H , Braunstein GD, Rasor J, et al. Maternal serum chorionic gonadotropic concentration and fetal sex predictions. Fertil Steril. 1980; 34: 336-40.
20. Batzer F. Hormonal Evaluation of Early Pregnancy. Fertil Steril. 1980; 34: 1-12.
21. Odell WD, Parlow AF. Cargille CM. Radioimmunoassay for human follicle-stimulating hormone: physiological studies. J Clin Invest. 1968; 47: 2551-62.
22. Moltz L, Schwartz U, Sorensen R, et al. Ovarian and adrenal vein steroids in patients with non-neoplastic hyperandrogenism: selective catheterization findings. Fertil Steril. 1984; 42: 69-75.

23. Selby C. Sex hormone binding globulin: origin, function and clinical significance. Ann Clin Biochem. 1990; 27: 532-41.

24. Vermeulen A, Goemaere S, Kaufman JM. Testosterone, body composition and aging. J Endocrinol Invest. 1999; 22: 110-16.

25. Saad F, Gooren LJ. The role of testosterone in the etiology and treatment of obesity, the metabolic Syndrome, and diabetes mellitus type 2. J Obes. 2011; pii:471584:10 pages.

26. Isidori AM, Giannetta E, Greco EA, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol. 2005; 63: 280-93.

27. Ohlsson C, Connor EB, Orwoll E, et al. High Serum Testosterone Is Associated With Reduced Risk of Cardiovascular Events in Elderly Men. J Am Coll Cardiol. 2011; 58: 1674-81.

28. Nordestgaard BG, Benn M, Schnohr P, et al. Non fasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA. 2007; 298: 299-308.

29. Bansal S, Buring JE, Rifai N, et al. Fasting compared with non-fasting triglycerides and risk of cardiovascular events in women. JAMA. 2007; 298: 309-16.

30. Proctor SD, Vine DF, Mamo JC. Arterial retention of apolipoprotein B (48)- and B(100)-containing lipoproteins in atherogenesis. Curr Opin Lipidol. 2002; 13: 461-70.

31. Kolovou GD, Anagnostopoulou KK, Daskalopoulou SS, et al. Clinical relevance of postprandial lipaemia. Curr Med Chem. 2005; 12: 1931-45.

32. Langer C, Gansz B, Goepfert C, et al. Testosterone up-regulates scavenger receptor BI and stimulates cholesterol efflux from macrophages. Biochem Biophys Res Commun. 2002; 296: 1051-7.

33. Malkin CJ, Pugh PJ, Jones TH, et al. Testosterone for secondary prevention in men with ischaemic heart disease? QJM. 2003; 96: 521-9.

34. Bahir BH, Al-Hadi AHM, Al-Shamma G. Why Not Post Prandial Serum Lipid? Znco J Med Sci. 2008; 22: 165- 8.

35. Ridker PM. Fasting versus Non fasting Triglycerides and the Prediction of Cardiovascular Risk: Do We Need to Revisit the Oral Triglyceride Tolerance Test? Clin Chem. 2008; 54: 111-3.

Correspondence to Zainab AA Al-Shamma
E-mail: z.alshamma@gmail.com

Received 19th Nov. 2012: Accepted 13th Feb. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Possible Role of Lymphotoxin α, β and their Receptor (TLβRs) in Promoting Liver Carcinogenesis during Infection with Hepatitis C Virus

Areej A Hussein[1] *BSc PhD*, **Ghazi R Auda**[2] *BVM PhD*

[1]Dept. of Microbiology, College of Medicine, Dayala University, Dayala, Iraq, [2]Division of Virology, National Institute for Biological Standards and control. UK

Abstract

Background Lymphotoxin α, β and their receptor play an important role in the control of lymphoid organ development and support of immune responses against pathogens.

Objective To investigate expression of the lymphotoxin α, β and their receptor TLβRs using immunohistochemistry technique in patients with chronic active hepatitis and hepatocellular carcinoma.

Methods Thirty five formalin fixed, paraffin embedded liver tissues, obtained from Liver and Digestive System Technical Hospital and private laboratories in Baghdad, were studied. In addition, thirteen apparently normal liver autopsies were collected from the Forensic Medicine Institute Archives after permission and used as control group.

Liver tissue sections were cut at 4 µm and placed on positively-charged slides, used for the detection of lymphotoxin α, β and receptor TLβRs by immunohistochemistry technique.

Results The expressions of lymphotoxin α, β and receptor TLβRs were detected in most patients infected with HCV, 88%, 84%, 76% respectively in patient with chronic active hepatitis and 80%, 70%, 90% respectively in patients with hepatocellular carcinoma while low level of expression of these markers was observed in healthy control group.

Conclusion Lymphotoxin α, β and their receptor TLβRs may play an important role in the development and progression of HCV associated liver pathology.

Key words lymphotoxin, TLR, carcinogenesis hepatocellular carcinoma.

Introduction

Inflammation is a defensive process initiated by innate and specific cellular and humoral immune component in response to an insulting agent, which in most instances an infectious agent [1,2]. Initiation of the inflammatory process is triggered by activation of the immune component through the release of vasoactive and chemotactic substances elicited due to trauma or infection [3].

Tumor necrosis factor (TNF) superfamily comprises several cytokines including, but not limited to, lymphotoxin (LT) α, β and their tumor necrosis factor receptor (TLRs). These factors are known to play a role in the induction of necrotizing activity of neoplastic cells [4].

Lymphotoxins α and β are known to be responsible for organogenesis and lymphoid tissue maintenance [5]. They are generally produced, under normal physiological circumstances, by activated T, B and NK

lymphocytes and other lymphoid tissue components [6].

The most common cause of chronic hepatitis is infection with HBV and HCV [7]. Persistent infections with these viruses are frequently associated with the development of hepatocellular carcinoma [8]. The role of these infections in the induction of neoplastic changes in liver tissues is still to be elucidated. The core protein of hepatitis C virus is known to have multifunctional features, including binding to the death domain of the tumor necrosis factor receptor type 1 (TNFR 1). It also known to bind to the cytoplasmic tail lymphotoxin β receptor, reflecting a possible involvement in the signaling pathways of apoptosis [9].

The over expression of certain cytotoxic cytokines has been implicated as a possible inducing factor for the progression towards hepatocellular carcinoma (HCC) [10].

This study aims to determine the extent of expression of lymphotoxin α, β and their receptor (TLRs) using immunohistochemistry techniques in patients with chronic HCV infection and hepatocellular carcinoma. The study also aims to elucidate the correlation between these cytokines expression and different clinicopathological variables such as age, gender, histopathological activity index (HAI), stage and grade.

Methods

Study population. Thirty five formalin-fixed, paraffin embedded liver tissue blocks were obtained from patients with confirmed cases of chronic HCV infection and hepatocellular carcinoma. The age of patients was ranged from 17 to 65 years. The histopathological types of hepatocellular carcinoma included in this study were moderately differentiated adenocarcinoma (4 cases) and poorly differentiated adeno-carcinoma (6 cases). All patients had positive test for anti-HCV antibodies (third-generation enzyme linked immunosorbent assay (ELISA). The patients' samples were collected during the period from January 2010 till December 2011 from the archives of histopathology laboratories of liver and digestive system technical hospital and private laboratories in Baghdad, but this research perform during 23, March 2012 till 20, September 2012.

Normal liver specimens were obtained from thirteen persons were collected from the Forensic Medicine Institute Archives.

Formalin-fixed, paraffin embedded tissue blocks were sectioned (4 μm) thickness, one section was stained with Haematoxylin and Eosin, and four sections were mounted on positively charged slides to be used for immunohistochemistry technique for the detection of lymphotoxin α, β and their receptor TLRs.

The histopathological diagnosis of the tissue blocks used in this study was primarily based on that obtained from histopathological records of liver biopsy samples and hospital laboratory records. Confirmatory histopathological re-evaluation of each obtained tissue blocks was done.

Immunohistochemical staining. Was carried out using mouse anti-human lymphotoxin alpha (US Biological- USA Cat. Number L2610-03B), mouse anti-human lymphotoxin beta (abcom-UK Cat. Number ab89568), mouse anti-human lymphotoxin beta receptor (US Biological- USA Cat. Number L8015-03L) and immune-histochemistry detection kit (US Biological/USA Cat. Number 17506).

The slides were deparaffinized by immersion two times in xylene for 5 minutes each time, and they were then rehydrated in serial alcohols in the following order: 100%, 95%, 70% and water for 5 minute each. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide for 30 minutes. Slides were then washed with distilled water followed by two times in phosphate-buffered saline for 5 minutes.

All of the slides were treated with 1% normal serum and incubated for 30 minutes at room temperature. Excess normal serum was tipped off slides before adding the primary antibody, dilution 1:250 for each lymphotoxin α and TLβRs, dilution 1:500 for lymphotoxin β, as recommended by manufacturer's instructions.

Slides were then incubated overnight at room temperature. In the next day the slides were rinsed gently two times with phosphate-buffer saline for 5 minutes and the slides were incubated with anti-mouse IgG biotin for 30 minutes at R.T then washed two times in phosphate-buffered saline for 5 minutes. Detection solution was added for 30 minutes at room temperature, and then slides were washed two times with phosphate-buffered saline for 5 minutes followed by the addition of the diluted liquid DAB for 20 minutes at room temperature. After soaking the tissue in water, it was counterstained with Hematoxylin for 30 sec. Slides washed well in running tap water for 30 sec, then dehydrated by serial alcohols 70%, 95%, 100%, 100% for 3 minutes each time and two times xylene for 5 minutes then mounted with permanent-mounted medium (DPX) and examined under light microscope at 400 magnification**.** The intensity of reactivity was graded as follows: 0 (absent), + (weak), ++ (moderate), +++ (intense) [11]. The Statistical analysis was performed using Fisher exact test.

Results

Patient's details: Thirty five cases were obtained from patients with chronic HCV infection 15 males (60%) and 10 females (40%) and hepatocellular carcinoma 10 males (100%). The mean age of patients with HCV infection was (37.6 ± 13.3 years) and patients with hepatocellular carcinoma was (44.5 ± 7.8 years). Histopathological typing for hepatocellular carcinoma revealed that 4 cases (40%) had moderately differentiated adenocarcinoma and 6 cases (60%) had poorly differentiated adenocarcinoma. Normal liver specimens were obtained from thirteen persons 8 males (61.53%) and 5 female (38.46%). The mean age was (55.2 ± 9.2 years).

Immunohistochemical staining: The current results revealed a significant increased in the cellular expression of lymphotoxin α and their receptor (TLRs) while non significant increased in expression of lymphotoxin β among the 35 investigated diseased liver samples as showed in Tables 1-3 and fig. 1- 3. On the other hand, there was low positive result among control groups.

Table 1. The Expression of LT- α in studied groups

Result of Immunohistochemistry		LT- α Expression	*P* value
Patients with Chronic HCV infection	Positive Negative Total	22 (88%) 3 (12%) 25 (100%)	< 0.001
HCC	Positive Negative Total	8 (80%) 2 (20%) 10 (100%)	0.001
Control Group	Positive Negative Total	1 (7.69%) 12 (92.30%) 13 (100%)	

Table 2. The Expression of LT-β in studied groups.

Result of Immunohistochemistry		LT-β Expression	*P* value
Patients with Chronic HCV infection	Positive Negative Total	21 (84%) 4 (16%) 25 (100%)	< 0.062
HCC	Positive Negative Total	7 (70%) 3 (30%) 10 (100%)	0.669
Control Group	Positive Negative Total	7 (53.84%) 6 (46.15%) 13 (100%)	

Table 3. The Expression of LTR in studied groups

Result of Immunohistochemistry		LTR Expression	*P* value
Patients with Chronic HCV infection	Positive Negative Total	19 (76%) 6 (24%) 25 (100%)	< 0.001
HCC	Positive Negative Total	9 (90%) 1 (10%) 10 (100%)	< 0.001
Control Group	Positive Negative Total	0 (0%) 13 (100%) 13 (100%)	

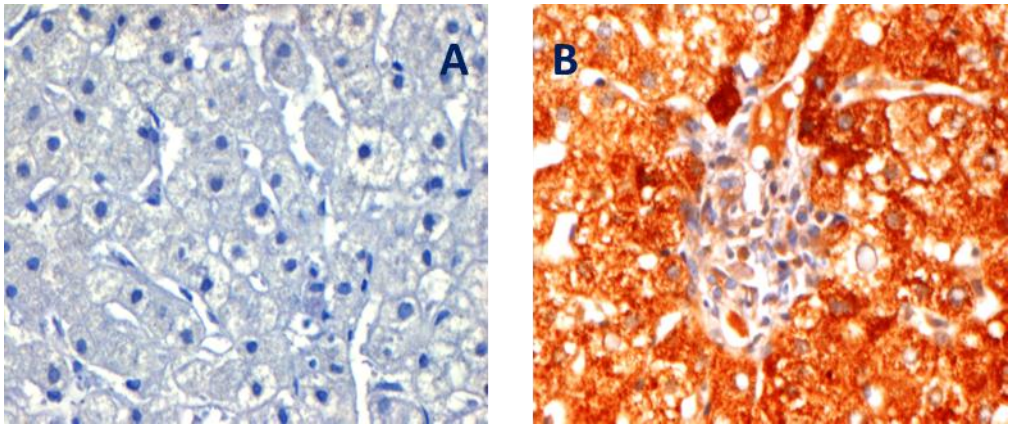


Fig. 1. Immunohistochemistry for LT-α in liver section with chronic HCV infection section, stained by DAB chromogen and counter stained with heamatoxylin. A: Negative expression, B: LT-α positive expression (400X).

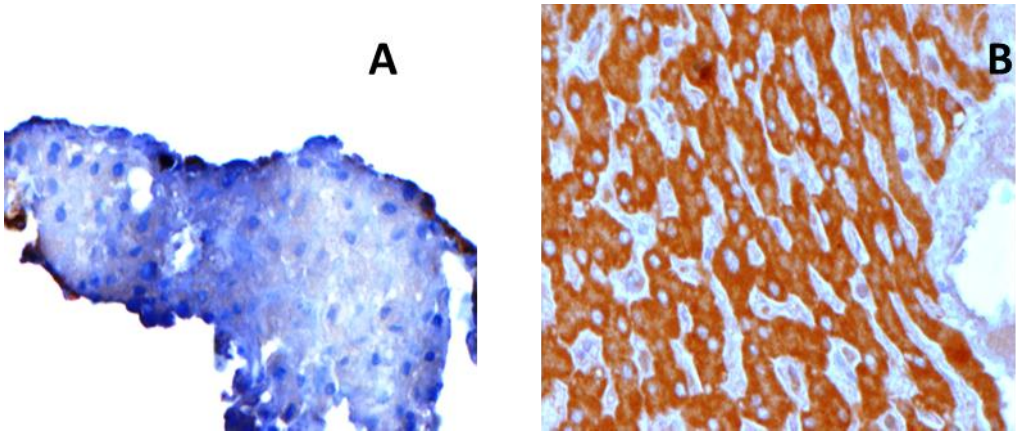


Fig. 2. Immunohistochemistry for LT- β in hepatocellar carcinoma infected section, stained by DAB chromogen and counter stained with heamatoxylin. A: Negative expression, B: LT- β positive expression (400X)

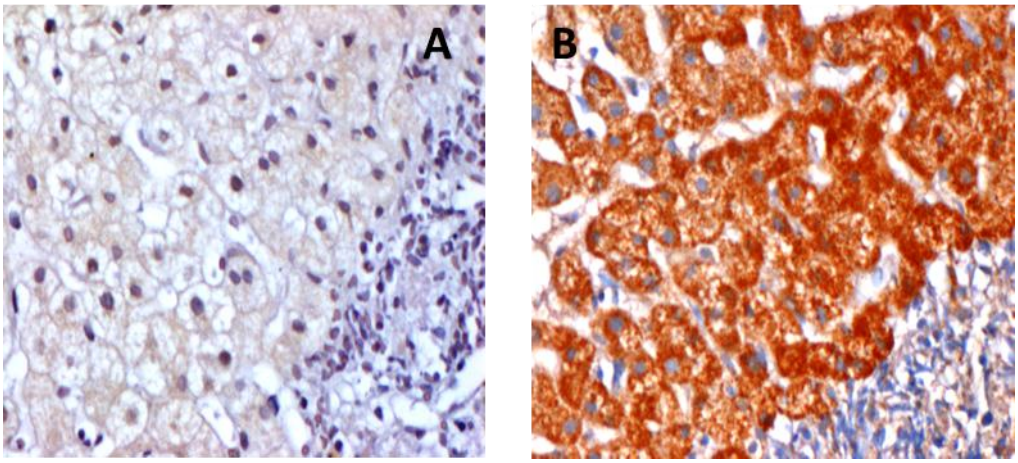


Fig. 3. Immunohistochemistry for LTβR in liver section with chronic HCV infection section stained by DAB chromogen and counter stained with heamatoxylin. A: Negative expression, B: LTβR positive expression (400X)

Tables 4-9 demonstrate correlation between expression of lymphotoxin α, β and their receptor (TLRs) with different variables. The results showed that there were no significant differences between expression of each lymphotoxin β with age, gender, grade and other while significant correlation between lymphotoxin α with age and receptor (TLRs) with disease stage of fibrosis. There was no correlation between hepatocellular carcinoma and the variables, which may be due to the limited sample size.

Table 4. Expression of LT- α in patients with chronic HCV infection and healthy control group

Variables		Expression of LT-α				*P* value
		Neg.	Low	Intermediate	High	
Age	≤ 40	0	2 (9.09%)	0	14 (63.63%)	0.037
	> 40	3 (100%)	2 (9.09%)	1 (4.54%)	3 (13.63%)	
Gender	Male	2 (66.6%)	2 (9.09%)	1 (4.54%)	10 (45.45%)	1.000
	Female	1 (33.3%)	2 (9.09%)	0	7 (31.81%)	
HAI	3/18	1 (33.3%)	0	0	1 (4.54%)	0.896
	4/18	1 (33.3%)	1 (4.54%)	0	5 (22.72%)	
	5/18	1 (33.3%)	2 (9.09%)	1 (4.54%)	6 (27.27%)	
	6/18	0	0	0	1 (4.54%)	
	7/18	0	0	0	1 (4.54%)	
	8/18	0	1 (4.54%)	0	2 (9.09%)	
	9/18	0	0	0	1 (4.54%)	
Stage	0	1 (33.3%)	0	0	2 (9.09%)	0.715
	1/6	0	2 (9.09%)	1 (4.54%)	3 (13.63%)	
	2/6	1 (33.3%)	0	0	3 (13.63%)	
	3/6	1 (33.3%)	1 (4.54%)	0	4 (18.18%)	
	4/6	0	0	0	1 (4.54%)	
	5/6	0	1 (4.54%)	0	1 (4.54%)	
	6/6	0	0	0	3 (13.63%)	
Control		12 (92.30%)	0	0	1 (7.69%)	

Table 5. Expression of LT- α in patients with hepatocellar carcinoma and healthy control group

Variables		Expression of LT-α			
		Negative	Low	Intermediate	High
Age	≤ 40	1 (50%)	1 (12.5%)	2 (25%)	1 (12.5%)
	> 40	1 (50%)	1 (12.5%)	2 (25%)	1 (12.5%)
Gender	Male	2 (100%)	2 (25%)	4 (50%)	2 (25%)
	Female	0	0	0	0
Grade	I	0	0	0	0
	II	0	2 (25%)	0	2 (25%)
	III	2 (100%)	0	4 (50%)	0
Control		12 (92.30%)	0	0	1 (7.69%)	

Table 6. Expression of LT-β in patients with chronic HCV infection and healthy control group

Variables		Expression of LT-β				*P* value
		Negative	Low	Intermediate	High	
Age	≤ 40	1 (25%)	1 (4.76%)	2 (9.52%)	12 (57.14%)	0.116
	> 40	3 (75%)	1 (4.76%)	2 (9.52%)	3 (14.28%)	
Gender	Male	4 (100%)	1 (4.76%)	2 (9.52%)	8 (38.09%)	0.125
	Female	0	1 (4.76%)	2 (9.52%)	7 (33.33%)	
HAI	3/18	1 (25%)	0	0	1 (4.76%)	0.452
	4/18	0	1 (4.76%)	0	6 (28.57%)	
	5/18	3 (75%)	0	2 (9.52%)	5 (23.80%)	
	6/18	0	0	1 (4.76%)	0	
	7/18	0	0	0	1 (4.76%)	
	8/18	0	1 (4.76%)	1 (4.76%)	1 (4.76%)	
	9/18	0	0	0	1 (4.76%)	
Stage	0	1 (25%)	0	0	2 (9.52%)	0.715
	1/6	1 (25%)	0	2 (9.52%)	3 (14.28%)	
	2/6	0	1 (4.54%)	1 (4.76%)	2 (9.52%)	
	3/6	2 (50%)	0	1.4.76%)	3 (14.28%)	
	4/6	0	0	0	1 (4.54%)	
	5/6	0	0	0	2 (9.52%)	
	6/6	0	1 (4.76%)	0	2 (9.52%)	
Control		6(46.15%)	5(38.46%)	2(15.38%)	0	

Table 7. Expression of LT-β in patients with hepatocellar carcinoma and healthy control group

Variables		Expression of LT-α			
		Negative	Low	Intermediate	High
Age	≤ 40	2 (66.6%)	0	1 (14.28%)	2 (28.57%)
	> 40	1 (33.3%)	2 (28.57%)	1 (14.28%)	1 (14.28%)
Gender	Male	3 (100%)	2 (28.57%)	2 (28.57%)	3 (42.85%)
	Female	0	0	0	0
Grade	I	0	0	0	0
	II	2 (66.6%)	0	2 (28.57%)	0
	III	1 (33.3%)	2	0	3 (42.85%)
Control		6 (46.15%)	5 (38.46%)	2 (15.38%)	0	

Table 8. Expression of LTβR in patients with chronic HCV infection and healthy control group

Variables		Expression of LT-βR				*P* value
		Negative	Low	Intermediate	High	
Age	≤ 40	4 (66.66%)	3 (15.78%)	3 (15.78%)	6 (31.57%)	1.00
	> 40	2 (33.33%)	2 (10.52%)	1 (5.26%)	4 (21.05%)	
Gender	Male	3 (50%)	2 (10.52%)	3 (15.78%)	7 (36.84%)	0.653
	Female	3 (50%)	3 (15.78%)	1 (5.26%)	3 (15.78%)	
HAI	3/18	0	0	0	2 (10.52%)	0.634
	4/18	1 (16.66%)	2 (10.52%)	1 (5.26%)	3 (15.78%)	
	5/18	4 (66.66%)	1 (5.26%)	2	3 (15.78%)	
	6/18	0	0	0	1 (5.26%)	
	7/18	0	0	0	1 (5.26%)	
	8/18	1 (16.66%)	1 (5.26%)	1 (5.26%)	0	
	9/18	0	1 (5.26%)	0	0	
Stage	0	0	0	0	3 (15.78%)	0.715
	1/6	5 (83.33%)	1 (5.26%)	0	0	
	2/6	0	1 (5.26%)	1 (5.26%)	2 (10.52%)	
	3/6	0	1 (5.26%)	2 (10.52%)	3 (15.78%)	
	4/6	0	0	0	1 (5.26%)	
	5/6	1 (16.66%)	1 (5.26%)	0	0	
	6/6	0	1 (5.26%)	1 (5.26%)	1 (5.26%)	
Control		13 (100%)	0	0	0	

Table 9. Expression of LTβR in patients with hepatocellar carcinoma and healthy control group

Variables		Expression of LT-βR			
		Negative	Low	Intermediate	High
Age	≤ 40	0	2 (22.22%)	1 (11.11%)	2 (22.22%)
	> 40	1 (100%)	2 (22.22%)	0	2 (22.22%)
Gender	Male	1 (100%)	4 (44.44%)	1 (11.11%)	4 (44.44%)
	Female	0	0	0	0
Grade	I	0	0	0	0
	II	0	0	0	4 (44.44%)
	III	1 (100%)	4 (44.44%)	1 (11.11%)	0
Control	13 (100%)	0	0	0	

Discussion

It has been established that signaling pathways of lymphotoxins can induce both canonical and noncanonical nuclear factor kappa B (NF-kB) cell survival system, whose role in controlling hepatic neoplasia remains controversial [10,12].

The current study had demonstrated that lymphotoxin α and β was over expressed in patients with chronic HCV infection and hepatocellular carcinoma (Table 1). This was in agreement with the finding of Haybaeck *et al* (2009) [14] who reported the role of persistent lymphotoxin pathways in the development of hepatocellular carcinoma. It has been shown, after studying the expression of lymphotoxin α, β and their receptor in HCV infected transgenic mice that overexpressing these proteins in

hepatocytes are more likely to develop chronic hepatitis followed by hepatocellular carcinoma.

Lymphotoxins are implicated indirectly in the induction of endothelogenesis, lymph-angiogenesis and inflammation by their direct action on NK cells to activate stromal cells and the production of Vascular endothelial growth factor A and C (VEGF-A, C) which are a crucial inflammatory mediator [14,15]. In addition, the production of chemokines and adhesion molecules by LT α could be implicated in the recruitment of macrophages which produce VEGF-C. On the other hand, tumor necrosis factor has been shown to up-regulate the expression of VEGF-C by macrophages [16].

Many investigations have documented the role of LTα in host defense mechanisms and reaction to infections, as mice deficient of this cytokine increases their susceptibility to infection with *Staphylococcus aureus* [17]. It was also reported that LTα is required for the granuloma formation and resistance to infection by *Mycobacterium*, *Leishmania*, *Plasmodium and Toxoplasma gondii* infections in mice [18-21]. In a study on transgenic mice it has been proposed that LTα plays a smaller role in the maintenance of lymphoid organs and has no direct involvement in the regulation of TNF [22].

In vivo and *in vitro* studies have indicated that infection with HBV or HCV leads to an increase in the LT expression in hepatocytes [23,24]. Another study performed *in vitro*, revealed that components of LTβR signaling pathway are required for HCV replication [25].

This study has revealed that there is no correlation between lymphotoxin α and the gender of patients, HAI or the stage of the disease. On the other hand, a significant correlation exists with that of the age of patients. This age related increase of incidence of hepatic neoplasia in HCV infected subjects appears to be a attributed to the inherent decline of the immune system and macrophage surveillance in old patients in addition to the increasing incidence of mutations of HCV infected hepatocytes [26].

Hepatocellular carcinoma is considered as the most common primary liver malignancy where the average age at diagnosis ranges from 60 to 80 years. During infection with HCV hepatic cirrhosis develops replacing injured liver cells. Formation of regenerative nodules is one of the healing processes that are usually happening in cases of hepatic cirrhosis and adenomatous hyperplasia. Development of the neoplasm is believed to evolve following cellular mutations that happen at the regenerative nodules which are then transform into malignancy [27].

The results of lymphotoxin β staining, shown in table 2, this result revealed increase in the expression of lymphotoxin β among studied group. This agrees with many authors who indicate that lymphotoxin β is expressed in chronic liver injury [25,28], and with Heliken-Walder *et al.*, (2005) [29] who indicate high expressed LTα and -β in the liver during analysis of two transgenic mouse lines. On the other hand this study did not reveal significant correlation between the positive signals of lymphotoxin β and different clinicopathological variable.

Lymphotoxin α is recognized by the same receptor of tumor necrosis factor and lymphotoxin beta is recognized by its receptor [30].

The current result demonstrated a significant increase in the cellular expression of lymphotoxin β receptor (TLβRs), among patients with chronic HCV infection and those with hepatocellular carcinoma. Previous studies demonstrated the role of LT-α1β2/LT-βR in the transduction of both apoptotic and non-apoptotic signaling pathways [31,32]. Moreover, it has also been reported that activation of LT-β receptor can induce inflammation through the production of chemokines and endothelial adhesion molecules necessary for recruitment of lymphocytes to sites of insult [33-35].

The work by Ruddell and colleagues demonstrated that LT β R signaling regulates hepatic stellate cell function and hepatic wound healing as well as controlling liver homeostasis in both health and disease [36].

Several reports point towards an interaction of the HCV core protein with the LT α R, leading to the modulation of the LT αR-signaling pathway [37-40]. The main finding of this study is that HCV infection activates the production of lymphotoxin α, β and their receptor TLRs in human liver tissues as evidenced by immunohistochemical studies.

Our result revealed there is no significant correlation between positive LTβR signaling with age of patients, grade and HAI, while significant correlation with stage of fibrosis of patients with chronic HCV infection, this may be related with activation of TLR-mediated signaling pathways initiating an early inflammatory response are indispensable for protecting the host against pathogenic organisms, an excessive and/or prolonged activation may lead to both acute and chronic inflammatory diseases. Therefore, the intensity and duration of TLR responses must be tightly regulated. Down regulation of TLR signaling, called TLR tolerance, as well as cross-tolerance among various TLR ligands might have been developed to prevent excessive inflammatory damage to the host [43].

Expression of LTβ-R on human fibroblasts and human carcinoma cell lines maintained in vitro has also been described, and stimulation of these cells through LTβ-R can produce growth stimulation, growth arrest, or cytokine production, depending on the cell type [42-44].

The expression of lymphotoxin α and β and their receptor TLRs has been reported by several researchers. The differences between the results of the these researchers and even with the results of present study could be related to many factors, like type of the tissue whether human or mice, sample size, stage of fibrosis, grade of the tumor, the methodology and affinity of the antibody, the duration of incubation, the sensitivity of detection system and lack of standardized technique because these factors also affect the expression of these markers. In conclusion, three members of tumor necrosis factor super family were over expressed in liver tissues and may be have critical role in the liver injures. Further studies are needed with large sample size to indicate the same results.

Acknowledgements

This work was performed at Division of Virology-National Institute for Biological Standards and Control, UK during the period from March-2012 till September-2012 at histopathology laboratory. Special thank for Belinda Dagg for kindly sectioning of the tissue blocks and great thanks for all staff of virology.

References

1. Foster SL, Medzhitov R. Gene-specific control of the TLR-induced inflammatory response. Clin Immunol. 2009; 130: 7-15.
2. Nathan C. Points of control in inflammation. Nature. 2002; 420: 846-52.
3. Poovassery JS, Vanden Bush TJ, Bishop GA. Antigen receptor signals rescue B cells from TLR tolerance. J Immunol. 2009; 183: 2974-83.
4. Wolf MJ, Seleznik GM, Zeller N, et al. The unexpected role of lymphotoxin β receptor signaling in carcinogenesis from lymphoid tissue formation to liver and prostate cancer development. Oncogene. 2010; 29: 5006-18.
5. Tumanov AV, Kuprash DV, Nedospasov SA. The role of lymphotoxin in development and maintenance of secondary lymphoid tissues. Cytokine Growth Factor Rev. 2003; 14: 275-88.
6. Ware CF. Network communications: lymphotoxins, LIGHT and TNF. Ann Rev Immunol. 2005; 23: 787-819.
7. Malhi H, Gores GJ, Lemasters JJ. Apoptosis and necrosis in the liver: a tale of two deaths. Hepatology. 2006; 43: S31-S44.
8. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007; 132: 2557-76.
9. Dar I, Sun-Lung T, Young-Mao C, et al. Activation of Nuclear Factor k β in Hepatitis C Virus Infection: Implications for Pathogenesis and Hepatocarcinogenesis. Hepatology. 2000; 31(3): 656-64.
10. Vainer GW, Pikarsky E, Ben-Neriah Y. Contradictory functions of NF-kappa β in liver physiology and cancer. Cancer Lett. 2008; 267: 182-8.
11. Boussaud V, Soler P, Moreau J, et al. Expression of three members of the TNF-R family of receptors (4-1BB, lymphotoxin-β receptor, and Fas) in human lung. Eur Respir J. 1998; 12: 926-31.
12. Maniatis T. Catalysis by a multiprotein IKB kinase complex. Science. 1997; 278: 818-9.

13. Haybaeck J, Zeller N, Wolf MK, et al. A Lymphotoxin-Driven Pathway to Hepatocellular Carcinoma. Cancer Cell. 2009; 16: 295-308.

14. Halin C, Tobler NE, Vigl B, et al. VEGF-A produced by chronically inflamed tissue induces lymphangiogenesis in draining lymph nodes. Blood. 2007; 110(9): 3158-67.

15. Angeli V, Ginhoux F, Llodra J, et al. B cell-driven lymphangiogenesis in inflamed lymph nodes enhances dendritic cell mobilization. Immunity. 2006; 24(2): 203-15.

16. Ristimaki A, Narko K, Enholm B, et al. Proinflammatory cytokines regulate expression of the lymphatic endothelial mitogen vascular endothelial growth factor-c. J Biol Chem. 1998; 273(14): 8413-8.

17. Hultgren O, Eugster HP, Sedgwick JD, et al. TNF/lymphotoxin-alpha double-mutant mice resist septic arthritis but display increased mortality in response to *Staphylococcus aureus*. J Immunol. 1998; 161(Suppl 11): 5937-42.

18. Roach DR, Briscoe H, Saunders B, et al. Secreted lymphotoxin-alpha is essential for the control of an intracellular bacterial infection. J Exp Med. 2001; 193(Suppl 2): 239-46.

19. Ehlers S, Hölscher C, Scheu S, et al. The lymphotoxin beta receptor is critically involved in controlling infections with the intracellular pathogens *Mycobacterium tuberculosis* and *Listeria monocytogenes*. J Immunol. 2003; 170(Suppl 10): 5210-8.

20. Roach DR, Briscoe H, Saunders BM, et al. Independent protective effects for tumor necrosis factor and lymphotoxin alpha in the host response to *Listeria monocytogenes* infection. Infect Immun. 2005; 73: 4787-92.

21. Schluter D, Kwok LY, Lutjen S, et al. Both lymphotoxin-alpha and TNF are crucial for control of *Toxoplasma gondii* in the central nervous system. J Immunol. 2003; 170: 6172-82.

22. Liepinsh DJ, Grivennikov SI, Klarmann KD, et al. Novel lymphotoxin alpha knockout mice with unperturbed tumor necrosis factor expression: reassessing LT alpha biological functions. Mol Cell Biol. 2006; 26(Suppl 11): 4214-25.

23. Lee SH, Park SG, Lim SO, et al. The hepatitis B virus X protein up-regulates lymphotoxin alpha expression in hepatocytes. Biochim Biophys Acta. 2005; 1741: 75-84.

24. Lowes KN, Croager EJ, Abraham LJ, et al. Upregulation of lymphotoxin beta expression in liver progenitor (oval) cells in chronic hepatitis C. Gut. 2003; 52: 1327-32.

25. Ng TI, Mo H, Pilot-Matias T, et al. Identification of host genes involved in hepatitis C virus replication by small interfering RNA technology. Hepatology. 2007; 45: 1413-21.

26. Burns EA, Leventhal EA. A immunity and cancer. Cancer Control. 2000; 7: 513-22.

27. Arakawa M, Kage M, Sugihara S, et al. Emergence of malignant lesions within an adenomatous hyperplastic nodule in a cirrhotic liver. Observations in five cases. Gastroenterology. 1986; 91: 198-208.

28. Voon DC, Subrata LS, Abraham LJ. Regulation of lymphotoxin β by tumor necrosis factor, phorbol myristate acetate, and ionomycin in Jurkat T cells. J Interferon Cytokine Res. 2001; 21: 921-30.

29. Heikenwalder M, Zeller N, Seeger H, et al. Chronic lymphocytic inflammation specifies the organ tropism of prions. Science. 2005; 307: 1107-10.

30. Cannella B, Sizing ID, Benjamin CD, et al. Antibodies to lymphotoxin alpha (LT alpha) and LT beta recognize different glial cell types in the central nervous system. J Neuroimmunol. 1997; 78(1-2): 172-9.

31. VanArsdale TL, VanArsdale SL, Force WR, et al. Lymphotoxin β receptor signaling complex: Role of tumor necrosis factor receptor-associated factor 3 recruitment in cell death and activation of NF-κB. Proc Natl Acad Sci USA. 1997; 94: 2460-5.

32. Nakano H, Oshima H, Chung W, et al. TRAF5, an activator of NF-κB and putative signal transducer for the lymphotoxin receptor. J Biol Chem. 1996; 271: 14661-4.

33. Wu Q, Wang Y, Wang J, et al. The requirement of membrane lymphotoxin for the presence of dendritic cells in lymphoid tissues. J Exp Med. 1999; 190: 629-38.

34. Degli-Esposti MA, Davis-Smith T, Din WS, et al. Activation of the lymphotoxin β receptor by cross-linking induces chemokine production and growth arrest in A375 melanoma cells. J Immunol. 1997; 158: 1756-62.

35. Ngo VN, Korner H, Gunn MD, et al. Lymphotoxin alpha/beta and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. J Exp Med. 1999; 189: 403-12.

36. Ruddell RG, Knight B, Tirnitz-Parker JE, et al. Lymphotoxin-beta receptor signaling regulates hepatic stellate cell function and wound healing in a murine model of chronic liver injury. Hepatology. 2009; 49: 227-39

37. Chen CM, You LR, Hwang LH, et al. Direct interaction of hepatitis C virus core protein with the cellular lymphotoxin-beta receptor modulates the signal pathway of the lymphotoxin-beta receptor. J Virol. 1997; 71: 9417-26.

38. Matsumoto M, Hsieh TY, Zhu N, et al. Hepatitis C virus core protein interacts with the cytoplasmic tail of lymphotoxin-beta receptor. J Virol. 1997; 71: 1301-9.

39. Zhu N, Khoshnan A, Schneider R, et al. Hepatitis C virus core protein binds to the cytoplasmic domain of tumor necrosis factor (TNF) receptor 1 and enhances TNF-induced apoptosis. J Virol. 1998; 72: 3691-7.

40. You LR, Chun-Ming C, Yan-Hwa WL. Hepatitis C Virus Core Protein Enhances NF-KB Signal Pathway

Triggering by Lymphotoxin-β Receptor Ligand and Tumor Necrosis Factor Alpha. J Virol. 1999; 73(2): 1672-81.

41. Wimmer N, Barbara H, Nicola B, et al. Lymphotoxin β Receptor Activation on Macrophages Induces Cross-Tolerance to TLR4 and TLR9 Ligands. J Immunol. 2012; 188: 3426-33.

42. Force WR, Walter BN, Hession C, et al. Mouse lymphotoxin-β receptor. Molecular genetics, ligand binding, and expression. J Immunol. 1995; 155: 5280-8.

43. Degli-Esposti MA, Davis-Smith T, Din WS, et al. Activation of the lymphotoxin β receptor by cross-linking induces chemokine production and growth arrest in A375 melanoma cells. J Immunol. 1997; 158: 1756-62.

44. Browning JL, Miakowski K, Sizing I, et al. Signaling through the lymphotoxin β receptor induces the death of some adenocarcinoma tumor lines. J Exp Med. 1996; 183: 867-78.

Correspondence to Dr. Areej A Hussein
E-mail: Dr.Areej.Hussein@medicine.uodiyala.equ.iq
Received 14th Jan. 2013: Accepted 27th Mar. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Medico-Legal Study of Non-Fatal Road Traffic Injuries

Nagi A Abdulla *PhD*

Forensic Medicine Unite- Para-Clinic Department, Faculty of Medicine and Health Sciences
Aden University - Yemen.

Abstract

Background Road traffic accidents are global problems, which represent one of the major causes of disability and death worldwide leading to unpleasant results on individuals, their families and their society.

Objective To study the incidence of road traffic accidents in relation to socio-demographic factors, and to find out the patterns and distribution of the resulting injuries.

Method This study included (375) victims of road traffic accidents whom were brought to the casualty room in AL-Gamhoria Teaching Hospital in Aden-Yemen for medical and legal evaluation during the period from 1st January up to 31 December 2010. Information collected consisted of personal identification data, and accident characteristics that were obtained from patients, close relatives, and other available persons who were present at the time of incidence.

Results Only 140 (37.4%) of victims were hospitalized. 32% of road traffic accidents (RTAs) was observed among those aged 21-30 years with predominance of male (82.9%). The illiterates were the victims in 38.7%, students (48%), and single (60%). Most of cases from middle and lower socio-economic classes (53.3% and 40%, respectively). Pedestrians (38.5%) and passengers (35.7%) were the victims in most of cases. Light motor vehicle was involved in 61.5%. Sideway and hit and run were the most common types of collisions seen in (33.3% and 30.2%, of all cases respectively). Most of the accidents occurred between 12.01pm to 06.00pm. Most of the accidents occurred during summer season, mainly in the weekend. Multiple superficial injuries like abrasions, contusions, and lacerations wee the commonest pattern of injuries. Limb injuries were the most (46.5%) followed by the head (26.4%).

Conclusion The middle aged group males were more common involved in road accidents. The pedestrians were mostly affected. The Injuries were mostly caused by light motor vehicles than other vehicles. Accidents were more common in the summer season and during weekend, in afternoon hours. Multiple superficial injuries were the commonest injury, and the head and limbs were the most common site of injuries among victims of non-fatal road traffic accidents.

Key words Non-fatal, road traffic accidents, injury patterns and distribution.

Introduction

Road traffic accidents (RTAs) are major but neglected global health problems. The death and injuries due to RTAs constitute a serious social problem worldwide [(1)].

Accident is an event, occurring suddenly, unexpectedly and inadvertently under unforeseen circumstances [(2)]. An accident has been defined as "an unexpected unplanned occurrence which may involve injury". A WHO advisory group in 1956 defined accident as "an unpremeditated event resulting in recognizable damage". According to another definition, an accident is that "occurrence in sequence of events which usually produces unintended injury, death or property damage [(3)].

The RTAs is defined as any vehicles accident occurring on a public road or highway and includes vehicle accidents where the place of occurrence is unspecified [(4)].

Worldwide the number of people killed in road traffic accidents each year is estimated at about

1.2 million, whereas those injured as high as 50 million [5]. During 1990 RTAs ranked ninth among the leading cause of death in world. It is projected to become second leading cause of death by the year 2020 next to Ischemic heart disease [3]. In 2009 the rate of road traffic death is 13 persons / hrs, according to National Crime Record Bureau (NCRB). Maximum cases reported between 15.00-18.00 hours in Asian countries and 60-80% RTAs occur in urban and semi urban regions [6]. All these figures are due to one, or more than one of the following factors, human, vehicles, road, and environment factors [2].

RTAs are no longer considered accidental but are part of the price we pay for the technological progress. The resulting injuries of which may involve head, neck, chest, abdomen, and extremities resulting death and deformity. Characterization of the commonly encountered injuries due to accidents, and establishment of corresponding precautions might reduce traffic accident related morbidity and mortality. Following the establishment of laws for use of seat belts and helmets worldwide, the frequency and severity of injuries particularly head traumas have decreased. Technological improvements in vehicles and inclusion of airbags are also regarded as protective factors. Also intense information campaigns through radio, television, and new papers have also been reported to reduce alcoholic driving profoundly [7].

The pattern of injury, fatal and otherwise, varies considerably depending upon whether the victim is a vehicle occupant, a motorcyclist – a pedal cyclist or a pedestrian [2]. The incidence of death in pedestrians is significantly higher than in car occupants or motorcyclists in road traffic accident, which are further increasing at an alarming rate [8].

The intention of our study is to search for the incidence of road traffic accidents in relation to socio-demographic factors, and to identify the patterns and distribution of injuries in case of road traffic accidents.

Method

This descriptive study was conducted at the casualty room in AL-Gamhoria Teaching Hospital in Aden-Yemen for evaluation of medical and legal aspects, comprised of 375 victims of road traffic accidents during the period from 1st January up to 31 December 2010.

Information collected were personal identification data, first including the socio-demographic factors as (sex, age, education status, occupation, marital state, socioeconomic status, and type of road user), next; accident characteristics as (type of vehicles, type of accident, cause of accident, time, day, and month of accidents, patterns of injuries and its distribution on the body parts and nature of treatment). Finally, the history of road traffic injuries was obtained from patient, close relatives and other available person who were present at the time of incidence. The data were manually analyzed.

Results

The distribution of study cases according to demographic profile is depicted in table 1. The sex distribution of the victims clearly showed a male predominance which constituted 311 (82.9%) of the total victims compared to only 64 (17.1%) females. Males outnumbered females in the ratio of 5:1. Age wise, the highest incidence of victims was seen in those belong to the age group (21-30 years) comprising 120 (32%) followed by age group 31-40 years having 103 (27.5%) and the lowest incidence was seen in extreme age groups, i.e. below 10 years and above 50 years, which represent 20 (5.3%) and 25 (6.7%) respectively.

About the education status, high numbers of the victims in the present study were Illiterate (38.7%) followed by those who were educated up to primary, secondary school level and university graduate. The occupation of the victims was highest among the students (48%). About 225 (60%) of victims were single. The people from middle and lower socio-economic class (53.3% and 40%) affected more than other class. Overall, the road user victims was divided

in to 3 categories, the pedestrians themselves constituted the largest group 144 (50.9 %) among all vulnerable RTAs. victims, followed by passengers 134 (35.7%) from them only 7% of drivers involved in RTAs. are learners while 93% were licensed.

Table 1. Demographic profile of victims in road traffic accidents

Demographic profile of victims			Number	Percentage
Sex	Males		311	82.9
	Female		64	17.1
Age (Years)	< 10		20	5.3
	11-20		48	12.8
	21-30		120	32
	31-40		103	27.5
	41-50		59	15.7
	> 50		25	6.7
Education status	Illiterate		145	38.7
	Primary school level		105	28
	Secondary school level		80	21.3
	Graduated level		45	12
Occupation	Unemployed		75	20
	Student		180	48
	Worker		105	28
	Housewife		15	4
Marital state	Single		225	60
	Married		105	28
	Divorced		15	4
	Widow		30	8
Socioeconomic status	Lower class		150	40
	Middle class		200	53.3
	Upper class		25	6.7
Type of road user	Pedestrian		144	38.5
	Vehicle occupants (N = 184)	Passenger	134	35.7
		Drivers	50	13.3
	Cyclist (N = 47)	Motor cycle	29	7.7
		Pedal cycle	18	4.8

In table 2 showing the distribution of study sample according to accident characteristics, It was observed that the most common offender vehicles being involved in the RTAs are the light motor vehicles (LMV) of all makes and models as (taxi, car, and minibus) represent 142 (61.5%) which is very high in comparison to accidents by others vehicles. Side way collision with hit and run was the most common type of accident seen in 125 (33.3%) and 113 (30.2%) cases respectively. It was seen that the high speed 98 (26%), congested road 79 (21.1%), and negligent road crossing 59 (15.7%) was the main causes in more than half of road traffic accident cases. According to the time of occurrence, the road traffic accidents were divided in to four slots; (12.01 am to 06.00 am), (06.01 am to 12.00 noon), (12.01 pm to 06.00 pm), and (06.01 pm to 12.00 midnight). It was seen that the highest percentage of RTAs reported in afternoon between 12.01 pm to 06.00 pm (40.8%) and the least percentage (13.3%) reported in morning between (12.01 am to 06.00 am).

Table 2. Distribution of study sample according to accident characteristics

Characteristic		Number	Percentage
Type of vehicles involved (N = 231)	Light motor vehicles	142	61.5
	Heavy motor vehicles	27	11.7
	Bicycles	44	19.0
	Others	18	7.8
Type of accident (collision) (N = 375)	Hit and run	113	30.2
	Run over	30	8.0
	Head on	20	5.3
	Side way	125	33.3
	Fall from bus	59	15.7
	Others	28	7.5
Cause of accident (event) (N = 375)	Negligent road crossing	59	15.7
	Negligent cycling	15	4.0
	Negligent driving	25	6.7
	Mechanical fault of vehicles	25	6.7
	Playing on road	38	10.1
	Congested road	79	21.1
	High speed	98	26.0
	Poor vision fingers	15	4.0
	Standing on doors (scoters)	21	5.6
Time of accident (N = 375)	12.01 am - 06.00 am	50	13.3
	06.01 am - 12.00 noon	90	24.0
	12.01 pm - 06.00 pm	153	40.8
	06.01 pm - 12.00 midnight	82	21.9

The highest percentage of road traffic accident occurred in summer season as June 50 (13.3%), July 49 (13.1%), and August 47 (12.5 %), while the lowers percentage occurred in winter season as January 12 (3.2%) and February 16 (4.2 %) respectively. There was a greater incidence of traffic injury during the weekend compared to mid-week-days as seen in fig. 1 and 2.

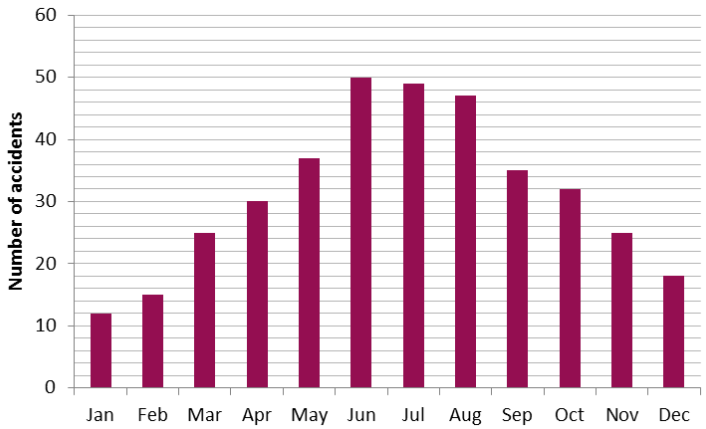


Fig. 1. Distribution of RTAs victims according to the months of the study

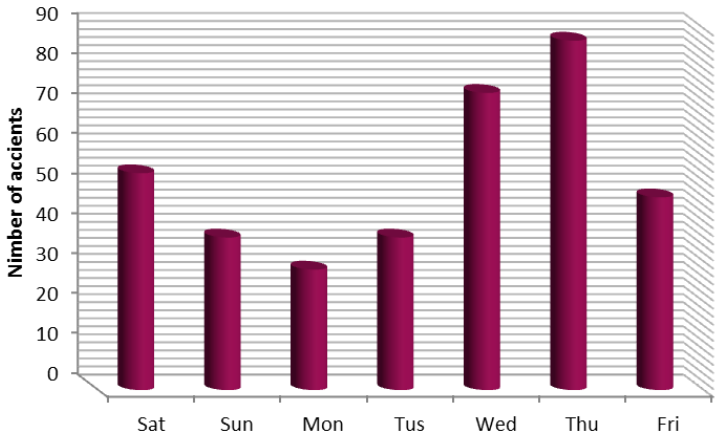


Fig. 2. Distribution of RTAs Victims according to the days of the week

Total number of all injuries seen in 375 RTA victims was 568, all type of injuries except incised wound were common. Multiple superficial injuries like abrasion 261 (46%), contusion 112 (19.7%) and laceration 100 (17.6%) were the common injury affected to the RTA victims followed by fracture of the bone 87(15.3%). The site of the body mostly affected by the road traffic injury included the head and face 150 (26.4%), lower and upper limb 135 (23.8%) 129 (22.7%) respectively, while the neck and spine injuries were much less common among the traffic victims as illustrated in table 3.

Table 3. Distribution of pattern of injuries on body site of RTAs victims

Site of injuries	Patterns of Injuries					Total
	Abrasion	Contusion	Incised	Laceration	Fracture	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Head and face	55 (9.7)	35 (6.2)	5 (0.9)	40 (7.0)	15 (2.6)	150 (26.4)
Neck and spine	2 (0.4)	2 (0.4)	0 (0.0)	3 (0.5)	2 (0.4)	9 (1.6)
Thorax	48 (8.5)	13 (2.3)	1 (0.2)	6 (1.1)	10 (1.8)	78 (13.7)
Abdomen	27 (4.8)	16 (2.8)	0 (0.0)	9 (1.6)	0 (0.0)	52 (9.2)
Upper limb	68 (12.0)	24 (4.2)	2 (0.4)	10 (1.8)	25 (4.4)	129 (22.7)
Lower limb	57 (10.0)	19 (3.3)	0 (0.0)	30 (5.3)	29 (5.1)	135 (23.8)
Pelvis	4 (0.7)	3 (0.5)	0 (0.0)	2 (0.4)	6 (1.1)	15 (2.6)
Total	261 (46.0)	112 (19.7)	8 (1.4)	100 (17.6)	87 (15.3)	568 (100)

All victims of road accidents suffered of same type of injuries to one or more of their body parts, the most frequent injured body parts are the limbs 130 (22.9%) and head 65 (11.4%) which are the most common areas affected among the pedestrian, while the drivers represent the second most common affected road user victim. The back seat occupants were much less commonly affected victims. Overall, the abrasion constituted the highest number injuries among all road user victims followed by laceration and fractures of bone 100 (17.6%) and 87 (15.3%) respectively, as shown in tables 4 and 5.

A Total of 235 (62.6%) road traffic victims were treated as out-patients clinic either conservative (no treatment) or by First Aid, while 140 (37.4%) road traffic victims needed admission for general or specific management. Regarding the type of treatment provided to the 140 admitted victims of road traffic accident, 100 victims (26.7%) received general management including First-Aid

and only 40 (10.7%) victims could receive specific management including major operation. It was observed from the study that 140 victims had sustained injuries, which required in-patient management; there were 40 (28.6) victims refer to privet hospital while the other admitted in different bed words within the same hospital as shown in tables 6 and 7.

Table 4. Anatomical distribution of injuries according to road user victims

Site of injuries	Type of road user victims					Total
	Pedestrian N = 144	Cyclist N = 47	Drivers N = 50	Front seat occupants N = 29	Back seat occupants N = 105	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Head and face	65 (11.4)	20 (3.5)	41 (7.2)	14 (2.5)	10 (1.8)	150 (26.4)
Neck and spine	2 (0.4)	1 (0.2)	3 (0.5)	1 (0.2)	2 (0.4)	9 (1.6)
Thorax	28 (4.9)	8 (1.4)	30 (5.3)	7 (1.2)	5 (0.9)	78 (13.7)
Abdomen	11 (1.9)	10 (1.8)	14 (2.5)	9 (1.6)	8 (1.4)	52 (9.2)
Upper limb	60 (10.6)	20 (3.5)	39 (6.9)	6 (1.1)	4 (0.7)	129 (22.7)
Lower limb	70 (12.3)	17 (3.0)	25 (4.4)	15 (2.6)	8 (1.4)	135 (23.8)
Pelvis	5 (0.9)	3 (0.5)	6 (1.1)	1 (0.2)	0 (0.0)	15 (2.6)
Total	241(42.4)	79(13.9)	158(27.8)	53 (9.3)	37 (6.5)	568 (100)

Table 5. Distribution of Injury patterns according to road user victims

Type of road user victims	Injury patterns					Total
	Abrasion	Contusion	Incised	Laceration	Fracture	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Pedestrian (N = 144)	150 (26.4)	52 (9.2)	0 (0.0)	55 (9.7)	41 (7.2)	298 (52.5)
Cyclist (N = 47)	38 (6.7)	8 (1.4)	2 (0.4)	12 (2.1)	12 (2.1)	72 (12.7)
Drivers (N = 50)	38 (6.7)	19 (3.3)	3 (0.5)	18 (3.2)	19 (3.3)	97 (17.1)
Front seat occupants (N=29)	20 (3.5)	16 (2.8)	2 (0.4)	9 (1.6)	10 (1.8)	57 (10.0)
Back seat occupants (N=105)	15 (2.6)	17 (3.0)	1 (0.2)	6 (1.1)	5 (0.9)	44 (7.7)
Total (N = 375)	261(46.0)	112(19.7)	8 (1.4)	100 (17.6)	87(15.3)	568 (100)

Table 6. The nature of treatment received among the victims

Treatment Receive		Number	Percentage
No treatment		184	49.0
Treated in casualty (First-Aid)		51	13.6
Treated in hospital (N = 140)	**General management**	100	26.7
	Specific management (major operation)	40	10.7
Total		375	100

Table 7. Distribution of victims according to the words of admission

Admitted in hospital ward	Number	Percentage
Emergency room	26	18.6
Intensive care unite	7	4.9
Orthopedic word	35	25
General surgery word	27	19.3
Pediatric word	5	3.6
Refer to other privet clinic	40	28.6
Total	140	100

Discussion

Road traffic accidents are global problem, which affects mainly young people [9]. They constitute the most common cause of traumas [10,11]. Human factors remains the leading cause of road traffic accident, such as over speeding, carelessness, not obeying traffic laws, under age driving, use of mobile, and fatigue [12].

Throughout the world as well as in Yemen, roads are bustling with cars, buses, trucks, and other types of vehicles. By making the transportation of good and people faster and more efficient, these vehicles support economic and social development in many countries. However, while motorized travel provides many benefits, it can also do serious harm unless safety is made a priority.

An accident usually takes place due to the interaction of a number of causes: bad driving is frequently a major reason but other factors may contribute to a variable extent; these include environmental conditions such as street lighting; the layout of crossroads, vehicle defects, alcoholic intoxication and natural disease of the driver [13].

In the current study, the male victims' outnumbered female victims, with male to female ratio as 5:1. This result comes in agreement with studies in the rest of the world [6,12,14-17]. It can be suggested that in our society males being the earning members of family are subjected to work related stress, and more exposed to outside environment as compared to female, in addition to cultural background.

Considering the age group analysis of the victims, it was also clear from the above study that the majority of RTAs victims were in the age group (21-30) years which represented (32%) of total number of victims, our observation closely match those of other researchers [6,14-16,18]. This age group represents the most active phase of life, physically and socially and the most productive age group of society suggesting huge economic loss to the country. This age group mainly consists of workers and students who usually travel by own vehicles or other. This situation can be improved by public education through the mass media and initiating road safety training campaign in school. Our observation closely matches those of other researchers [20]. The age group of 10 year and above the age group of 50 years, the proportion of victims was low, this finding were similar to the study reported by *Jha et al* (2003) [19] but other study found that age group between 45-64 is the highest age group [17].

Also, regarding the education status of victims, it was observe that the victims were either illiterate or had education only up to primary school level, and also are more affected in this study which gives the indication that lack of road traffic senses resulting either from illiteracy or poor literacy. Our observation closely matches those of other researchers [20].

About the occupation status of the victims in the present study the highest number of the victims were student, the reason for this may be attributed to the fact that those classes of persons are most often required to move out on the roads in the process of their studies and work. Our observation closely matches those of other researchers [20].

Regarding the type of road user victims status, it was found that more than one-third of victims were pedestrians (38.4%) followed by vehicle passengers, vehicle drivers, and cyclist respectively, this finding has been reported and conducted by various researchers [2,14], but other studies found that the vehicle passengers mainly drivers were more involved than pedestrians [6,21,22]. This difference is due to that the pedestrian in our country are the frequent road users and footpaths meant for their use are occupied by hawkers to such an extent that pedestrian are forced to walk onto the roads and ignoring traffic rules such as zebra crossing and waiting for green lights.

Considering the types of vehicle involved in road traffic accidents, our study showed that the LMV (61.5%) were more common offending vehicle involved in RTA than others, this finding coincides with the results of different studies [23], and contradicts with other which the HMV are common vehicles involved in their studies [16,24]. This could be attributed to the fact that the present study is carried out in an urban area where these types of vehicles are most common.

Analyzing the time at which RTA was sustained, it is found that, incidents were highest in afternoon hours between 12.01 pm to 06.00 pm (40.8%). Similar observation were made in other studies [6,12,17,21,25,26], but contradicts with other which the higher incidence occur in morning hours between 06.01 am -12.00 noon [2,16,17]. One of the reasons for the high rate during this time period could be the rush home for the main meal of the day when work finishes and schools close also due to heavy traffic during peak hours.

Regarding monthly distribution of accident victims, the present study revealed high incidence occurred in summer month (June, July, and August), similar finding were observed in other studies[18,26-28] while this finding contradicts with other which the higher incidence in rainy season (January to march) [14], also some studies showed the majority of accident occurred during winter season [25,29,30]. This may explain that the hot weather is on its peak in these months, in addition that the traffic is highly crowded making the traffic movement worse, and the holiday season in our country.

In our study, we found that most of vehicle accidents occur at the weekend (Thursday and Wednesday) 21.3% and 20% respectively. This finding differs from other studies found in Nepal where the highest numbers were in Sunday and the lowest on Monday [26], also in Iraq the highest numbers were in Saturday and the lowest on Tuesday [17], while in India which the highest numbers of accidents on Saturday, however, in a national injury mortality surveillance system in 2004 reported on Saturday and Sunday 20.8% and 17.1% respectively [25].

This study clearly revealed that head was injured in most of the cases followed by lower limbs and upper limbs (26.4%, 23.8%, and 22.7%) respectively, these results correlate with the works of others literature [14,17,31,32]. The most common pattern of injuries on the body of road user were multiple superficial injuries as (abrasion, contusion and laceration) than fractures on different body parts. This finding differs to those obtained by other studies which found the predominant pattern of injury were fractures [23,31,32].

As reported in our study, 49% of injured victims did not need medical treatment while the remaining victims 51% had taken medical treatment, from this 37.4% of victims admitted in hospital words mainly in orthopedic and general surgical words, these results correlate with the work of another study done in Nairobi [33].

Reference

1. Fujita Y, Shibata A. Relationship between traffic fatalities and drunk driving in Japan. Traffic Inj Prevent. 2006; 7: 325-27.
2. Singh YN, Bairagi KK, Das KC. An epidemiological study of road traffic accident victims in medico-legal autopsies. J Indian Acad Forensic Med. 2005; 27(3): 166-9.
3. Park Textbook of social and preventive medicine. 15th ed. Jabalpur: Banarsi Das Bhanot; 1997. p. 298-501.
4. Kaul A, Sinha US, Kapoor AK, et al. An epidemiological study of fatal road traffic accidents in Allahabad

region. Indian Internet J Forensic Med Toxicol. 2005; 3(1): 4-11.
5. Dhillon S, Sekhon HS. Pattern and distribution of injuries in fatal road traffic accidents in Shimla (Himachal Pradesh). Medico-Legal Update. 2007; 7(4): 115-8.
6. Patel DJ, Agnihotram G. Study of road traffic accident deaths in and around Bastar Region of Chhattisgarh. J Indian Acad Forensic Med. 2010; 32(2): 110-2.
7. Yildirim A, Ulusoy H, Cetin I, et al. Fracture Frequency and Variation in Fatal Traffic Accidents. Turkish J Forensic Sci. 2009; 8(2): 23-27.
8. Singh H, Dhattarwal S, Mittal S, et al. A review of pedestrian traffic fatalities. J Indian Acad Forensic Med. 2007; 29(4): 55-8.
9. WHO. World Report on Road Traffic Injury Prevention. Geneva: WHO, 2004; p. 3-29.
10. Stended WL, Corbus F, Feger SK. Epidemiology and Prevention of Fatal Head Injuries in Germany-Trends and the Impact of the Reunification. Acta Neurochir. 2005; 147: 231-47.
11. Vaca PJ. Fatal national highway traffic safety administration (NHTSA) notes. Ann Emerge Med. 2006; 48: 739-48.
12. Khan ZU, Al-Asiri KM, Iqbal J. Injury patterns from road traffic accidents. Pakistan J Med Sci. 2010; 26(2): 394-7.
13. Alissa A. Road Traffic Injuries: A descriptive analysis of cases received at the Emergency Department / King Hussein Medical Center. J Res Med Sci. 2006; 13(1): 35-8.
14. Shalaby SA, Akeed YA, Fawzi MM, et al. Prospective study of some medico-legal aspects of road traffic accidents in great Cairo 2007. Inter J Acad Res. 2010; 2(2): 127-37.
15. Rao D, Mukerjee S. A study of pattern of injuries in road traffic collisions. J Punjab Acad Forensic Med Toxicol. 2010; 10: 14-6.
16. Putul M. Study of abdomino-pelvic injuries in victim of road traffic accident. Inter J Med Toxicol Legal Med. 2010; 12(3): 25-9.
17. Al-Qazzaz MA. Medico-legal study of fatal road traffic injuries in Baghdad governorate. Board thesis, Iraq Council for Medical Specialization, 2002; p. 27.
18. Swarnkar M, Singh PK, Dwivedi S. Pattern of trauma in central India: an epidemiological study with special reference to mode of injury. Internet J Epidemiol. 2010; 9(1): 35-41.
19. Jha N, Srinivasa DK, Roy G, et al. Injury pattern among road traffic accident cases. A Study from South India. Indian J Commun Med. 2003; 23: 85-90.
20. Verma PK, Kov T. Epidemiology of RTAs in Delhi, result of survey. Regional Health Forum WHO. South East Asia Region. 2004; 8(1).
21. Patil SS, Kakade RV, Durgawale PM, et al. Pattern of road traffic injuries: A Study from Western Maharashtra. Indian J Commu Med. 2008; 33(1): 56-7.
22. http://www.4ishgd.valencia.upv.es/index_archivos/62.pdf. 4th international symposium on highway design. Analysis of accident patterns in Lebanon.
23. Ganveer GB, Tiwari RR. Injury Pattern among Non-Fatal Road Traffic Accident Cases: A Cross-Sectional Study in Central India. Indian J Med Sci. 2005; 59(1): 9-12.
24. Kual A, Sinha US, Pathak YK, et al. Fatal road traffic accidents, study of distribution, nature and type of injury. J Indian Acad Forensic Med. 2005; 27(2): 71-6.
25. Kumar A, Lalwani S, Agrawal D, et al. Fatal road traffic accidents and their relationship with head injuries: an epidemiological survey of five years. Indian J Neurotrauma. 2008; 5(2): 63-7.
26. Mishra B, Sinha ND, Sukhla SK, et al. Epidemiological study of road traffic accident cases from western nepal. Indian J Commu Med. 2010; 35(1): 115-21.
27. Stomp W, Fidler V, Duis HJ, et al. Relation of the weather and the lunar cycle with the incidence of trauma in the Groningen region over a 36- year period. J Trauma. 2009; 67(5): 1103-8.
28. Fried KA, Roesler JS, Azam A, et al. Predicting trauma admission: the effect of weather, weekday, and other variables. Minnesota Med. 2009; 92(11): 47-49.
29. Lahiri SK, Mandal P, Mandi P, et al. A study on cases contracting road traffic accidents in rural area and attending the Tarakeswar Rural Hospital, West Bengal. Indian J Prevent Soc Med. 2005; 36(3,4): 94-101.
30. Singh H, Aggarwal AD. Fatal road traffic accidents among young children. J Indian Acad Forensic Med. 2010; 32(4): 286-8.
31. Aggarwal KK, Oberoi SS, Kumar R, et al. Pattern and distribution of injuries in fatal road traffic accidental cases. J Punjab Acad Forensic Med Toxicol. 2010; 9(2): 71-5.
32. Oberoi SS, Aggarwal KK, Bhullar DS, et al. Pattern and distribution of injuries in fatal two wheeler accidental cases. J Punjab Acad Forensic Med Toxicol. 2011; 11(1): 31-3.
33. Gichuhi K. Injury pattern among non-fatal road traffic crash victims. East Afr Ortho J. 2007; 1: 23-5.

Correspondence to Dr. Nagi A Abdulla
E-mail: nagiali1966@hotmail.com
Received 3rd Dec. 2012: Accepted 20th Jan. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Serum Leptin Level in Severe Preeclampsia

Maad M Shalal *MBChB FICOG*, **Anwar N Al-Bassam** *MBChB CABGO*, **Isra T Hassan** *MBChB*

Dept. of Obstetrics and Genecology, Baghdad Teaching Hospital, Medical City Complex, Baghdad, Iraq

Abstract

Background Preeclampsia is a major cause of maternal morbidity and mortality with unknown aetiology. Placental hypoperfusion and diffuse endothelial cell injury are considered the central pathological process. Many adipocyte hormones like leptin play an important role in the inflammatory and atherosclerotic process and may be used as a marker for preeclampsia.

Objective To find the role of serum leptin measurement in pregnant women as a marker of preeclampsia.

Methods Seventy six primigravida women in their 3rd trimester of pregnancy were studied; 44 of them with severe preeclampsia, while the other 32 women with normal blood pressure without any history of previous diseases. Blood samples were taken for serum leptin, uric acid and creatinine levels, urine samples were collected for albumin. Serum leptin level was measured by ELISA kits.

Result Serum leptin and uric acid levels but not the creatinine was different in eclamptic group than control group. Mean age, height and weight were not different between the two groups. The systolic and diastolic blood pressures were also different between the two groups. 26 cases (59.1%) had proteinuria of 3+ albumin and 18 cases (40.9%) with 4+.

Conclusion Elevated serum leptin level can be used as a marker in the assessment of preeclampsia.

Key words Primigravida, preeclampsia, serum lipten.

Introduction

Preeclampsia is a systemic disease characterized by hypertension and proteinuria; and it continues to be an important cause of maternal morbidity and mortality. The cause is not yet clear; it includes immunological, genetic, environmental and placental abnormality. The final result of all of these is endothelial dysfunction, characteristic of preeclampsia [(1)]. Preeclampsia refers to the onset of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive woman.

The clinical manifestations of preeclampsia can appear anytime from the second trimester to the first few weeks postpartum; however, the initial pathological changes begin in the late first trimester and consist of abnormal remodeling of the spiral arteries [(2)]. Because the only cure is delivery, preeclampsia is associated with a high maternal and neonatal morbidity and mortality, so preeclampsia is believed to account for 15% of premature delivery and 17.6% of maternal death worldwide [(3,4)]. Preeclampsia is the 3rd leading cause of maternal mortality and can complicate 3-14% of all pregnancies [(5,6)]. The disease is mild in 75% of cases and severe in 25% [(7-9)].

A good test for predicting women who will develop preeclampsia should be simple, rapid, noninvasive, inexpensive, and easy to perform and should not expose the patient to discomfort

or risk. Ideally, it should provide an opportunity for intervention to prevent development of the disease, or at least result in better maternal and/or fetal outcomes [10].

Leptin (from the Greek "leptos"; meaning thin) is a protein hormone with important effects in regulating body weight, metabolism and reproductive functions [11]. The protein has 167 amino acid sequence containing one disulphide bond, it's molecular weight is about 16 KDs and has four helix bundle with one very short strand segment and two relative intermitting loops [12]. In pregnant women, leptin is synthesized in and secreted from placental trophoblast into maternal circulation at a considerable amount comparable with those in non pregnant woman [13]. Leptin is also produced by a culture of human choriocarcinoma cell line. Plasma leptin level is also markedly elevated in patient with Hydatidiform mole and choriocarcinoma, indicating that gestational trophoblastic neoplasms are leptin producing tumors. It has been demonstrated that placental production of leptin is augmented in women with severe preeclampsia [14].

Ouyang *et al*. [15] did a case control study between women with severe preeclampsia and normotensive women regarding serum leptin and found a significant elevation of serum leptin in women with severe preeclampsia. This finding pointed to the importance of leptin in the pathophysiology of preeclampsia and their involvement in the pathogenesis of the disease. As leptin causes oxidative stress in endothelial cells and has a calcifying effect on these cells, it has been suggested that leptin promote atherogenesis.

So, in pregnancy induced hypertension, placental ischemia is responsible for increased leptin level with increase in the inflammatory cytokines such as TNF alpha and IL-6 [16]. The aim of this work is to study the correlation between serum Leptin and severe preeclampsia.

Methods

This cross-sectional age-control study was done in the Department of Obstetrics and Gynecology/Baghdad Teaching Hospital-Medical City during the period from January 2010 to August 2010. A total number of 76 primipara in their third trimester where included in this study. Women with preexisting chronic hypertension, Diabetes mellitus, multiple pregnancies, chronic renal disease, chronic liver disease, and those with history of hyperuricemia were excluded from the study.

After taking detailed obstetrical and medical history 32 patients were having normal blood pressure without any history of prior hospitalization; while other 44 patients were presented with severe hypertension; diagnosed as systolic blood pressure of 160 mmHg and more and diastolic blood pressure of 110 mmHg and more, with a marked proteinuria on dipstick test in a random urine samples. After counseling and affordability of investigation, their blood samples were drawn for serum creatinine and uric acid.

Other samples were collected to obtain and clarify sera. Those samples were left to stand at room temperature for at least 30 minutes to allow the blood to clot, then centrifuged for 5 minutes, frozen at (-20 °C) and kept there without thawing till the day of testing. Then, serum leptin was measured using ELISA sandwich kits with the range of the assay from 0 to 100 ng/dl, also urine samples were taken for proteinuria by dipstick.

Results

Table 1a & b show that there was no statistically significant differences regarding body mass index between the two groups, while there were statistically significant differences regarding systolic and diastolic blood pressure ($P < 0.0001$). Their albumin in urine dipsticks on random urine samples show proteinuria in all cases of severe PET group (100%), while it was nil in all cases of the control group (100%).

There is statistically significant difference regarding serum leptin and serum uric acid between the two groups ($P = 0.0001$) While serum creatinine show mean±SD of 0.92 ± 0.18 in the severe PET group which is the upper

normal value, and of 0.86 ± 0.15 in the controls group which shows no statistical significant difference between them (Table 2).

Table 3 shows the correlation between serum leptin and serum uric acid in the severe PET group (r = 0.511). In addition, there was correlation between serum leptin and serum creatinine in the severe PET group (r = 0.724).

Table 1a. The Demographic Criteria of Patients with Severe Preeclampsia and the Control Group

Parameter		PET	Control group
Age (years)		23.95 ± 3.24	23.78 ± 3.23
BMI (Kg/m2)		30.33 ± 4.64	30.23 ± 4.96
Blood Pressure (mmHg)	Systolic BP	171.59 ± 14.3	111.72 ± 7.68*
	Diastolic BP	119.55 ± 8.88	68.44 ± 7.98*

* = $P < 0.0001$

Table 1b. The Demographic Criteria of Patients with Severe Preeclampsia and the Control Group

		PET		Control	
Parameter		Number	%	Number	%
Age (years)	< 20	6	13.6	5	15.6
	20-24	18	40.9	13	40.6
	25-29	20	45.5	14	43.8
Albumin	Nil	-	-	32	100
	+	-	-	-	-
	+++	26	59.1	-	-
	++++	18	40.9	-	-

Table 2. The distribution of Serum Leptin, Creatinine and uric acid in Severe Preeclamtic Group and the Control group

Parameter	PET		Control group	
	Mean±SD	Range	Mean±SD	Range
Leptin (ng/dl)	73.65 ± 38.13	7.0 - 140.0	23.08 ± 13.87	6.9 - 48.0*
Creatinine (mg/dl)	0.92 ± 0.18	0.7 - 1.3	0.86 ± 0.15	0.7 - 1.1
Uric acid (mg/dl)	5.53 ± 0.95	3.7 - 7.0	3.85 ± 0.86	3.0 - 6.0*

* = $P < 0.0001$

Table 3. The Correlations of Serum Leptin with Different Parameters in Both Control and PET Group

Parameter	Leptin (ng/dl)		Level of Significance
	Control	PET	
Age (years)	0.604	0.451	r
	0.0001	0.002	*P*
BMI (Kg/m^2)	0.801	0.098	r
	0.0001	0.527	*P*
Systolic BP (mmHg)	0.016	0.267	r
	0.931	0.079	*P*
Diastolic BP (mmHg)	0.142	0.014	r
	0.438	0.929	*P*
Uric acid (mg/dl)	0.258	0.511	r
	0.154	0.0001	*P*
Creatinine (mg/dl)	0.397	0.724	r
	0.024	0.0001	*P*

Discussion

Our study shows that all patients with severe preeclampsia have upper normal creatinine level which may indicate that the patient start to have a defect in glomerular function; in that the serum creatinine level still does not exceed upper normal level, and this may explain why there is accumulation of serum uric acid and leptin which depend on glomerular filtration in spite of normal creatinine level.

This may be due to high sensitivity of leptin and uric acid to the early changes in glomerular function than serum creatinine [15], so leptin can be used possibly as an indicator of severity of preeclampsia, which indicates that the patient started to have affected glomerular filteration by severe preeclampsia. This is in agreement with the study done by Laivuori *et al*. [17], where they explain that as leptin is eliminated mainly through the kidney and preeclampsia can be accompanied at least by histological renal changes [18]. The correlation between serum leptin, serum creatinine and serum uric acid suggest an association, either direct or indirect, between elevated serum leptin and renal changes in preeclampsia. Finally it could be concluded that elevated serum leptin level can be used as a marker in the assessment of severe preeclampsia.

References

1. Ozkan S, Erel CT, Madazli R, et al. Serum leptin levels in hypertensive disorder of pregnancy. Eur J Obstet Reprod Biol. 2005; 120: 158-63.
2. Report of the National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy. Am J Obstet Gynecol. 2000; 183: S1-S22.
3. Koonin LM, Mackay AP, Berg CJ, et al. Pregnancy-related mortality surveillance-united states, 1987-1990. Morbidity and Mortality weekly report. Center for Disease Control Surveillance Summary. 1997 Aug; 46(4): 17-36.
4. Berg CJ, Chang J, Challaghan WM, et al. Pregnancy-related mortality in the United States, 1991-1997. Obstet Gynecol. 2003; 101: 287-96.
5. Lewis G, Drife J, Botting B, et al. Why mothers die 2000-2002. In: Nelson JP, Pre-eclampsia and Eclampsia. London: RCQ press, 2004. p. 79.
6. Knight M. National Perinatal Epidemiology Unit, University of Oxford, UK Eclampsia in the United Kingdom, 2005. BJOG. 2007 Sept; 114(9): 1072-8.
7. Villar J, Betran AP, Gulmezoglu M. Epidemiological basis for the planing of maternal health services. WHO/ RHR, 2001.
8. Khedun SM, Moolley J, Naicker T, et al. Drug managment of hypertension disorder of pregnancy. Pharmacal Ther. 1997; 74(2): 221-58.
9. Livingstone JC, Livingstone LW, Ramseg R, et al. Magnesium sulfate in women with mild preeclampsia: a randomized controlled trial. Obstet Gynecol, 2003 Feb; 101(2): 217-20.
10. NICE Guidelines CG6 Antenatal care-Routine care for the healthy pregnant women, London; October 2003.
11. Cherhab FF, Mounzih K, Lu R, et al. Early onset of reproductive function in normal female mice treated with leptin. Science. 1997; 275: 88-90.
12. Zhang Y, proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372: 425-32.
13. Harvey J, Shanley LJ, O'Malley D, et al. Leptin: a potential cognitive enhancer? Biochem Soc Trans. 2005; 33: 1029-32.
14. Lyon CJ, Law RE, Hsuehw A. Mini review: adiopocity, inflammatory and atherogenesis. Endocrinology. 2003; 144: 2195-200.
15. Ouyang Y, Chen H, Chen H. Reduced plasma adiponectin and elevated leptin in preeclampsia. Internat J Gynecol Obstet. 2007; 98(2): 110-4.
16. Haque Z, Lakho GR, Nafeco M, et al. Serum leptin level correlation to high blood pressure in adult females. J Coll physician Surgpak. 2006; 16(7): 450-4.
17. Laivuoria H, Kaajia R, Koistinen H, et al. Leptin during and after preeclamptic or normal pregnancy: it's relation to serum insulin and insulin sensitivity. Metabo J. 2000; 49: 259-63.
18. Spargo B, Lichtig C, Luger A, et al. Renal lesion in preeclampsia: Examination by light-electron and immunofluorescence microscopy. In: Lindheimer M, katz A, Zuspan F (eds). Hypertension in pregnancy. New York: Wiley; 1976. p. 123-9.

Correspondence to Dr. Anwar N. Al-Bassam
E-mail: laith_alhadad@yahoo.com
Received 11th Mar. 2012: Accepted 31st Mar. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Gonadal Dysfunction with Postprandial Hypertriglyceridemia is Risk Predictor of Cardiovascular Disease in Men with Type 2 Diabetes Mellitus

Zainab AA Al-Shamma[1] *MSc*, **Yahya YZ Farid**[1] *PhD*, **Hashim M Hashim**[2] *MRCP*

[1]Dept. of Chemistry & Biochemistry, [2]Dept. Medicine, College of Medicine, Al-Nahrain University, Baghdad - Iraq.

Abstract

Background The association of type 2 diabetes mellitus and risk of cardiovascular disease is well documented. Insulin resistance is the hallmark feature of type 2 diabetes and there is evidence to suggest that testosterone is an important regulator of insulin sensitivity in men, with a role for testosterone in lipid metabolism and specially the triglyceride fraction.

Objective To emphasize the association of low level of total testosterone with that of the postprandial triglyceride in male patients' with type 2 diabetes mellitus.

Methods Forty two type 2 diabetes mellitus male patients and 42 healthy controls of age range between 30-60 years**,** during the period from December 2011 to June 2012. Postprandial venous blood used for random blood glucose, lipid profile, urea and creatinine measurement. Luteinizing hormone, follicle stimulating hormone, testosterone, and sex hormone binding globulin was done using Enzyme-Linked Immuno Sorbent Assay (Sandwich assay).

Results A negative correlation between testosterone, and postprandial triglyceride, in both type 2 diabetes mellitus and control groups with a significant difference in testosterone between the two groups. The sex hormone binding globulin was also correlated negatively with postprandial triglyceride in only the control group.

Conclusion Hypogonodism in male (decline in testosterone level) leads to increased postprandial hypertriglyceridemia, which could, both, be considered of predictors for cardiovascular disease risk factors in male patients with type2 diabetes mellitus.

Key words Postprandial triglycerides, type2 diabetes mellitus, testosterone.

Introduction

Dyslipidemia caused by insulin resistance is characterized by hyper-triglyceridemia with low HDL-cholesterol (HDL-c), two important risk factors for the development of diabetes mellitus [(1,2)]. Testosterone was reported to have important metabolic actions in men, affecting body composition and exerting direct effects on insulin sensitivity and lipid metabolism [(3,4)]. Hypogonadism is either primary or secondary. Hypogonadism that accompanies most chronic systemic diseases and aging is primary and is characterized by low testosterone levels and high gonadotropin with a significant association with insulin resistance and development of diabetes mellitus. The low level of sex hormone binding globulin (SHBG), which associates low testosterone concentration, has also been considered a risk predictor of Type 2 DM; prospective studies have shown that men with higher testosterone levels had a 42% lower risk of type 2 diabetes [(5-9)]. The Massachusetts

Male Aging Study (MMAS) and the Multiple Risk Factor Intervention Trial (MRFIT) have shown that low levels of total testosterone and SHBG (which is associated with insulin resistance) were both independent risk factors in middle-aged men who later developed diabetes [10,11]. The present study was undertaken to emphasize the relationship between serum testosterone levels and postprandial hypertriglyceridemia, on one hand, and their relation to the development of cardiovascular disease (CVD) risk in male diabetic patients, on the other.

Methods

This study included 42 male patients with T_2DM of age ranged between 30-60 years and disease duration of 1-8 years, who were attending the Diabetic Clinic at Al-Kadhimyia Teaching Hospital, during the period from December 2011 to June 2012. The study also included 42 normal male volunteers matching in their age and body mass index (BMI) with the patient group. Type 1 DM and thyroid disease patients were excluded from the study.

Ten ml were withdrawn from each patient and control subject between 2-4 hours after meal (postprandial state for lipid test) in a plain tube and centrifuged for 15 minutes at 3000 rpm after being allowed to clot at room temperature for 30 minutes. The separated sera were divided into aliquots and stored frozen at -20^{o}C, and then used for measurement of hormones. Random blood glucose, postprandial lipid profile, urea and creatinine were done immediately after separation of the serum using the available routine methods. The determination of patients' sex hormones (leutinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and SHBG) was done by Sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA assay). The oral consent had been taken from all patients and controls for blood collection.

Statistical analysis

All values were expressed as mean ± standard deviation (mean ± SD). All Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS version 15.0). Independent student t-test was performed to assess differences between two means. Pearson correlation coefficient was used to determine the correlation between quantitative data. *P* value < 0.05 was considered significant [12].

Results

Table 1 shows significant differences in the mean ± SD values between the diabetic and control groups in testosterone, SHBG ($P < 0.001$; $P < 0.004$, respectively) and in LH ($P < 0.0001$), while there was no significant differences in mean values of FSH.

There is also significant differences in the mean ± SD values of glucose, TG, T-cholesterol, HDL-c ($P < 0.0001$); LDL ($P = 0.0002$) and atherogenic index $P = 0.0004$, while no significant differences in the mean values of age, BMI, urea and creatinine there were observed. In both control and patient groups serum testosterone showed inverse correlations with the postprandial TG (Figs. 1 and 2).

Discussion

The lower serum testosterone and SHBG in the present diabetic patients confirm previous studies, which have suggested a role for low androgenic activity in the development of obesity, insulin resistance and Type 2 DM in men [13-17]. Obesity is a factor, which complicates the picture of Type 2 DM. The hypothesis of hypogonadal-obesity cycle originally suggested by Cohen in 1999 stated that testosterone inhibits adipocytes lipoprotein lipase activity [18]. In cases of low testosterone, which may result from increased aromatase activity, there is an increase in the adiposity and fat deposition which may cause a decline in testosterone level. The lower SHBG in the sera of the present diabetics could be attributed to high insulin levels which decrease the release of SHBG from hepatocytes [19,20]. However, other reports attributed this decline in SHBG to high glucose or fructose concentrations, which suppress its expression in the hepatocytes [21]. The impairment in the feedback inhibition, which is normally present between testosterone and LH, is the cause of high serum LH in the present diabetics [22].

Table 1. Demographic parameters of the diabetic patients and the control group

Parameters	Diabetic Patients N = 42	Control Group N = 42	*P* value
Age (yr)	48.88 ± 8.73	45.55 ± 7.05	0.0576
BMI (kg/m^2)	31.07 ± 5.26	30.4 ± 5.28	0.5635
Glucose (mmol/l)	12.46 ± 5.12	5.5 ± 1.24	<0.0001
TG (mmol/l)	2.71 ± 0.89	1.77 ± 0.53	<0.0001
Tc (mmol/l)	5.63 ± 0.73	4.46 ± 0.74	<0.0001
HDL-c (mmol/l)	0.92 ± 0.2	1.15 ± 0.2	<0.0001
LDL-c (mmol/l)	3.49 ± 0.71	2.43 ± 0.69	0.0002
Atherogenic index	3.98 ± 1.35	2.23 ± 0.87	0.0004
Urea (mmol/l)	5.93 ± 0.94	5.97 ± 0.67	0.8462
Creatinine (µmol/l)	75.48 ± 7.51	74.1 ± 7.7	0.4068
FSH (IU/l)	9.3 ± 6.67	7.71 ± 5.66	0.242
LH (IU/l)	7.81 ± 2.46	5.62 ± 2.02	<0.0001
Testosterone (nmol/l)	11.38 ± 3.8	14.62 ± 5.04	0.0013
SHBG (nmol/l)	24.31 ± 13.53	33.67 ± 16.01	0.0049

BMI = Body Mass Index, TG = Triglycerides, Tc = Total Cholesterol, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, FSH = Follicular stimulating hormone, LH = luteinizing hormone, SHBG = sex hormone binding globulin

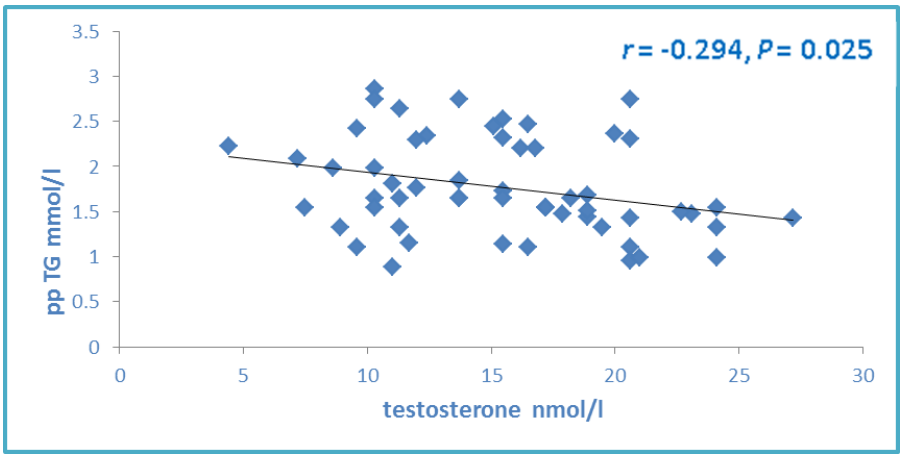


Fig. 1. Correlation between testosterone and postprandial triglycerides in the control group

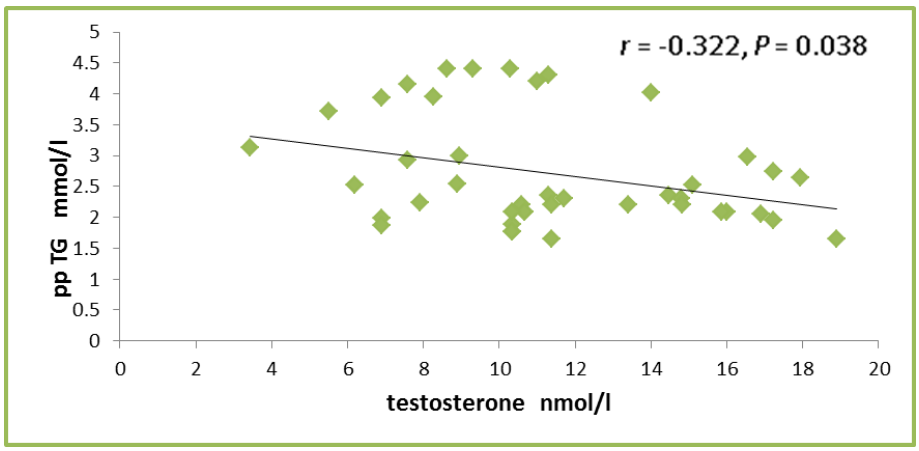


Fig. 2. Correlation between testosterone and postprandial triglycerides in diabetic patients

Also In control group of this research there was significantly negative correlations between post prandial (pp) TG with testosterone as in figure 1. This consider as risk factor for developing many systematic disease e.g. Type 2 DM and CVD in which the dyslipidemia is the main characteristic feature, and this agreed with Iraqi clinical study [23].

Elevated serum triglyceride level is a common dyslipidemic feature that accompanies Type 2 DM and pre-diabetic states [24], a picture which can be seen better with the postprandial TG than the fasting TG [25]. This point clarifies the reason for using the postprandial TG in many of the recent reports on DM or CVD [23,26-28]. Elevated levels of postprandial TG indicate the presence of increased levels of remnants from chylomicrons and very LDL-c. The cholesterol-containing, triglyceride-rich lipoproteins penetrate the arterial endothelium and may get trapped within the sub-endothelial space, potentially leading to the development of atherosclerosis [29].

The rise of this predictor pp TG of atherosclerosis risk associates the decline in serum testosterone level in the present diabetics and their control which implies the importance of both factors in this respect and necessitates their improvement to decrease the risk of atherosclerosis and CVD.

References

1. Wood PA. How Fat Works. Cambridge, Massachusetts: Harvard University Press; 2006. Chapters 5, 15, 16.
2. Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev. 2007; 87: 507-20.
3. Wu FC, von Eckardstein A. Androgens and coronary artery disease. Endocr Rev. 2003; 24: 183-217.
4. Ding EL, Song Y, Malik VS, et al. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2006; 295: 1288-99.
5. Spark RF. Testosterone, diabetes mellitus, and the metabolic syndrome. Curr Urol Rep. 2007; 8: 467-71.
6. Kalyani RR, Dobs AS. Androgen deficiency, diabetes, and the metabolic syndrome in men. Curr Opin Endocrinol Diabetes Obes. 2007; 14: 226-34.
7. Fukui M, Kitagawa Y, Ose H, et al. Role of endogenous androgen against insulin resistance and athero-sclerosis in men with type 2 diabetes. Curr Diabetes Rev. 2007; 3: 25-31.
8. Tivesten A, Mellström D, Jutberger H, et al. Low serum testosterone and high serum estradiol associate with lower extremity peripheral arterial disease in elderly men. J Am Coll Cardiol. 2007; 50: 1070-6.
9. Flack KD, Davy KP, Hulver MW, et al. Aging, resistance training, and diabetes prevention. Aging Res. 2011; ID 127315, 12 pages.
10. Stellato RK, Feldman HA, Hamdy O, et al. Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. Diabetes Care. 2000; 23: 490-94.
11. Oh JY, Barrett-Connor E, Wedick NM, et al. Endogenous sex Hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. Diabetes Care. 2002; 25: 55-60.
12. Elliott AC, Woodward WA. Statistical analysis quick reference guidebook: with SPSS examples. USA: SAGE Publications; 2007. p. 47-75.
13. Muller M. Endogenous sex hormones and metabolic syndrome in aging men. J Clin Endocrinol Metab. 2005; 90: 2618-23.
14. Chang TC, Tung CC, Hsiao YL. Hormonal changes in elderly men with non-insulin-dependent diabetes mellitus and the hormonal relationships to abdominal adiposity. Gerontology. 1994; 40: 260-7.
15. Giagulli VA, Kaufman JM, Vermeulen A. Pathogenesis of the decreased androgen levels in obese men. J Clin Endocrinol Metab. 1994; 79: 997-1000.
16. Pasquali R, Macor C, Vicennati V, et al. Effects of acute hyperinsulinemia on testosterone serum concentrations in adult obese and normal-weight men. Metabolism. 1997; 46: 526-9.
17. Pitteloud N, Mootha VK, Dwyer AA, et al. Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. Diabetes Care. 2005; 28: 1636-42.
18. Cohen PG. The hypogonadal-obesity cycle. Med Hypotheses 1999; 52: 49-51.
19. Plymate SR, Jones RE, Matej LA, et al. Regulation of sex hormone binding globulin (SHBG) production in Hep G2 cells by insulin. Steroids. 1988; 52: 339-340.
20. Crave JC, Lejeune H, Brébant C, et al. Differential effects of insulin and insulin-like growth factor I on the production of plasma steroid-binding globulins by human hepatoblastoma-derived (Hep G2) cells. J Clin Endocrinol Metab. 1995; 80: 1283-9.
21. Selva DM, Hogeveen KN, Innis SM, et al. Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone-binding globulin gene. J Clin Invest. 2007; 117: 3979-87.
22. Matsumoto AM. Andropause: Clinical Implications of the Decline in Serum Testosterone Levels with Aging in Men. J Gerontol Med Sci. 2002; 57: M76-M99.

23. Bahir BH, Al-Hadi AHM, Al-Shamma GA. Why Not Post Prandial Serum Lipid? Zinco J Med Sci. 2008; 22: 165-8.
24. Ginsberg HN, Zhang YL, Hernandez-Ono A. Regulation of plasma triglycerides in insulin resistance and diabetes. Arch Med Res. 2005; 36: 232-40.
25. Lee SH, Lee VB, Won HK, et al. Postprandial Triglyceride Is Associated with Fasting Triglyceride and HOMA-IR in Korean Subjects with Type 2 Diabetes. Diabetes Metab J. 2011; 35: 404-10.
26. Kolovou GD, Anagnostopoulou KK, Daskalopoulou SS, et al. Clinical relevance of postprandial lipaemia. Curr Med Chem. 2005; 12: 1931-45.
27. Nordestgaard BG, Benn M, Schnohr P, et al. Non-fasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA. 2007; 298: 299-308.
28. Ridker PM. Fasting versus non-fasting Triglycerides and the Prediction of Cardiovascular Risk: Do We Need to Revisit the Oral Triglyceride Tolerance Test? Clin Chem. 2008; 54: 111-13.
29. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, et al. Non fasting Triglycerides and Risk of Ischemic Stroke in the General Population. JAMA. 2008; 300: 2142-52.

Correspondence to Zainab AA Al-Shamma
E-mail: z.alshamma@gmail.com
Received 7th Jan. 2013: 15th Mar. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

The Conversion Rate in Laporascopic Cholecystectomy in Patients Complaining of Acute and Chronic Cholecystitis

Saad AR Al-Shammari *MBCHB FICMS*
Dept. Surgery, AL-Kindy College of Medicine, Baghdad, Iraq

Abstract

Background Laparoscopic cholecystectomy is the gold standard in the treatment of cholelithiasis, but there are still some patients requiring conversion to open cholecystectomy for several factors.

Objective To estimate the conversion rate and evaluate preoperative risk factors for conversion from laparoscopic to open cholecystectomy.

Methods 140 laparoscopic cholecystectomies were carried out from January 2008 to January 2011 at Al-Kindy Teaching Hospital. Preoperative clinical, laboratory and radiographic parameters for these patients assessed and analyzed prospectively.

Results Conversion to open cholecystectomy was needed in 30 patients (21.4%). Multivariate analysis identified male sex, with positive Murphy's sign, gall bladder wall thickness > 3 mm, a history of acute cholecystitis and time from the onset of symptoms till the time of surgery > 3 days as independent predictors of conversion rate to open cholecystectomy.

Conclusion The identification of certain risk factors for conversion from laporascopic to open cholecystectomy preoperatively such as male gender, age more than 40 years, onset of symptoms, gallbladder wall thickness can help the surgeon to plan and counsel the patients about the conversion rate.

Keywords Acute cholecystitis, laporascopic cholecystectomy, open cholecystectomy

Introduction

Gallstone disease is a global health problem. Most patients are asymptomatic, and gallstones are generally detected by ultrasonography during the evaluation of unrelated medical conditions [(1)]. Cholelithiasis affects approximat-ely 10% of the adult population in the United States and every year, approximately 500,000 cholecystectomies are performed [(2)].

It has been well demonstrated that the incidence of gall stones increases with age, an estimated 20% of adults over 40 years of age and 30% of those over the age of 70 years have biliary calculi. During the reproductive years, the female-to-male ratio is about 4:1, with the sex discrepancy narrowing in the older population to near equality. The risk factors predisposing to gallstone formation include obesity, diabetes mellitus, estrogen and pregnancy, hemolytic diseases, and cirrhosis [(2)].

Over the past two decades, laparoscopic cholecystectomy (LC) has become the gold standard for the surgical treatment of gallbladder disease. A shorter hospital stay (and, thus, a more rapid return to normal activity and work), less postoperative pain, a faster recovery, better cosmesis, and lower cost are some of the advantages of LC over open surgery [(3)]. The potential for conversion from a laparoscopic to an open procedure has been reported in the literatures with a high degree of variability,

ranging from 0% to 20% [4]. This variability is reflective of surgeon experience (including patient selection) and patient-specific risk factors often cited in the literature, including male sex, older age, acute cholecystitis and previous upper abdominal surgery [5,6]. Male sex is often cited as a risk factor for conversion to the open procedure [7].

Acute cholecystitis (AC) often requires emergency admission to the hospital. The traditional treatment of AC was conservative followed by cholecystectomy, usually 6 weeks to 8 weeks after discharge, although early cholecystectomy in patients with AC was shown to be safe and effective many years ago [8].

The two main controversies regarding the treatment of acute cholecystitis in patients fit for surgery are the timing of cholecystectomy (either initial conservative treatment followed by delayed cholecystectomy or planned early cholecystectomy), and the selection of the surgical procedure for cholecystectomy (either laparoscopic surgery or laparotomy) [9]. The currently available evidence predominantly supports immediate cholecystectomy on the basis that early surgery does not increase the risk of operative morbidity and mortality associated with early cholecystectomy [10] and that such a measure reduces the hospital stay for each patient by up to ten days, in contrast to conservative (late) cholecystectomy [11].

Laparoscopic cholecystectomy for an acutely inflamed gallbladder is technically more demanding than surgery for acute biliary pain without inflammation (biliary colic) because of severe inflammatory adhesions and distortion of the biliary anatomy; and the time interval from admission to surgery may affect conversion rates [12]. Since its introduction, LC has quickly become the most widely used treatment for gallstone disease, because of substantially less post-operative pain and a shorter recovery time compared to open cholecystectomy (OC) [13]. Randomized trials have also shown that early LC (within 72 hours of admission) for the treatment of AC is safe, feasible, and associated with a shorter hospital stay [14].

Methods

A cross sectional study with analytic content was carried out at Al-Kindy Teaching Hospital from the period between January 2008 to January 2011. One handred and forty patients were admitted to Alkindy teaching hospital and Dar Al-Najat Private Hospital. The patients who were included in this study were divided into 2 groups:

Group 1: included 90 patients presented with signs and symptoms and radiological features of chronic cholecystitis(recurrent attack of pain at right upper quadrent of the abdomen and some times vomiting and by abdominal ultrasound there is single or multiple stones with or without increase thickness of the wall of the gallbladder).

Group 2: included 50 patients presented with signs and symptoms and radiological features of acute cholecystitis, presented with right upper quadrant pain which persist for more than twelve hours, fever (temp. > 37.5 ^{o}C), vomiting and some of them jaundice, tenderness, muscle guarding and positive Murphy's sign and some cases boas sign was positive, The clinical diagnosis was supported by ultrasonic features of acute cholecystitis which includes increases in the thickness and edema of the wall, gallbladder distension with the presence of non floating gallstones impacted in the Hartman pouch. They received intravenous fluid, antibiotics and analgesia and nasogastric tube when necessary.

All patients admitted to the hospital and prepared for laparoscopic cholecystectomy. Investigations done for all patients including full blood count, random blood sugar, blood urea and serum creatinine, general urine examination, abdominal ultra-sound, chest x-ray, and electrocardiography in patients more than 35 years old.

All patients were operated upon under general anesthesia and endotracheal intubation, classical four ports laporascopic cholecystectomy was planned and when there was a need for conversion it was done through right subcostal incision.

The main reason for the conversion was inability to safely display and identify anatomical

structures of Calot's triangle correctly secondary to severe inflammation or dense adhesions.
Postoperative follow up for all patients was done and postoperative events were recorded for every patient on preforma, statistical analysis was done by the measurement of the *P* value. A *P* value below 0.05 was regarded as significant.

Results

There were 140 patients in this study 90 patients in group 1, 13 (14.4%) of them converted to open cholecystectomy and 50 patients in group 2, 17 (34%) of them converted to open. Males show a significantly higher conversion rate than females in patients with chronic cholecystitis as shown in table 1.

Table 1. Relation between the sex and conversion into OC

Sex	Lapchole		Conversion	
	No.	%	No.	%
Male	25	27.8	9	69.2
Female	65	72.2	4	30.8
Total	90	100	13	100

P = 0.003

While the age showed no significant difference in the conversion rate as shown in table 2.

Table 2. Relation between age and conversion

Age	Lapchole		Conversion	
	No.	%	No.	%
≥ 40 years	48	53.3	8	61.5
< 40 years	42	46.7	5	38.5
Total	90	100	13	100

Table 3. Relation between clinical history and conversion

Clinical history		Lapchole		Conversion		*P* value
		No.	%	No.	%	
Pain	Positive	80	88.9	11	13.8	0.653
	Negative	10	11.1	2	20	
Murphy's sign	Positive	60	66.7	9	15	0.854
	Negative	30	33.3	4	13.3	
Temp. > 37.5 °C	Positive	8	8.9	1	12.5	0.886
	Negative	82	91.1	12	14.6	
Onset of symptoms	> 3 days	70	77.8	11	15.7	0.574
	< 3 days	20	22.2	2	10	

Table 4. Relation between the results of abdominal ultrasound and conversion rate

US results		Lapchole		Conversion		*P* value
		No.	%	No.	%	
Wall thickness	> 3mm	20	22.2	8	40	0.003
	< 3mm	7	77.8	5	7.1	
Distended gallbladder	Positive	50	55.6	6	12	0.525
	Negative	40	44.4	7	17.5	
Number of gallstones	Multiple	67	74.4	8	11.9	0.328
	Single	23	25.6	5	21.7	

There was no significant statistical difference in the conversion rate in relation to the clinical history as shown in table 3. There was statistically significant difference in conversion rate in relation to ultrasonic findings of increased wall thickness of the gall bladder as shown in table 4.

In patients with acute cholecystitis (group 2), there was a significant increase in the conversion rate in males as in table 5. While the age shows no significant effect on the conversion rate as in table 6.

Table 5. Relation between the sex and conversion

Sex	Lapchole		Conversion	
	No.	%	No.	%
Male	18	36	11	64.7
Female	32	64	6	35.3
Total	50	100	19	100

P = 0.039

Table 6. Relation between age and conversion

Age	Lapchole		Conversion	
	No.	%	No.	%
≥ 40 years	31	62	7	41.2
< 40 years	19	38	10	58.8
Total	50	100	13	100

There was a significant increase in the conversion rate in relation to the time between the onset of symptoms to the time of operation while other clinical features did not have any significance in this respect (Table 7). An increase in the wall thickness of gall bladder was found also to be a significant risk factor for conversion from LC to OC as shown in table 8.

Discussion

This study showed the conversion from laporascopic cholecystectomy to open cholecystectomy in group1was 14.4% and 34% in group 2, while the study of Lim et al showed that the conversion rates in cases with acute cholecystitis were reported in the literature to reach up to 27.7% [15] and Fried et al who found that in acute cholecystitis, the conversion rate can be as high as 30% [16], and the study of Habib et al who found that the rate of conversion to open surgery in cases of severe cholecystitis is 8.7-35% [17].

In this study, the conversion rate was found to be highly related to male gender 69.2% in in group 1 and 64.7% in group 2 with a statistically significant difference (*P* value 0.003 and 0.039 respectively). This coincides with the study of Volkan Genc *et al* who found that male gender was found to be the only statistically significant risk factor for conversion with a conversion rate of 2.5-fold in men than in women [18]. Ballal *et al* also found that the patient-related factors who were good predictors of conversion included male sex, emergency admission, old age, and complicated gallstone disease (*P* < 0.001) [19]. The study of Shamim *et al* showed that the conversion rate was higher in male patients (16.45% males vs. 5.09% female) [20]. This association may be due to the increased severity of gallstone disease in men [21].

Regarding the age we found that the patients aged more than or equal to 40 years have higher conversion rate than those less than 40 years (61.5% versus 38.5%) in group 1 and (41.2% versus 58.8%) in group 2 with no statistically significant difference , and this goes with Fried et al study who found that advanced age may be associated with increased postoperative complications and high conversion rates [22], while Shamim et al study found no risk of conversion was associated with increasing age(Age-wise conversion rates were: 2.31% in 20s, 8.06% in 30s, 7.49% in 40s, 7.98% in 50s, 4.21% in 60s, and 4% in 70s) [20].

Concerning the clinical history and conversion rate, we found that there is no significant risk for conversion in group 1 regarding the pain, morphy's sign, temperature and onset of symptoms, while in group 2 the pain was present in all patients 100% and 70% of patients complained for more than 3 days converted to open cholecystectomy with highly significant p-value(0.05) and this is similar to the study of Khan et al who found that conversion rate was significantly low (zero versus 32%: *P* = 0.01) if the procedure was performed within 48 hours from the onset of symptoms [23].

Regarding preoperative ultrasounic findings, this study showed that 40% of patients with gallbladder wall thickness more than 3mm in group 1 are converted to open cholecystectomy

in comparison with 7.1% with those with less than 3mm gallbladder wall thickness with highly significant p-value (0.003) while in group 2, 40% of patients with gallbladder wall thickness more than 3mm and 12.9% of them less than 3mm wall thickness were converted to open with significant p-value (0.033) and this is parallel to the study of Brodsky *et al* who found that the gallbladder wall thickness associated with a conversion rate of 58% [24] while study of Khan *et al* found that the conversion rate was significantly high (33% versus zero; *P* = 0.01) if the gallbladder wall was thickened [23].

In conclusion, the identification of certain risk factors for conversion from laporascopic to open cholecystectomy preoperatively such as male gender, age more than 40 years, onset of symptoms, gallbladder wall thickness can help the surgeon to plan and counsel the patients about the conversion rate.

Table 7. Relation between clinical history and conversion in acute cholecystitis

Clinical history		Lapchole		Conversion		*P* value
		No.	%	No.	%	
Pain	Positive	50	100	17	100	
	Negative	0	0	0	0	
Murphy's sign	Positive	45	90	14	31.1	0.401
	Negative	5	10	3	60	
Temp. > 37.5°C	Positive	67	74.4	8	11.9	0.328
	Negative	23	25.6	5	21.7	
Onset of symptoms	> 3 days	10	20	7	70	0.05
	< 3 days	40	80	10	25	

Table 8. Relation between the results of abdominal ultrasound and conversion

US results		Lapchole		Conversion		*P* value
		No.	%	No.	%	
Wall thickness	> 3mm	20	22.2	8	40	0.033
	< 3mm	70	77.8	9	12.9	
Distended gallbladder	Positive	50	55.6	10	20	0.803
	Negative	40	44.4	7	17.5	
Number of gallstones	Multiple	67	74.4	12	17.9	0.740
	Single	23	25.6	5	21.7	

References

1. Bittner R. Laparoscopic surgery: 15 years after clinical introduction. World J Surg. 2006; 30: 1190-203.
2. Schirmer BD, Winters KL, Edlich RF. Cholelithiasis and cholecystitis. J Long Term Eff Med Implants. 2005; 15(3): 329-38.
3. Ros A, Gustafsson L, Krook H, et al. Laparoscopic cholecystectomy versus mini-laparotomy cholecystectomy: a prospective, randomized, single blinded study. Ann Surg. 2001; 234: 741-9.
4. Kama NA, Doganay M, Dolapci M, et al. Risk factors resulting in conversion of laparoscopic cholecystectomy to open surgery: Surg Endosc. 2001; 15: 965-8.
5. Fried G, Barkun J, Sigman H, et al. Factors determining conversion to laparotomy in patients undergoing laparoscopic cholecystectomy: Am J Surg. 1994; 167: 35-9.
6. Livingston EH, Rege RV. A nationwide study of conversion from laparoscopic to open cholecystectomy: Am J Surg. 2004; 188: 205-11.
7. Schirmer BD, Dix J, Schmieg RE, et al. The impact of previous abdominal surgery on outcome following laparoscopic cholecystectomy. Surg Endosc. 1995; 9: 1085-9.
8. Norrby S, Herlin P, Holmin T, et al. Early or delayed cholecystectomy in acute cholecystitis? A clinical trial: Br J Surg. 1983; 70: 163-5.

9. Fowkes FG, Gunn AA. The management of acute cholecystitis and its hospital cost: Br J Surg. 1980; 67: 613-7.

10. Papi C, Catarci M, D'Ambrosio L, et al. Timing of cholecystectomy for acute calculous cholecystitis: a meta-analysis: Am J Gastroenterol. 2004; 99: 147-55.

11. Harper DR. Disease cost in a surgical ward: Br Med J. 1979; 1: 647-9.

12. Rattner DW, Ferguson C, Warshaw AL. Factors associated with successful laparoscopic cholecystectomy for acute cholecystitis: Ann Surg. 1993; 217: 233-6.

13. National Institutes of Health Consensus Development Conference Statement on Gallstones and Laparoscopic Cholecystectomy: Am J Surg. 1993; 165: 390-8.

14. Nanez B, Mutter D, Russier Y, et al. Safety of laparoscopic approach for acute cholecystitis: retrospective study of 609 cases: World J Surg. 2001; 25: 1352-6.

15. Lim KR, Ibrahim S, Tan NC, et al. Risk factors for conversion to open surgery in patients with acute cholecystitis undergoing interval laparoscopic cholecystectomy: Ann Acad Med Singapore. 2007; 36: 631-5.

16. Fried GM, Barkun JS, Sigman HH, et al. Factors determining conversion to laparotomy in patients undergoing laparoscopic cholecystectomy: Am J Surg. 1994 Jan; 167(1): 35-9.

17. Habib FA, Kolachalam RB, Khilnani R, et al. Role of laparoscopic cholecystectomy in the management of gangrenous cholecystitis: Am J Surg 2001; 181:71-5.

18. Genc V, Sulaimanov M, Cipe G, et al. What necessitates the conversion to open cholecystectomy? A retrospective analysis of 5164 consecutive laparoscopic operations. Clinics (Sao Paulo). 2011 Mar; 66(3): 417-20.

19. Ballal M, David G, Willmott S, et al. Conversion after laparoscopic cholecystectomy in England. Surg Endosc. 2009; 23: 2338-44.

20. Shamim M, Memon AS, Bhutto AA, et al. Reasons of conversion of laparoscopic to open cholecystectomy in a tertiary care institution. J Pakistan Med Assoc. 2009 Jul; 59: 456-60.

21. Lein HH, Huang CS. Male gender: risk factor for severe symptomatic cholelithiasis World J Surg. 2002; 26: 598-601.

22. Fried GM, Clas D, Meakins JL. Minimally invasive surgery in the elderly patient. Surg Clin North Am. 1994; 74: 375-87.

23. Khan IA, El-Tinay OE. Laparoscopic cholecystectomy for acute cholecystitis. Can preoperative factors predict conversion? Saudi Med J. 2004 Mar; 25(3): 299-302.

24. Brodsky A, Matter I, Sabo E, et al. Laparoscopic cholecystectomy for acute cholecystitis: can the need for conversion and the probability of complications be predicted? A prospective study. Surg Endosc. 2000 Aug; 14 (8): 755-60.

Correspondence to Dr. Saad AR Al-Shammari
E-mail: saadahumari@yahoo.com
Cell phone: +964 7813809350
Received 13th Sep. 2012: Accepted 22nd Apr. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Ligasure *versus* Clamp and Tie Technique to Achieve Hemostasis in Thyroidectomy for Benign Diseases

Tariq I Al-Aubaidi *FIBMS*, **Muayad A Fadhel** *FIBMS*, **Basim R Gadban** *FIBMS*

Dept. of Surgery, College of Medicine, Baghdad University

Abstract

Background Occurrence of adverse effects and advantages of the Ligasure diathermy system (or Ligasure vessel sealing system) in thyroidectomy have not been tested in prospective randomized studies comparing its use with that of the time-saving clamp-and-tie technique to ligate and divide thyroid vessels. The effectiveness of Ligasure in achieving vessel division and hemostasis remains dependent on vessel diameter, and the risk of damage to adjacent structures cannot be completely excluded.

Objective To evaluate the operative time, hospital stay and postoperative complications that achieved by ligasure in versus with clamp-tie technique in subtotal and near total thyroidectomy.

Methods One hundred patients with benign multinodular goiter underwent subtotal and near total thyroidectomy. Subtotal and near total thyroidectomy was performed in 45 patients using ligasure and in 55 patients using the clamp-and tie technique.

Results Postoperative complication rate was 2.7-7% overall in ligasure group (group one) vs. 7-31% clamp-and-tie technique (group two) including all temporary postoperative disturbances. There are no permanent complications but statistically, there was difference in their incidence. The mean hospitalization time was 1.4 day in group one vs. 2.5 day in group two. Mean operative time was shorter in the Ligasure group with minimal time difference of 17 minutes.

Conclusion The use of Ligasure is safe and effective at vessel division and homeostasis than the clamp-and-tie technique, with a statistically significant decrease in mean operative time and hospitalization time. Because of that decrease in operative time and low complication rate, this would allow more patients to undergo thyroidectomy by this technique.

Key words Ligasure, clamp and tie, thyroidectomy, hemostasis

Introduction

Thyroidectomies for goitreous patients are known to be more blood spattered operations that requires careful and accurate hemostasis than other operations performed. The conventional technique by clamp and tie of vessels for hemostasis can be done safely but it is a time consuming. Recently, a vessel sealing system was developed and started to be used in various fields of surgery including urological, gynecological, gastric, and laparoscopic and anorectal procedures [1-6]. Hemostasis is extremely important in thyroid surgery to avoid post-operative complications, but it requires meticulous technique, various devices as electrosurgical devices use heat energy to denature proteins and heating of surgical field due to lateral dispersion may easily

damage vital structures. Research has been looking for new instruments with less thermal spread in the effort to reduce both operating time and complications [7,8]. Ligasure enable sealing and division of a vessel which disperses less heat to surrounding tissue than classical bipolar or monopolar electro coagulation methods. Ligasure system produces a consistent permanent autologous seal to veins, arteries and tissue bundles up to 7 mm in diameter, melting the tissue's collagen and elastin. It incorporates intelligent sensor within the diathermy forceps that provide audible tones once a complete seal cycle is accomplished [9-10]. The objectives were to evaluate the operative time, hospital stay and postoperative complications that achieved by ligasure in versus with clamp-tie technique in subtotal and near total thyroidectomy.

Methods

This cross sectional study on sample of 100 patients with multinodular goiter who were admitted to the first surgical unit in Baghdad Teaching Hospital at Medical City between first of June 2009 to first of June 2010. We divided the patients in to two groups, in group one a 45 patients were involved and underwent thyroidectomy by using a bipolar vessel ligation system (ligasure) and it was the choice of modality for hemostasis and group two as 55 patients involved and a clamp and tie suture technique was used for hemostasis. A thorough history and clinical examination were done and investigations were sent and the diagnosis of goiter was established, the surgery was done by using these two methods of hemostasis. According to pre-operative clinical, radiological and laboratory evaluations of 30 patients (30%) were hyperthyroid with diagnosis of toxic multinodular goiter.

Out of these 30 hyperthyroid patients a 14 patients (47%) were in ligasure group and 16 patients (53%) were in a clamp and group. All hyperthyroid patients had been receiving antithyroid medication pre-operatively to provide an euthyroid state. Treatment with propranolol tablet with initial dose of 40-60 mg/day and carbimazol tablet in dose of 10-30 mg/day, which were reduced gradually to maintain euthyroid state, as serum thyroid hormone concentrations declined. Patients received this treatment for a minimum of 2 weeks before operation. The indications for surgical treatment of these hyperthyroid patients were as follow, patients were inconvenienced for medical treatment (20 patients), large goiter (10 patients), while the main indications for surgery of all euthyroid patients (70 patients) were large goiters that caused compressive effects. None of these patients in the current study groups had been receiving either any medications known to have any side effects on coagulation, or any anti-coagulative drugs, and none of them had been diagnosed of any coagulopathic disorders formerly.

Patients were assessed for early post-operative complications as recurrent laryngeal nerve paralysis, hypoparathyroidism, hemorrhage, operating time and duration of post-operative hospital stay. Indirect laryngoscopic examination was applied to carry in all patients. Post-operative cord palsy was defined as the presence of an immobile vocal cord or decreased movements of cords during phonation. Classical thyroidectomy is done by performing a collar incision, the subcutaneous tissue and platysma were divided, and skin flaps were developed by monopolar electrocautery. The strap muscles were divided in the midline and retracted laterally, in group one all middle thyroid veins and vessels of superior and inferior thyroidal poles were sealed with ligasure regardless of their size, the ligasure was used only if the distance was wider than 2 mm between the tip of the device and recurrent laryngeal nerve., after bleeding control, suction drains were placed in all patients. After surgical intervention, Histopathological examinations were performed for all patients.

Patients were followed for 6 months post-operatively. In group two, we used a clamp and tie technique for hemostasis for superior and inferior poles. In all patients, thyroidectomy was

performed after identification of recurrent laryngeal nerves and at least one parathyroid gland on each side.

Statistical analysis

The data were analyzed using SPSS 11.0 for windows; comparisons of the data were done by wilcoxon and chi-square tests. The results were expressed as mean ± SD and $P < 0.05$ was accepted to be statistically significant.

Results

The mean age of the 100 patients was found to be 48 ± 11 years (range 18-69 years). The female/ male ratio was calculated to be 8.5/1.5 (n=85/15). Among all patients, 46 patients underwent bilateral near total thyroidectomy and 54 patients underwent bilateral subtotal thyroidectomy. Here was no mortality in both groups in this study. The evaluation of the patients with/ without ligasure usage, the operative time and duration of the hospital stay in group one was (58 min.), (1.4 days) respectively were significantly lower than those in group two (75 min.),(2.5days) as $P < 0.05$ as shown in table 1.

The complication rates of ligasure group (group one) were significantly lower than those in clamp and tie technique (group two) ($P < 0.005$), permanent vocal cord palsy and permanent hypoparathyroidism were not encountered in this study. Patients had postoperative incidence of temporary hypoparathyroidism (n=3) 6.6% in group one while (n=5) 9% in group two. Temporary recurrent laryngeal nerve palsy (n=1) 2.2% in group one while (n=10) 20% in group two, seroma (n=2) 4% in group one while (n=5) 9% in group two, hemorrhage (n=0) in group one while (n=3) 6% in group two, wound infection (n=2) 4% in group one while (n=5) 9% in group two and cervical hematoma (n=1) 2.2% in group one while (n=3) 6% in group two as shown in table 2.

Table 1. Demographic features of ligasure group and clamp and tie surgical technique group

Feature	Group I N = 45	Group II N = 55	*P* Value
Age (years)	47 (21-69)	41 (18-67)	0.066
Mean operation time (minutes)	58	75	0.0001
Mean hospital stay (days)	1.4 (1-3)	2.5 (1-5)	0.001

Group I = Ligasure group, Group II = Clamp and tie technical group

Table 2. Postoperative complications that occurred in ligasure group and clamp and tie surgical technique group

Complication	Group I N = 45	Group II N = 55	*P* Value
Temporary hypoparathyroidism	3 (6.6%)	5 (9%)	0.004
Permanent hypoparathyroidism	0	0	-
Temporary recurrent Laryngeal nerve palsy	1 (2.2%)	10 (18.18%)	0.003
Permanent recurrent Laryngeal nerve palsy	0	0	-
Seroma	2 (4%)	7 (13%)	0.004
Hemorrhage	0	3 (6%)	0.003
Wound infection	2 (4%)	5 (9%)	ns
Cervical hematoma	1(2.2%)	3 (6%)	ns

Group I = Ligasure group, Group II = Clamp and tie technical group

The evaluation of patients according to thyroid hormone status is shown in table 3. Among all 100 patients, 30 patients (30%) were hyperthyroidism and 70 patients (70%) were euthyroid. Out of the 30 hyperthyroidic patients, 14 patients (46%) underwent thyroidectomy with ligasure, and clamp and tie technique was performed to remaining 16 patients (53%).

Out of 70 euthyroidic patients, 31 patients (44.3%) were operated by use of ligasure and 39 patients (55.7%) underwent thyroidectomy with clamp and tie surgical technique. There was no significant difference due to age and gender, while there were significantly reduce in mean operative time, mean hospital stay and complications rate in group one in comparison with group two ($P < 0.05$), in hyperthyroidism patients the mean operative time in group one was 60 min. while in group two was 80 min. , the mean hospital stay was 1.2 day in group one while it was 2.2 days in group two and complications rate was 2.7% in group one while it was 5.8% in group two regarding the euthyroid state while it was 7% in group one and 31% in group two regarding hyperthyroid state. Hospital stay between ligasure group and clamp and tie group was significant ($P < 0.05$) as shown in table 3.

Table 3. Demographic features of the patients according to thyroid hormone status

Feature	Hyperthyroid		*P* value	Euthyroid		*P* value
	Group I	Group II		Group I	Group II	
Number of patients	14 (46%)	16 (54%)	-	31 (44.3%)	39 (55.7%)	-
Mean age (years)	45 (24-61)	48 (18-65)	0.074	48 (18-65)	49 (20-69)	0.062
Female/male	11/3	12/4	0.460	32/4	30/4	0.920
Mean operative time (min.)	60	80	0.001	58	75	0.001
Mean hospital stay (days)	1.2 (1-3)	2.2 (1-4)	-	1.2 (1-3)	2.2 (1-5) 2/34	0.001
Complication rates	1/14 (7%)	5/16 (31%)	-	1/36(2.7%)	(5.8%)	0.002

Group I = Ligasure group, Group II = Clamp and tie technical group

The mean operating time for subtotal and near total thyroidectomy subgroups of ligasure group were significantly shorter when compared with that of clamp and tie group, 53.38 versus 65.8 minutes ($P = 0.005$) and 62.5 versus 84.89 minutes ($P = 0.001$) respectively as shown in table 4.

Table 4. Duration of operations according to the extent of procedure

Surgical Procedure	Duration of operation (minutes)				*P* value
	Ligasure group		Clamp and tie group		
	No.	Mean	No.	Mean	
Subtotal thyroidectomy	30 (66%)	53.38 min	38 (69%)	65.80 min	0.005
Near total thyroidectomy	15 (22%)	62.50 min	17 (31%)	84.89 min	0.001

Discussion

The ligasure technique enables surgeons to apply high current (4 A) and low voltage (200 V) to achieve simultaneous vessel sealing and division. The device acts through denaturation of the collagen and elastin in the vessel wall. The pressure applied by the scissors opposes the walls to allow the proteins to form a seal. Microscopically, it is possible to verify that internal elastic lamina is preserved and collagen bundles form across the previous lumen [(2)]. The device has received acceptance worldwide in several surgical fields. It is claimed to be safe and effective because it allows vessel sealing and division without dispersion of electric power and with a little or no production of heat.

In thyroid surgery, there is an additional reason to use it in near or subtotal thyroidectomy [2]. Petrakis *et al* [11] in a retrospective case-control study reported fewer complications and shorter operative and hospitalization times in ligasure group. Other retrospective and prospective but not randomized studies did not find any differences between complication rates and hospitalization times [2,3,10]). Operative time was substantially reduced in studies by Kirdak and Shen *et al* [2,3] but not by Kiriakopoulos *et al* [12]. In our study, the use of ligasure technique was safe, less time consuming, less hospital stay and less post-operative complications in comparison with clamp and tie technique.

The complication rate in our study was 7% in ligasure group (hyperthyroid) and 2.7% (euthyroid). In our study, 2.2% of patients in ligasure group and 9% of patients in the clamp and tie technique group had transient complications involving recurrent laryngeal nerves, and 6.6% of patients in ligasure group and 9% of patients in clamp and tie group had temporary hypoparathyroidism. Harold *et al* [13] and Kahky *et al* [14] had reported that temporary hypoparathyroidism may be noted in 13.4% of patients when only clinical symptoms are considered. In study of Delbridge *et al* [15] in 20% of patients who underwent near total thyroidectomy required calcium supplementation for 3-6 weeks after surgery. Transient recurrent nerve paralysis has been observed in 8.7% to 39% of patients [16,17] and is not completely avoidable even with systemic laryngeal nerve identification. There were no permanent complications after near total in our series including permanent hypocalcaemia and permanent recurrent nerve lesions with no statistical difference between the two study groups. The vessel sealing used to prevent inadvertent damage to recurrent nerve by systemic. Careful identification and minimized long term effects of inadvertent damage to parathyroid glands.

From the current study one may conclude that the usage of ligasure is a safe technique in thyroidectomy for benign diseases, and it is recommended to use over the clamp and tie technique.

References

1. Dilek ON, Yilmaz S, Degirmenci B, et al. The use of a vessel sealing system in thyroid surgery. Acta Chir Belg. 2005; 105(4): 369-72.
2. Kirdak T, Korun N, Ozguc H. Use of ligasure in thyroidectomy procedures: results of a prospective comparative study. World J Surg. 2005; 29: 771-4.
3. Shen WT, Baumbusch MA, Kebebew E, et al. Use of the electrothermal vessel sealing system versus standard vesselligation in thyroidectomy. Asian J Surg. 2005; 28(2): 86-9.
4. Sosa JA, Bowman HM, Tielsch JM, et al. The importance of surgeon experience for clinical and economic outcomes from thyroidectomy. Ann Surg. 1998; 228: 320-30.
5. Landman J, Kerbl K, Rehman J, et al. Evaluation of a vessel sealing system, bipolar electrosurgery, harmonic scalpel, titanium clips, endoscopic gastrointestinal anastomosis vascular staples and sutures for arterial and venous ligation in a porcine model. J Urol. 2003; 169: 697-700.
6. Matthews BD, Pratt BL, Backus CL, et al. Effectiveness of the ultrasonic coagulating shears, Ligasure vessel sealer, and surgical clip application in biliary surgery: a comparative analysis. Am Surg. 2001; 67: 901-6.
7. Jayne DG, Botterill I, Ambrose NS, et al. Randomized clinical trial of Ligasure versus clamp-and-tie diathermy for haemorrhoidectomy. Br J Surg 2002; 89:428-432.
8. Sandonato L, Cipolla C, Gracefta G, et al. Bipolar electrothermic coagulation (Ligasure bipolar vessel sealing system) in thyroid surgery. Chir Ital. 2003; 55(3): 411-5.
9. Lachanas VA, Prokopakis EP, Mpenakis AA, et al. The use of Ligasure Vessel Sealing System in thyroid surgery. Otolaryngology Head Neck Surg 2005; 132(3): 487-489.
10. Dilek ON, Yilmaz S, Degirmenci B, et al. The use of vessel sealing system in thyroid surgery. Acta Chir Belg. 2005; 105: 369-72.
11. Petrakis IE, Kogerakis NE, Lasithiotakis KG, et al. Ligasure versus clamp-and-tie thyroidectomy for benign nodular disease. Head Neck. 2004; 26: 903-9.
12. Kiriakopoulos A, Dimitrios T, Dimitrios L. Use of a diathermy system in thyroidsurgery. Arch Surg. 2004; 139: 997-1000.
13. Harold KL, Pollinger H, Matthews BD, et al. Comparison of ultrasonic energy, bipolar thermal energy, and vascular clips for hemostasis of small-, medium-, and large-sized arteries. Surg Endosc. 2003; 17: 1228-30.
14. Kahky MP, Weber RS. Complications of surgery of the thyroid and parathyroid glands. Surg Clin North Am. 1993; 73: 307-21.

15. Delbridge LW, Younes NA, Guinea AI, et al. Surgery for primary hyperparathyroidism 1962-1996: indications and outcomes. Med J Aust. 1998; 168: 153-6.
16. Wade JS. Thyroidectomy. Br J Hosp Med. 1978; 20: 456-68.
17. Reeve T, Thompson NW. Complications of thyroid surgery: how to avoid them, how to manage them, and observations on their possible effect on the whole patient. World J Surg. 2000; 24: 971-5.

Correspondence to Dr. Basim R Gadban
E-mail: basimgadban@yahoo.com
Received 15th Jan. 2013: Accepted 3rd Mar. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Microdebrider Technique for Management of Inferior Turbinate Hypertrophy

Adil N Razooqi *MBChB FICMS*, **Firdaws F Ridha** *MBChB FICMS*, **Ali AH Fadhil** *MBChB DLO*

Section of ENT, Dept. of Surgery, Al-Kadhimyia Teaching Hospital, Baghdad, Iraq

Abstract

Background Microdebrider-assisted turbinoplasty of inferior turbinate is a surgical procedure for reducing the size of turbinate and achieve patent nasal airways in a condition where an enlarged turbinate cause chronic nasal obstruction not responding to medical treatment.

Objective To assess the results of microdebrider technique in the management of patients with chronic nasal obstruction due to inferior turbinate hypertrophy.

Methods From January 2010 to March 2011, forty patients with chronic nasal obstruction due to inferior turbinate hypertrophy not responding to medical treatment were treated with microdebrider-assisted turbinoplasty. They were subjected to detailed study of medical history and clinical examination. Post-operative follow up was performed for subjective and objective evaluation up to one year.

Results Thirty seven patients (92.5 %) had good airway passage during the first two weeks after operation and these results continued up to twelve months. The complications encountered with this procedure were limited to postoperative bleeding with no crusting or adhesions.

Conclusion Microdebrider-assisted turbinoplasty is a safe procedure for achieving turbinate size reduction with acceptable morbidity in patients with nasal obstruction due to turbinate hypertrophy. Bleeding is a minimal complication. Preservation of mucosa leads to early healing and absence of crusting.

Keywords Turbinate, partial turbinectomy, microdebrider

Introduction

Disturbances of nasal air flow occur in about 30% of the population causing nasal obstruction; one of the major causes of chronic nasal obstruction is diseases of inferior turbinate commonly inferior turbinate hypertrophy [(1)]; Chronic hypertrophic rhinitis, both allergic and non-allergic, in which there is swelling of the sub mucosa due to dilatation of the sub mucosal venous sinusoid [(2)], sometime, there is sub mucosal fibrosis [(3)]. There is almost always compensatory structural hypertrophy of inferior turbinate on the concave side of the sepal deviation, which sometimes does not respond to medical treatment and needs surgery. Different surgical methods have been achieved for inferior turbinate hypertrophy. The efficacy of the surgical techniques in treating turbinate hypertrophy should be judged by two basic criteria: to diminish the complaints and to preserve the function of the turbinate; so, this endoscopic powered modification of the classic techniques is the quite acceptable from morphological and physiological point of view [(4)]. This study aimed to assess the results of microdebrider technique in the management of patients with chronic nasal obstruction due to inferior turbinate hypertrophy.

Methods

This prospective study enrolled 40 patients who attended department of otolaryngology in Al-Kadhimyia Teaching Hospital, their age ranged from 11-50 years. All patients involved in this study were complaining of nasal obstruction due to bilateral inferior turbinate hypertrophy provided that no other cause of nasal obstruction (no nasal polyposis, almost straight nasal septum, no concha bullosa, patent postnasal space, no bony hypertrophy). The study is established over a period of 1 year, and the 40 patients with bilateral inferior turbinate hypertrophy were examined clinically with anterior rhinoscopy, endoscopically with nasopharyngoscopy and radiologically with CT scan. Full preoperative investigations were done. We classified inferior turbinate hypertrophy into three grades:-

Grade1: Normal size inferior turbinate, not atrophic without nasal obstruction.

Grade2: Moderate size inferior turbinate, not touching the septum, with nasal obstruction that responds to local decongestant.

Grade3: Large mulberry turbinate, touching the septum, with nasal obstruction that not responds to local decongestant.

The operation is done in all cases of grade 3 and some cases of grade 2 with nasal obstruction that does not respond permanently to medical treatment. The rhinomanometric examination was carried out one day preoperatively, and 6-12 weeks postoperatively using anterior rhinomanometer type 300 by ATMOS.

Microdebrider-assisted inferior turbinoplasty procedure was performed under general anesthesia. The anterior, inferior and posterior borders were infiltrated with 2% lignocaine and 1/100,000 adrenalin, an antero-inferior incision and sub mucosal pocket on inferior turbinate with a conventional 15 blade and freer elevator. The microdebrider unit was set at 4000-rpm oscillating mode, with inferior turbinate blade 4 mm size introduced in the sub mucosal pocket. A great care was taken to stay in the sub mucosal pocket and more lateral to avoid mucosal perforation. Light nasal packing was done for 24 hours.

Postoperatively patients instructed to use nasal irrigation with sodium bicarbonate 2% three times daily for one week, analgesia, and antibiotic. The first visit was on the 7th day postoperatively, then monthly for 3 months, then at 6th month and at one year. At each visit the nose was examined for any bleeding, crusts which were removed, mucosal tear, nasal airway patency, and adhesion. Also asking about sneezing, nasal discharge, facial pain and patients smell.

Results

A total of 40 patients, 24 (60%) males and 16 (40%) females underwent microdebrider-assisted inferior turbinoplasty. In this study the age ranged from 11-50 years with a mean age of 34 years table 1.

Table 1. Age Distribution of the patients

Age Group (year)	No.	%
11-20	8	20
21-30	10	25
31-40	18	45
41-50	4	10
Total	40	100

The 40 patients had suffered from nasal obstruction due to inferior turbinate hypertrophy also those patients were suffering from mouth breathing; while 26 of these patients (65%) suffered from nasal discharge; 12 patients (30%) suffered from sneezing; 20 patients (50%) complaining from snoring; 4 patients (10%) suffered from hyposmia and 4 patients (10%) complaining from facial pain table 2.

CT scan of paranasal sinuses revealed mucosal thickening of maxillary sinuses in 30 patients (75%), thickening of ethmoidal sinuses in 16 patients (40%), and mucosal thickening of frontal sinuses in 4 patients (10%). Ten patients (25%) had no changes. Soft tissue shadow of

inferior turbinate hypertrophy was found in 40 patients (100%).

Table 2. Patients' Complaint

Complaint	No.	%
Nasal obstruction	40	100
Mouth breathing	40	100
Sneezing	12	30
Snoring	20	50
Nasal discharge	26	65
Hyposmia	20	50
Facial pain	4	10

Subjective symptoms such as nasal obstruction, sneezing, nasal discharge, bleeding, hyposmia and facial pain, were evaluated on the 7th day and in the first, second, third, sixth and twelve months after the procedure. The microdebrider-assisted inferior turbinoplasty was perfectly tolerated by the patients. No crusting and synechia were observed, and no post-operative bleeding except few drops after removal of packing and stopped spontaneously.

Table 3. Duration of Postoperative Airway Patency

Duration	No.	%
1 week	30	75
1 month	37	92.5
3 month	37	92.5
6 month	37	92.5
12 month	37	92.5

The severity of nasal obstruction improved significantly during the first week after operation, 30 patients with good airway, and 7 patients had moderate improvement, while 3 patients had no improvement. After one month, 37 patients had good airway passages and persisted till 12th month after operation, while 3 patients with mild improvement. This improvement was observed clinically (table 3).
The severity of nasal discharge, sneezing, facial pain and hyposmia had significantly improved in the 1st week of operation and persisted in the 12th month after the operation table 4. Three patients developed mucosal tear but no mucosal loss and those managed by sodium bicarbonate 2% nasal douche and antibiotic for one week table 5. Table 6 shows the mean and the median of resistance preoperatively and postoperatively. The rhinomanometric examination was carried out one- day preoperatively, and 6–12 weeks postoperatively table 7.

Table 4. Preoperative and postoperative subjective and objective Data

Complaint	Preoperative	Postoperative improvement (%)	
		Yes	No
Nasal obstruction	100	7.5	92.5
Sneezing	30	0	100
Hyposmia	10	0	100
Nasal discharge	65	0	100
Facial pain	10	0	100

Table 5. Postoperative Complications

Complications	No.	%
Bleeding	0	0
Mucosal tear	3	7.5
Crusting	0	0
Synechia	0	0
Dryness	0	0

Table 6. Preoperative and Postoperative resistance (Pa/cm^3/s)

Site	Preoperative		Postoperative	
	Mean	Median	Mean	Median
Right side	2.84	1.20	0.72	0.41
Left side	2.37	1.18	0.70	0.45
Total	0.82	0.52	0.26	0.21

Discussion

In this study, we treated 40 patients with chronic nasal obstruction due to inferior turbinate hypertrophy by microdebrider technique and we found that the nasal airway was significantly improved in 92.5% of the patients, with no crustation or adhesion. Most of the patients

developed headache postoperatively that disappeared after removing the pack from the nasal cavity. Improvement of nasal discharge, facial pain, sneezing and hyposmia were significantly good from second week to the ends of twelfth months after operation.

Table 7. Preoperative and postoperative measurement of airflow (cm^3/s)

Air flow	Preoperative	Postoperative
Mean	266	730
Median	248	686

P = < 0.001 (Wilcoxon test)

Van Delden *et al* [5] after performing microdebrider assisted turbinoplasty for 100 patients during 1994-1997 found postoperative improvement in nasal patency occurred in 93% of patient. Friedman *et al* [6] at 1999, studied 112 patient who underwent bilateral microdebrider–assisted turbinoplasty and suggested that microdebrider usage in turbinoplasty is a safe method for achieving turbinate size reduction with acceptable morbidity in patient with nasal obstruction and bleeding is a rare complication; while preservation of mucosa leads to early healing and absence of crusting and bone exposure. The microdebrider technique lends itself to precise tissue removal with satisfactory reduction of tissue, but also 5% developed synechia.

Lee *et al* [7] at 2004 during a study of 29 patients with microdebrider –assisted turbinoplasty found that the nasal obstruction improvement was 91%. Joniau *et al* [8] at 2006 performed their study on 19 patients. They did powered turbinoplasty on one side and sub mucosal diathermy on other side, and they found that powered turbinoplasty (microdebrider) was superior to sub mucosal diathermy. Hegazi *et al* [9] at 2007 observed that 10% of the patients developed mild crustation after microdebrider turbinoplasty and saw complete resolution of nasal obstruction in 80% of patients and mild nasal obstruction in 20% of patients two months after Microdebrider-Assisted turbinoplasty. Chen *et al* [10] at 2007, during a study of 120 patients with chronic nasal obstruction, had divided them into 2 groups, one treated with microdebrider – assisted turbinoplasty and the other with sub mucosal resection. They had found that they are equally effective (both subjectively and objectively) in determining nasal obstruction in patients with hypertrophic inferior turbinate; however, microdebrider –assisted turbinoplasty was superior to sub mucosal resection due to more significant preservation of nasal mucosa resulting from definitive, controlled volume reduction of inferior turbinate sub mucosa.

Liu *et al* [11] at 2009 noted that microdebrider turbinoplasty and related symptom such as nasal obstruction, sneezing, rhinorhea and snoring significantly decreased from 6 months to 3 years after surgery, and also observed crustation and adhesion in 7 patients of 60 patients. Cingi *et al* [12] at 2009 found that the nasal obstruction significantly improved after microdebrider – assisted turbinoplasty on seventh day and persist after 3 months from surgery. Finally, Bahandarkar *et al* [13] at 2010 found that microdebrider-assisted turbinoplasty is a trend toward procedures that are mucosal sparing and may offer better long term outcome than radiofrequency ablation.

In conclusion, microdebrider - assisted turbinoplasty is a safe method for achieving turbinate size reduction with acceptable morbidity in patients with nasal airway obstruction secondary to turbinate hypertrophy; with bleeding as a minimal complication. Preservation of mucosa leads to early healing and absence of crusting.

References

1. Luczaj J. Submucosal bipolar radiofrequency therapy for treatment of inferior turbinate hypertrophy. Otolaryngol Polska. 2007; 61(3): 290-4.
2. Cumming CW, Paul W, Lee A, et al. Cummings Otolaryngology Head and Neck Surgery. 4th ed. Mosby; 2005. p. 1008-9.
3. Richarson J. Turbinate treatment in vasomotor Rhinitis. Laryngoscope. 1985; 58: 834-47.
4. Hol MK, Huizing EH. Treatment of inferior turbinate pathology: a review and critical evaluation of different techniques. Rhinology. 2000; 38: 157-66.

5. Van Delden MR, Cook PR, Davis WE. Endoscopic surgery, partial inferior turbinoplasty. Otolaryngol Head Neck. 1999; 121: 406-9.
6. Friedman M, Tanyeri H, Lim J. A safe, alternative technique for inferior turbinate reduction. Laryngoscope. 1999; 109: 1834-7.
7. Lee CF, Chen TA. Power microdebrider–assisted modification of endoscopic inferior turbinopasty; a preliminary report. Chang Gung Med J. 2004; 27: 359-65.
8. Joniau S, Wong I, Rajapaksa S, et al. Long term comparison between sub mucosal cauterization and power reduction of inferior turbinate. Laryngoscope. 2006; 116(9): 1612-16.
9. Hegazy HM, EL-Badawy MR, Hassan AA. Endoscopic sub mucous inferior turbinate reduction with microdebrider-A study of 50 cases. Tanta Med Sci J. 2007; 2: 194-9.
10. Chen Y, Liu C, Huang H. Comparison of microdebrider – assisted inferior turbinoplasty and sub mucosal resection of children with hypertrophic turbinate. Internat J Pediat Otolaryngol. 2007; 71: 921-7.
11. Liu C, Tan C, Lee F, et al. Microdebrider–assisted versus radiofrequency inferior turbinoplasty. Laryngoscope. 2009; 119: 414-8.
12. Cing C, Ure B, Cakli H, et al. Microdebrider –assisted versus radiofrequency assisted inferior turbinoplasty; prospective study with objective and subjective measure. ACTA Otorhinolaryngologica Italica. 2010; 30(3): 139.
13. Bhandarkar ND, Smith TL. Outcomes of surgery for inferior turbinate hypertrophy. Curr Opin Otolaryngol. 2010; 18: 49-53.

Correspondence to Dr. Adil N Razooqi
Mobile + 964 (0) 7706656060
E-mail: dradilnaeem@yahoo.com
Received 17th Sep. 2012: Accepted 28th Feb. 2013

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Serum Creatine Kinase and its Isoenzyme CK-MB in the Prediction of Tubal Ectopic Pregnancy

Hala A Abdullateef *MBChB CABOG*

Dept. of Obstetrics and Gynecology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Ectopic pregnancy is a major cause of maternal morbidity and mortality. Creatine kinase is an enzyme that its increase reflects tissue injury and could be useful in the diagnosis of tubal pregnancy.

Objectives To evaluate the diagnostic value of total creatine kinase in women with ectopic pregnancy, tubal rupture ectopic pregnancy, spontaneous abortion, and normal pregnancy and to investigate the possible discriminatory ability of creatine kinase-MB for diagnosis of tubal rupture ectopic pregnancy.

Methods Forty women with ectopic pregnancy, 17 with intrauterine abortion and 24 women with normal gestation were studied. The diagnosis of ectopic pregnancy was based on clinical assessment and transvaginal ultrasonography. Serum human chorionic gonadotropin levels were measured by enzyme linked immuno-sorbent assay. Total serum creatine kinase and creatine kinase-MB values were determined by spectrophotometrical analysis.

Results Creatine kinase and creatine kinase-MB levels were significantly higher in tubal ectopic pregnancy compared with both intrauterine abortions and normal gestations. When using creatine kinase-MB of 4.55 IU/ml as a cut-off value for the diagnosis of tubal ectopic pregnancy from control groups, sensitivity 81.64%, specificity 84.3%, positive predictive value 88.5% and negative predictive value 71.4%. Creatine kinase level in the ruptured ectopic pregnancy group was significantly higher than in the unruptured ectopic pregnancy, and normal pregnancy. When using creatine kinase of 29.43 IU/ml as a cut-off value for the diagnosis of ruptured ectopic pregnancy from unruptured groups, sensitivity 92%, specificity 100%, positive predictive value 100%, negative predictive value 96% and efficiency 97.4%.

Conclusions Women with ectopic had a significantly higher levels of creatine kinase-MB compared with women with normal pregnancy or intrauterine abortion and it has a high discriminatory ability for diagnosis of tubal rupture ectopic pregnancy.

Keywords Ectopic pregnancy, creatine kinase-MB

Introduction

Ectopic pregnancy (EP) is a major cause of maternal morbidity and responsible for 6% of pregnancy deaths [1]. Distinguishing normal from abnormal pregnancies is a clinical challenge because there is no definitive noninvasive diagnostic test available before visualization on ultrasonography. Clinicians must therefore follow patients over the course of several days to weeks for diagnosis [1,2], a time in which there is some potential for the ectopic pregnancy to rupture and result in life-threatening intra-abdominal hemorrhage. Early treatment may also allow for tubal-conserving procedures to be used, which is important for a patient's future fertility [3,4]. Therefore,

development of a serum test to diagnose an ectopic pregnancy with high accuracy would be of great clinical significance.

Creatine kinase (CK) is an intracellular enzyme that catalyzes the formation of adenosine triphosphate (ATP) from creatine phosphate and adenosine diphosphate (ADP). It is therefore abundant in metabolically active tissues with significant energy demands, specifically skeletal and smooth muscle, myocardium, and brain [5].

Three distinct isoenzyme forms of CK have been identified, namely, CK-MM, MB, and BB (M: muscle, B: brain) [5]. Because an increase of CK plasma concentration always reflects injury to a tissue of high CK activity, CK measurements are particularly useful in the diagnosis of acute myocardial infarction, in which determination of CK-MB isoenzyme levels is much more specific than total CK [6,7].

In tubal pregnancy, the zygote penetrates the tubal epithelium and lies next to the muscular layer as the fallopian tube lacks a submucosal layer. This invasion into the muscle causes an increase in muscle cell creatine kinase (CK) in blood [8].

The extent of penetration into the muscle will depend upon the site of implantation. In 1993, Lavie *et al* [9] reported that an initial maternal serum CK was predictive of tubal pregnancy in first trimester. Subsequently, three studies [10-12], were able to reproduce their findings.

Another study by Kurzel *et al* [13] found an elevated mean CK level but with questionable clinical utility and four studies [14-17] reported no elevation in serum CK in tubal pregnancy.

The current study was designed: (a) to further evaluate the diagnostic value of total CK in women with EP, spontaneous abortion, and normal pregnancy; (b) To determine, whether serum CK level might be a marker for diagnosis of tubal rupture ectopic pregnancy; and (c) To measure CK-MB isoenzyme concentrations in the previously mentioned samples and to investigate the possible discriminatory ability of MB fraction.

Methods

Forty women with EP, 17 intrauterine abortive and 24 women with normal intrauterine gestation (controls) were followed up at Al-Kadhimyia Teaching Hospital, Baghdad, Iraq, between November 2010 and June 2011. Descriptive characteristics such as the age, height and weight of the patients were taken.

Diagnosis of EP was based on clinical assessment and transvaginal ultrasonography. All EP were treated by laparotomy and confirmed by histopathology. From all women, blood was drawn by routine venipuncture. Blood samples were centrifuged at 3000 rpm and sera were stored at -20 ^{o}C.

Exclusion criteria were the absence of any medical disorder that would raise the serum CK. Inclusion criteria were the confirmation of intrauterine pregnancies in the control group and abortive group with a positive serum human chorionic gonadotropin (hCG). For the cases with ectopic pregnancy; diagnosis should be confirmed by transvaginal ultrasound and a positive hCG.

Human chorionic gonadotropin (hCG) levels were measured by monoclonal antibody Enzyme Linked Immuno-Sorbent Assay (ELISA) techniques for follow up or confirmation of the diagnosis. Total serum creatine kinase and CK-MB values were determined by spectrophotometric analysis.

Values are presented as mean ± standard error for mean (S.E.M.). Comparison of means between different groups was performed with Student's t test.

Receiver Operator Characteristic (ROC) curves was constructed to plot sensitivity against specificity.

The areas under the ROC curves (AUC) were calculated and compared with the AUC (0.5) of the non-diagnostic test (the line with slope of 1). For cut-off values of significant sensitivity and specificity (> 70%), contingency tables (cross-tabs) were constructed for the calculation of positive and negative predictive values. Confidence intervals of sensitivity, specificity,

positive and negative predicted values were calculated.

Statistical analyses were performed by SPSS software (v. 11.5) and also Excel 2007. P value < 0.05 level of significance was considered statistically significant.

Results

The basic anthropometric and clinical parameters of the women studied are presented in table 1. In our study, there were no statistical significant differences between the groups regarding maternal age and body mass index (BMI). The age of tubal ectopic pregnancy (group A) was 28.97 ± 0.957 years, it was 32.05 ± 1.95 years in IU abortion (group B) while, in normal pregnancy (group C) was 25.8 ± 1.139 years. The BMI was 26.27 ± 0.735 Kg/m^2 in group (A) while it was (28.47 ± 1.08 and 24.98 ± 1.278 Kg/$m^{2)}$ in groups B and C, respectively. In gestational age there were no statistical significant differences between the groups between women with tubal EP and women with normal pregnancy, and there were statistical significant differences (P < 0.001) between tubal EP and women with IU abortive pregnancy. The gestational age was 6.425 ± 0.142, 10.394 ± 0.6 and 6.54 ± 0.26 in groups A, B and C, respectively.

Table 1. Basic anthropometric and clinical parameters of the studied women

Parameter	Ectopic pregnancy N = 40	IU abortion N = 17	IU normal N = 24
Age (yrs)	28.97 ± 0.957	32.05 ± 1.95	25.8 ± 1.139
BMI (kg/m2) Pregnancy	26.27 ± 0.735	28.47 ± 1.08	24.98 ± 1.278
period (weeks)	6.425 ± 0.142	10.394±0.6*	6.54±0.226

IU = intrauterine, * = P < 0.05 (ectopic pregnancy *Vs* IU abortion)

The ROC curves shown in table 2 and figure 2 demonstrated a significant discriminatory ability of increased total creatine kinase levels for the diagnosis of tubal ectopic pregnancy. The AUC for total creatine kinase was 0.903 (95%CI: 0.831–0.975). A significant difference was found in EP (P < 0.001).

When using total creatine kinase concentration of 22.22 IU/ml as a cut-off value for the diagnosis of ectopic pregnancy from control groups, sensitivity was 68.4%, specificity 100%, the positive predictive value was 100% and the negative predictive value 66.66%.

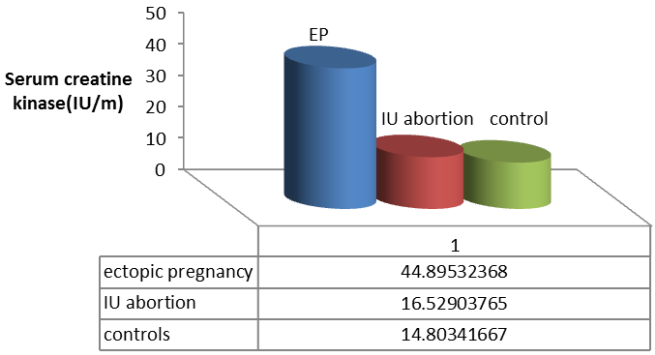
Table 2. AUC for ROC analysis of CK and CK-MB with testing for statistical differences

Enzyme	AUC ± SEM	95% CI*	*P value*
CK	0.903 ± 0.037	0.831 - 0.975	P < 0.001
CK-MB	0.938 ± 0.031	0.878 - 0.998	P < 0.001

* CI= Indicate to confidence interval.

Fig. 3 shows that the mean S.CK-MB levels were significantly higher in women with tubal EP compared with those of women with normal pregnancy (P < 0.0001) and (P < 0.001) IU abortion. A slight difference was also observed between women with abortive IU pregnancy and controls (P < 0.05).

The ROC curves demonstrated a significant discriminatory ability of increased CK-MB levels for the diagnosis of tubal ectopic pregnancy. The AUC for CK-MB was 0.983 (95%CI: 0.878–0.998). A significant difference was found in tubal EP (P < 0.001) as shown in fig. 4 and table 2.



	1
ectopic pregnancy	44.89532368
IU abortion	16.52903765
controls	14.80341667

Fig. 1. Levels of serum creatine kinase in tubal EP, IU abortion and control groups

When using CK-MB concentration of 4.55 IU/ml as a cut-off value for the diagnosis of tubal ectopic pregnancy from control groups, sensitivity was 81.64%, specificity 84.3%, the positive predictive value was 88.5% and the negative predictive value 71.4%.

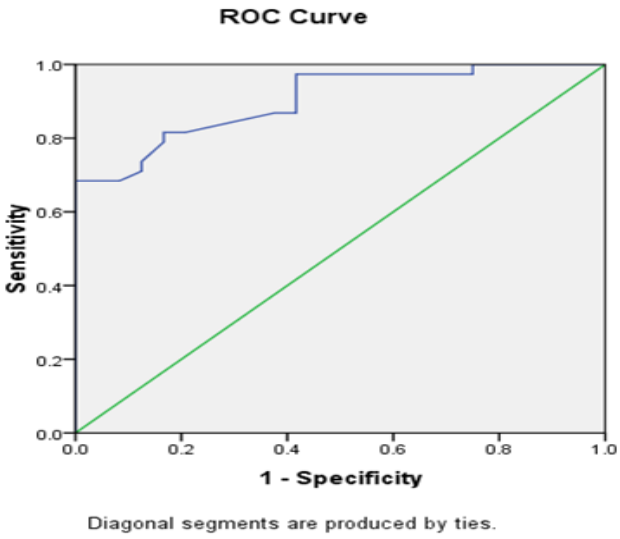
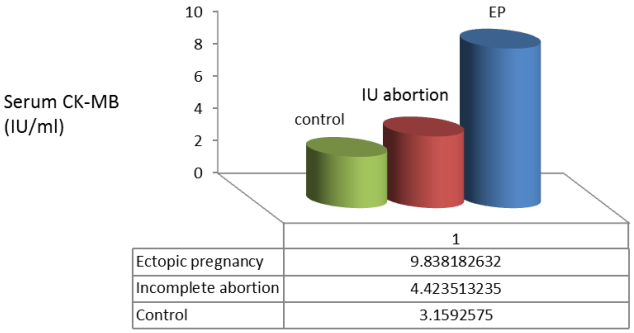


Fig. 2. Receiver Operator Characteristic (ROC) curves of increased total creatine kinase levels as diagnostic tests for ectopic pregnancy from control groups.

The concentration of creatine kinase (CK) and serum β-hCG levels in ruptured and unruptured of tubal ectopic pregnancy (EP) and control groups.

Fig. 5 shows the mean serum creatine kinase level in the ruptured ectopic pregnancy group was significantly higher than the levels in the unruptured ectopic pregnancy (P = 0.0001), and normal pregnancy (P < 0.0001) groups. No significant difference in β-hCG levels between ruptured and unruptured ectopic pregnancies.

The ROC curves demonstrated a significant discriminatory ability of increased CK levels in ruptured ectopic pregnancy from unruptured. The AUC for CK in ruptured was 0.974 (95% CI: 0.926–1.022). A significant difference was found in ruptured EP (P < 0.001).



	1
Ectopic pregnancy	9.838182632
Incomplete abortion	4.423513235
Control	3.1592575

Fig. 3. Levels of serum CK-MB in EP, IU abortion and control groups.

When using CK ruptured concentration of 29.43 IU/ml as a cut-off value for the diagnosis of ruptured ectopic pregnancy from unruptured groups, sensitivity was 92%, specificity 100%, the positive predictive value was 100%, the negative predictive value 96% and efficiency 97.4%.

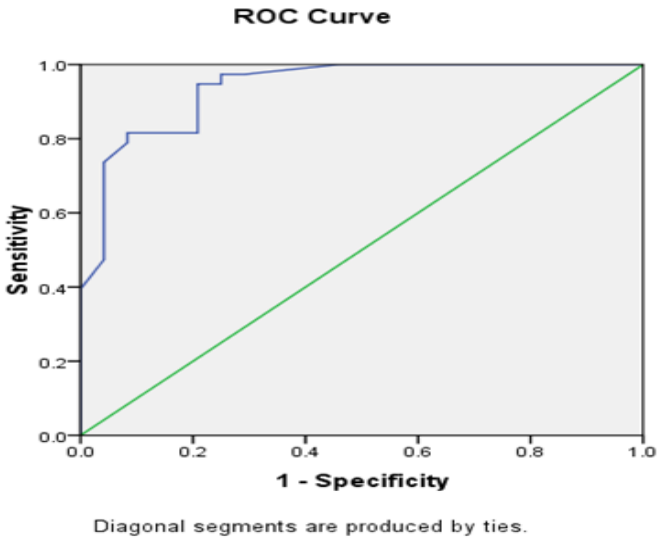
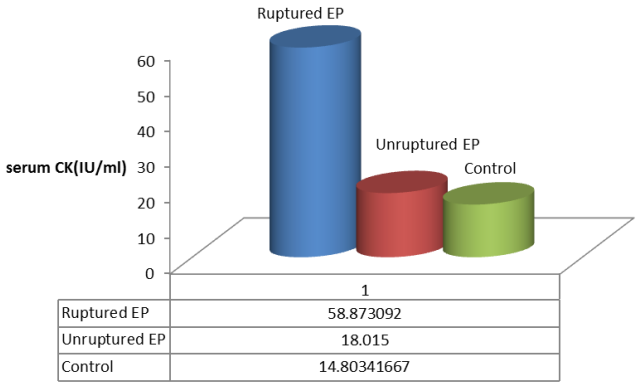


Fig. 4. Receiver Operator Characteristic (ROC) curves of increased CK-MB levels as diagnostic tests for tubal ectopic (EP) from control groups.

The ROC curves demonstrated a significant discriminatory ability of increased CK levels in ruptured ectopic pregnancy from control group. The AUC for CK in ruptured was 0.988 (95%CI: 0.964–1.013). A significant difference from the control was found in ruptured EP ($P < 0.001$).



	1
Ruptured EP	58.873092
Unruptured EP	18.015
Control	14.80341667

Fig. 5. Levels of serum CK in ruptured, unruptured EPs and control groups.

When using CK ruptured concentration of 23.3 IU/ml as a cut-off value for the diagnosis of ruptured ectopic pregnancy from unruptured groups, sensitivity was 96%, specificity 100%, the positive predictive value was 100%, the negative predictive value 96.9% and efficiency 97.9%.

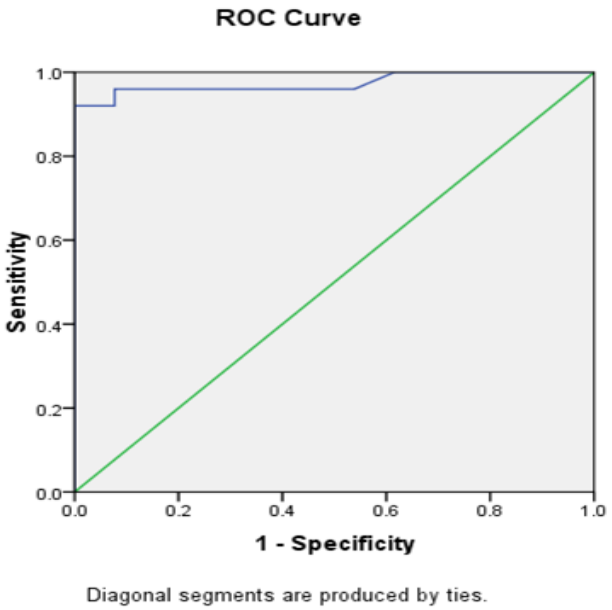


Fig. 6. Receiver Operator Characteristic (ROC) curves of increased CK in ruptured ectopic pregnancy levels from unruptured groups.

Discussion

Creatine kinase (CK) is an enzyme that is released when muscle becomes damaged [18]. The first study of CK as a marker of Fallopian tube damage produced some encouraging results [9].

In the current research, CK levels were significantly increased in women with tubal EP compared with both women with IU abortion ($P < 0.00$) and those with normal gestation ($P < 0.00$).

A rise in serum CK level is natural in tubal gestation, because the zygote penetrates the tubal epithelium and lies adjacent to the muscle layer which lacks a sub mucosa [8,10]. Due to invasion of the muscle layer by trophoblasts, the maternal blood vessels are eroded and blood leaks through the growing trophoblasts and damaged muscle layer giving a rise in muscle cell product like CK [8,10]. The pathology in missed abortions is different and there is no rise in serum creatine kinase [10], as is also evident from our results.

The present results are in agreement with Lavie *et al* [9], who studied the role of maternal serum CK levels as a predictor of tubal pregnancy. They found that serum CK levels were significantly higher in the tubal pregnancy group than in spontaneous abortion and normal pregnancy. Similar results were obtained by Saha *et al* [11] in their comparative study of 20 patients evaluated and endorsed the positive role of serum CK as a possible marker of tubal pregnancy. Develioglu and coworkers [19] conducted a comparative study on 32 cases and their results revealed that serum CK levels can be taken as an adjuvant tool in ruling out ectopic pregnancy, particularly if it was ruptured ectopic pregnancy. Yet another comparative study by Singh *et al* [8] on 15 patients revealed that CK levels were higher in tubal pregnancy than normal intra-uterine pregnancy. Several authors have, however, found conflicting results from Qasim *et al* and Vitoratos [14,17].

Also in this study serum CK concentrations were significantly higher in the patients with ruptured tubal ectopic pregnancy compared with

unruptured tubal ectopic pregnancy (P < 0.0001).

It is possible therefore that increased levels of creatine kinase associated with muscular damage may precede rupture of the tube. The positive correlation between gestational age and creatine kinase levels in ruptured tubal ectopic pregnancy is some evidence for this proposition as it shows that the increase in creatine kinase levels, and in the extent of tubal muscular damage that it marks, is a function of time, and not simply an end result of the eventual disruption of the tubal wall.

All the women, with ruptured tubal pregnancy had significant tubal damage and raised values of CK. These indicate that tubal rupture is associated with an increase in creatine kinase levels. These results are in agreement with Singh *et al* [8] in their study suggested that maternal CK levels are significantly higher in women with tubal pregnancy and are reliable in the diagnosis of a tubal pregnancy.

Because the differential expression of CK-MB isoenzymes varies significantly between different tissues [5,6], then it might be useful to estimate CK-MB levels separately. Notably, no previous studies on CK-MB fractions in tubal EP were found in the literature. Intriguingly, in present study women with EP were significantly higher CK-MB levels compared with the intrauterine abortion (P < 0.001) and normal intrauterine pregnancy (P < 0.0001).

Estimation of CK-MB with a cut-off value of 4.55 IU/L produces a good sensitivity 81.64%, specificity 84.3 % and 80.9 % efficiency, the positive predictive value was 88.5 % and the negative predictive value 71.4 % in diagnosis of tubal ectopic pregnancy.

Conclusively, the current study is the first to demonstrate that women with EP have a significantly higher of CK-MB levels compared with women with IU normal or abortive pregnancy. The exact reasons for the increase CK-MB relative level in tubal EP are at present unknown and remain to be elucidated.

References

1. Barnhart KT. Clinical practice. Ectopic pregnancy. N Engl J Med. 2009; 361: 379-87.
2. Kirk E, Papageorghiou AT, Condous G, et al. The diagnostic effectiveness of an initial transvaginal scans in detecting ectopic pregnancy. Hum Reprod. 2007; 22: 2824-8.
3. Bangsgaard N, Lund CO, Ottesen B, et al. Improved fertility following conservative surgical treatment of ectopic pregnancy. BJOG. 2003; 110: 765-70.
4. Seror V, Gelfucci F, Gerbaud L, et al. Care pathways for ectopic pregnancy: a population-based cost-effectiveness analysis. Fertil Steril. 2007; 87: 737-48.
5. Lott JA, Abbott LB. Creatine kinase isoenzymes. Clin Lab Med. 1986; 6: 547-76.
6. Malasky BR, Alpert JS. Diagnosis of myocardial injury by biochemical markers: problems and promises. Cardiol Rev. 2002; 10: 306-17.
7. Mongiardo A, Ferraro A, Ceravolo R, et al. Mechanism of troponin and CK-MB release after percutaneous coronary interventions. Ital Heart J. 2002; 3: 270-4.
8. Singh A, Bansal S. Serum Creatinine kinase - an alternative diagnostic marker in ruptured tubal pregnancy. J Obstet Gynecol India. 2005; 55(5): 448-50.
9. Lavie O, Beller U, Neuman M, et al. Maternal serum creatinine kinase: a possible predictor of tubal pregnancy. Am J Obstet Gynecol. 1993; 169:1149-50.
10. Chandra L, Jain A. Maternal serum creatine kinase as a biochemical marker of tubal pregnancy. Int J Gynecol Obstet. 1995; 49: 21-3.
11. Saha PK, Gupta I, Ganguly NK. Evaluation of serum creatine kinase as a diagnostic marker for tubal pregnancy. Aust NZJ Obstet Gynecol 1999; 39: 366-7.
12. Birkhahn RH, Gaeta TJ, Leo PJ, et al. The utility of maternal creatine kinase in the evaluation of ectopic pregnancy. Am J Emerg Med 2000; 18:695-7.
13. Kurzel RB, Mazdisnian F, Paige S, et al. Serum creatine kinase is not a reliable indicator of ectopic pregnancy. Int J Fertil Women's Med 2001; 46:300-3.
14. Qasim SM, Trias A, Sachdev R, et al. Evaluation of serum creatine kinase levels in ectopic pregnancy. Fertil Steril. 1996; 65: 443-5.
15. Korhonen J, Alfthan H, Stenman, U et al. Failure of creatine kinase to predict ectopic pregnancy. Fertil Steril. 1996; 65: 922-4.
16. Darai E, Vlastos G, Benifla J, et al. Is maternal serum creatine kinase actually a marker for early diagnosis of ectopic pregnancy? Eur J Obstet Gynecol Reprod Biol. 1996; 68: 25-7
17. Vitoratos N, Gregariou O, Papadias C, et al. Clinical value of creatinine kinase in diagnosis of ectopic pregnancy. Gynecol Obstet Invest. 1998; 46: 80-3.
18. Costa TN, Cassaro Stunz CM, Nicolau JM, et al. Comparison of MB fraction of creatine kinase mass and troponin I serum levels with necropsy findings in acute myocardial infarction. Am J Cardiol. 2008; 101 311-4.
19. Develioglu OH, Askalli C, Uncu G, et al. Evaluation of serum creatine kinase in ectopic pregnancy with reference to tubal status and histopathology. BJOG 2002; 109(2): 121-8.

Mobile +964 7901 332901
E-mail: drhala1966@yahoo.com
Received 23rd Apr. 2012: Accepted 8th Oct. 2012

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Effect of Maternal Hemoglobin on Anthropometric Measurements of Full Term Newly Born Babies

Sabah M Ali[1] *MBChB FICPS*, **Ali M Murad**[2] *MBChB, FICOG*, **Adiba M Murad**[2] *MBChB CABP*

[1]Dept. of Pediatrics, College of Medicine, Dayala University, Dayala, [2]Dept. of Gynecology & Obstetrics, [3] Dept. of Pediatrics, College of Medicine, Al-Mustansiriya University, Baghdad.

Abstract

Background Hemoglobin of the mother during pregnancy is well established to be contributors to abnormal prenatal development and pregnancy outcomes.

Objectives To study the effect of maternal anemia on anthropometric measurement of full term newly born babies.

Methods Two hundred pregnant women at time of delivery were investigated for their Hemoglobin. Their newborns were investigated for anthropometric measurement (weight, length, head circumference and chest circumference) immediately after birth. The questionnaire involved questions about age, parity, economy, educational level, and antenatal care of the mothers, and also sex, gestational age, anthropometric measures (weight, length, head circumference and chest circumference) and outcomes of the newborns.

Results From 200 pregnant women who were included in our study, 115 (57.5%) of them delivered by normal vaginal delivery and 85 (42.5%) delivered by caesarian section. Sixty (30%) of mothers were anemic. Five (2.5%) of the newborns were small for gestational age all of them from anemic mothers.

Conclusion Anemia affects neonatal outcomes in full term babies; full term babies of anemic mothers were in the normal anthropometric range, but they were much lower than babies of normal mothers.

Keywords Pregnancy, Anemia, Small for gestational age

Introduction

Anemia is one of most prevalent nutritional deficiency problem afflicting pregnant women [(1)]. This is particularly a major health problem in developing countries, where nutritional deficiency, malaria and worm infestation are common. Prevalence in non-industrialized countries varies between 35 - 75 %, with the average being 56 % [(2)].

Maternal anemia is considered a risk factor for adverse pregnancy outcome [(3)]. It is responsible for 40-60% of maternal deaths in developing countries. Anemia that complicates pregnancy threatens the life of both the mother and the fetus [(4)]. Anemia is hemoglobin (Hb) concentration below 110 g/l [(5)]. The prevalence is higher among the primigravidae than multiparous women [(6)]. Maternal nutritional state is an important predictor of perinatal results. This concept has gained more importance in the recent years as there is now growing acceptance of the 'fetal origin of adult disease' hypothesis [(7)]. The objectives was to study the effect of maternal Hb on anthropometric measurements of full term baby.

Methods

This study is a cross-sectional study included 200 pregnant women who attended to the obstetrical ward in Al-Yarmook Teaching

Hospital, in the period between the 1st of March to the 1st of May 2010; one hundred fifteen delivered by normal vaginal delivery (NVD), and eighty five were delivered by caesarian section (C/S).

All these 200 pregnant women were studied have full term babies, and all pregnant women with chronic diseases had been excluded from the study. For each mother, blood sample was aspirated, before delivery and sent for hemoglobin analysis (PCV was done) at the laboratory in Al-Yarmook Teaching Hospital.

Regarding the newborn of each mother only full term were included in the study, all preterm were excluded as well as, post date and any baby with obvious dimorphic feature .

Four measurements took for each baby (weight, length, head circumference and chest circumference) which were done immediately after birth in the neonatal intense care unit in AL-Yarmook Teaching Hospital.

I. Weight measurement of baby used digital scale.
II. Lengths measurement of baby by Infantometer (studiometery) done by fix baby inside the box in supine position fix the head & lower limbs then attach the lower end of infantometer to sole of the feet.
III. Head Circumference measurements done according the following steps:
   1. Use non-stretch tape, such as flexible metal tape measure.
   2. Warp the tape around the widest part of the child head.
   3. Move the tape around a bit and record the largest possible measurement.

Measure the circumference of the head at the level of the plane passing above the glabella (the most anterior protrusion of the forehead) and over the opisthrocranion (the most posterior protrution from glabella on the back of the head), per-pendicular to the mid-sagittal plane.

Three different measurements take for each baby and record the largest one.

IV. Chest Circumference measurement of baby. The infant lies on back. With an automated tape device, measure the circumference of the chest at the level of the nipples during normal breathing.

Analysis of data was carried out using the available statistical package of SPSS-18 (Statistical Packages for Social Sciences- version 18 "PASW" Statistics). 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) was tested using student-t-test for difference between two independent means, while different percentages (qualitative data) were tested using Pearson Chi-square test. Statistical significance was considered whenever the P value was less than 0.05.

Results

From 200 pregnant women who were included in this study, 115 (57.5%) of them delivered by NVD and 85 (42.5%) delivered by C/S. One hundred forty (69.5%) of women with normal Hb, 60 women (30.5%) with anemia, 58 women (29.5%) with mild anemia and 2 women (1%) of them with moderate anemia; we make as anemic and no anemic group figure 1.

The mean of (weight, length and chest circumference) show significant difference between anemic and non-anemic (P = 0.031, P = 0.009, P = 0.004) while head circumference was not significantly affected (P = 0.054) as it is shown in table 1. There was a statistically significant difference in the anthropometrics measurement of new born of anemic and non-anemic groups. The study showed that the (weight, length, head circumference and chest circumference) of neonates in anemic group was less than non-anemic group weight (180 g), length (0.8 cm), head circumference (0.38 cm), chest circumference (0.7 cm) show in table 1.

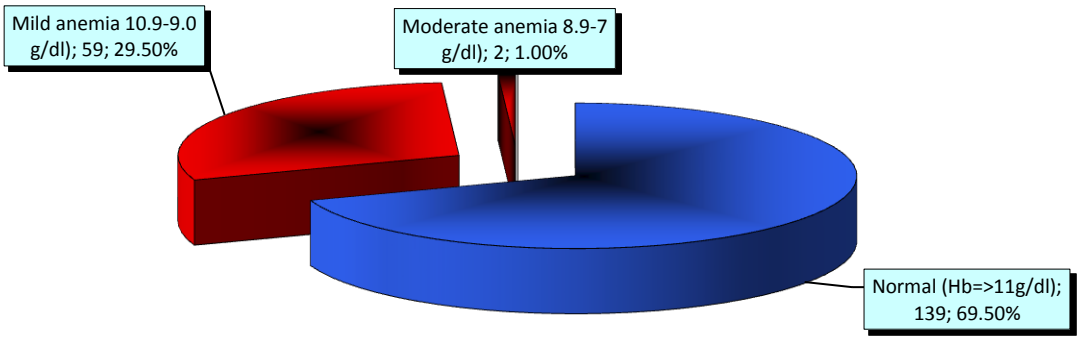


Figure 1. Hemoglobin status of the mothers of new born baby

Table 1. Relation of maternal Hb level and anthropometric measurements of new born

Parameter	Normal (Hb≥11.0)		Anemic (Hb<11.0)		P value
	Mean ± SD	Range	Mean ± SD	Range	
Hemoglobin (g/dl)	11.86 ± 0.92	11.00 - 15.80	10.08 ± 0.57	8.20 - 10.80	0.0001
Weight (Kg)	3.47 ± 0.48	2.75 - 5.00	3.29 ± 0.63	2.25 - 4.50	0.031
Length (cm)	49.52 ± 1.81	45.00 - 55.00	48.72 ± 2.31	42.00 - 54.00	0.009
OFC (cm)	34.46 ± 1.19	31.00 - 38.00	34.08 ± 1.45	30.00 - 37.50	0.054
Chest circumference (cm)	33.13 ± 1.50	30.00 - 38.00	32.43 ± 1.63	28.00 - 35.00	0.004

*Significant difference using t-test for two independent means at 0.05 level of significance

The percentile of (weight for age and length for age) was statistically significantly affected (P = 0.001, P = 0.02), while weight for length and head circumference for age was not significantly affected (P = 0.215, P = 0.063) as show in tables 2 through 5.

Table 2. Relation of weight for age percentile of new born with Hemoglobin of the mother

Weight for age percentile	Normal (Hb≥11.0)		Anemic (Hb<11.0)		P value
	No	%	No	%	
<3rd	1	0.7	4	6.7	0.001*
3rd-50th	74	52.9	40	66.7	
50th-97th	60	42.9	11	18.3	
>97th	5	3.6	5	8.3	

*Significant difference using chi square test for two independent means at 0.05 level of significance

Table 3. Relation of length for age percentile of new born with Hemoglobin of the mother

Length for age percentile	Normal (Hb≥11.0)		Anemic (Hb<11.0)		P value
	No	%	No	%	
$<3^{rd}$	2	1.4	6	10.0	0.02*
3^{rd}-50^{th}	77	55.0	35	58.3	
50^{th}-97^{th}	59	42.1	19	31.7	
$>97^{th}$	2	1.4	0	0	

*Significant difference using chi square test for two independent means at 0.05 level of significance.

Table 4. Relation of weight for length percentile of new born with Hemoglobin of the mother

Weight for length percentile	Normal (Hb≥11.0)		Anemic (Hb<11.0)		P value
	No	%	No	%	
<3rd	2	1.4	4	6.7	0.215
3rd-50th	53	37.5	19	31.7	
50th-97th	65	46.4	27	45.0	
>97th	20	14.3	10	16.7	

Table 5. Relation of OFC for age percentile of new born with Hemoglobin of the mother

OFC for age percentile	Normal (Hb≥11.0)		Anemic (Hb<11.0)		P value
	No	%	No	%	
<3rd	1	0.7	3	5.0	0.063
3rd-50th	92	65.7	43	71.7	
50th-97th	47	33.6	14	23.3	
>97th	-	-	-	-	

Discussion

In this study anemia prevalence in pregnant women was found to be 30.5%. In India the overall prevalence of anemia among pregnant women was estimated to be 72.5 %[8], in Nigeria (61.2%) [9], in Turkey 43% in [10], in Bangladesh (36%) [11], in India another study (34.4%) [12], in New Zealand (13%) [13].

This study focused on the relationship between maternal anemia and perinatal outcome where we found that maternal anemia during pregnancy was associated with low birth weight, affect length and chest circumference but not head circumference that related to duration of anemia first, second or third trimester anemia.

In a study done in Turkey between January 2005 and December 2006[10], on two groups of pregnant women (first group with anemia and second group without anemia), of 3688 pregnant women 1588 (43%) were found to be anemic, the anthropometric measurements (weight, length, head circumference and chest circumference) of newborn of anemic and non-anemic mother groups showed statistically significant difference [10].

In a study done in Pakistan, from January 2004 to December 2005[14], from 860 pregnant women, 402 (46.7%) were anemic, perinatal outcome include preterm delivery, low birth weight and intrauterine growth retardation, low birth weight among anemic women was 1.8 time more than non-anemic [14].

In a study done in Pakistan from October 2001 to October 2002) [16], on 629 pregnant women of these 313 were anemic the risk of low birth weight was 1.9 higher among anemic women [15].

In a study done in India on 102 pregnant women show that (34.3%) of pregnant women were anemic, the maternal hemoglobin concentration showed significant correlation with birth weight ($P = 0.01$) [16].

In a study done in Sri Lanka on 817 pregnant women, about (7.1%) were anemic the study show that anemia during pregnancy was not adversely associated with any of pregnancy outcome, hemoglobin level of > 13.9 g/dl was adversely associated with low birth weight [17].

In a study done in Norway, 877 pregnant women, with low hemoglobin levels at term were closely associated with increased frequency of newborn in heavy weight for date [18].

In this study, the effect of anemia first affect the weight and then affect the length and chest circumference then affect head circumference that may explain why head circumference not affected either due to anemia in last trimester or treated anemia during pregnancy.

In conclusion, anemia affects neonatal outcomes in full terms baby, full term babies of anemic mothers were in the normal anthropometric range, but they were much lower than babies of normal mothers.

References

1. Thangaleela T, Vijayalakshmi P. Impact of anaemia in pregnancy. Indian J Nutr Diet 1994a; 31, 9251-6.
2. World Health Organization. WHO Global Database. Geneva: WHO, 1997.
3. Brabin L, Nicholas S, Gogate A, et al. A high prevalence of anemia among women in Mumbai, India. Food Nutr Bull. 1998; 19: 205-9.
4. Viteri FE. The consequences of iron deficiency and anemia in pregnancy. Adv Exp Med Biol. 1994; 352: 127-39.
5. Thangaleela T, Vijayalakshmi P. Impact of anaemia in pregnancy. Indian J Nutr Diet. 1994b; 31, 26-32.
6. Gambling L, Andersen HS, McArdle HJ. Iron and copper, and their interactions during development. Biochem Soc Trans. 2008 Dec; 36(Pt 6): 1258-61.
7. Wjst M, Popescu M, Trepka MJ, et al., Pulmonary function in children with initial low birth weight. Pediatr Allergy Immunol. 1998; 9: 80-90.
8. Malhota M, Sharma JB, Barta S, et al. Maternal and perinatal outcome in varying degrees of anemia. Int J Gynaecol Obstet. 2002; 79: 93-100.
9. Akinola OI, Fabamwo AO, Tayo AO, et al. Maternal Hemoglobin and Fetal Birth Weight any Relationship? The Experience in Lagos, South Western Nigeria. Nigerian Med Pract. 2008; 54(3-4): 50-52.
10. Telatar B, Comert S, Vitrinel A, et al. The effect of maternal anemia on anthropometric measurements of newborns. Saudi Med J 2009; 30(3):409-412.
11. Akhter S, Momen MA, Rahman MM, et al. Effect of maternal anemia on fetal outcome. Mymensingh Med J 2010 Jul; 19(3):391-8.
12. Singla PN, Tyagi M, Kumar A, et al. Fetal growth in maternal anemia. J Trop Pediatr. 1997 Apr; 43(2): 89-92.
13. Brough L, Rees GA, Crawford MA, et al. Effect of multiple-micronutrient supplementation on maternal nutrient status, infant birth weight and gestational age at birth in a low-income, multi-ethnic population. Br J Nutr. 2010 Aug; 104(3): 437-45.
14. Bakhtiar UJ, Khan Y, Nasar R. Relation between maternal hemoglobin and perinatal outcome. Rawal Med J. 2007 Jul-Dec; 32(2): 102-4.
15. Lone FW, Qureshi RN, Emanuel F. Maternal anemia and its impact on perinatal outcome. Top Med Int Health. 2004 Apr; 9(4): 486-90.
16. Rusia U, Madan N, Agarwal N, et al. Effect of maternal iron deficiency anemia on fetal outcome. Indian J Pathol Microbiol. 1995 Jul; 38(3): 273-9.
17. Abeysena C, Jayawardana P, de A Seneviratne R. Maternal hemoglobin level at booking visit and its effect on adverse pregnancy outcome. Aust N Z J Obstet Gynaecol. 2010 Oct; 50(5): 423-7.
18. Sagen N, Nilsen ST, Kim HC, et al. Maternal hemoglobin concentration is closely related to birth weight in normal pregnancies. Acta Obest Gyne Scand. 1984; 63(3): 245-248.

Correspondence to Dr. Sabah M Ali
E-mail: sabahma2002@yahoo.com
Received 9th Feb. 2012: Accepted 17th Dec. 2012.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq/

The Pattern of Bacterial Pathogens & their Antibiotics Sensitivity among Patients with Respiratory Tract Infections

Abdul-Munim N Mohammed *MSc*

Dept. of Microbiology, College of Medicine, Al -Mustansiriya University, Baghdad, Iraq

Abstract

Background Knowing the bacterial pathogens and their antibiotic sensitivity is an important way of establishing a suitable guideline of treatment of infection.

Objectives To isolate bacterial pathogens from patients with respiratory tract infections (RTI), and to determine the antibiotic sensitivity of isolates.

Methods Sputum specimens were collected from 145 patients with RTI admitted to Al-Kindy Teaching Hospital from March 2011 to January 2012. Out of these, 88 (60.7%) patients (age rang 17-59 years) had an established bacterial etiology, and of these, 57 (64.8%) were males and 31 (35.2%) females. All isolates were diagnosed according to bacteriological and biochemical standard methods. For identified of antimicrobial susceptibility used from Kirby Bauer method according to (NCCLS).

Results *Klebsiella* species and *Pseudomonas aeruginosa* were the most common isolates among the Gram negative pathogens (26.2% and 11.7% respectively), followed by *Escherichia coli* and *Proteus* species, while *Streptococcus pneumonia* was the most common isolate among the Gram positive organisms, identified in (15.2%) followed by *Staphylococcus aureus* and Streptococcus pyogenes. High rates of resistance to Amoxicillin and Cephalothin were demonstrated by all bacteria, whereas most isolates were found to be highly sensitive to Amikacin, Ciprofloxacin and Tobramycin. In contrast, Cefotaxim, Tetracyciln, Gentamycin and Erythromicin were less effect against most of isolates.

Conclusions *Klebsiella* spp. was the most common pathogens, whereas *Streptococcus pneumonia* which ranks as second common pathogens from patients with RTI in the present study. Amikacin, Ciprofloxacin and Tobramycin were the most effect antibiotics in *vitro* against tested bacteria. Conversely, no or less effect of other antibiotic agents was obtained making them not to be considered the drugs of choice in treatment of patients with RTI.

Keywords Bacterial pathogens, Antibiotics resistance, Patients RTIs.

Introduction

Respiratory tract infection (RTI) is defined as any infectious disease of the upper or lower respiratory tract. Upper respiratory tract infections (URTIs) include the common cold, laryngitis, pharyngitis/tonsillitis, acute rhinitis, acute rhinosinusitis and acute otitis media. Lower respiratory tract infections (LRTIs) include acute bronchitis, bronchiolitis, pneumonia and tracheitis [(1)]. The Centers for Disease Control and Prevention (CDC), World Health Organization (WHO) and Institute of Medicine have identified antimicrobial resistance as a major public health threat [(2-4)]. Antibiotic is credited with dramatic reduction in the morbidity and mortality associated with many bacterial infections, its abuse has resulted in the rapid emergence of resistant strains that reduce the effectiveness of many antibiotics [(5)].

Antibiotics are commonly prescribed for RTIs in adults and children in primary care. General Practice Consultation Rates (GPCR) in England and Wales show that a quarter of the population will visit their GPCR because of an RTI each year [6]. Therapy for community acquired respiratory tract infections is often empirical. However, increasing antibiotic resistance in frequently isolated respiratory tract pathogens complicated the selection process of antimicrobial agents [7]. Pharmaco-economic analyses have confirmed that bacteriologically more effective antibiotics can reduce overall management costs. Particularly with respect to consequential morbidity and hospital admission. Application of these principles would positively benefit therapeutic outcomes, resistance avoidance and management costs and will more accurately guide antibiotic choices by individuals, formulary, and guideline committees [8].

Methods

Sputum specimens were collected from 145 patients with RTI admitted to Al-Kindy Teaching Hospital from March 2011 to January 2012. Out of these, 88 (60.7%) patients (age rang 17-59 years) had an established bacterial etiology, and of these, 57 (64.8%) were males and 31 (35.2%) females.

The sputum samples were collected in sterile universal plastic containers and sent to the Diagnostic Microbiology Laboratory of Al-Kindy Teaching Hospital were analyzed. All isolates were diagnosed according to well-known established bacteriological methods [9]. Biochemical identification of bacterial species was performed by standard methods [10].

Antimicrobial susceptibility test: The isolates were subjected to susceptibility testing to the commonly used antimicrobial agents by Kirby - Bauer method according to criteria of National Committee for Clinical Laboratory Standard (NCCLS) [11], and their results of zone growth inhibition were compared to that in table 1.

Statistics: Descriptive statistical analysis (number and percentage) were used to calculate for type of bacterial isolates and their sensitivity results.

Table 1. Zone size and their interpretation (National Committee for clinical laboratory Standard (NCCLS)

Antimicrobial agent (symbol)	Disc potency	Diameter of zone of inhibition (mm)		
		Sensitive	Intermediate	Resistant
Amikacin (AN)	30 µg	≥ 17	15-16	≤14
Amoxicillin (AMX)	10 µg	≥18	14-17	≤ 13
Ciprofloxacin (CIP)	5 µg	≥21	16-20	≤ 15
Gentamycin (GM) Tobramycin	10 µg	≥15	13-14	≤ 12
(TM)	10 µg	≥15	13-14	≤12
Cephalothin (CF)	30 µg	≥ 18	15-17	≤ 14
Cefotaxim (CTX)	30 µg	≥19	15-18	≤ 14
Tetracycline (TE)	30 µg	≥ 19	15-18	≤ 14
Erythromicin (ER)	15 µg	≥ 23	14-22	≤ 13

Results

A total of 145 patients with RTI were examined, the bacterial etiology agents were identified in 88(60.7%) patients. In our study since the number of males was higher than females (64.8%), so the number of isolates was comparatively higher in males than females.

From 88 positive cases with RTI, 103 bacterial strains were isolated. Out of these, 64 (62.1%) were Gram-negative bacilli and 39 (37.9%) were Gram-positive cocci. Indeed some sputum samples contained more than one bacterium. These results as shown in figure 1.

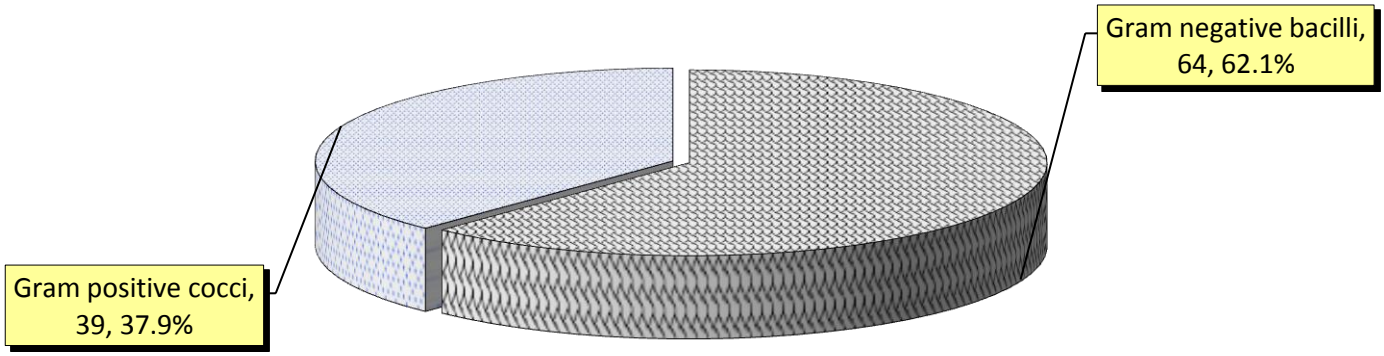


Figure 1. Distribution of the Microorganism isolates from 88 positive cases

Table 2 shows the Klebsiella species and Pseudomonas aeruginosa were the most prevalent (36.9% and 16.5% respectively) among the Gram-negative bacilli, followed by Escherichia coli (5.8%) and Proteus species (2.9%). Streptococcus pneumonia was the most prevalent among Gram-positive cocci (21.3%), followed by Staphylococcus aureus and Streptococcus pyogenes (11.7% and 4.9% respectively).

Table 2. Distribution of the Bacterial isolates from sputum of patients with RTI

Bacterial species		No. of isolates	%
Gram-negative *bacilli*	*Klebsiella species*	38	36.9
	Pseudomonas aeruginosa	17	16.5
	Escherichia coli	6	5.8
	Proteus species	3	2.9
Gram-positive *cocci*	*Streptococcus pneumonia*	22	21.3
	Staphylococcus aureus	12	11.7
	Streptococcus pyogenes	5	4.9

The drug sensitivity to bacterial pathogen isolates from patients with RTIs:

High rates of resistance to Amoxicillin and Cephalothin was demonstrated by all bacteria, whereas most isolates were found to be highly sensitive to Amikacin, Ciprofloxacin and Tobramycin. Klebsiella species showed high resistance to most of antibiotic agents except Amikacin, Ciprofloxacin and Tobramycin were the most potent activity against this strain.

*Streptococcus pneumonia*e, showed moderate to high resistance against Cephalothin, Tetracycline and Erythromicin, while good effect to other antibiotic agents, which were used in this study.

Pseudomonas aeruginosa, *Streptococcus* pyogenes and *Escherichia coli* isolates exhibited strong resistance to most tested antibiotic types except Amikacin, Ciprofloxacin and Tobramycin revealed good efficacy.

Most of isolates showed good susceptibility to Cefotaxim and Gentamicin except Klebsiella species (18.4%) and Escherichia coli (33.3%) which were poor efficacy to these antibiotics agents. Majority of isolates were highly resistance to Tetracycline and Erythromicin except Proteus species and Staphylococusaureus showed fully sensitive to these antibiotic agents. These results, as presented in figures (2-8).

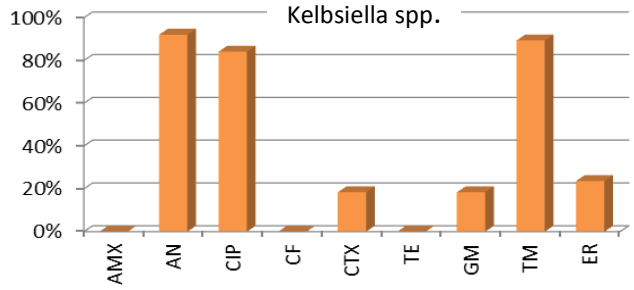


Figure 2. Susceptibility of Klebsiella *species* to antibiotics

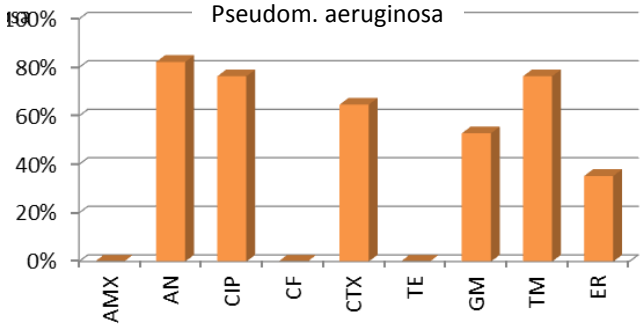


Figure 3: Susceptibility of *Pseudomonas aeruginosa* to antibiotics

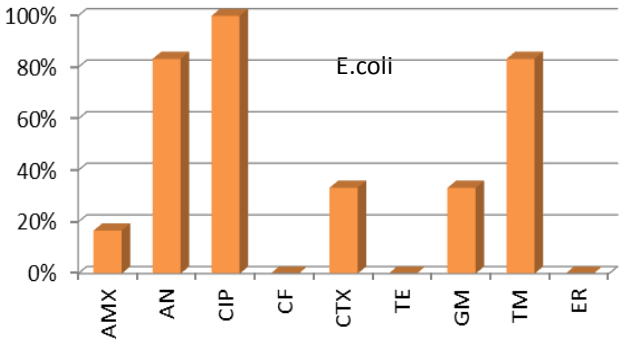


Figure 4. Susceptibility of E.coli *to* antibiotics

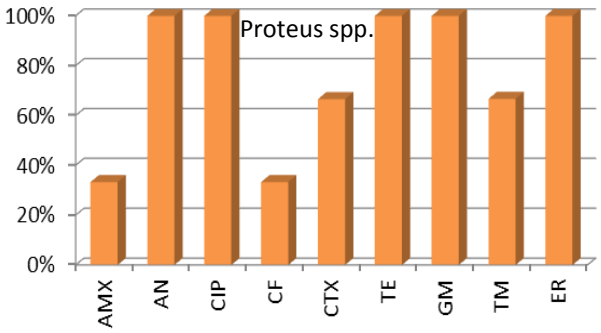


Figure 5. Susceptibility of *Proteus species* to antibiotics

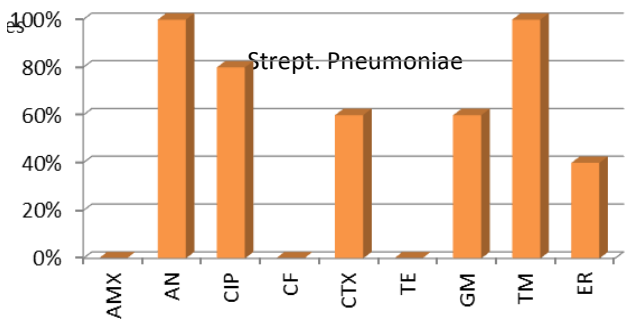


Figure 6: Susceptibility of *Streptococcus pneumoniae to* antibiotics

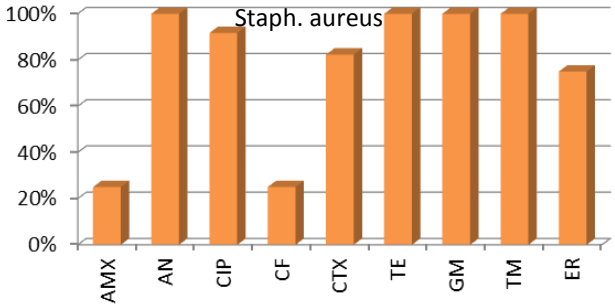


Figure 7. Susceptibility of *Staphylococcus aureus* to antibiotics

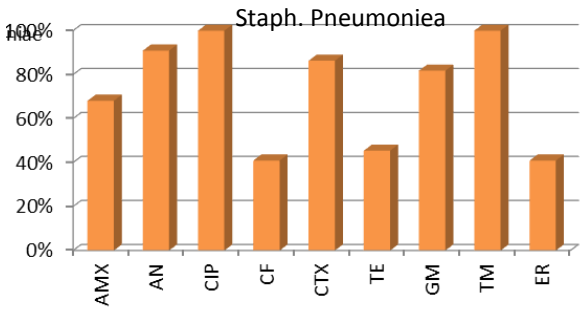


Figure 8: Susceptibility of Streptococcus pyogenes to antibiotics

Discussion

The current study showed a high percentage of Gram-negative bacteria (62.1%) among patients with RTIs. This finding was higher than that reported by Schneeberger *et al*. [12] (8%), while it was lower than that reported by Okesola and Ige [13], (93%). These differences in these results may be due to the same patients were under antimicrobial treatment at the time of specimens collection.

It is clear from this work that the *Klebsiella* species and *Pseudomonas aeruginosa* were the most prevalent among the Gram negative pathogens (36.9% and 16.5% respectively), followed by *Escherichia coli* (5.8%) and *Proteus* species (2.9%). *Streptococcus pneumonia* was the most prevalent among the Gram-positive organisms identified in (21.3%) followed by *Staphylococcus aureus* and *Streptococcus pyogenes* (11.7 % and 4.9% respectively). These results are approximately in agreement with Okesola and Ige, [13] but it was different with the finding reported by other researchers ([14,15]).

High rates of resistance to AMX and CF were demonstrated by all bacteria, while most isolates were found to be highly susceptible to AN, CIP and TM. In contrast, (CTX, TE, GM and ER) were less effect against most of frequently isolates. Antimicrobial resistance by respiratory tract infections has increased worldwide due to excessive use of antimicrobial agents. However, increasing antibiotic resistance in frequently isolated respiratory tract pathogens complicated the selection process of antimicrobial agents [7,8]. *Klebsiella* species being the high resistance to most of antibiotic agents except AN, CIP and TM were the most potent activity against this strain. This finding is different with respect to what was mentioned by most previous studies [13,16]. *Streptococcus pneumoniae* showed moderate to high resistance against CF, TE and ER, while good effect to other antibiotic agents, which were used in this study. These results are approximately in agreement with other research [13]. TE showed the poor efficacy against *Streptococcus pneumonia* (45.4%). This result was higher than that reported by author [17], while it was lower than that reported by other [18].

Pseudomonas aeruginosa isolates showed complete resistance to each of AMX, CF and TE. This finding was in consistent with study of Levy [19],who proved that some strains of *Pseudomonas aeruginosa* were resistant to most every antibiotic now available. *Pseudomonas aeruginosa* also, showed low resistance to GM, CTX, CIP and TM. These results were in disagreement with reported by many other studies [17,20,21]. AN showed the most potent activity against *Pseudomonas aeruginosa* (82.3%). This result was compatible with other reported [20], while lower prevalent of resistant (10%) to this agent was proved by [21].

Based on the findings of our study, we conclude that Klebsiella species and *Streptococcus pneumonia*e can be considered an important etiology agent of respiratory tract infections, having a high rate of drug resistance. AN, CIP and TM were the most effect antibiotics in *vitro* against tested bacteria. Conversely, no or less effect of other antibiotic agents which were used in this study thus should not be considered the drugs of choice in the treatment of patients with RTI in our study.

References

1. Eccles MP, Grimshaw JM, Johnston M, *et al.* Applying psychological theories to evidence-based clinical practice: Identifying factors predictive of managing upper respiratory tract infections without antibiotics". Implement Sci. 2007; 2: 26.
2. US Department of Health and Human Services. Preventing emerging infectious diseases: A strategy for the 21st century. MMWR Morb Mortal Wkly Rep 1998; 47 (No.RR-15). CDC Web site. http://www. cdc. gov /MMWR /pdf/rr/rr4715. pdf. Accessed March 5, 2009.
3. World Health Organization. Drug resistance threatens to reverse medical progress. WHO Web site. http://www.who.int/inf-pr- 2000/en/pr- 2000-41.html. Accessed March 5, 2009.
4. Institute of Medicine. Microbial threats to health: emergence, detection, and response. IOM Web site. http://www.iom.edu/CMS3783/3919/5381/6146.aspx. Accessed March 5, 2009.
5. Lim VKE. Antibiotic resistance and its control in the Far East. Antibiotics Chemotherapy. J Antimicrob Chemothe. 2001 Sep; 5(2): 1-3.
6. Ashworth M, Charlton J, Ballard K, *et al*. Variations in antibiotic prescribing and consultation rates for acute respiratory infection in UK general practices 1995-2000. Br J General Pract. 2005; 55: 603-8.
7. Guthrie R. Community acquired lower respiratory tract infections: etiology and treatment. Chest 2001; 20: 2021-34.
8. Ball P, Baquero F, Cars O, et al. Concensus group on resistance and prescribing in respiratory tract infection. Antibiotic therapy of community respiratory tract infection: strategies for optimal outcome and

minimized resistance emergence. J Antimicrob Chemothe 2002; 49: 31-40.

9. Collee JG, Marr W. Culture of Bacteria. In: Collee JG, Fraser AG, Marmion BP, et al. (eds). Mackie and McCartney Practical Medical Microbiology. 14th ed. Churchill Livingstone; 1996. p. 113-29.
10. Baron EJ, Peterson LR, Finegold SM. Bailey and Scotts. Diagnostic Microbiology, 9th ed, Mosby, 1995; p. 333-52.
11. Bauer AW, Kirby WM, Sherrie JG, et al. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 1996; 45: 493-96.
12. Schneeberger PM, Dorigo ZJW, van DZA, et al. Diagnosis of atypical pathogens in patients hospitalized with community-acquired respiratory infection. Scand J Infect Dis. 2004; 36(4): 269-73.
13. Okesola AO, Ige OM. Trends in Bacterial pathogens of lower Respiratory infections. Indian J Chest Dis Allied Sci. 2007 Sep; 20: 269-72.
14. Ozylimaz ZE, Akan OA, Gulhan M, et al. Major bacteria of community acquired respiratory tract infections in Turkey. Jpn J infect Dis. 2005; 58: 50-2.
15. Liebowitz LD, Slabbert M, Huisamen A. National surveillance programmer on susceptibility patterns of respiratory pathogens in South Africa: amoxifloxacin compared with eight other antimicrobial agents. J Clin Pathol. 2003; 56: 344-47.
16. Quale JM, Landman D, Bradford PA, et al. Molecular epidemiology of a citywide outbreak of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae infection. Clin Infect Dis 2002; 35(7): 834-41.
17. Reynolds R, Potz N, Colman M, et al. Antimicrobial susceptibility of the pathogens of bacteraemia in the UK and Ireland 2001–2002: the BSAC Bacteraemia Resistance Surveillance Programme. J Antimicrob Chemothe. 2004; 53: 1018-32.
18. Shimada K, Nakano K, Igari J, et al. Susceptibilities of bacteria isolated from patients with lower respiratory infectious diseases to antibiotics. Jpn J Antibiot. 2004; 57(3): 213-45.
19. Levy SB. The challenge of antibiotic resistance. Sci Am. 1998; 278: 46-53.
20. Gonlugur U, Bakici MZ, Ozdemir L, et al. Retrospective analysis of antibiotic susceptibility patterns of respiratory isolates of Pseudomonas *aeruginosa* in a Turkish University Hospital. http://www.ann-clin.microb .com. /content2003; /2/1/5.
21. Pitt TL, Sparrow M, Warner M, et al. Survey of resistance of *Pseudomonas aeruginosa* from UK patients with cystic fibrosis to six commonly prescribed antimicrobial agents. Thorax. 2003; 58: 794-6.

E-mail: dr. abdulmunim@yahoo.com
Mobile: 07715194462
Received 31st Jan: 2012: Accepted 12th Sept. 2012.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Protective Role of Vitamin E and/or Methionine against Lead-Induce Changes on Hematological Parameters in Rabbits

Majeda AJ Alqayim *PhD*, **Sadat A Asis** *MSc*

Dept. of Physiology and Pharmacology, College of Veterinary Medicine, Baghdad University

Abstract

Background The importance of lead as a toxic metal and environmental pollutant has long been recognized to human and animal health. In Iraq, lead pollution was documented in Baghdad and in Sulaimaniya city.

Objective To explore the protective role of vitamin E alone or in combination with the amino acid methionine against lead acetate side effects on hematological parameters of adult male rabbits.

Methods Thirty male adult rabbits were divided equally into five groups four of them administered lead acetate for 90 days, as sub-chronic exposure, and the fifth was considered as control. Three of these groups were treated with Vitamin E and/or methionine for 90 days. At the end of experiment, blood and liver samples were collected for either hematological analysis or histopathological examination.

Results Lead caused a significant decrease in lymphocytes and erythrocyte indices; and a significant increase in reticulocytes and neutrophils. At the same time, Vit. E, alone or mixed with methionine, corrected these values to semi normal values.

Conclusion Decreased erythrocyte indices and reticulocytosis that is found in the present study, demonstrates regenerative anemia in rabbits that had administered lead acetate. Vitamin E, alone or mixed with methionine, was efficient in reducing the side effects of Lead on hematological parameters; while, methionine had little or no effect when administered alone against lead which may be attributed to the dose or duration of the treatment.

Keywords Lead; vitamin E; methionine; hematological changes

Introduction

The importance of lead as a toxic metal and environmental pollutant has long been recognized to human and animal health [1,2] in Iraq as well as other parts of the world. Lead pollution was documented, particularly in Baghdad [3].

The mechanism by which lead affects the human body is extremely complex. On an atomic level, lead can induce a wide range of adverse effects in humans depending on the dose and duration of exposure [4,5]. Lead induces the production of reactive oxygen species (ROS) that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems [6]. The toxic lead effects on the hematologic system [5,7], thus, cause a slowly developing hypochromic normocytic or microcytic anemia [8].

Vitamin E is naturally occurring antioxidants that play important roles in health by inactivating harmful free radicals produced through normal cellular activity and from various stressors [9]. The protective mechanism of vitamin E against lead toxicity could be attributed to its antioxidant property or its location in the cell membrane and its ability to stabilize membrane

by interacting with unsaturated fatty acid chain [10-13].

The essential amino acid methionine shows antioxidant properties in various models of oxidative stress [14]. The mechanisms responsible for the observed methionine-induced cytoprotection are not yet fully understood. The free radical scavenging activities of methionine can only partially be explained by the chelating function of its sulfur moiety [15]. There has been increased interest among researchers to use antioxidant nutrients and medicinal plants with antioxidant activity for protection against lead toxicity [16].

In an effort to decrease the severity of lead exposure side effects on hematological parameters, the present study was designed to explore the protective role of vitamin E alone or in combination with the amino acid methionine against lead acetate side effects on hematological parameters of adult male rabbit for 90 days, as sub chronic exposure.

Methods

Experimental design:

Thirty adult male rabbits of local breed were divided randomly into equal five groups, each group of six animals treated as follow: **Group 1**: Control group that were orally administered with tap water daily. **Group 2**: Orally administered with 2.5 mg/Kg B.W. lead acetate (250 mg/100 ml) dissolved in tap water daily. **Group 3**: Orally administered with 2.5 mg/Kg B.W. lead acetate (250 mg/100 ml) dissolved in tap water + 100 mg/Kg B.W. methionine dissolved in 2 ml of tap water daily. **Group 4**: Orally administered with 2.5 mg/Kg B.W. lead acetate (250 mg/100 ml) dissolved in tap water + 100 IU/Kg B.W. vitamin E daily. **Group 5**: Orally administered with 2.5 mg/Kg B.W. lead acetate (250 mg/100 ml) dissolved in tap water + 100 IU/Kg B.W. vitamin E+ 100 mg/Kg B.W. methionine \dissolved in 2 ml of tap water daily. The experiment lasts for 90 days, meanwhile, animals were observed daily for their behavior and health performance. At the end of the experiment all the experimental animals were sacrificed and 6-8 ml of blood samples was collected into EDTA tube for immediate hematological measurements and reticulocytes count.

Hematological and biochemical changes:

Total red blood cells (RBC) count, hemoglobin (Hb) concentration, packed cell volume % (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), Platelet count, total and differential WBC as well as lymphocyte and neutrophil count were accomplished by using heamatological analyzer (Hycel Hematology analyzer, version B, ver2.5x.) at Damawand general laboratory for processing in both hematology and biochemistry/ Sulaimaniya city. Reticulocytes were counted in 1000 cells of the total RBCs and expressed as percentage.

Serum iron: Serum iron concentration was enzymatically measured using enzymatic assay kit (Biolabo SA, Maizy-France).

Serum Ferritin: was measured by Ferritin enzyme immunoassay test Kit (Linear chemicals, Barcelona-Spain) using DANA 3200 ELISA Reader.

Histopathological changes:

Liver tissues preserved in 10% neutral formalin buffer solution. After fixation, the tissue was trimmed and the specimens were washed with saline for (1-2 hrs) and transferred to following steps: **1. Dehydration:** specimens were passed through ascending grades of ethanol alcohol (70%, 80%, 90%, 100%) for 1 hour in each concentration. **2. Clearing:** two solutions of xylol commonly used for clearing. The specimens rested 1 hour in each step. **3. Impregnation with Paraffin wax**, **4. Blocking**, **5. Sectioning** and **6. Staining with Prussian blue stain** for hemosidrine.

Statistical analysis

Data are shown as the mean ± stander error (SE). When a significant interaction between major factors was identified by ANOVA. SPSS version 11.5, Duncan's new multiple range test was used post-ANOVA to identify significant differences between mean values at probability level of (P< 0.05) taken as significant.

Results

Observations of the experimental animals during the present study revealed that rabbits treated with lead acetate suffered from mild anorexia, easy hair shading especially during handling and dullness. Also these signs were observed in group 3, 4 and 5 especially at the end of the experiment in comparison with the control group. The 90 days duration of the experiment indicates the sub-chronic exposure for experimental animals to lead acetate.

Hematological and Biochemical changes:

The protective role of the oral administration of vitamin E and/or methionine against side effects of lead on the hematological parameters of experimental animals, i.e., levels of RBCs, Hb, PCV%, MCV, MCH, MCHC and reticulocyte % in adult male rabbits for 90 days, were shown in table 1.

Results revealed that Lead exposure led to non-significant reduction of Hb concentration, PCV%, and RBCs ($\times 10^6$/µl) count; significant reductions of MCH, MCV, MCHC in comparison with control group (69.130 ± 0.85 pg, 21.05 ± 0.25 fl, 30.48 ± 0.27 g/dl; versus 12.05 ± 0.51 pg, 36.61 ± 1.13 fl and 5.02 ± 0.09 g/dl; respectively) ($P< 0.05$); and significant elevation of reticulocyte % (1.60 ± 0.25%). The same results were observed from rabbits administered methionine against lead.

Administration of vitamin E alone against lead for 90 days has led to non-significant reduction of Hb, PCV and total RBCs (11.06 ± 0.46 g/dl, 34.34 ± 1.6 %, 4.85 ± 0.25 $\times 10^6$RBCs/µl; respectively); significant elevation of MCV, MCH and MCHC (71.11 ± 1.4 pg, 22.90 ± 0.54 fl and 32.25 ± 0.27 g/dl); and significant decrease in reticulocyte % (3.61±0.31%) in comparison with group administered lead.

On the other hand administration of methionine mixed with vit. E against Lead had succeeded to return back Hb, PCV, RBCs, MCHC, and reticulocyte % to the semi normal values, (12.16 ± 0.50 g/dl, 37.71 ± 1.75%, 5.54 ± 0.27 $\times 10^6$ RBCs/µl, 32.30 ± 0.36 g/dl, 3.45 ± 0.25 %; respectively (Table 1).

Table 1. Lead Induced Changes on Hb, PCV, RBC Count and Indices and Reticulocytes Count

Parameter	Rabbit Groups				
	G1 (N = 6)	G2 (N = 6)	G3 (N = 6)	G4 (N = 6)	G5 (N = 6)
Hb (g/dl)	12.050±0.517^{a}	11.017±0.485^{a}	11.450±0.287^{a}	11.067±0.467^{a}	12.167±0.508^{a}
PCV (%)	36.617±1.138^{a}	36.167±1.427^{a}	36.833±0.610^{a}	34.433±1.631^{a}	37.717±1.751^{a}
RBCs ($\times 10^6$/µl)	5.025±0.099^{ab}	5.252±0.251^{ab}	5.350±0.127^{ab}	4.850±0.250^{b}	5.548±0.270^{a}
MCV (fl)	72.850±1.058^{a}	69.133±0.852^{b}	69.767±0.426^{b}	71.117±1.455^{ab}	^{A}68.083±0.812^{b}
MCH (pg)	23.967±0.775^{a}	21.050±0.275^{c}	21.550±0.148^{bc}	22.900±0.542^{ab}	22.000±0.284^{bc}
MCHC (g/dl)	32.867±0.656^{a}	30.483±0.387^{b}	30.833±0.275^{b}	32.250±0.279^{a}	32.300±0.361^{a}
Reticulocytes (%)	1.602±0.252^{d}	7.793±0.351^{a}	5.337±0.292^{b}	3.617±0.315^{c}	3.458±0.250^{c}

Different small letters horizontally denotes significant Differences between groups at P < 0.05

Table 2 illustrated analysis of the data obtained from the present study, showing the non significant changes of total WBC count between different groups. On the other hand, after administration of lead there was a significant elevation of Neutrophils which correlated with a significant reduction of Lymphocytes ($P< 0.05$). These abnormal changes were corrected in groups treated with Vit. E and/or methionine. After 90 days of lead administration, serum iron increased significantly in the four studied groups (G2, G3, G4 and G5): (299.45 ± 6.56, 211.5 ± 7.36, 226.90 ± 10.66 and 270 ± 8.69 µg/dl; respectively) in comparison with control group (193.25 ± 6.56 µg/dl). Marginal reduction of Ferritin concentration after lead administration was observed in group 1 (0.15 ± 0.03 µg/dl) in comparison with control (2.200 ± 0.15 µg/dl); however, this reduction was corrected by the

administration of vit.E and/or methionine as shown in the other groups (Table 3).

Table 2. Lead-Induced Change on WBC, Lymphocyte, neutrophil, and Platelets and control group

Groups	WBC ($\times10^3/\mu l$)	Lymphocyte ($\times10^3/\mu l$)	Neutrophil ($\times10^3/\mu l$)	Platelets ($\times10^3/\mu l$)
G1	6.550 ± 0.161^{a}	3.500 ± 0.141^{a}	1.833 ± 0.276^{a}	188.667 ± 1.745^{b}
G2	6.083 ± 0.156^{a}	2.417 ± 0.098^{b}	1.867 ± 0.161^{a}	225.333 ± 12.478^{a}
G3	6.467 ± 0.338^{a}	3.833 ± 0.343^{a}	1.367 ± 0.042^{ab}	190.833 ± 4.045^{b}
G4	6.383 ± 0.421^{a}	4.100 ± 0.545^{a}	1.117 ± 0.172^{b}	202.000 ± 10.132^{b}
G5	6.367 ± 0.288^{a}	3.800 ± 0.363^{a}	1.133 ± 0.126^{b}	181.833 ± 5.558^{b}

Different small letters horizontally denotes significant Differences between groups at $P < 0.05$

Table 3. Lead Induced Changes on Iron and Ferritin and in the control group

Groups	Iron (µg/dl)	Ferritin (µg/dl)
G1	193.250 ± 6.564^{b}	2.200 ± 0.157^{a}
G2	299.450 ± 15.178^{a}	0.150 ± 0.034^{c}
G3	211.050 ± 7.362^{ab}	1.367 ± 0.076^{b}
G4	226.900 ± 10.662^{ab}	1.567 ± 0.143^{b}
G5	270.333 ± 8.693^{a}	1.667 ± 0.115^{b}

Different small letters horizontally denotes significant Differences between groups at $P < 0.05$

Histopathological changes:

Examination of liver histopathological sections from rabbits received lead revealed a heavy deposition of iron blue-stained hemosidrine in periportal hepatocellular kupffer cells and portal tracts macrophages; while iron deposition was clear in the middle and periportal zone (Figure 1), when compare this picture with liver section of control rabbits (Figure 2).

This heavy deposition of iron was reduced into mild one in pan lobular hepatocyte, kupffer cells and portal tract macrophages in rabbits administering vit.E and/or methionine along with lead after 90 days of the experiment (Figures 3, 4 and 5).

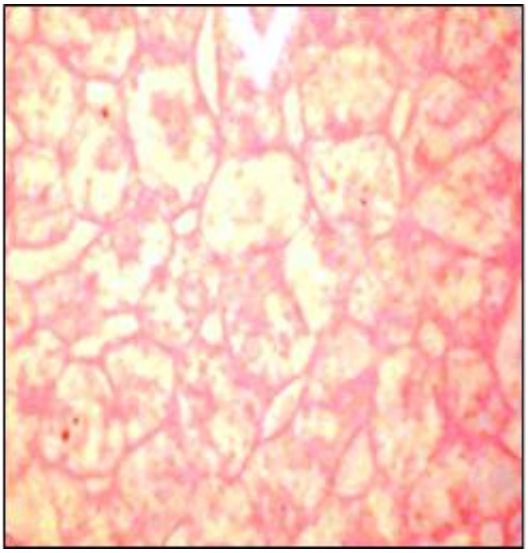


Fig. 1. Liver section form control rabbits, stained with Prussian blue showing no iron deposition (400×).

Discussion

In the present study, the role of vitamin E and/or methionine on hematological changes in lead-administered male rabbits for 90 days was investigated. The dose and the sub-chronic exposure of adult male rabbits to lead acetate were designed to produce cumulative effects on hematological system. The main findings of the present study regarding the changes in RBCs count, Hb concentration and Erythrocytes indices are showed in table 1.

The results referred to the type of anemia induced by lead, the most common form was microcytic-hypochromic. The significant reduction of MCH and MCHC caused by lead for

90 days might be due to reduced Hb production. Lead inhibits ferrochelatase, the enzyme that catalyzes the incorporation of iron into the porphyrin ring leading to reduced iron incorporation in Hb [10,17], and might result in reduced oxygen transfer by RBCs. This was compensated for by increased number of these cells with smaller volume [18], and was confirmed by the significant reduction of MCH and MCHC. This is in agreement with observations in this study regarding RBC and reticulocyte count and MCV, suggesting that bone marrow could overcome lead toxicity because of sub-chronic exposure which was not at high dose, but suppressed the production of Hb [19] unless it would indicate impaired marrow function or lack of erythropoietin stimulus [20,21].

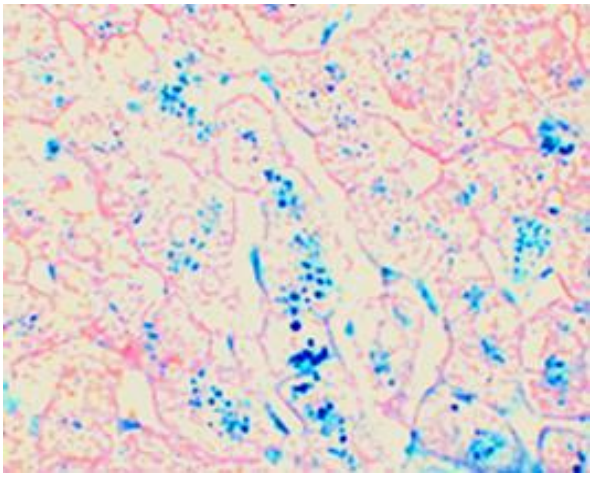


Fig. 2. Liver section from rabbits administered lead was stained with Prussian blue, showing heavy deposition of iron in periportal hepatocelluler, kupffer cells and portal tracts macrophages in middle and periportal zone (400×)

On the other hand, results of this study demonstrated that vitamin E and methionine played an important role in improvement of Hb biosynthesis, RBCs production, which was confirmed by the significant elevation of total RBCs count, Hb, and MCHC. This protective mechanism of vitamin E and methionine against lead adverse effects could be explained by the direct effect of vitamin E on improving the morphology of RBCs by its ability to stabilize their membrane [22], and the free radical scavenger activities of methionine residues as powerful sulfur-containing endogenous antioxidant V [17]. In addition, methionine is a very important nutrient during the Hb biosynthesis and RBC production in bone marrow.

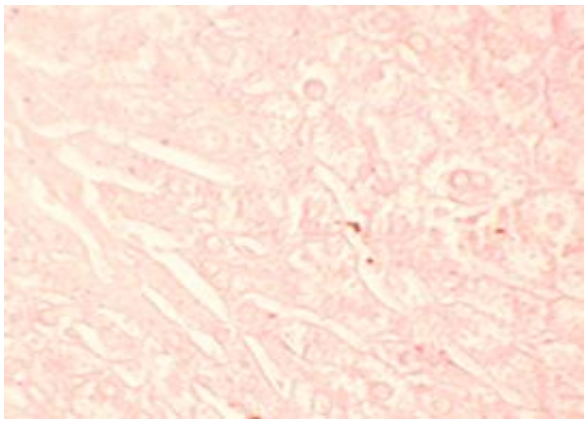


Fig. 3. Liver section form rabbits received lead+methionine stained with Prussian blue, showing a heavy deposition of iron in periportal hepato-celluler, kupffer cells and portal tracts macrophages (400×).

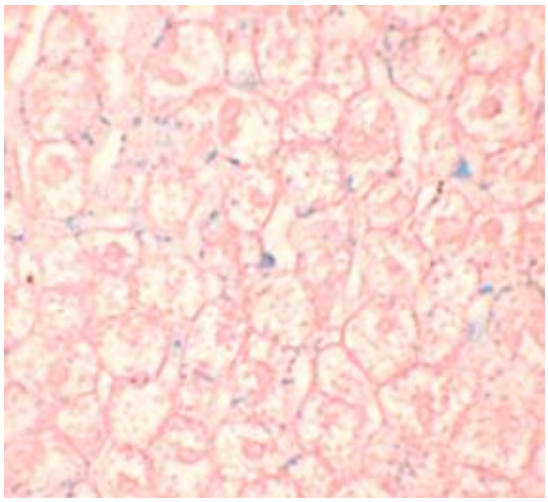


Fig. 4. Liver section form rabbits received lead+vit.E stained with Prussian blue, showing mild panlobuler hepatocyte and kupffer cell iron deposition, iron present in portal tract macrophage (400×).

The increased platelet count found in the present study (Table 2), refer to other

hematological disturbances induced by lead administration, which could be attributed to either hyper stimulation of thrombopoiesis in the bone marrow as a response to peripheral loss of platelets caused by increased platelet aggregation and adhesion [4,10], or as response to decreased Hb concentration caused by lead acetate administration. This result is in agreement with the results of other researchers who found that accelerated platelet aggregation when there is lowered Hb concentration [23]. On the other hand, results of the present study reflected the protective role of methionine and vitamin E against changes induced by lead administration. There were no changes in platelet count in rabbits received vit.E and methionine (Table 2). This protective effect could be explained by the direct action of vit.E and methionine on reducing the aggregation and adhesion of platelets in peripheral blood vessels, keeping normal blood level of thrombocytes in rabbits. Further, Vit.E supplementation also proved to be effective in significantly decreasing the already raised values of platelets [24].

Although results in table 2 indicated a significant decrease in lymphocyte count, which was correlated with a significant increase in neutrophil count; however, the mean total WBC count was relatively unchanged. Despite that, not all researchers agree on the effects of lead on total and differential WBCs [18,25]. The damaging effects of the reactive oxygen species on living systems as a result of lead intoxication required more phagocytic functions faced by increase of neutrophils. These damaging effects were prevented by the protective action of vitamin E, thus, neutrophil count returned back to semi normal values in rabbits administered vit. E alone or mixed with methionine reflecting either the antioxidant property and/or the protective role of vit. E on bone marrow [12,22,24,25].

After 90 days of the experiment, results revealed a significant increase in serum iron faced by the significant decrease in serum ferritin in rabbits administering lead acetate as compared with the control and other groups (table-3). Lead inhibits ferrochelatase enzyme that catalyzes the incorporation of iron into the porphyrin ring causing reduced iron incorporation in Hb and elevation of serum iron [10,27]. Moreover, competition between lead and iron, since lead is similar to some other ions, enables lead to take the place of iron in heme molecule of Hb, leading to increased serum iron [28]. The decreased serum ferritin may be due to the accumulation of the ferritin in the form of the hemosiderin, resulting in hemosiderosis and hemochromatosis, both of which are associated with excessive levels of serum iron and % saturation of the transferrin with iron and decreased serum ferritin levels [29]. The hemosiderosis observed in the liver of rats administering lead acetate (Figure 2) may be due to increased serum iron released from the heme liberated from the premature hemolysed RBCs. This excess iron cannot be utilized for Hb synthesis, because of the toxic effects of lead. Increased intracellular iron, increased ferritin expression, deposed as hemosiderin in liver [5,20,30]. The severity of these findings were decreased in groups administered vit. E alone or mixed with methionine (Figures 3, 4 and 5), reflecting again the protective role of vit. E against lead toxic effects. These finding could be explained that prevention of the preliminary RBCs elimination decreases phagosytosis and consequently decreases iron deposition, as well as the antioxidant effect of vit. E in reducing liver parenchymal damage and ferritin release to the circulation. L-methionine produces an increase in ferritin protein expression, thus activation of endogenous iron sequestration could be an important mechanism by which methionine increases the cellular defense against oxidative injury [11,17].

In conclusion, decreased MCV, MCH, and MCHC and reticulocytosis demonstrate regenerative anemia in rabbits administering lead acetate; since reticulocytes are still synthesizing Hb and bone marrow tries to compensate these changes by an increase in the reticulocytes release while lead causes increased iron in circulation and intra cellular deposition, vit. E alone or mixed

with methionine, prevent the occurrence of these changes. Results of this study, also, provide direct evidence that combined treatment of Pb-exposed animals with vit.E, whether alone or mixed with methionine has a protective role represented by the significant improvement of MCV, MCH and MCHC; with reticulocyte production within normal values. Therefore, it can be concluded that vitamin E can effectively mitigate most of the adverse effects induced by lead exposure. However, further studies are necessary to determine the mechanisms involved in the synergistic role of dietary nutrients against lead adverse effects.

References

1. Gambrell J. Lead poisoning outbreak causes emergency In Nigeria. Huffpost Social News. The Internet Newspaper—Personalized New York, NY· huffingtonpost.com. 2011 Febr, 22.
2. Birgit P, Poppenga RH. Lead and zinc intoxication in companion birds. Compendiumvet.com. 2009 Jan; 31(1): E1-E12.
3. Al-Bassam A. Survey of lead pollution In Baghdad: Water, air and soil pollution, 1983; 19(1): 3-14.
4. Mugahi MN, Heidari Z, Sagheb AHM, et al. effects of chronic lead acetate intoxication on blood indices of male adult rat. DARU. 2003; 11(4): 147-51.
5. Klaassen CD. Casarett and Doulls Toxicology, the basic science of poisons. 7th ed. New York: Mcgraw-Hill Medical Publishing Division; 2008. p. 137-138, 146-150, 464, 944-945, 1158,
6. Çaylak E, Haldfeoglu D, Aydin S, et al. The effects of sulfur-containing compounds on total antioxidant capacity levels of liver, kidney and brain in lead-exposed rats. Turkiye Klinikleri J Med Sci. 2007; 27: 823-8.
7. Moussa SA, Bashandy SA. Biophysical and biochemical changes in the blood of rats exposed to lead toxicity. Romani J Biophys. 2008; 18(2): 123-33.
8. Kosnett MJ. Lead. In: Ford MD, Delaney KA, Ling LJ, et al. (eds.) Clinical toxicology. 1st ed. Philadelphia: W. B. Saunders Company; 2007. Chapter 89.
9. Hassan NS, Awad SM. Reverse effect of vitamin e on oxidative stress, derivatives and conductivity changes of hemoglobin induced by exposure to cadmium. J Appl Sci Res. 2007; 3(6): 437-43.
10. Casas JS, Sordo J. Lead chemistry, analytical aspects, environmental impact and health effects. 1st ed. London: Elsevier BV; 2006. p. 17, 32, 169, 174-80, 185, 200, 214.
11. Gaurav D, Preet S, Dua KK. chronic cadmium toxicity in rats: treatment with combined administration of vitamins, amino acids, antioxidants and essential metals. J Food Drug Anal. 2010; 18,(6): 464-70.
12. Flora SJ, Mittal M, Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J Med Res. 2008; 128(4): 501-23.
13. Xiang HL, Dadin F, Nabil HL, et al. δ-tocotrienol protects mouse and human hematopoietic progenitors from γ-irradiation through extracellular signal-regulated kinase/mammalian target of rapamycin signaling Haematologica. 2010; 95: 3324-32.
14. Erdmann K, Grosser N, Schröder Hl. Methionine reduces oxidant stress in endothelial cells: role of heme oxygenase-1, ferritin, and nitric oxide. AAPS J. 2005; 7(1): 195-200.
15. Carson FL. Histotechnology. American Society for Clinical Pathology, Chicago; 1997.
16. El-Nekeety AA, El-Kady AA, Soliman MS, et al. Protective effects of aquilegia vulgaris (l) against lead acetate – induced oxidative stress in rats. Food Chem Toxicol. 2009; 47: 2209-15.
17. Ozsoy SY, Ozsoy B, Ozyildiz Z, et al. Protective effect of l-carnitine on experimental lead toxicity in rats: a clinical, histopathological and immunohistochemical study. Biotech Histochem. 2011; 86(6): 436-43.
18. Patil AJ, Bhagwat VR, Patil JA, et al. Effect of lead (pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (bmw) of western maharashtra (india) with reference to heme biosynthesis. Internat J Environ Res Public Health. 2006; 3(4): 329-37.
19. Golalipour MJ, Roshandel D, Roshandel G, et al. Effect of lead intoxication and d-penicillamine treatment on hematological indices in rats. Int J Morphol. 2007; 25(4): 717-22.
20. Nagaraja H, Tan J, Srikumar C, et al. Protective effects of alpha-polic acid against lead acetate – inducwd oxidative stress in the bone marrow of rats. Internat J Pharmacol. 2011; 7(2): 217-27.
21. Hoffbrand AV, Catovsky D, Tuddenham EGD. Postgraduate haematology. 5th ed. Massachusetts: Blackwell Publishing Ltd; 2005. p. 27, 34-35, 40.
22. Kalia K, Flora SJS. Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning. J Occup Health. 2005; 47(1): 1-21.
23. Scharbert G, Wetzel L, Berlinger L, et al. Effect of anemia on coagulation and platelet function: a whole blood in vitro study. Crit Care. 2011; 15(Suppl 1): P445.
24. Garg DP, Bansal AK, Malhotra A, et al. Methomyl induced hematological and biochemical alterations – protection by vitamin E. Pesticide Biochem Physiol. 2009 Mar; 93(3): 127-32.
25. Bravo Y, Quiroz Y, Ferrebuz A, et al. Mycophenolate mofetil administration reduces renal inflammation, oxidative stress, and arterial pressure in rats with lead-

induced hypertension. Am J Physiol Renal Physiol. 2007; 293(2): 616-23.

26. Sajitha GR, Jose R, Andrews A, et al. Garlic oil and vitamin E prevent the adverse effects of lead acetate and ethanol separately as well as in combination in the drinking water of rats. Indian J Clin Biochem Jul. 2010; 25(3): 280-8.

27. Harvey JW. The Erythrocyte: Physiology, Metabolism, and Biochemical Disorders. Chapter Seventh. In: Kaneko JJ, Harvey JW, Bruss ML (eds.): Clinical biochemistry of domestic animals. 5th ed. Academic Press; 1997. p. 157-233.

28. Blake S. Vitamins and minerals demystified. New York: Mcgraw-Hill Companies, Inc.; 2008. p. 111-121, 275.

29. Arneson W, Brickell J. Clinical Chemistry: A laboratory perspective. Philadelphia: FA Davis Company; 2007. p. 183-8.

30. Nordberg GF, Fowler BA, Nordberg M, et al. The Handbook on the toxicology of metals. 3rd ed. Copenhagen: Academic Press, Inc.; 2005. p.119, 269, 600-43.

Corresponded to Majida AJ Alqayim

E-mail: jaafer59@yahoo.com

Received 15th Apr. 2012: Accepted 10th Mar. 2013.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Is Pediatric Appendicitis Score Sufficient to Make the Diagnosis of Acute Appendicitis Among Children?

Ali F Abdullah[1] *MBChB FICMS*, **Firas M Katoof**[1] *MBChB FICMS*, **Basim Sh Ahmed**[2] *MBChB FICMS*

[1]Dept. of Surgery, [2]Dept. of Pathology, College of Medicine, Al-Mustansyriah University, Baghdad

Abstract

Background Children with acute abdominal pain represent most of the admitted cases to the pediatric surgical department, one third of these cases are acute appendicitis. Early diagnosis of "no appendicitis" or "appendicitis" on the basis of pediatric appendicitis score could potentially save emergency department's time and resource use and could avoid time cost and risks for further evaluation.

Objective Evaluation of Samuel scoring system in diagnosing children with acute appendicitis and their need for surgery.

Methods One hundred and twelve patients aged between 5 to 15 years who presented with abdominal pain suggestive of acute appendicitis were studied. A complete data from patients were analyzed by using Samuel score. The clinical findings used by previously mentioned scoring system were analyzed to determine reliability of pediatric appendicitis score (PAS). The Final diagnosis was determined by histopathological report for patients' undergone appendicectomy.

Results The mean (median, SD) score for children with acute appendicitis and non-acute appendicitis were 4.9 (5, 1.8) and 4.6 (5, 1.7) respectively. No variable (of the known signs and symptoms regarded as pathognomonic for acute appendicitis) shows a significant value in the diagnosis of acute appendicitis. A PAS of ≥ 4 had a sensitivity, specificity, Positive predictive value (PPV), and Negative predictive value (NPV) of 0.78, 0.27, 0.87, and 0.16 respectively.

Conclusion The diagnosis of acute appendicitis and the need for surgery is still a matter of clinical judgment which can be built with practice, and although the PAS could provide useful diagnostic information in children with suspected acute appendicitis, it cannot be used as sole method for determining the need for surgery.

Key words Pediatric Appendicitis Score (PAS), acute appendicitis, appendicectomy.

Introduction

Children with acute abdominal pain represent most of the admitted cases to the pediatric surgical department; one third of these cases being acute appendicitis [(1)]. Time and patience are required to evaluate child with acute abdominal pain, morbidity result from late diagnosis or negative appendicectomy. Definitive diagnosis of acute appendicitis is made in only 50-70% of children at the time of initial assessment [(2)]. This reflects the proportion of appendices that are normal on histological studies and negative appendicectomy rate of 10-30% [(3-5)]. CT scan had been used to decrease the rate of negative appendicectomy, but this carries a significant risk as a result of increased exposure to ionizing radiation and may result in increased health care costs [(6-8)].

The "MANTRALS" score was proposed by Alvarado in 1986 as a method to predict acute appendicitis in adult [9], and a lot of modified scores had been used to predict acute appendicitis in children. Recently, Samuel from England published a simple pediatric appendicitis score (PAS); in 2002, on the basis of a cohort of children 4 to 15 years old. The PAS ranges from 0 to 10 [10]. Early diagnosis of "no appendicitis" or "appendicitis" on the basis of PAS potentially could decrease emergency department time and resource use and could avoid time, cost, and risks for further evaluation [11-14].

The intention of our study is to evaluation Samuel scoring system in diagnosing children with acute appendicitis and their need for surgery.

Methods

A prospective observational study was conducted from January 2012 to October 2012 at Al-Yarmook Teaching Hospital and Central Teaching Hospital for Pediatrics in Baghdad. The study included 112 patients aged between 5 to 15 years, who presented with abdominal pain suggestive of acute appendicitis. A complete data from patients were analyzed by using Samuel score (Table 1).

Table 1. Samuel Score system

Variables		Score point value
Anorexia		1
Nausea/vomiting		1
Right lower quadrant tenderness		2
Cough/hopping/percussion/tenderness in the right lower quadrant		2
Migration of pain		1
Elevation in temperature ("Pyrexia,"≥37.3°C)		1
Leukocytosis ≥10 000 cells/mm^3		1
Differential WBC with 75% polymorphonuclear cells or *ANC ≥ 7500 cell/mm^3		1
Score system	not appendicitis with recommendation of observation	≤5
	appendicitis with recommendation of surgery	≥6

*ANC: absolute neutrophil count

A written informed consent was taken from parents of patients below 7years while informed agreement was obtained from children older than this age. Patients who had appendicular mass with periappendiceal abscess, history of previous abdominal surgery (including Appendicectomy), chronic medical illness, nonverbal children & had previous abdominal radiological imaging (within previous 2 weeks) were excluded from the study. Two independent evaluations of clinical findings used by previously mentioned scoring system were done to determine inter observer reliability; the period between the two assessments was 15 minutes to eliminate changes in the patient's condition.

Final diagnosis was determined by histopathological report for patients undergone appendicectomy. Statistical analyses for all data obtained in this study were carried out using Pearson Chi-square test at 0.05 level of significance and Student-t-test.

Results

One hundred and twelve patients had been recruited to the current study that fulfilled the inclusion and exclusion criteria with their age ranging from 5-15 years. Ninety seven patients (86.6%) had pathologically proven acute appendicitis, while 15 patients (13.4%) had no acute appendicitis according to the

histopathological results. The age and sex distributions were illustrated in table 2. The mean (median, SD) score for children with acute appendicitis and non-acute appendicitis were 4.9 (5, 1.8) and 4.6 (5, 1.7) respectively. No studied variable (of the known signs and symptoms) shows a significant value in the diagnosis of acute appendicitis (Table 3).

Table 2. The demographic characteristics of patients included in the study

Variables	Appendicitis patients N = 97	Non Appendicitis patients N = 15
PAS mean ± S.D (Range)	5 ± 1.9 (1-9)	4.7 ± 1.8 (1-7)
Nausea and Vomiting	70 (72%)	11 (73%)
Anorexia	61 (63%)	11 (73%)
Migratory Pain	49 (50.5%)	9 (60%)
RIF tenderness on palpation	59 (61%)	8 (53%)
RIF tenderness on Coughing/hopping/percussion	47 (48%)	5 (33%)
Fever ≥ 37.3^{o}C	50 (51%)	7 (47%)
Leukocytosis WBCs. ≥ 10000 cell/mm^{3}	55 (57%)	9 (60%)

RIF = right iliac fossa

To establish a cutoff point for the diagnosis of acute appendicitis, a score of 4 showed that 76 patients with acute appendicitis undergone appendectomy (78.3%), while 21 patients (21.7%) were sent home (they have histopathologically confirmed acute appendicitis) as an end result. On the other hand, 11 patients (73.7%) who had negative histopathological results undergone appendectomy (Table 4). These results were obtained when applying ROC curve (Fig. 1) which revealed an area under the curve of 0.542 (95% confidence interval [CI], 0.393-0.691).

Table 3. The value of variables with regards to the histopathological results

Variables		Total		Histo-pathologically positive	
		No.	%	No.	%
Age (years)	5 – 9	44	39.3	39	88.6
	≥ 10	68	60.7	56	86.2
Gender	Male	66	58.9	60	90.9
	Female	46	41.1	37	80.4

Table 4. The PAS according to histopathological findings

PAS score	Histopathologically positive		Histopathologically negative	
	No	%	No	%
1	2	2.1	1	6.7
2	12	12.4	1	6.7
3	7	7.2	2	13.4
4	15	15.4	2	13.4
5	21	21.6	3	20.0
6	17	17.5	4	26.6
7	16	16.5	2	13.4
8	5	5.2	-	-
9	2	2.1	-	-
Total	97	100%	15	100%

χ2=3.795; d.f.=8; P value=0.875 (Not significant)

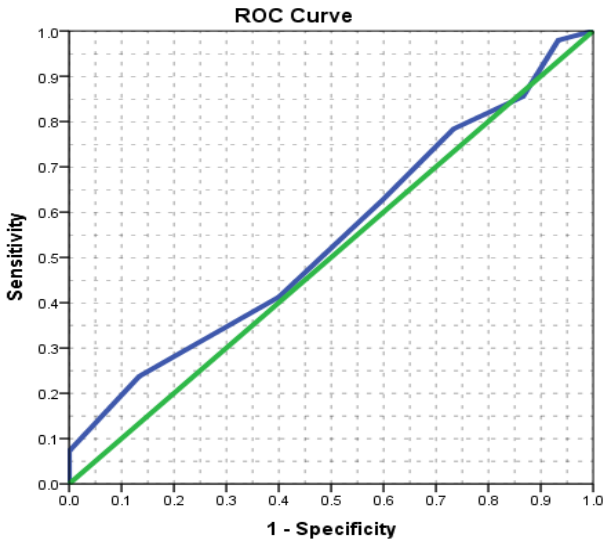


Fig. 1. The ROC curve for PAS and the area under the curve results

Discussion

The PAS developed by Samuel in 2002 using symptoms, signs, and laboratory findings, the score range from 0-10, and the cutoff value for the appendicectomy was equal or greater than 6. From that date a few studies had been conducted and they show different result. In our study we exclude the ANC from the score due to laboratory limitation so our score range from 0-9.

In this study there was no significance for each variable in the diagnosis of acute appendicitis, and there was no identified significant cutoff value for the indication of appendicectomy. These results are due to many limitations which consist of the sample evaluated for possible acute appendicitis, as determined by pediatric emergency physician, which differs from other study sample. We should keep in mind that symptoms, signs, and labratory results are affected by the time of presentation and duration of symptoms; so, any comparison should account for it [15]. Ultimately the value of scoring depends on clinicians' experience in assessing children, and therefore, always involves some subjectivity and interpretation. The experience of clinicians and their individual threshold to declare the presence of signs will always allow variability [15].

In this study, we found that the best cutoff value as an indication for appendicectomy is equal or greater than 4, which had a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 0.78, 0.27, 0.87, and 0.16 respectively. In other studies the cutoff value was different than the original one by Samuel [10]. In Schneider *et al* [15] they found that the same cutoff score of 6 or greater had a PPV of 54%, a sensitivity of 82% and specificity of 65%; while, in Bhatt *et al* [16] found a sensitivity of 92.8%, specificity of 69.3%. In contrast Goldman et al [17] found that a PAS of 7 or greater (rather than 6) gave a sensitivity of 94%, and a specificity of 98%. Katherine *et al* [18] found a score of 6 had a sensitivity of 88.4%, a specificity of 50%, a PPV of 67%, and NPV of 97%.

In comparing the appendicitis group from non-appendicitis group (according to histopathological results), we found that the PAS mean ± SD (range) were 5 ± 1.9 (1-9), 4.7 ± 1.8 (1-7), had no significant value ($P > 0.05$). The area under the ROC curve was 0.542 (95% CI, 0.393-0.691) and it was not significant.

In conclusion, the diagnosis of acute appendicitis and the need for surgery is still a matter of clinical judgment which can be built with practice, and although the PAS could provide a useful diagnostic information in children with suspected acute appendicitis, it cannot be used as a sole method for determining the need for surgery.

References

1. Ludwig S, Fleisher GR, Henretig FM, et al. Pediatric emergency medicine. Philadelphia (Pa): Williams & Wilkins; 2006. p. 1607.
2. Balthazar EJ. Appendicitis: Prospective evaluation with high resolution CT. Radiology. 1991; 180: 21-4.
3. Surana R, O'Donnell B, Puri P. Appendicitis diagnosed following active observation does not increase morbidity in children. Pediatr Surg Int. 1995; 10: 76-8.
4. Pearl RH, Hale DA, Molloy M, et al. Pediatric appendectomy. J Pediatr Surg. 1995; 30: 173-81.
5. Douglas CD, Macpherson NE, Davidson PM, et al. Randomized controlled trial of ultrasonography in diagnosis of acute appendicitis, incorporating the Alvarado score. Br Med J. 2000; 321: 919-22.

6. Lee SL, Walsh AJ, Ho HS. Computed tomography and ultrasonography do not improve and may delay the diagnosis and treatment of acute appendicitis. Arch Surg. 2001; 136: 556-62.
7. Safran DB, Pilati D, Folz E, et al. Is appendiceal CT scan overused for evaluating patients with right lower quadrant pain? Am J Emerg Med. 2001; 19: 199-203.
8. Brenner D, Elliston C, Hall E, et al. Estimated risks of radiation-induced fatal cancer from pediatric CT. AJR Am J Roentgenol. 2001; 176: 289-96.
9. Alvarado A. A practical score for the early diagnosis of acute appendicitis. Ann Emerg Med. 1986; 15: 557-64.
10. Samuel M. Pediatric appendicitis score. J Pediatr Surg. 2002; 37(6): 872-81.
11. Curran TJ, Muenchow SK. The treatment of complicated appendicitis in children using peritoneal drainage: results from a public hospital. J Pediatr Surg. 1993; 28: 204-8.
12. McAnena OJ, Austin O, O'Connell PR, et al. Laparoscopic versus open appendicectomy: a prospective evaluation. Br J Surg. 1992; 179: 818-20.
13. McLean AD, Stonebridge PA, Bradbury AW, et al. Time of presentation, time of operation, and unnecessary appendicectomy. BMJ. 1993; 306: 307.
14. Surana R, O'Donnell B, Puri P. Appendicitis diagnosed following active observation does not increase morbidity in children. Pediatr Surg Int. 1995; 10: 76-8.
15. Schneider C, Kharbanda A, Bachur R. Evaluating appendicitis scoring systems using a prospective pediatric cohort. Ann Emerg Med 2007; 49: 778-84.
16. Bhatt M, Joseph L, Ducharme FM, et al. Prospective validation of the pediatric appendicitis score in a Canadian Pediatric Emergency Department. Acad Emerg Med. 2009; 16: 591-6.
17. Goldman RD, Carter S, Stephens D, et al. Prospective validation of the pediatric appendicitis score. J Pediatr. 2008; 153: 278-82.
18. Mandeville K, Pottker T, Bulloch B, et al. Using appendicitis scores in the pediatric ED. Am J Emerg Med. 2011; 29: 972–7.

Correspondences to Dr. Ali F Abdullah
E mail: dr.ali.f.abdullah@gmail.com
Mobile: +964 7901353184
Received 8th Jan. 2013: Accepted 20th May. 2013

المجلد الحادي عشر، العدد الثاني، 1434 هـ، 2013م

المجلة العراقية للعلوم الطبية

عنون المراسلات إلى المجلة العراقية للعلوم الطبية، صندوق بريد 14222 بغداد، العراق. تلفون وفاكس (964-1-5224368).
رقم الإيداع في دار الكتب والوثائق ببغداد 709 لسنة 2000