Volume 12 (1) 2014 ISSN 1681-6579

IRAQI JOURNAL OF MEDICAL SCIENCES

Editorial Board Members

Iraqi Journal of Medical Sciences

Aims and Scope

Iraqi Journal of Medical Sciencesis published by College of Medicine, Al-NahrainUniversity. 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 Sciencespublishes 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 (Design + Setting), 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, methods, results, discussion& Conclusion.
- **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.
- **Acknowledgments:**

Collate acknowledgments in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

- **Conflict of interest:**

All authors must disclose any financial and personal relayionships with other people or organisations that could inappropriately influence (bias) their work. **Example** of potential conflicts of interest include employment , consultancies, stock ownership , honoraria , paid expert testimony , patent applications \ registrations , and grants or other funding . see also http://www.elsevier.com\conflictsofinterest .

Please complete and upload the conflict of interest and author declaration form with your manuscript.

- **Author contributions:**

Each author is required to declare his or her individual contribution to the article:all authors must have materially participated in the research and \ or article preparation, so roles for all authors should be described. The statement that all authors have approved the final author's article should be true and included article in the disclosure.

- **Role of the funding source :**

You are requested to identify who provided financial support for the conduct of the research and\or preparation of the article and to briefly describe the role of the sponsor (s),if any , in study design; in the collection , analysis and interpretation of data;in the writing of the report; and in the decision to submit the article for publication . If the funding source (s) had no such involvement then this should be stated.

- **List of abbreviation**

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

Take Care of Journal with Fake Impact Factor

Haider S. Kadhim *MBChB, PhD*

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

The importance of impact factor (IF) came from its use as an indicator for journal as having high scientific value in comparison to non-impact factor journal. Unfortunately none of our Iraqi journals have IF, hope to get IF for our journal in near future. Still there is a conflict of doing high scientific value researches and publishing in an average IF journal with all of costs and payments. Here I would to focus on the importance of IF, mainly with the help of Wikipedia, the free encyclopedia and another important website namely, science editing and publishing (http://sci-edit.net) of how to get information about the IF and how to know journals with IF.

The IF of an academic journal is a measure that reflects the average number of citations to recent articles published in that journal. It is frequently used to know the relative importance of a journal within its field, as journals with higher impact factors consider to be more important than those with lower ones. The IF was devised by Eugene Garfield, the founder of the Institute for Scientific Information. IFs are calculated yearly starting from the year 1975 for those journals that are indexed in the *Journal Citation Reports* (JCR).

Calculation

The IF of a journal, in any given year, is the average number of citations received per paper published in that journal during the two preceding years [(1)]. For example, if a journal has an impact factor of 3 in 2008, then its papers published in 2006 and 2007 received 3 citations each on average in 2008. The 2008 impact factor of a journal would be calculated as follows:

> *A* = the number of times that articles published in that journal in 2006 and 2007, were cited by articles in indexed journals during 2008.
> *B* = the total number of "citable items" published by that journal in 2006 and 2007. ("Citable items" are usually articles, reviews, proceedings, or notes; not editorials or letters to the editor). 2008 impact factor = *A*/*B*.

New journals, which are indexed from their first published issue, will receive an IF after two years of indexing; in this case, the citations to the year prior to Volume 1, and the number of articles published in the year prior to Volume 1 are known zero values. Journals that are indexed starting with a volume other than the first volume will not get an IF until they have been indexed for three years. Annuals and other irregular publications sometimes publish no items in a particular year, affecting the count. The IF relates to a specific time period; it is possible to calculate it for any desired period, and the JCR also includes a five-year IF [(2)]. The JCR shows rankings of journals by IF, if desired by discipline, such as organic chemistry or psychiatry.

Use

The IF is used to compare different journals within a certain field. The ISI Web of Knowledge indexes more than 11,000 science and social science journals [3,4].

Validity as a measure of importance

The IF is highly dependent on the academic discipline, possibly on the speed with which papers get cited in a field. The percentage of total citations occurring in the first two years after publication varies highly among disciplines from 1-3% in the mathematical and physical sciences to 5-8% in the biological sciences [5-7]. Thus, IFs cannot be used to compare journals across disciplines.

The IF is based on the arithmetic mean number of citations per paper, yet citation counts follow a Bradford distribution (i.e., a power law distribution) and therefore the arithmetic mean is a statistically inappropriate measure [8]. For example, about 90% of *Nature*'s 2004 IF was based on only a quarter of its publications, and thus the importance of any one publication will be different from, and in most cases less than, the overall number [9]. Furthermore, the strength of the relationship between IFs of journals and the citation rates of the papers therein has been steadily decreasing since articles began to be available digitally [10].

This problem was exacerbated when the use of IFs is extended to evaluate not only the journals, but the papers therein. The Higher Education Funding Council for England was urged by the House of Commons Science and Technology Select Committee to remind Research Assessment Exercise panels that are obliged to assess the quality of the content of individual articles, not the reputation of the journal in which they are published [11]. The effect of outliers can be seen in the case of the article "A short history of SHELX", which included this sentence: "This paper could serve as a general literature citation when one or more of the open-source SHELX programs (and the Bruker AXS version SHELXTL) are employed in the course of a crystal-structure determination". This article received more than 6,600 citations. As a consequence, the IF of the journal *Acta Crystallographica Section A* rose from 2.051 in 2008 to 49.926 in 2009, more than *Nature* (at 31.434) and *Science* (at 28.103) [12]. The second-most cited article in *Acta Crystallographica Section A* in 2008 only had 28 citations [13].

Finally, journal rankings constructed based solely on IFs only moderately correlate with those compiled from the results of expert surveys [14].

It is important to note that IF is a journal metric and should not be used to assess individual researchers or institutions [15,16].

Take care of fake impact factors

There are more than 20000 Journals showing FAKE IMPAC FACTOR on their site. Be aware of them. So how to know, if Impact Factor of certain journal is Fake:

1. Visit SCI site http://ip-science.thomsonreuters.com/mjl/
2. Enter title of the journal in Search. If it is indexed it will appear. Click on coverage, if it says "Science Citation Index" it means the journal is covered and has some IF that you cannot see unless you buy their "Journal Citation Report". If journal does not appear in list means it is not indexed with them, still the journal site says some IF, and no doubt it was fake one. Stay away from these journals if you think your research work has some quality.

So IF of journals will appear, at least 3 years later after publishing at least 5-6 issues consistently, however, getting indexed in SCI is not easy they demand very high quality in publication [17].

References

1. Introducing the Impact Factor. Retrieved 2009-08-26.
2. JCR with Eigenfactor. Retrieved 2009-08-26.
3. Web of Knowledge > Real Facts > Quality and Quantity". Retrieved 2010-05-05.
4. Thomson Reuters Master Journal List. Thomson Reuters. Retrieved 2013-06-20.
5. Rossner M, Van Epps H, Hill E. Show me the data. J Cell Biol 2007; 179(6):1091-2.
6. European Association of Science Editors (EASE) Statement on Inappropriate Use of Impact Factors. Retrieved 2012-07-23.

7. Nierop E. Why Do Statistics Journals Have Low Impact Factors? *Statistica Neerlandica* 2009; 63(1):52-62.

8. Joint Committee on Quantitative Assessment of Research. Citation Statistics (PDF). International Mathematical Union (12 June 2008).

9. Not-so-deep impact. Nature 2005; 435 (7045):1003-1004.

10. George LA, Vincent L, Yves G. The weakening relationship between the impact factor and papers' citations in the digital age. J Am Soc Inform Sci Technol 2012; 63(11):2140.

11. Bob G. New impact factors yield surprises. The Scientist 2010. Retrieved 31 March 2011.

12. What does it mean to be #2 in Impact?, Thomson Reuters Community.

13. Serenko A, Dohan M. Comparing the expert survey and citation impact journal ranking methods: Example from the field of Artificial Intelligence. J Inform 2011; 5(4):629-648.

14. Seglen PO. Why the impact factor of journals should not be used for evaluating research. BMJ 1997; 314(7079):498-502.

15. *EASE Statement on Inappropriate Use of Impact Factors*. European Association of Science Editors. November 2007. Retrieved 2013-04-13.

16. http://sci-edit.net/journals/impactfactor.pdf. Retrieved 2014-03-14.

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 Relationship of Interictal Epileptic Discharges with Duration of Illness in Epileptic Patients

Shaymaa J. Mohammed *MSc, PhD*

Dept. of Physiology, College of Medicine, Basra University, Basra, Iraq

Abstract

Background Epileptic discharges generally initiated at specific locations and spread after time in preferred directions along specific pathways, this spread will simultaneously change the dynamics of system in which it spread and cause epileptic destabilization of neuronal circuits.

Objectives To study the relationship between single focal epileptic discharge or multiple focal epileptic discharges and the duration of their disease.

Methods Ninety six epileptic patients with partial epilepsy and mean age of 12.5±7.5 years were studied, no one of patients receive antiepileptic drugs or were on irregular treatment. They had been divided according to duration of illness into two groups; those with more than one year illness and those with less than one year illness. Electroencephalography recording were obtained via 10-20 system using bipolar and referential montage with a thirty minutes record for each patient, accordingly. Patients were divided into those with single focal epileptic discharge (unifocal) & those with multifocal (multiple focal) epileptic discharge.

Results A significant difference in mean duration of illness between patients with unifocal and multifocal epileptic discharge were found. Those with multifocal epileptic discharge show higher mean duration of illness (17.8±9.05) months as compared to those with unifocal epileptic discharge (9.1±6) months. Significant positive linear correlation was found (P = 0.01), and the duration of illness increased more in patients with multifocal epileptic discharge.

Conclusion We found that longer duration of exposure to epileptic discharge could lead to generation of new foci not exist previously and that may possibly be due to kindling phenomena and triggering more spread of epileptic discharge.

Key words Epileptic discharge, Kindling phenomena, electroencephalography.

List of Abbreviation: AEDs = Antiepileptic drugs, EEG = Electroencephalography, ED = Epileptic discharge, MRI = Magnetic resonance imaging, CNS = central nervous system.

Introduction

Brain regions vary considerably in their capacity to participate in different forms of epileptic activities. Epileptic discharges generally initiated at specific locations and spread after time in preferred directions along specific pathways [(1)]. In human as well as in experimental animals, cortical epilepsy can begin with abnormal activity at seizure focus followed by synchronization and subsequent spread through the cortex [(2)].

The process of spread of epileptic discharges in central nervous system (CNS) can occur as a propagation of signals in neuronal network or as a process of dynamic changes in neuronal circuits and nets [(3)]. As the spatial extent of the neuronal population involved in seizures is

increased so the question raised weather this spread causing generation of new focuses were not exist before seizure onset [1].

Some researchers suggest that the after discharge will spread in base of space and time from one area like hippocampus to adjacent areas like entorhineal cortex. This spread will simultaneously change the dynamics of system in which it spread and causing epileptic destabilization of neuronal circuits [4].

Aside from the triggers that may potentially induce an epileptic seizure, the spread of epileptic discharge in human is still under debate. Since then, much work has been done in utilizing kindling and its proposed effects on neural plasticity, one of the fundamental properties of neurophysiology [5]. Since neural plasticity is a feature considered universal throughout the CNS, this suggests that changes prompted by kindling within one part of the CNS may occur throughout the body as well [6]. With this in mind, many have viewed kindling as most notably affecting the neuronal cells of the hippocampus and via a Hebbian Learning mechanism (synaptic strength is increased the more frequently it is active), these cells become altered permanently [7], this may be the mechanism by which spontaneous seizures are generated. Although kindling-induced alterations are seen most strikingly in the hippocampus and the limbic system, repeated stimulation of the pathways of the limbic, cortical, subcortical and brain stem regions (either chemically or electrically) induces a progressive sequence of long-lasting cellular and molecular alterations at all levels of biological organization in neural circuits, from gene transcription to patterns of neuronal connectivity [8]. Therefore, the objective of this research was to study the effect of kindling mechanisms on spreading of epileptic discharges in human cerebral cortex by one way or another [6,8].

Methods

This retrospective study was conducted in Al-Sader Teaching Hospital in Basra in the period from march/2012 up to April/2013 designed to evaluate the epileptic discharge in a group of ninety six patients with partial epilepsy with an age range from 5-20 years and mean age (12.5±7.5years). They were divided according to duration of illness in to two groups:

A: Those with duration of illness twelve months or less.

B: Those with duration of illness more than twelve months.

No one of patients had history of head trauma, meningitis, encephalitis or chronic illness. We included only patients with partial epilepsy; those with Absence, myoclonic or tonic clonic seizure were excluded. All patients show normal neurological exam. Patients were on no treatment or on very irregular treatment. Patients (or the relatives of children patients) were informed about the aim of study and their acceptance obtained. This work is in agreement with the medical ethics provided from the ethical committee in Basra collage of medicine and the Department of Training & Improving Skill - Research & Educational facilities in Al-Sader Teaching Hospital.

A digital electroencephalography (EEG) machine used to examine all patients according to standard 10-20 system, two montages used to read each record, a bipolar and referential montage, and the EEG record obtained after thirty minutes of exam carefully interpreted and patients divided according to EEG results into two groups:

1. Patients with unifocal (single focal epileptic discharge).
2. Patients with multifocal (multiple focal) epileptic discharge.

Data analysis was done by using SPSS version 20 computer software. Descriptive statistics for all data of each set was expressed as mean ± 2SD. The difference in mean duration of illness between groups was assessed by independent sample t-test, $P < 0.05$ considered statistically significant, spearman test used to assess the correlation between duration of illness and interictal epileptic discharge.

Results

Out of the total 96 patients included in the study, 54 (56.3%) were females and 42 (43.8 %) were males, no significant difference found in epileptic discharges (ED) weather unifocal or multifocal between male and female as illustrated in table 1.

Table 1. The sexual distribution of patients with unifocal and multifocal epileptic discharges

Epileptic Discharges	Males		Females		Total
	No.	%	No.	%	
Unifocal	8	19	15	27.8	23
Multifocal	34	81	39	72.2	73
Total	42	100	54	100	96

Twenty three patients (23.95%) were found to have unifocal ED and seventy three (76.05%) have multifocal ED (Table 2).

Table 2. Frequency distribution of the two study groups according to epileptic discharge

Epileptic Discharges	Group A		Group B		Total	
	No.	%	No.	%	No.	%
Unifocal	15	34.9	8	15.1	23	23.95
Multifocal	28	64.9	45	84.9	73	76.05
Total	34	100	53	100	96	100

Patients in group A were further subdivided in to two groups; group A1: Those with duration of illness ≤ 6 months and A2: Those with duration of illness from >6 months up to 12 months. According to these classifications we found that patients with multifocal ED were more in group A2 than in group A1 (Table 3).

Table 3. The number and percentage of unifocal and multifocal epileptic discharge in group A1 and A2 patients

Epileptic Discharges	Group A1		Group A2		Total
	No.	%	No.	%	
Unifocal	10	62.5	5	18.5	15
Multifocal	6	37.5	22	81.5	28
Total	16	100	27	100	43

On taking the patients as a whole or separately into group A and B, the mean duration of illness was significantly longer in those with multifocal as compared to those with unifocal epileptic discharge as illustrated in table 4.

Table 4. The mean duration of illness in patients with unifocal and multifocal epileptic discharges

ED	Group A		Group B		Group A & B	
	N	mean ±SD	N	Mean±SD	N	mean±SD
Unifocal	15	5±2	8	16.6±2.77	22	9.09±6
Multifocal	28	8±2.4	45	23.9±5.5	73	17.8±9.05

ED = epileptic discharges, *P* = 0.01 (for all groups)

For patients in group (A2), the mean duration of illness was significantly longer (*P* = 0.01) in those with multifocal epileptic discharge versus those with unifocal epileptic discharge, whereas there was no difference in the mean duration of illness in those patients belongs to group A1 (Table 5).

Table 5. Comparison of epileptic discharges in between group A patients

Epileptic Discharges	Group A		Group B	
	No.	mean±SD	No.	mean±SD
Unifocal	10	4±1.6	5	7.4±5.5*
Multifocal	6	4±1.3	22	8.95±1.12

* *P* = 0.01

A significant positive linear relation (*P* = 0.01) was found between mean duration of illness and number of ED (Fig. 1).

Discussion

The results of this study showed that patients with multifocal epileptic discharges have longer duration of illness as compared to those with unifocal epileptic discharge. Which suppose that the development of new epileptic foci is time related and as the brain exposed to epileptic activity for long time their development will be more possible [19].

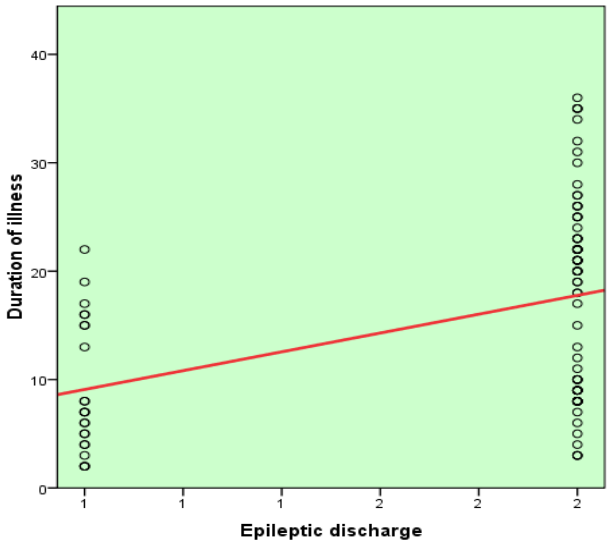

Fig. 1. Correlation between epileptic discharges and duration of illness in epileptic patients

The data of this study also showed a positive relation between mean duration of illness and the development of multifocal ED.

The outcomes obtained in this study agree with findings of other researchers who examined the development of mirror foci in patients who had medically refractory partial seizures secondary to brain tumors that were presumed to represent a single primary epileptogenic lesion[6].

A longer duration of epilepsy and more number of epileptic seizures prior to surgery correlated with failure of these secondary epileptogenic regions to resolve postoperatively.

The mechanisms beyond this spread still under dispute but the most prevalent is the kindling phenomena which assumes that repeated subconvulsive stimulation of a site in one hemisphere leads to increased excitability and abnormal electrical activity at that site and at the homologous site in the opposite hemisphere which will progress through a complex neuronal connectivity [3,11].

These secondary epileptic foci can be developed in patients with partial epilepsy due to kindling like mechanisms and the duration of exposure to such kindling influences can determine whether secondary epileptogenesis become irreversible [9,10].

Also many studied series clearly demonstrated a positive relation between longer duration of an epileptogenic condition prior to medical control and a poorer prognosis [9].

In the study that held by Blume (2007)[10], about secondary bilateral synchrony and its EEG correlate, he found that 91% of patients with duration of illness more than two years have the phenomena of secondary bilateral synchrony which was explained in view of complex interaction of multiple potentially epileptogenic regions and since some forms of epilepsy are progressive [12]; so this progression can be halt by AEDs and prevent continuous sub-threshold activation of adjacent neuronal network.

Furthermore, other readings assume that kindling mechanisms cause progression of epileptic symptoms with time and might interfere with normal cerebral activity.

Since seizure may predispose to further seizures so effective treatment maybe important to prevent evolution into chronic and more intractable multifocal epilepsy [5,7,6].

In conclusion, the duration of epilepsy is very important to determine the spread of epileptic discharge from one focus to other foci in the brain, and since multiple focal epilepsy associated with more adverse effects on brain functions (and development in children), so early treatment of epilepsy is essential to prevent the change of unifocal to multifocal epilepsy.

Acknowledgments

Great thanks to Dr. Mazin Al-Owath, the neurologist in Basra general hospital for his great help throughout the study and to the staff of the neurophysiologic unit in Al-Sader teaching hospital for their great help and collaboration.

Conflict of interest

There is no conflict of interest that could influence the objectivity of the research reported.

Funding

No specific grant from any funding agency in the public or commercial sector was obtained.

References

1. McCracken B, Roberts D. A single evoked after discharge produces rapid time-dependent changes in connexin36 protein expression in adult rat dorsal hippocampus. Neurosci Let. 2006; 405(1-2): 84-8.
2. Goda M, Kovac S, Speckmann JE, et al. Glutamate and dopamine receptors contribute to the lateral spread of epileptiform discharges in rat neocortical slices. Epilepsia. 2008; 49(2): 237-47.
3. Gotman J. Epileptic networks studied with EEG-fMRI. Epilepsia. 2008; 49(Suppl. 3): 42-51.
4. Gotman J, Pittau F. Combining EEG and fMRI in the study of epileptic discharges. Epilepsia. 2011; 52(Suppl. 4): 38-42.
5. Zahoruk R. Kindling: Origin of Epilepsy? The Meducator, 2005; 1(5): 55-62.
6. Engel J, Dichter MA, Schwartzkroin PA. Basic mechanisms of human epilepsy. In: Engel J, Pedley TA. (eds). Epilepsy: a comprehensive textbook. 2nd ed. Philadelphia: Lippincott Williams and Willkins; 2008. p. 495-507.
7. Bertram E. The Relevance of Kindling for Human Epilepsy. Epilepsia. 2007; 48(Suppl. 2): 65-74.
8. Sutula T. Mechanisms of epilepsy progression: current theories and perspectives from neuroplasticity in adulthood and development. Epilepsy Res. 2004; 60(2-3): 161-71.
9. Helmstaedter C, Kurthen M, Lux S, et al. Chronic epilepsy and cognition: A longitudinal study in temporal lobe epilepsy. Epilepsia. 2003; 28(2): 237-47.
10. Blume TW, Pillay N. Electrographic and Clinical Correlates of Secondary Bilateral Synchrony. Arch Neurol. 2007; 53(3): 228-32.
11. Trabka W. Studies of the mechanisms of spreading of epileptic discharges in the central nervous system. Hippocampus. 2008; 18(10): 1021-33.
12. Tschuluun N, Wenzel J, Katleba K, et al. Initiation and spread of epileptiform discharges in the methylazoxy methanol acetate rat model of cortical dysplasia: functional and structural connectivity between CA1 heterotopia and hippocampus/neocortex. Epilepsy Res. 1999; 36(2-3): 165-88.
13. Gabor A, Marsan AC. Co-existence of focal and bilateral diffuse paroxysmal discharges in epileptics: Clinical-electro graphic study. Epilepsia. 2007; 10(4): 453-72.
14. Jokeit H, Ebner A. Effects of chronic epilepsy on intellectual functions. Ann Neurol. 2003; 54: 425-32.
15. Falconer M, Kennedy W. Epilepsy due to small focal temporal lesions with bilateral independent spike-discharging foci. A study of seven cases relieved by operation. Adv Neurol. 2004; 44: 303-18.
16. Orman R, Von Gizycki H, Lytton WW, et al. Local axon collaterals of area CA1 support spread of epileptiform discharges within CA1, but propagation is unidirectional. Neuroscience. 2005; 133(1): 327-42.
17. Ben-Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. Lancet Neurol. 2006; 5(12): 1055-63.
18. McNamara J. Kindling: An animal model of complex partial epilepsy. Ann Neurol. 1984; 16(Suppl): S72-S76.
19. Gilbert T, Campbell Teskey G. Conventional anticonvulsant drugs in the guinea-pig kindling model of partial seizures: effects of repeated administration. Exp Brain Res. 2007; 178(1): 115-25.
20. Blumenfeld H, Rivera M, Vasquez JG, et al. Neocortical and thalamic spread of amygdala kindled seizures. Epilepsia. 2007; 48(2): 254-62.
21. Singh S, He X, McNamara J, et al. Morphological changes among hippocampal dentate granule cells exposed to early kindling-epileptogenesis. Hippocampus. 2013; 23(12): 1309-20.

E-mail: shaymaaneuro@yahoo.com
Received 6th Nov. 2013: Accepted 19th Feb. 2014

Iraqi JMS

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

Environmental Risk Factors for Congenital Cardiovascular Defects among Infants and Children in Basra, Southern Iraq

Ghada M. Abood[1] *FICMS*, **Meaad K. Hassan**[2] *CABP*

[1]Dept. of Pediatrics, Thi-Qar Medical School, [2]Dept. of Pediatrics, Basra Medical College, Iraq

Abstract

Background In Basra, Southern Iraq, an increased prevalence of congenital cardiovascular defects was reported. Although genetic and environmental factors predispose to these defects, little information is available concerning the non-inherited modifiable factors that may cause these defects.

Objectives To determine the environmental risk factors for congenital cardiovascular defects in infants and children.

Methods A total of 109 patients with congenital cardiovascular defects and 252 infants and children without congenital cardiovascular defects were studied. Their age ranged from 1 day-14 years. History included residence, family history of congenital heart diseases, maternal factors, employment, maternal exposure to drugs and radiation during pregnancy, and maternal illnesses and potential paternal risk factors.

Results A significant association between maternal age (less than 20 years or more than 34 years) (odd ratio, OR 4.65), influenza (OR 4.25), maternal phenobarbital intake (OR 1.54) was demonstrated with congenital cardiovascular defects. On the other hand, lower birth order (OR 0.412), absence of maternal exposure to air pollution like carbon monoxide, nitrogen dioxide, ozone, sulfur dioxide, and particulate matter (OR 0.852), and maternal stressful events (OR 0.822) were associated with a reduced risk for congenital cardiovascular defects.

Conclusions Birth order, maternal age, maternal exposure to air pollutions, maternal stressful events, influenza and phenobarbital therapy are independent risk factors for congenital cardiovascular defects.

Keywords Congenital cardiovascular defect, children, Basra

List of abbreviation: CCVDs = congenital cardiovascular defects, ACEI = angiotensin-converting enzyme inhibitors, NSAID = non-steroidal anti-inflammatory drugs, ECG = electrocardiography.

Introduction

Congenital cardiovascular defects (CCVDs) represent some of the more prevalent malformations among live births and remain the leading cause of death from congenital malformations [1,2]. Disease prevention has been limited by a lack of information about modifiable risk factors for abnormalities in cardiac development [1].

Heart defects at birth may occur as an isolated malformation, but may also be associated with other anomalies or occur as part of a syndrome [3].

Nearly a two-fold increase in the reported rate of heart defects since the early 1970s was described [4]. In Basra, which is located in the extreme south of Iraq and an estimated population of 2,531,997, a hospital-based study has found that the relative risk of CCVDs for the years 1991-1994, 1995-98, 1999-2000 in comparison to 1990, was 2.4, 5.8, and 8.3, respectively. In 1999-2000 the reported prevalence of the CCVDs in Basra was 14/10, 000 [5,6].

The cause of most CCVDs is unknown [2,3]. Most cases of CCVDs are thought to be multifactorial

and result from a combination of genetic predisposition and environmental stimulus [2,7]. A small fraction of cases, perhaps 15%, can be traced to a known cause, even when including environmental teratogens with genetic and chromosomal conditions [4].

Some types of CCVDs can be related to an abnormality of an infant's chromosomes (5-6%), single gene defects (3-5%), or environmental factors (2-4%) [2,4]. As CCVDs may result in significant lifelong morbidity, and are an important cause of mortality attributed to birth defects, the development of effective prevention interventions is very important forward step. This study was carried out to study the potential environmental risk factors associated with CCVDs.

Methods

This study is a case-control study; infants and children with CCVDs who have been admitted to pediatric wards or referred to Echocardiography Clinic at Basra Maternity and Children Hospital, over the period from the 15th of February 2008 till the end of June 2008, were recruited (excluding those with chromosomal abnormalities like Down syndrome and multiple congenital anomalies). A total of 109 patients aged 1 day -14 years were included in the study.

A total of 252 age and sex matched infants and children without CCVDs consulting the outpatient department of the same hospital for minor illnesses were considered as control group.

Information taken included age, sex, birth order, family history of CCVDs, and diagnosis, which depended on clinical data, chest x-ray findings, electrocardiography (ECG), and Echocardiogram.

Maternal factors included: age, abnormal pregnancy outcome (previous miscarriage, still birth, preterm birth), employment (either unemployed, or employed). If employed; the type of employment is considered as without risk or with risk of occupational exposure to organic solvents like dyes, lacquers, paints, mineral oil products, maternal employment in agricultural industry, and maternal exposure to herbicides, rodenticides, pesticides, and insecticides [8-10].

The residence was also reported; Basra Center, Northern Area which includes (Al-Garma, Al-Qurna, Al-Hartha, Al-Madina), Western Area (Al-Zubair District), Southern Area (Fao, Abu-Al-Khaseeb), Eastern Area (Shatt Al-Arab and Shalamjah) [11].

Maternal illnesses and drugs used during the first trimester; including angiotensin-converting enzyme inhibitors (ACEI), aspirin, ibuprofen, diclofenate, phenytoin, phenobarbital, valproic acid, carbamazepine, metronidazole, cotrimoxazole, clomiphene (before pregnancy), vitamin A, corticosteroids, folic acid, and multivitamins containing folic acid (women were considered if they take folate and multivitamin supplements regularly from 3 months before conception through the third month of pregnancy, while women who started to take drug after they become pregnant were considered as not taking the drug) [3].

Maternal exposure to radiation (exposure in occupational settings or as part of medical or dental evaluations). Maternal water consumption, habits (smoking, coffee, and tea) and stressful events like close relative death, divorce or separation, and job loss were also assessed.

Paternal factors included: age, occupation (occupation at risk include jewelry making, welding, lead soldering, ionizing radiation, and paint stripping) [12] and habits (smoking and alcohol drinking).

Environmental factors included maternal exposure to air pollutants (distance 8.6 km-14.2 km which is the distance from air pollution source to the maternal residence) like carbon monoxide, nitrogen dioxide, ozone, sulfur dioxide, and particulate matter (such as dust, ash, and smoke) during first trimester of pregnancy [13,14]. These pollutants released by incomplete combustion of fuels such as coal, gas, and oil. In Basra these factories located in Um-Qasir, Al-Zubair, Khur Al-Zubair, Abu Al-Khaseeb, AL-Hartha, and AL-Najibia.

An informed consent was obtained from one of the parents, usually the mother before

recruitment in the study. The study approved by the Ethical Committee of Basra Medical College.
Statistical analysis was done using SPSS program (version 11), data were expressed and comparisons of proportions was performed using chi square and / or Exact Fisher`s test when appropriate. Logistic regression analysis was also done for the analysis of different potential risk factors. *P* value of <0.05 was considered as statistically significant.

Results

Ventricular septal defect (VSD) was the most common type of CCVDs detected in 35 (32.1%), followed by Tetralogy of Fallot in 12 (11%), VSD and pulmonary stenosis in 11 (10.1%), patent ductus arteriosus in 7(6.4%), atrial septal defect in 6(5.5%), hypertrophic cardiomyopathy in 6(5.5%) followed by transposition of great arteries in 5(4.6%). Other types of CCVDs were less common and accounted for (24.8%) in this study.
A significantly higher number of patients with CCVDs have a sibling, mother or father with CCVD, ($P < 0.01$). In addition, it was found that the frequency of CCVDs increases significantly with increasing birth order of the child ($P < 0.001$) as shown in Table 1.

Table 1. Distribution of cases according to age and sex, birth order and family history of congenital cardiovascular defects

Parameter		Patient Group N = 109		Control Group N = 252		*P* Value
		No.	%	No.	%	
Age (years)	<1	60	55	161	63.9	> 0.05
	1-4	29	26.6	70	27.8	
	5-9	15	13.8	17	6.7	
	10-14	5	4.6	4	1.6	
Sex	Male	56	51.4	146	57.9	> 0.05
	Female	53	48.6	106	42.1	
Family history of CCVDs in first degree relatives	None	101	92.7	249	98.8	< 0.01
	Any*	8	7.3	3	1.2	
Birth order	1st	7	6.4	50	19.8	< 0.001
	2nd	9	8.3	70	27.8	
	3rd	10	9.2	44	17.5	
	4th	10	9.2	43	17	
	5th	13	11.9	16	6.3	
	6th	15	13.7	11	4.4	
	7th	17	15.6	9	3.6	
	8th	18	16.5	6	2.4	
	≥9th	10	9.2	3	1.2	

*Any; CCVD in siblings, mother or father

Table 2 demonstrate significantly higher number of mothers of children with CCVDs were either younger than 20 years of age (30.3%) or older than 34 years (41.3%) compared to mothers of control group (6% and 13.5%) respectively, ($P < 0.001$). In addition, a significantly higher number of mothers of children with CCVDs have a history of reproductive problems (35.8%) than mothers of the control group (21.4%) ($P < 0.05$), and 23.9% of mothers of children with CCVDs have a history of stressful events during the

periconceptional period, compared with mothers of control group (6.4%), ($P < 0.001$).
The study also has revealed that a significantly higher number of mothers of children with CCVDs did not take folic acid 102 (93.6%) and multivitamins 105 (96.3%) during peri-conceptional period; whereas, mothers in the control group reported a significantly higher frequency of intake of folic acid 72 (28.6%) and multivitamins 64 (25.4%), ($P < 0.001$).
Pre-gestational diabetes, gestational diabetes, fever, influenza, and epilepsy were reported in a significantly higher number of mothers of children with CCVDs None of mothers in both groups reported history suggestive of rubella.

Table 2. Selected maternal characteristics among patients andcontrol group

Variable		Patient Group N = 109		Control Group N = 252		*P* Value
		No.	%	No.	%	
Age (years)	<20	33	30.3	15	6.0	< 0.001
	20-34	31	28.4	203	80.5	
	≥35	45	41.3	34	13.5	
History of abnormal Pregnancy outcomes	No	70	64.2	198	78.6	< 0.05
	Miscarriage	33	30.3	48	19.0	
	Stillbirth	2	1.8	3	1.2	
	Preterm birth	4	3.7	3	1.2	
Maternal stress event	No	83	76.1	236	93.6	< 0.001
	Close relative death	19	17.4	15	6.0	
	Job loss	7	6.5	1	0.4	

The study revealed that a significantly higher number of mothers in the control group (81.3%) were not taking drugs during pregnancy ($P < 0.001$), while mothers of children with CCVDs showed a highly significant association between ibuprofen ($P < 0.01$), clomiphene ($P < 0.001$), phenobarbital ($P < 0.01$), and cotrimoxazole intake ($P < 0.05$) and CCVDs. None of mothers in both groups gave a history of intake of angiotensin-converting enzyme inhibitors, phenytoin, carbamazepine, valproic acid, and vitamin A (Table 3).
Most of mothers of both groups were not consuming coffee (93.5% and 95.5%), and there was no significant difference among them concerning tea consumption. None of mothers in both groups were smokers. A significantly higher number of mothers in the control group (66.3%) were living in Basra center, while a significantly higher number of mothers of children with CCVDs were living in North, West and South of Basra, compared with mothers of the control group, ($P < 0.001$). A statistically significant association between the sources of air pollution in Basra (oil refineries, natural gas company, cement factory, petrochemical factory, electrical power station, and fertilizer factory) and CCVDs in children ($P < 0.001$) .
Regarding maternal employment; (14.9%) from all mothers of children with CCVDs and control group were employed in occupations without risk of exposure. None of mothers in both groups gave a history of exposure to radiation during pregnancy (Table 4).
Concerning paternal risk factors; young paternal age < 25 years and advanced paternal age ≥40 years were significantly higher among children with CCVDs, ($P < 0.01$) and a significantly higher number of fathers in the control group have no occupational risk (93.3%), compared with fathers of children with CCVDs (87.2%) ($P < 0.05$) as noticed in Table-5. None of fathers in both groups gave a history of alcohol drinking.

Table 3. Potential Maternal risk factors during pregnancy

Variable			Patient Group N = 109		Control group N = 252		OR	95% CI	P value
			No.	%	No.	%			
Maternal illness	No		58	53.2	218	86.6			< 0.001
	Fever		20	18.3	16	6.3	3.313.	1.64-6.68	< 0.001
	Influenza		21	19.3	16	6.3	52	1.75-7.05	< 0.001
	Epilepsy*		2	1.8	0	0.0	3.35	2.86-3.93	< 0.05
	GD		6	5.5	2	0.8	7.28	1.44-36.6	< 0.01
	Pre-GD*	Type 1	1	0.9	0	0.0			< 0.05
		Type 2	1	0.9	0	0.0			
Maternal drug ingestion during pregnancy	No		55	50.4	205	81.3			< 0.001
	Clomiphene		24	22	18	7.1	3.68	1.74-3.72	< 0.001
	Phenobarbital		5	4.6	1	0.4	0.154	1.37-1.72	< 0.01
	Cotrimoxazole		6	5.5	3	1.2	4.83	1.18-19.70	< 0.05
	Metronidazole		5	4.6	13	5.2	0.88	0.30-2.54	> 0.05
	Corticosteroids		3	2.8	6	2.4	1.16	0.28-4.72	> 0.05
	Aspirin	1st TM	2	1.8	2	0.8			> 0.05
		2nd TM	1	0.9	1	0.4			
	Diclofenac sodium	1st TM	1	0.9	1	0.4			> 0.05
		2nd TM	1	0.9	1	0.4			
	Ibuprofen	1st TM	1	5.5	1	0.4			< 0.01

GD = gestational diabetes, TM = trimester, * P-value of pre-gestational diabetes and epilepsy were calculated by Fisher's Exact Test.

Table 4. Potential environmental risk factors during pregnancy

Risk factors		Patient Group N = 109		Control Group N = 252		*P* Value
		No.	%	No.	%	
Residence	Basra center	50	45.9	167	66.3	< 0.001
	North of Basra	23	21.1	31	12.3	
	South of Basra	11	10.1	13	5.2	
	West of Basra	23	21.1	27	10.7	
	East of Basra	2	1.8	14	5.6	
Maternal exposure to air pollutants	No	71	65.1	229	90.9	< 0.001
	Oil refineries	3	2.8	2	0.8	
	Natural gas company	5	4.6	4	1.6	
	Cement factory	5	4.6	2	0.8	
	Petrochemical factory	5	4.6	3	1.2	
	Electrical power station	11	10.1	8	3.2	
	Fertilizer factory	9	8.3	4	1.6	
Employment	Yes	8	7.4	19	7.5	> 0.05
	No	101	92.6	233	92.5	
Maternal water consumption	Home tap water	10	9.2	18	7.2	> 0.05
	Bottled water	99	90.8	234	92.8	

Table 5. Paternal risk factors

Risk factors			Patient Group N = 109		Control Group N = 252		*P* Value
			No.	%	No.	%	
Age (years)	<25		24	22.0	31	12.3	< 0.01
	25-29		12	11.0	58	23.0	
	30-34		20	18.3	66	26.2	
	35-39		15	13.7	49	19.4	
	40-44		17	15.6	23	9.1	
	≥ 45		21	19.2	25	9.9	
Paternal habits	No		53	48.6	129	51.2	> 0.05
	Smoking	< 20/day	10	9.2	6	2.4	
		20-40	36	33.0	105	41.6	
		> 40	10	9.2	12	4.8	
Occupation	With risk		14	12.8	17	6.7	< 0.05
	Without risk		95	87.2	235	93.3	

The whole variables included in the study were subjected to logistic regression analysis to know the variables that are associated with CCVDs. It was observed that the higher birth order, young or advanced maternal age, maternal influenza, and maternal phenobarbital intake were found to be independent significant risk factors for CCVDs. On the other hand, lower birth order, absence of maternal exposure to air pollution, and maternal stressful events confer a protection against the development of CCVDs (Table 6).

Table 6. Independent variables associated with CCVDs

Variables	B*	SE*	OR	95% CI	*P* value
Birth order	0.888	0 .111	0 .412	0.331-0.511	< 0.001
Maternal age	1.53	0 .374	4.65	2.23-9.68	< 0.001
Influenza	1.44	0.538	4.25	1.48-12.22	< 0.01
Phenobarbital	0.432	0 .056	1.54	1.37-1.72	< 0.01
Absence of Maternal exposure to air pollution	0.160	0 .036	0 .852	0.79-0.91	< 0.001
Absence of Maternal stressful events	0.196	0 .052	0.822	0.74-0.91	< 0.001

B*: regression coefficient, SE*: standard error

Discussion

This case-control study describes the potential environmental risk factors for CCVDs in Basra. The current study reported significantly higher frequency of CCVDs in first degree relatives of patients compared to control group (7.3% and 1.2% respectively). A significant association among first degree relatives of patients with CCVDs was also reported by Bassili *et al* in Egypt [(15)], Stoll *et al* in France [(16)], and Correa *et al* in Baltimore [(17)]. Increased incidence of CCVDs in the same family suggests genetic influences [(4)], or because the family is exposed to the same environmental factors.

Higher birth order was significantly associated with higher risk of CCVDs; this is in agreement with that reported by Taksande *et al* in India [(18)] and Materna-Kiryluk *et al* in Poland [(19)]. This finding provides indirect evidence of environmental influence in the causation of CCVDs, which are known to be inherited in a multifactorial manner [(19)].

A significant association between CCVDs and young maternal age < 20 years and advanced maternal > 34 years was reported, similar results were found by Ferencz *et al* in Baltimore [20], Reefhuis *et al* in Atlanta [21]. Advanced age is associated with chromosomal anomalies, which could be the underlying cause for these associations.

History of abnormal pregnancy outcomes were significantly associated with increased risk of CCVDs, similar results were reported also by Ferencz *et al* in Baltimore [20], Cedergren *et al* in Sweden [7], and Pradat in Sweden [22]. Whether a history of reproductive problems represents a proxy for teratogenic exposures or for an inherent increased susceptibility for CCVDs is unclear [1].

Maternal stressful events including close relative death, and job loss were reported in a significantly higher percent of mothers of children with CCVDs, similar result was reported by Carmichael *et al* in California [23]. The exact mechanism is not known but it was presumed that increased catecholamine production due to stress, leads to decreased uterine blood flow and increased fetal hypoxia that could result in different types of birth defects [24].

Maternal periconceptional intake of multi-vitamin and folic acid was significantly associated with reduced risk for CCVDs in their offspring's, a similar results were reported by Beynum *et al* in Netherlands [3], Botto *et al* in USA [25], and Scanlon *et al* in Atlanta [26]. In contrast, a hospital-based case control study by Werler *et al* in Boston didn't report such association [27]. The maternal methylene-tetrahydro folate reductase 677TT genotype is associated with two folds increased risk of CCVDs in offspring, especially for a conotruncal heart defects if mothers did not use folate supplements [3].

Among maternal illnesses during pregnancy, there was a significant association with maternal diabetes. This is in agreement with other studies in different countries [7,20,28]. Both human and animal studies have demonstrated that diabetic embryopathy is associated with hyperglycemia during organogenesis. The precise pathogenic mechanisms remain unclear. Abnormal glucose levels disrupt expression of a regulatory gene in the embryo. Oxidative stress with generation of free radicals is another possible mechanism [4].

Maternal fever and influenza are also important risk factors for CCVDs, similar result was reported by ACS *et al* in Hungary [29], and Botto *et al* in Atlanta [30]. Both fever and infection have documented biological effects on specific developmental pathways. Altered apoptosis is a possible mechanism for this association [4].

Maternal epilepsy was significantly associated with CCVDs in this study; similar result was reported by Pradat in Sweden [22]. It has been difficult to determine whether maternal seizures are independently associated with an increased risk of heart defects [22,31].

Among medication intake during early pregnancy, this study showed a statistically significant association of CCVDs with maternal intake of Ibuprofen, a similar result was reported by Wilson *et al* in Baltimore [32]. In contrast, a study reported by Nielson *et al* in Denmark [33] has concluded that there is no evidence that any NSAID is teratogenic. The use of NSAID during pregnancy poses a potential threat to the myocardium. Persistent pulmonary hypertension and premature closure of the ductus arteriosus were reported in infants whose mothers took NSAID toward the end of pregnancy [8].

Maternal use of clomiphene was found to be significantly associated with an increased risk of CCVDs; similar results were reported by Bassili *et al* in Egypt [15], and Ferencz *et al* in Baltimore [20]. In contrast, a study by Niebyl *et al* in USA showed no association between maternal use of clomiphene with cardiac defects [9].

Folic acid antagonists (cotrimoxazole) intake during early pregnancy were also significantly associated with CCVDs, a similar result was found by Diaz *et al* in USA [31] and Czeizel *et al* in Hungary [34]. Folic acid antagonists act through different mechanisms including dihydrofolate reductase inhibitors, impairing absorption of folate, increasing the degradation of folate, or

affect various other enzymes in folate metabolism [31].

For antiepileptic drug, a significant association of CCVDs with phenobarbital intake during early pregnancy was found, a similar result was reported by Cedergren *et al* in Sweden [7], and Diaz *et al* in USA [31]. These findings are consistent with the view that phenobarbital may exert a teratogenic effect through mechanisms other than the depletion of folic acid, and a direct toxic effect has been proposed [31].

Drinking tea and coffee by mothers during pregnancy were not significantly associated with CCVDs in their offspring's; similar result was reported by Olsen *et al* in Denmark [35].

Maternal exposure to ambient air pollutions during early pregnancy is among the important environmental triggers of CCVDs, similar result was reported by Gilboa *et al* in Texas [13] and Ritz *et al* in California [14], who confirmed that increased ambient air levels of pollutants (carbon monoxide, ozone, sulfur dioxide, nitrogen dioxide, and particulate matter) are associated with increased risk of CCVDs. It was observed that mutations in fetal DNA may follow exposure to air toxics during pregnancy [13]. This could explain the significantly higher percent of children with CCVDs living in the north, west, and south of Basra where factories like petrochemical factory, Natural Gas Company, oil refineries, electrical power station, cement factory, and fertilizer factory are located.

There was a significant association between young paternal age <25years and advanced paternal age ≥45years with CCVDs in their offspring, similar results were obtained by Bassili *et al* in Egypt [15], and Yang et al in USA [36] possibly through dominant mutations. However, Cedregren *et al* in Sweden did not find certain paternal age effect [7].

The current study did not reveal a significant association between paternal smoking and CCVDs in their offspring's. While a study reported by Cresci *et al* in Italy [37] and Kuciene et *al* in Kaunas [38] had found an increased risk of CCVDs with paternal smoking. In addition, a significant association was identified between paternal occupational exposure and CCVDs in their offspring's, similar result was reported by Snijder *et al* in Netherlands [12].

From this study, it can be concluded that birth order, maternal age, maternal exposure to air pollutions; maternal stressful events, influenza, and phenobarbital therapy are independent risk factors for congenital cardiovascular defects.

Acknowledgment

We like to thank Dr. Asaad Yahya, Department of Animal Production, College of Agriculture for his indispensable help in the statistical effort of this work.

Author Contribution

Meaad Hasan designed the study and co-writes the manuscript, Ghada M Abboud collected and analyzed the data and write the paper

Conflict of Interest

Authors disclose no conflicts of Interest

Funding

The research was supported by the Iraqi Commission of Medical Specialization.

References

1. Jenkins KJ, Correa A, Feinstein JA, et al. Non-inherited Risk Factors and Congenital Cardiovascular Defects: Current Knowledge: A Scientific Statement From the American Heart Council on Cardiovascular Disease in the Young. Circulation. 2007; 115(23): 2995-3014.
2. Bernstein D. Congenital heart disease In: Behrman RE, Kliegman RM, Jenson HB (eds). Nelson's Textbook of Pediatrics. 17ed. Philadelphia: WB Saunders CO; 2004. p. 1499-554.
3. Beynum IM, Kapusta L, Heijer M, et al. Maternal MTHFR 677CT is a risk factor for congenital heart defects: effect modification by periconceptional folate supplementation. Eur Heart J. 2006; 27(8): 981-7.
4. Botto LD, Correa A. Decreasing the burden of congenital anomalies: an epidemiologic evaluation of risk factors and survival. Prog Pediat Cardiol. 2003; 18(2): 111-21.
5. Al-Sadoon I, Hasan GG, Yacoub AA. Depleted uranium and health of people in Basrah: epidemiological evidence. Incidence and Pattern of congenital anomalies among births in Basrah during the period 1990-1998. Med J Basrah Univ. 1999; 17(1 and 2): 27-34.
6. Hindin R, Brugge D, Panikkar B. Teratogenicity of depleted uranium aerosols: A review from an epidemiological perspective. Environ Health. 2005; 4: 17. doi: 10.1186/1476-069X-4-17.

7. Cedergren MI, Selbing AJ, Kallen BAJ. Risk factors for cardiovascular malformation-a study based on prospectively collected data. Scand J Work Environ Health. 2002; 28(1): 12-7.
8. Mone SM, Gillman MW, Miller TL, et al. Effects of environmental exposures on the cardiovascular system: prenatal period through adolescence. Pediatrics. 2004; 113(4): 1058-69.
9. Niebyl JR, Simpson JL. Teratology and drugs in pregnancy. Glob Libr Women's Med, 2008; ISSN 1756-2228. Accessed from http://www.glowm.com/section_view/heading/Teratology%20and%20Drugs%20in%20Pregnancy/item/96
10. Shaw GM, Nelson V, Iovannisci DM, et al. Maternal occupational chemical exposures and biotransformation genotypes as risk factors for selected congenital anomalies. Am J Epidemiol. 2003; 157: 475-84.
11. Habeeb OS, Al-Ali JK, Al-Wiswasi MK, et al. Cancer Registration in Basra 2005: Preliminary Results. Asian Pacific J Cancer Prev. 2007; 8: 187-90.
12. Snijder CA, Vlot IJ, Burdorf A, et al. Congenital heart defects and parental occupational exposure to chemicals. Hum Reprod. 2012; 27(5): 1510-7.
13. Gilboa SM, Mendola P, Olshan AF, et al. Relation between ambient air quality and selected birth defects, seven county study, Texas, 1997-2000. Am J Epidemiol. 2005; 162(3): 238-52.
14. Ritz B, Yu F, Fruin S, et al. Ambient air pollution and risk of birth defects in southern California. Am J Epidemiol. 2002; 155: 17-25.
15. Bassili A, Mokhtar SA, Dabous NI, et al. Risk factors for congenital heart diseases in Alexandria, Egypt. Euro J Epidemiol. 2000; 16(9): 805-14.
16. Stoll C, Alembik Y, Roth MP, et al. Risk factors in congenital heart disease. Eur J Epidemiol. 2004; 5(3): 382-91.
17. Correa A, Ferencz C, Neill CA, et al. Ebstein's malformation of the tricuspid valve: genetic and environmental factors. The Baltimore-Washington Infant Study Group. Teratology. 1994; 50(2): 137-47.
18. Taksande A, Vilhekar K, Chaturvedi P, et al. Congenital malformations at birth in central India:A rural medical college hospital based data. Indian J Hum Genet. 2010; 16(3): 159-63.
19. Materna-Kiryluk A, Wieckowska B, Wisniewska K, et al. Maternal reproductive history and the risk of isolated congenital malformations. Pediatr Perinat Epidemiol. 2011; 25(2): 135-43.
20. Ferencz C, Loffredo CA, Correa A, et al. Genetic and Environmental risk factors of major cardiovascular malformations: the Baltimore-Washington study: 1981-1989. Perspec Pediatr Cardiol. 1997; 5: 79-86.
21. Reefhuis J, Honein MA. Maternal age and non-chromosomal birth defects, Atlanta-1968-2000: teenager or thirty-something, who is at risk? Birth Defects Research part A. Clin Mol Teratol. 2004; 70(9): 572-9.
22. Pradat P. A case-control study of major congenital heart defects in Sweden 1981-1986. Eur J Epidemiol. 1992; 8(6): 789-96.
23. Carmichael SL, Shaw GM. Maternal Life Event Stress and Congenital Anomalies. Epidemiology. 2000; 11(1): 30-5.
24. Carmichael SL, Shaw GM, Lammer EJ. Maternal Stressful Life Events and Risks of Birth Defects. Epidemiology. 2007; 18(3): 356-61.
25. Botto LD, Mulinare J, Erickson JD. Occurrence of congenital heart Defects in relation to maternal multivitamin use. Am J Epidemiol 2000; 151: 878-84.
26. Scanlon KS, Ferencz C, Loffredo CA, et al. Periconceptional folate intake and malformations of the cardiac outflow tract . Epidemiology. 1998; 9: 95-98.
27. Werler MM, Hayes C, Louik C, et al. Multivitamin supplementation and risk of birth defects. Am J Epidemiol. 1999; 150(7): 675-82.
28. Abu-Sulaiman RM, Subaih B. Congenital heart disease in infants of diabetic mothers: echocardiographic study. Pediatr Cardiol. 2004; 25(2): 137-40.
29. Acs N, Banhidy F, Puho E, et al. Maternal influenza during pregnancy and risk of congenital abnormalities in offspring. Birth Defects Research Part A. Clin Mol Teratol. 2005; 73(12): 989-96.
30. Botto LD, Lynberg MC, Erickson JD. Congenital Heart Defects, Maternal febrile illness, and Multivitamin use: A population-Based study. Epidemiol. 2001; 12(5): 485-90.
31. Diaz SH, Werler MM, Walker AM, et al. Folic Acid Antagonists during Pregnancy and the Risk of Birth Defects. N Engl J Med. 2000; 343(22): 1608-14.
32. Wilson PD, Loffredo CA, Correa A, et al. Attributable fraction for cardiac malformations. Am J Epidemiol. 1998; 148(5): 414-23.
33. Nielsen GL, Sorensen HT, Larsen H, et al. Risk of adverse birth outcome and miscarriage in pregnant users of non-steroidal anti-inflammatory drugs: population based observational study and case- control study. BMJ. 2001; 322: 266-70.
34. Czeizel AE, Rockenbauer M, Sorensen HT, et al. The teratogenic risk of trimethoprim-sulfonamides: a population based case-control study. Repro Toxicol. 2001; 15(6): 637-46.
35. Olsen J, Overvad K, Frische G. Coffee consumption, birth weight, and reproductive failures. Epidemiology. 1991; 2(5): 370-74.
36. Yang Q, Wen SW, Leader A, et al. Paternal age and birth defects: how strong is the association? Hum Reprod. 2007; 22(3): 696-701.
37. Cresci M, Foffa I, Ait-Ali L, et al. Maternal and paternal environmental risk factors, Metabolizing GSTM1 and GSTT1 polymorphisms, and congenital heart disease. Am J Cardiol. 2011; 108(11): 1625-31.
38. Kuciene R, Dulskiene V. Parental cigarette smoking and the risk of congenital heart septal defects. Medicina. 2010; 46(9): 635-41.

Correspondence to Dr. Meaad K. Hassan
E-mail: alasfoor_mk@yahoo.com
Tel. +964 7801000174
Received 3rd Apr. 2013: Accepted 27th Jan. 2014

Iraqi JMS

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

Role of Vitamin E, L-Carnitine and Melatonin in Management of β-Thalassemia Major

Ahmed M.T. Al-Mosawi[1] *BSc MSc*, **Faruk H. Al-Jawad**[2] *MBChB PhD*, **Safaa A. Al-Badri**[3] *MBChB FIBMS*

[1]Dept. of Pharmacology & Therapeutics, College of Medicine, Wasit University, [2]Dept. of Pharmacology & Therapeutics, College of Medicine, Al-Nahrain University, [3]Welfare Children Teaching Hospital, Baghdad Medical City Complex, Ministry of Health, Baghdad, Iraq

Abstract

Background β-thalassemia major is an inherited disease resulting from decrease or total lack of β globin chains. Patients with this disease need repeated blood transfusion for survival. This may cause oxidative stress and tissue injury due to iron overload and depletion of antioxidant enzymes.

Objective Evaluation the role of vitamin E, L- carnitine and melatonin supplementation in management of β-thalassemia major patients.

Methods Forty five patients with β-thalassemia major were allocated to three groups A, B and C treated with vitamin E, L-carnitine and melatonin respectively. Serum malondialdehyde, serum reduced glutathione, serum ferritin, Hb, PCV, MCV, MCH, and MCHC levels and RBCs count were measured before and after treatment.

Results A significant decrease was observed in serum malondialdehyde and ferritin level after therapy in all treated groups; whereas, no significant ($P > 0.05$) changes in glutathione level after treatment in all groups. Hb level and RBC count increased significantly in group A (vitamin E), whereas, PCV, MCV, MCH and MCHC levels did not change significantly in all treated groups.

Conclusion Vitamin E, L- carnitine and melatonin have beneficial effects of in reducing lipid peroxidation and iron overload in patients with β-thalassemia major. These antioxidants may increase the life span of RBCs, which manifested by significant increase in Hb level in vitamin E treated group and significant decrease in serum ferritin level in all treated groups.

Keywords Beta-thalassemia, malondialdehyde, glutathione, ferritin, hematological parameters, vitamin E, L-carnitine, melatonin.

List of Abbreviation: HbF = fetal hemoglobin, SOD = superoxide dismutase, HOCl = hypochlorous acid, MDA = malondialdehyde, GSH = glutathione, TBA = thiobarbituric acid, RBC = red blood cell count, Hb = hemoglobin, PCV = packed cell volume, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

Introduction

Beta thalassemia is a hereditary anemia resulting from defects in the production of β-globin chains. Depending on clinical severity, three forms are distinguished, namely, thalassemia major, thalassemia intermedia and thalassemia minor [(1)]. β-Thalassemia major, is a clinically severe disorder that results in the transfusion dependent state which creates a state of iron overload [(2,3)]. Once reticulo-endothelial stores saturate, iron deposition increases in parenchymal tissues such as endocrine glands, hepatocytes, and myocardium [(4)]. The toxicity of iron is attributed to its ability to catalyze free-radical reactions that have life-threatening consequences. Iron could catalyze the oxidative breakdown of most biomolecules such as lipids, sugars, amino acids, DNA etc. [(1)].
Iron overload induces extra ferritin protein synthesis but the protein is overfilled with the extra iron that damages ferritin, with conversion

to toxic hemosiderin [5]. Vitamin E, is an important lipid-soluble antioxidant in humans, has been used as a potential agent to help protection against oxidative stress in thalassemia patients [6]. Due to increased consumption in homozygous β-thlassemia, low plasma levels of α-tocopherol, may induce lipid peroxidation within the red blood cells and consequently hemolysis [7]. Poor physical fitness is a common problem among thalassemic patients. L- carnitine plays an essential role in fatty acid beta-oxidation. In a clinical study, it was found that L-carnitine seems to be a safe and effective adjunctive therapeutic approach in thalassemic patients, and improves their cardiac performance and physical fitness [8]. In the treatment of thalassemia, newer approaches have been tried as alternative to standard therapy. Butyrate analogues such as L-carnitine have been found to increase fetal hemoglobin (HbF) synthesis and hence used in treatment of β- thalassemia. It also protects erythrocytes from oxidative stress, stabilizes the cell membrane, increasing the life span of red blood cells and is found to inhibit apoptosis in different diseases and that, thalassemic children had significant DNA double-strand breaks in their leukocytes and can be ameliorated by L-carnitine supplementation [1]. In a study conducted by Merchant *et al*, L-carnitine levels were found to be lower in thalassemics as compared to age matched controls [9]. Melatonin is a potent scavenger especially for hydroxyl and peroxyl radicals, inhibits apoptosis in normal cells, influences activities and cellular mRNA levels of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase, and glutathione reductase [10]. Recent preliminary results have shown that melatonin can prevent hypochlorous acid (HOCl) mediated heme destruction in hemoglobin and highlights a new mean by which melatonin can exhibit its protective effect [11].

The aim of this study was to evaluate the effects of vitamin E, L- Carnitine and melatonin supplementation on the levels of oxidative stress (malondialdehyde and glutathione), ferritin and hematological parameters in β-thalassemia major patients.

Methods

Patients Selection and drugs treatment:

This study was carried out between June 2011 and May 2012 on 45 patients with β-thalassemia major in Thalassemia Center in Al-Kut Hospital after giving the informed consent of the patients and agreement of the hospital, the patient age ranges were 10-34 years (mean ± SD = 19.12 ± 6.29), all the selected patients s were under regular blood transfusion and regular chelation therapy with deferoxamine (Desferal®, and they did not take any other antioxidants preparations) at the time of study.

The patients were allocated to three groups and were treated as follow:

Group A: 15 Patients (9 females and 6 males), were treated orally with 200 mg/day vitamin E (United pharmaceuticals, Jordan), 100 mg at morning and 100 mg at night for three months, in addition to other drugs prescribed according to center drug policy.

Group B: 15 Patients (8 females and 7 males), were treated orally with 30 mg/kg/day **L-carnitine (**Ultimate nutrition, USA), taken daily in two divided doses at morning and night for three months, in addition to other drugs prescribed according to center drug policy.

Group C: 15 Patients (9 females and 6 males) were treated orally with 3 mg/day **melatonin** soft gels (Vitane pharmaceuticals Inc., USA) taken at night at bedtime for two months with a break for one week between the two months, in addition to other drugs prescribed according to center drug policy. (**Melatonin** was administered to patients with ages of eighteen years old and above, since the safety of melatonin in subjects below eighteen years old has not been established.

Sample collection and preparation:

Five milliliters of blood were obtained from each patients by venipuncture, taken on routine visit to the thalassemia center within the first hour of visit, and before starting blood transfusion,

which considered (baseline or before treatment), and then at every visit to check the changes in the liver enzymes (to ensure the safety of administered drug), and at the end of treatment period (after treatment).

The period of treatment was three months, except for melatonin, which administered for two months separated by one week free interval.

All blood samples were collected in plain tubes; erythrocytes were separated by centrifugation at 3000 rpm for 10 minutes, the serum obtained was used for biochemical analysis.

Assay methods

1- Measurement of serum malondialdehyde (MDA) level: MDA measurement is based on the reaction of thiobarbituric acid (TBA) (BDH chemicals, Ltd. Poole, England) with MDA forming a pink colored MDA-TBA adduct that its light absorbance measured at 532 nm [(12)].

2- Measurement of serum glutathione (GSH) level: Reduced glutathione was determined based on the reaction of GSH with DTNB (5,5-Dithiobis (2- nitrobenzoic acid)) (BDH chemicals, Ltd. Poole, England) at pH 8 to produce a colored complex which absorb light at 412 nm and it is directlyproportional to GSH concentration [(13)].

3- Measurement of serum Ferritin: Serum ferritin level was measured by using minividas kit (Biomerieux SA, France).

4- Measurement of hematological parameters: RBC (Red Blood Cell count), Hb (Hemoglobin concentration), Hematocrit or PCV (packed cell volume), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin) and MCHC (Mean Corpuscular Hemoglobin Concentration) were measured in whole blood on the day of collection using Micros (60), blood automated Coulter counter **(**Horiba ABX, France**).**

Statistical analysis

Numerical data are presented as mean ± SD. The paired Student t-test was used to compare data obtained from each group before and after treatment, P-value less than 0.05 considered significant.

Results

Out of the 45 total numbers of patients involved in the study, 39 patients completed the study. While six patients were excluded, one patient due to marked elevation of liver enzymes (hepatitis C), two patients due to poor compliance, two patients due to gastrointestinal side effects (abdominal pain) and one patient due to sedative effect of melatonin.

In all treated groups, post treatment serum MDA level was significantly decreased as compared to pretreatment (3.9 ± 2.9 µM/L versus 1.74 ± 0.83 µM/L in group A; 3.44 ± 1.7 µM/L versus 2.07 ± 0.93 µM/L in group B; and 3.46 ± 1.3µM/L versus 2.33 ± 1.2 µM/L in group C).

Similarly, post treatment serum ferritin level was significantly decreased in all treated groups (6029.2 ± 3250 ng ml versus 4831 ± 3014 ng/ml in group A; 5435.9 ± 2905 ng/ml versus 4102.8 ± 1645 ng/mL in group B; and 5435.9 ± 2905 ng/mL versus 4391.6 ± 2517 ng/ mL in group C).

On the contrary, post treatment Hb level and RBC count were increased significantly in group A only (7.9 ± 1.5 g/dL versus 8.5 ± 1.2 g/dL; 3 ± 0.6 $x10^{12}$/L versus 3.3±0.45 $x10^{12}$/L, respectively). Concerning post treatment GSH level, PCV, MCV, MCH and MCHC, no significant change was observed versus pretreatment in all treated groups (Tables 1 through 3).

Discussion

Malondialdehyde (MDA), a terminal compound of lipid peroxidation, is used widely as an index of oxidative status. Increased plasma MDA levels have been observed in patients affected by β-thalassemia major [(14)].

Results of present study showed that vitamin E significantly ($P < 0.05$) decrease serum MDA level and these results are in agreement with results obtained by Palasuwan *et al* [(6)]. Vitamin E can transfer its phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA, thereby breaking the radical chain reaction and preventing the peroxidation of PUFA in cellular and subcellular membrane phospholipids [(15)]. Although no clinical studies were found about the effect of L-carnitine and melatonin on MDA

and some parameters that investigated in this study in β-thalassemia major.

Table 1. Effect of vitamin E on serum MDA, GSH, ferritin, hemoglobin (Hb), PCV, RBCs, MCV, MCH and MCHC levels in β- thalassemia major patients

Parameters	Effect of Vitamin E	
	Pretreatment	Post treatment
Malondialdehyde (µM/L)	3.9 ± 2.9	1.74 ± 0.83*
GSH(µM/L)	1.77 ± 0.49	1.51 ± 0.15
Serum Ferritin (ng/mL)	6029.2 ± 3250	4831 ± 3014**
Hemoglobin (g/dL)	7.9 ± 1.5	8.5 ± 1.2*
PCV (L/L)	24.6 ± 5.4	26.5 ± 3.6*
RBC ($x10^{12}$/L)	3.0 ± 0.6	3.3 ± 0.45
MCV (fL)	79.3 ± 5.1	78.1 ± 3.6
MCH (pg/cell)	26.46 ± 2.53	25.85 ± 3.34
MCHC (g/dL)	32.33 ± 2.002	32.3 ± 2.38

* = $P < 0.05$, ** = $P < 0.001$

Table 2. Effect of L-carnitine on serum MDA, GSH, ferritin, hemoglobin (Hb), PCV, RBCs, MCV, MCH and MCHC levels in β- thalassemia major patients

Parameters	Effect of L-Carnitine	
	Pretreatment	Post treatment
Malondialdehyde (µM/L)	3.44 ± 1.7	2.07 ± 0.93*
GSH(µM/L)	1.54 ± 0.23	1.57 ± 0.29
Serum Ferritin (ng/mL)	6259.7 ± 2269	4102.8 ± 1645**
Hemoglobin (g/dL)	8.7 ± 0.87	8.8 ± 1.5
PCV (L/L)	27.8 ± 2.7	28.5 ± 4.9
RBC ($x10^{12}$/L)	3.58 ± 0.39	3.65 ± 0.68
MCV (fL)	78 ± 5.8	78.4 ± 7.9
MCH (pg/cell)	24.4 ± 2	24.1 ± 2.6
MCHC (g/dL)	31.12 ± 1.177	31.09 ± 0.8964

* = $P < 0.05$, ** = $P < 0.001$

Table 3. Effect of melatonin on serum MDA, GSH, ferritin, hemoglobin (Hb), PCV, RBCs, MCV, MCH and MCHC levels in β- thalassemia major patients

Parameters	Effect of Vitamin E	
	Pretreatment	Post treatment
Malondialdehyde (µM/L)	3.46 ± 1.3	2.33 ± 1.2**
GSH (µM/L)	1.38 ± 0.28	1.35 ± 0.46
Serum Ferritin (ng/mL)	5435.9 ± 2905	4391.6 ± 2517**
Hemoglobin (g/dL)	7.4 ± 0.85	7.7 ± 0.81
PCV (L/L)	24.1 ± 3.3	24.9 ± 2.8
RBC ($x10^{12}$/L)	3.23 ± 0.62	3.35 ± 0.53
MCV (fL)	75.1 ± 3.8	74.3 ± 4.5
MCH (pg/cell)	23.4 ± 2.5	24.2 ± 2.1
MCHC (g/dL)	31.38 ± 1.92	31.25 ± 1.17

** = $P < 0.001$

The results also demonstrated that oral administration of L-carnitine significantly (P < 0.001) decreases serum MDA level, in study done by Ates *et al* to determine the antioxidant properties of the L-carnitine in the treatment of patients with age-related macular degeneration. The MDA level was significantly reduced at the end of the 3-month period (P < 0.001) [16]. The present results also found that administration of melatonin significantly (P < 0.001) decreases serum MDA level. In one study, Herrera *et al* reported that melatonin prevents the oxidative stress changes induced by intravenous administration of iron and erythropoietin in doses commonly used to treat anemia in chronic hemodialysis patients and had no adverse side effects, and that oral dose of melatonin (0.3 mg/kg) prevents the increase in MDA level [17]. In addition to that, it was found that melatonin administration (5 mg daily for 30 days), resulted in a significant reduction in the MDA level in elderly primary essential hypertensive patients [18].

The results of present study have demonstrated a significant decrease in serum ferritin level after treatment with antioxidants in all groups. These results were in agreement with results obtained by Attia *et al* who reported that administration of vitamins E, C and A to homozygous β-thalassemic patients for twelve months results in significant (P < 0.05) decreases in ferritin values [7]. Glutathione is the most abundant erythrocyte thiol and a principal reducing agent for sulphydryl enzymes and hemoglobin, the loss of function of hemoglobin due to autoxidation is restored by the intervention of reduced glutathione [19]. The glutathione level did not show significant (P > 0.05) difference in all treated groups when compared to before treatment level.

Red cells from β-thalassaemia patients generally display a shortened life span with overt haemolysis. In this condition, the red cell membrane is under increased oxidant stress with a threat to membrane integrity. Reduced glutathione (GSH) prevents oxidative damage to red blood cells [16].

Hb level and RBC count showed significant (P < 0.05) increase after treatment with vitamin E only, Attia *et al* found that the value of Hb improve after treatment vitamins (E, C and A) for twelve months in homozygous β-thalassemic patients [7]. In addition to that, Tesoriere *et al* showed that vitamin E significantly (P < 0.05) increased the RBC count in β-thalassemia intermedia patients [20]. While in groups B and C, there was non-significant increase in Hb level, El-Beshlawy *et al* found a significant increase in the blood transfusion intervals after L-carnitine administration and improvement in the cardiac performance and physical fitness in patients with thalassemia major, and however, there was no significant change in hemoglobin concentration [8].

The results also did not show significant (P > 0.05) differences in PCV, MCV, MCH and MCHC levels after treatment in all treated groups. In a study, Palasuwan *et al* reported that PCV and MCV did not change significantly after treatment with vitamin E 200 I.U. daily for 3 months in hemoglobin-E carriers [21]. While in another study, Karimi et al. demonstrated that the combination of L-carnitine with hydroxyurea could be more effective in improving hematologic parameters in patients with β-thalassemia intermedia than hydroxyurea alone [22]. Di Iorio *et al* have demonstrated that L-carnitine administration was effective as adjunctive treatment of anemia associated with chronic kidney disease in many β-thalassemic patients [23]. The effect of L-carnitine supplementation in the present study showed good activity against MDA serum ferritin level, but with little activity in improving the hematological parameters, which may require increasing the dose or prolonging the treatment period as were seen in some clinical studies. The administration of melatonin improved several hematological parameters, but only the Hb value showed significant increase. Tesoriere *et al* investigated the antioxidant activity of melatonin in human erythrocytes, exposed to oxidative stress by cumene hydroperoxide (cumOOH), and found that melatonin was

actively taken up into erythrocytes under oxidative stress, and is consumed in the defense of the cell, delaying Hb denaturation and release of hemin. RBCs are highly exposed to oxygen and can be a site for radical formation, under pathological conditions, which results in their destruction. A protective role of melatonin should be explored in hemolytic diseases [24].

Arushanian *et al* reported that melatonin leads to a significant increase in the hemoglobin level and erythrocyte number [25]. In addition to that, Maulood *et al* found that melatonin could improve hematological complications in bleomycin treated rats [26]. Melatonin also was effective to reverse the deleterious effects of radiation on the blood parameters in rats in study by Ozmerdivenli et *al* [27]. Finally, the thalassemic patients those had completed the present study were well tolerated the antioxidant agents, with few adverse effects, which were mild and limited, no allergic reactions were seen to any of the antioxidant agents.

In conclusion, iron overload in β-thalassemia major, causes tissue injury and organ damage. The poor compliance of patients with iron chelation therapy, made the iron chelaters alone in β-thalassemia major is not sufficient to neutralize the da8mage induced by free radicals. The marked role of antioxidants given in present study in decreasing lipid peroxidation (through significant reduction of MDA), serum ferritin and the improvement in some hematological parameters were encouraging to suggest the addition of these antioxidants to the classical drug regimen of β-thalassemia major.

Acknowledgment

We thank Dr. Muntather Al-Saidee and Dr. Laith Sami Dr. Suhad Faleh for their great support and help.

Author Contribution

Dr. Ahmed M. Taqi Al-Mosawi collected the data and participated the statistical analysis and drafting of the article. Dr. Faruk H. Al-Jawad contributed in the designing of the proposal, analysis and interpretation of the data; Dr. Safaa A. Al-Badri participated in the follow up of patients during period of study and in drafting of the article.

Conflict of Interest

The authors declare no conflict of interest.

Funding

Personal funding

References

1. Ragab SM, Mahfouz RG. L-Carnitine ameliorates the iron mediated DNA degradation in peripheral leukocytes of β- thalassemic children. Egypt J Med Hum Genet. 2010; 11(1): 17-31.
2. Thein SL. Pathophysiology of {beta} thalassemia: a guide to molecular therapies. Hematology (Am Soc Hematol Educ Program). 2005: 31–7.
3. Hoffman R. Hematology: Basic Principles and Practice. 4th ed. Elsevier: Churchill Livingstone; 2005. p. 262-275.
4. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and desferoxamine. Haematologica. 2004; 89: 1187-93.
5. Theil E. Ferritin iron minerals are chelator targets, antioxidants, and coated, dietary iron. Ann N Y Acad Sci. 2010; 3(4): 197-204.
6. Palasuwan A, Sriprapun M, Dahlan W, et al. Antioxidant protection in hemoglobin E trait subjects after vitamin E supplementation. Southeast Asian J Trop Med Public. 2008; 39(1): 82-.9
7. Attia MM, Sayed AM, Ibrahim FA, et al. Effect of antioxidant vitamins on the oxidant/antioxidant status and liver function in homozygous beta-thalassemia. Romanian J Biophys. 2011; 21(2):93-106.
8. El-Beshlawy A, El-Accaoui R, Abd El-Sattar M, et al. Effect of L-carnitine on the physical fitness of thalassemic patients. Ann Hematol. 2007; 86(1): 31-4.
9. Merchant R, Jain A, Udani A, et al. L-carnitine in beta-thalassemia. Indian Pediatr. 2010; 47(2): 165-7.
10. Sener A, Ozsavici D, Bingol-Ozakpinar O, et al. Oxidized-LDL and Fe3+/ascorbic acid-induced oxidative modifications and phosphatidyl serine exposure in human platelets are reduced by melatonin. Folia Biologica. 2009; 55: 45-52.
11. Galijasevic S, Abdulhamid I, Abu-Soud HM. Melatonin is a potent inhibitor for myeloperoxidase Biochemistry, 2008; 47: 2668-77.
12. Stocks J, Dormandy TL. The auto-oxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol. 1971; 20: 95-111.

13. Godin DV, Wahaieb SA, Garent ME. Antioxidant enzyme alteration in experimental and clinical diabetes. Mol Cell Biochem. 1988; 84: 223-31.

14. Gunarsih A, Amalia P, Boediman I. Variables associated with malondialdehyde level in thalassemia major patients. Paediatr Indones. 2012; 52(3): 125-31.

15. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002; 18: 872-9.

16. Ates OH, Alp HH, Mumcu U, et al. The Effect of L-carnitine treatment on levels of malondialdehyde and glutathione in patients with age related macular degeneration. Eurasian J Med. 2008; 40: 1-5.

17. Herrera J, Nava M, Romero F, et al. Melatonin prevents oxidative stress resulting from iron and erythropoietin administration. Am J Kidney Dis. 2001; 37(4): 750-7.

18. Kedziora-Kornatowska K, Szewczyk-Golec K, Czuczejko J, et al. Antioxidative effects of melatonin administration in elderly primary essential hypertension patients. J Pineal Res. 2008; 45(3): 312-7.

19. Ponnazhagan S, Sarkar R. Enzymes of the pentose phosphate pathway in glutathione-regulated membrane protection in β-Thalassaemia. Eur J Clin Chem Clin Biochem. 1992; 30: 481-4.

20. Tesoriere L, D'Arpa D, Butera D, et al. Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce oxidative damage to LDL and erythrocytes in beta-thalassemia intermedia patients. Free Radic Res. 2001; 34(5): 529-40.

21. Palasuwan A, Soogarun S, Wiwanitkit V, et al. Preliminary study of the effect of vitamin E supplementation on the antioxidant status of hemoglobin-E carriers. Southeast Asian J Trop Med Public Health. 2006; 37(3): 184-9.

22. Karimi M, Mohammadi F, Behmanesh F, et al. Effect of combination therapy of hydroxyurea with L-carnitine and magnesium chloride on hematologic parameters and cardiac function of patients with beta-thalassemia intermedia. Eur J Haematol. 2010; 84(1): 52-8.

23. Di Iorio BR, Guastaferro P, Cillo N, et al. Long-term L-Carnitine Administration reduces Erythropoietin Resistance in Chronic Hemodialysis Thalassemia Minor Patients. Drug Target Insights. 2007; 2: 1-7.

24. Tesoriere L, D'Arpa D, Conti S, et al. Melatonin protects human red blood cells from oxidative hemolysis: new insights into the radical-scavenging activity. J Pineal Res. 1999; 27(2): 95-105.

25. Arushanian SA, Beier EB, Mastiagina OA, et al. Melatonin effect on the hematological indices of healthy humans. Eksp Klin Farmako. 2006; 69: 36-8.

26. Maulood IM. Effect of low molecular weight heparin, melatonin and their combination on plasma endothelin -1, serum electrolytes and some hematological variables in bleomycin treated rats. J Duhok Univ. 2009; 12(1): 161-6.

27. Ozmerdivenli R, Karacabey K, Gundogdu C, et al. Protective role of melatonin on blood parameters following irradiation in rat. Afr J Biotechnol. 2011; 10(80): 18564-8.

Correspondence to Ahmed MT. Al-Mosawi
E-mail: Safafaraj@yahoo.com
Received 10th Mar. 2013: Accepted 27th Jan. 2014

Iraqi JMS

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

Gender Selection by Ericsson Method in Intrauterine Insemination for Infertile Couples

Saad S. Al-Dujaily *PhD*, **Shighaf E. Al-Dahan** *MBCHB, DGO, HDART*

Dept. of Clinical Reproductive Physiology, High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq.

Abstract

Background Ericsson method used to determine whether enriched sperm samples would result in offspring of a desired gender. It is used in approximately 50 centers in the United States and in many centers worldwide when scientists and andrologist discovered that sperm samples with high concentrations of either X or Y bearing sperm could be obtained.

Objective To examine the effect of Ericsson method on gender selection following intra-uterine insemination for infertile couples.

Methods One hundred infertile couples were included in this study. A programmed ovulation induction for women was done. Luteinizing hormone and estradiol hormone level were measured. Ultrasonography was used to detect the number and diameter of follicles and endometrial thickness at menstruation cycle 1 day before human chorionic gonadotropin injection. Semen analysis was done for all husbands and density gradient technique with 7% and 17% albumin concentration was performed for sperm selection *in vitro*. Intra-uterine insemination was accomplished and pregnancy test was done 14 days following insemination to detect the level of human chorionic gonadotropin in blood. The gender of fetus was recorded 4 months following pregnancy by ultrasonography.

Results Twenty two (22%) out of one hundred women become pregnant. According to gender, live birth babies distributed into thirteen male babies (76.64%) and only four female babies (23.36%). One pregnant woman delivered twins following intra-uterine insemination.

Conclusion It is concluded that Ericson method is a simple and effective technique for gender selection when infertile couples seek to have a baby by intra-uterine insemination.

Key Words Gender selection, Infertile couples, Intra-uterine insemination, Ericsson method.

List of Abbreviation: E2 = estradiol, FSH = follicular stimulating hormone, hCG = human chorionic gonadotropin, LH = luteinizing hormone, IUI = intrauterine insemination, IVF = in vitro fertilization, PGD = preimplantation genetic diagnosis, TSH = thyroid stimulating hormone.

Introduction

Over centuries, couples and individuals with or without their partner's knowledge have tried to influence the sex of their babies. Following the ancient Chinese astrological birth chart, couples matched the mother's time of birth to the month of proposed conception to select a baby's gender. Ancient China still used today [(1)]. The other theory was founded by *Hippocrates, Greek Physician* who believed that male develop on the right side of the *uterus*, and females on the left. Women were instructed to have sex lying on their right side, if they desire a boy [(2)].

The other old theory described by *Leviticus, the Talmud* was instructed the woman to have an *orgasm* before the man to conceive a male, and vice versa for a female baby. Early Greeks

(Anaxagoras *-500 to 428 BCE)* often tied off the left testicle with twine during intercourse, hoping to produce a son, and the right testicle, a daughter [3]. In 20th century, scientists prescribed a high-protein diet for women wishing to conceive a boy [4]. Sperm sorting utilizes the technique of flow cytometry to analyze and 'sort 'spermatozoa. During the early to mid 1980s, Johnson *et al* [5,6] was the first to sort viable whole human and animal spermatozoa using a flow cytometer and utilized the sorted motile sperm for artificial insemination .

Recently, two major types of pre-implantation methods can be used for social gender selection. The Ericsson method, which is used to determine whether enriched sperm samples would result in offspring of a desired gender, was first applied in a clinical setting in the 1970s by Dr. Ronald J. Ericsson [7], and *in vitro* fertilization (IVF)/preimplantation genetic diagnosis (PGD) technique [8] in which, the embryos of the desired gender are implanted back in the mother's uterus. Thus, the goal of this work was to examine the effectiveness of Ericsson method to determine the desire gender following IUI of infertile couples.

Methods

This study was carried out in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, through October 2011 till December 2012. One hundred infertile couples were included in this study for gender selection. Couples were carefully managed, through detailed history (IVF indicated cases were excluded) viewing all previous investigations. Men with normozoospermia and mild male infertility factors (those with mild oligozoospermia, mild asthenozoospermia, and those with leucocytospemia) were all included. The spouses with anovulatory causes (except resistant cases of polycystic ovary syndrome and endometriosis), unexplained infertility and hostile post coital test were involved in this study.

Female investigation

The average women age that included in this work was 27.7±3.4 years old (ranged between 21 to 36 years). All the women had full detailed history, and complete physical examination done by the gynecologist. Hormonal analysis was done through menstrual cycle at days 2-3, 11-12 and 21-22 includes serum prolactine hormone, luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating hormone (TSH) and estradiol hormone (E_2). Then at mid luteal phase, progesterone level was measured too. A serial vaginal ultrasonography was carried out to check the ovulation status, size of the uterus, endometrial thickness, size of each ovary, number and size of antral follicles, on day 12-14 of the cycle. The tubal patency was examined by hysterosalpingography and /or diagnostic laparoscopy [9].

Male examination

The husbands were examined by a consultant urologist. Semen sample (100) was obtained via masturbation after an abstinence period of 3-5 days, collected directly into a clean, dry and sterile disposable Petri dish. Each sample was transported to the semen examination laboratory immediately and allowed to liquefy in an incubator at 37 °C. After complete liquefaction, the semen was analyzed by a macroscopic and microscopic examination using the standards of World Health Organization [10].

In vitro preparation of semen for gender selection

The semen of infertile men complaining of mild oligoasthenozoospermia was prepared *in vitro* using albumin discontinuous density gradients. The two albumin density gradients 7% and 17% were prepared by the dilution of human albumin 20 % (Biotast Pharma GmbH, Germany) with Hams-F12 medium (Sigma-Aldrich-USA). The procedure of semen preparation *in vitro* was depending on Ericsson method [7] with a modification in using centrifugation at 4000 RPM for 20 minutes. Microscopic examination was done using 10 µl of last fraction from the top of tube and the results of certain sperm characters were reported.

Intra-uterine Insemination

Hundred spouses who prepared for IUI were involved in this study. About 0.3-0.5 ml of the prepared semen was aspirated into 1 ml syringe and attached to endo-cervical catheter (Gynetics, Belgium) and used for IUI. The IUI was done as described by Vermeylen *et al* [11]. All the women instructed to be in supine position on the side of ovulating ovary for 30 minutes. Luteal support was started from the next day after insemination by using progesterone tablets (Duphaston® 10 mg; Solvay- Holland) twice daily for 2 weeks then a blood sample was obtained from the female to test for human chorionic gonadotropin (ß-hCG). The pregnant women were followed for fetal gender determination from 16 week gestation on ward and the number of delivered babies was recorded thereafter.

Statistical analysis

This was performed using SPSS (Statistical Package of Social Science; version 17.0 LED Technology, USA) and Microsoft Excel Work Sheet 2007). The results were expressed as mean ± standard error of the mean (SEM). Paired sample t-test and Chi-square were used to compare between the results depending on the nature of data. The differences between the values were considered statistically significant if the *P* was lower than 0.05 [12].

Results

Table 1 showed different certain sperm function parameters before and after activation using albumin discontinuous density gradient of 7% and 17%. The mean of sperm concentration after *in vitro* activation was significantly ($P < 0.05$) decreased compared to before activation. There was a significant ($P < 0.05$) increase in active sperm motility grade A and grade B following *in vitro* activation compared to before activation. The percentage of sperm motility grade C and grade D was significantly ($P < 0.05$) decreased after activation than that of before activation. The percentage of morphologically normal sperm after activation (68.37 ± 1.68) was significantly ($P < 0.05$) higher than that of before activation (44.78 ± 1.29).

Table 1. Sperm parameters of men semen whose spouses become pregnant after *in vitro* activation by discontinuous density gradient technique

Parameters		*In vitro* activation by 7%-17% density gradient technique		*T* test value
		Before	After	
Sperm concentration (x 10^6/ml)		51.75 ± 1.87	25.28 ± 1.33	10.37 *
Sperm motility (%)	Grade A	10.15 ± 1.26	57.70 ± 2.05	6.849 *
	Grade B	31.36 ± 1.44	32.58 ± 1.18	5.010 *
	Grade C	29.44 ± 0.72	6.71 ± 0.68	7.925 *
	Grade D	29.05 ± 1.55	3.01 ± 0.52	3.103 *
Morphologically normal sperm (%)		44.78 ± 1.29	68.37 ± 1.68	5.076 *
* (P<0.05)				

Table 2 show the rate of pregnancy following IUI of 100 women involved in this study. There were twenty two out of one hundred women become pregnant (22%) and the seventy eight women did not get pregnant. The statistical analysis found a significant difference ($P < 0.01$) between them.

The total male babies was thirteen and the percentage of male sex selection was (76.64%), when one women pregnant with twins males babies, while only four female babies delivered which give 23.36% percentage (Table 3).

Table 2. Pregnancy rate following IUI of 100 women

Status	No.	%
Pregnant	**22**	**22**
Non- Pregnant	**78**	**78**
Total	**100**	**100**
Chi-square value = 9.644 **		

Sixteen pregnant women reach full term and delivered 17 babies representing 73.9%, while the remaining six sustained trimester abortion.

Table 3. Distribution of Live birth according to gender

Gender	No.	Percentage (%)
Male	**13***	**76.64**
Female	**4**	**23.36**
Total	**16**	**100**
Chi-square value = 9.25 **		

*One pregnant women delivered twins male, ** = $P < 0.01$

There was a significant ($P < 0.01$) higher percentage of term pregnancy compared to abortion out of the twenty two pregnant patients (Fig. 1).

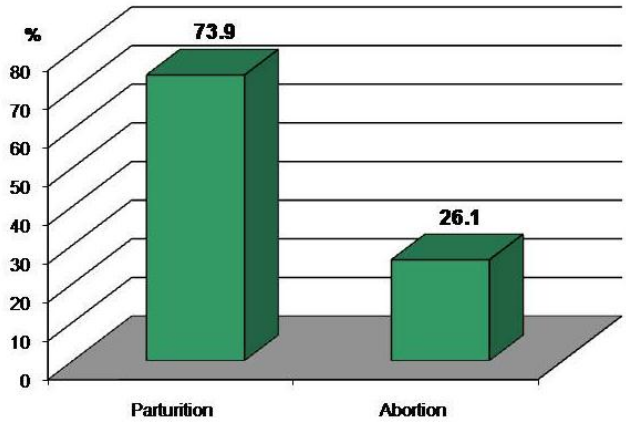

Fig. 1. Percentage distribution of pregnant status according to parturition and abortion

Discussion

The sperm preparation and activation *in vitro* by discontinuous density gradient technique using 7% and 17% albumin concentrations showed a significant improvement in sperm active motility grades A and B. This improvement in active sperm motility may be resulted from the effect of the technique, which removed the seminal plasma, pus cells and agglutinated spermatozoa [13]. This (7% and 17%) albumin concentration for IUI was also used by Ericsson method for sex selection [14].

In this work, there was a modification in Ericsson method by using discontinuous density gradient procedure. This technique is used in different fertility centers worldwide because of the best results obtained in the last fraction of sperm with high active motility and morphologically normal sperm percentage, free from debris, round cells, immotile spermatozoa [15]. Therefore, the procedure of preparation by this technique improves both the sperm motility and percentage of morphologically normal sperms [16]. In addition to that, using human serum albumin for sperm preparation will enrich the medium with high amount of adenine monophosphate, which enhance sperm motility by fueling its energy and increase the number of highly active motile sperms [17]. Furthermore, albumin act as antioxidant in a way that it did not protect against DNA damage induced by NADPH, but is extremely effective at preventing DNA fragmentation arising from the suppression of glutathione peroxidase activity with mercapto-succinate [17].

Out of the one hundred couples inseminated only twenty two got pregnant (22%), six ends with trimester abortion while the others got full term pregnancy. The pregnancy success rate following IUI was higher than that of IUI results in other studies [18-20] which range from 14%-21%.

The higher successful rate of pregnancy in this study could be attributed to the following facts:

1. the use of human serum albumin technique as centrifugation media, which was not used in those studies that may give lower pregnancy outcome [21].
2. Preparation of the women for IUI in this study e.g. Ovulation induction and timing of insemination used) depend on criteria of previous study conducted in the Institute by Al-Dujaily and Abo-Regheef [22] which gave a high pregnancy rate too.

3. The average ages of women got IUI was 30 years old. It has been found that age of the patient is a significant influencing factor in the success of IUI [23]. In those women over the age of 40, the pregnancy rate following IUI is very low [24].

Gender selection outcome: The pregnancy success rate was 22%; six cases ends with first trimester abortion, 75% of the delivered babies were male, while only 25% of the delivered babies were female babies. These results obtained match the results obtained by Dr. Ericsson method for sex selection [7]. The high incidence of male gender results can be attributed to the fact that discontinuous albumin density gradient result in clean fraction of concentrated sperms free from debris, round cells, immotile spermatozoa [25]. Moreover, Y chromosomes will be concentrated at the bottom layer of the centrifuge as the light Y chromosome can move rapidly down the different layers of albumin concentration in contrast to X chromosome which has higher molecular weight and hence cannot emerge easily from the high albumin concentration used in the discontinuous density gradient centrifugation. This gives high yield of male babies delivered to women inseminated using this method. However, the most important factor that may halt the increase in male sex selection in this study not more than 75% with percentage of 22% pregnancy rate is that all the couples are infertile which in turn interfere with the results of insemination by a sufficient number of male sperms [26]. As most of the semen samples were obtained from infertile men, this will increase the incidence of low number of male sperms before density gradient technique compared to fertile men semen.

On the other hand, out of the twenty two pregnant patients only two patients got twin pregnancy (9%) and one of them was aborted in the first trimester. The incidence of multiple pregnancies (twins) may result from fertilization of more than one oocyte due to ovulation induction programs. A major concern for couples undergoing any fertility treatment is the risk of multiple pregnancies. For those couples undergoing ovulation induction with clomiphene alone, the risk of twins is about 11% [27]. For those couples treated with superovulation/IUI, the risk of twins is about 16% [28].

The present study concluded that gender selection by Ericsson method is easy and effective procedure to enhance the desire of the infertile couples.

Authors' contribution

The proposal design was suggested by Prof. Al-Dujaily. The performance of work and writing the manuscript were collectively done with Dr. Shighaf Al-Dahan.

Conflict of interest

The authors declare no conflict of interest

Funding

The high institute of infertility diagnosis and assisted reproductive technologies - Al-Nahrain University has granted the research

References

1. Dawson WR. The Egyptian medical papyri. In: brothwell DR, Sandison AT, (eds). Diseases in Antiquity. London: Charles C Thomas; 1967. p. 98-111.
2. King H. Hippocrates` Woman: Reading the female body in Ancient Greece. New York: Routledge, 1998.
3. Sanders JB. Transitions from Ancient Egyptian to Greek medicine Lawrence: University of Kansas Press, 1963.
4. Morice p, Josset p, Chapron C, et al. History of infertility. Hum Reprod Update. 1995; 1: 497-504.
5. Johnson LA, Flook JP, Hawk HW. Sex selection in rabbits. Live births from X and Y sperm separated by DNA and cell sorting. Biol Reprod. 1989; 41: 199-203.
6. Johnson LA, Welch GR, Keyvanfa K, *et al.* Gender preselection in humans. Flow cytometric separation of X and Y spermatozoa for the prevention of X- linked disease. Hum Reprod. 1993; 8: 1733-9.
7. Ericsson RJ, Langevin CN, Nishino M. Isolation of fractions rich in human Y sperm. Nature (London). 1973; 246: 421-4.
8. Haydyside AH, Kontogianni EH, Hardy K, *et al*. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature (London). 1990; 244: 768-70.
9. Edmonds DK. Dewhursts Textbook of obstetrics and gynaecology, 7th ed. USA: Blackwell publishing; 2007. p. 69-80.
10. World Health Organization. WHO laboratory Manual for the examination human semen and sperm-cervical mucus interaction. 4th ed. Cambridge university press; 1999. p. 1-33.
11. Vermeylen MD, Hooghe T, Debrock S, *et al*. The type of catheter has no impact on the pregnancy rate after

intrauterine insemination: a randomized study. Hum Reprod. 2006; 21: 2364-7.
12. Sorli D. Medical biostatistics and epidemiology. 1st ed. Connecticut, USA: Appleton and Lang; 1995. p. 47-88.
13. Chapman M, Miskry T. The use of intrauterine insemination in Australia and New Zealand. Hum Reprod. 2002; 17(4): 956-9
14. Beernink FJ, Drnowski WP, Ericsson RJ. Sex preselection through albumin separation of sperms. Fertil Steril. 1993; 95: 382-6.
15. Boomsma CM, Heineman MJ, Cohlen BJ, *et al*. Semen preparation techniques for intrauterine insemination. Cochrane Database Syst Rev. 2007; 4:CD0045074.
16. Ahinko K. Successful intrauterine insemination treatment. 1st ed. Finland: University of Tampere press; 2009. p. 24-5.
17. Ding DC, Liou SM, Huang LY, et al. Effects of four methods of sperm preparation on motion characteristics and nitric oxide concentration in laboratory-prepared oligospermia. Zhonghua Yi Xue Za Zhi (Taipei). 2000; 63(11): 822-7.
18. Mohamed EE, Mohamed MA. Effect of sperm chromatin condensation on the outcome of intrauterine insemination in patients with male factor infertility. J Reprod Med. 2012; 57(9-10): 421-6.
19. Nuojua-Huttunen S, Gissler M, Martikainen H, *et al*. Obstetric and perinatal outcome of pregnancies after intrauterine insemination. Hum Reprod 1999; 14(8): 2110-5.
20. Elnashar AM. Intrauterine insemination. MEFSJ. 2004; 9(2): 101-6.
21. Ombelet W, Campo R, Bosmans E, et al. Intrauterine insemination (IUI) as a first-line treatment in developing countries and methodological aspects that might influence IUI success. ESHRE Monogr. 2008; 1: 64-72.
22. Al-Dujaily SS, Abo-Regheef MJ. Correlation between *in vitro* preparation technique, endometrial thickness, hormonal profile and pregnancy rate with IUI outcome. Iraqi J Emb Infertil Res. 2012; 2(4): 34-8.
23. Guzick DS, Carson SA, Coutifaris C, *et al*. Efficacy of superovulation and intrauterine insemination in the treatment of infertility. National Cooperative Reproductive Medicine Network. N Engl J Med. 2005; 340: 177-83.
24. Wang B, Hu Y, Sun H, et al. Investigation of correlative factors affecting successful intrauterine insemination. Zhonghua Nan Ke Xue. 2004; 10(7): 526-9.
25. Sallam HN, Makrigiannakis A. Infertility and assisted reproduction. Cambridge: Cambridge University Press: 2008. p. 416-27.
26. Manigart Y, Rozenberg S , Barlow P, et al. ART outcome in HIV-infected patients. Hum Reprod. 2007; 21(11): 2935-40.
27. Cohlen B, Cantineau A, and te Velde E. Multiple pregnancy after assisted reproduction. Lancet. 2005; 366: 452-3.
28. Gleicher N, Oleske DM, Tur-Kaspa I, *et al*. reducing the risk of high-order multiple pregnancy after ovarian stimulation with gonadotropins. *N Engl J Med* 2000; 343: 2213-7.

Correspondence to Prof. Dr. Saad S. Al-Dujaily
E-mail: aldujaily8@yahoo.com
Received 26th Sep. 2013: Accepted 15th Jan. 2014

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 adding Neostigmine to Lidocaine on the Onset of Epidural Anaesthesia

Sabah N. Al-Sa'ad[1] *FICMS CABA*, **Tariq T. Atta**[2] *CABA*, **Zinah M. Mnati**[2] *FICMS*

[1]Dept. of Anaesthesia & Intensive Care Unit, Baghdad Teaching Hospital, Medical City, [2]Dept. of Anaesthesia & Intensive Care Unit, Al-Yarmouk Teaching Hospital, Baghdad, Iraq.

Abstract

Background Shortening the onset time of sensory block is a practical goal to improve the quality of epidural anesthesia. The addition of Neostigmine to a local anesthetic solution is one of the ways used during epidural anesthesia to perform this goal.

Objective To examine the onset time of sensory block and intensity of motor block during epidural lidocaine anesthesia with and without Neostigmine addition to the epidural solution and to compare haemodynamic changes and any associated side effects.

Methods We made two groups of twenty patients, each of both the sexes ranging from 20-80 years age group of American Society of Anaesthesia (ASA) Grade I & II, selected for abdominal surgery; group I (epidural administration of 17 ml of 2% Lidocaine plus 1 ml of normal saline); group II (epidural administration of 17 mL of 2% Lidocaine plus 500 µg Neostigmine in 1 ml of normal saline). The sensory block was assessed by pinprick method; the motor block was assessed by using Bromage scale. The hemodynamic changes, post epidural shivering, and side effects of epidural Neostigmine were also recorded.

Results The onset time of sensory block up to T_{10} dermatome was significantly more rapid in the group II (8.95±2.44 minutes) than that of the group I (25±4.32 minutes). The upper level of sensory block was also significantly higher in group II, regarding intense motor block it was significantly in group II (13.11± 5.52 minutes) while in group I it was 30.2± 6.4 minutes; this represents the stage of just being able to flex knee but full flexion of foot (Bromage Scale). Post epidural arterial blood pressures and heart rates were not statistically different between both groups. No significant difference was also noticed considering associated side effect (nausea, vomiting, hypotention and shivering).

Conclusions Addition of Neostigmine 500µg to 2% Lidocaine shortened the onset of sensory block with rapid cephaled spread with more potent motor block without increasing side effect.

Key Words Epidural, Neostigmine, Lidocaine, Onset of sensory block and Motor block.

List of Abbreviation: ASA: American society of Anesthesia, L_2:Second lumbar dermatomal nerve supply, L2: Second lumber inter-vertebral space, L3: Third lumber inter-vertebral space, L4: Fourth lumber inter-vertebral space, LA: Local anesthetic, S_5: Fifth sacral dermatomal nerve supply, T_{10}: Tenth thoracic dermatomal nerve supply, T_6: Sixth thoracic dermatomal nerve supply.

Introduction

Local anaesthetic agents can produce unwanted side effects such as motor and autonomic block. Their onset may be slow and have limited duration of action. At higher doses, there is a risk of cardiotoxicity and central nervous system side effects. For these reasons, other drugs are sometimes co-administered to utilize their synergistic analgesic properties and to limit the local anesthetic dose requirement [(1)]. A variety of drugs have been studied more recently to try to improve the quality of neuraxial blockade, and speed the onset of action. Neostigmine, a cholinesterase inhibitor, is more recent addition to the list of epidural

anesthesia for analgesia. Recently, epidural Neostigmine was studied for analgesia during labor [2-4]. It acts by inhibiting acetyl cholinesterase and preventing the breakdown of acetylcholine, increases the concentration of acetylcholine available to bind muscarinic and nicotinc receptors, in the dorsal horn of spinal cord provides analgesia, and also it enhances the duration and intensity of epidural anesthesia [5].

All previous studies were designed to evaluate the effects of adding Neostigmine to mixture of Lidocaine for epidural analgesia. The onset of action is 10-15 minutes [6] (10-20 minutes) [7]. Alkalizations of local anesthetic solutions has also been used to increase the speed of onset of local anesthetic by increasing the concentration of the nonionic form of the drug; more drugs are available to penetrate the lipid nerve cell membrane to produce more rapid intramural diffusion [5].

Methods

This prospective randomized clinical study was conducted at Al-Yarmouk Teaching Hospital, Baghdad, Iraq in the period of 1st of November 2012 to 1st of March 2013 on 40 patients ASA classes I, II of either sex, age ranged 20-80 years old, height 160-180 cm. and weight 60-100 Kg. Scheduled for elective operation under epidural anaesthesia.

Selection of subjects was made after excluding patients who had: absolutecontra indications for epidural anaesthesia such as, coagulation disorder, spine deformities, allergy or anaphylaxis to drugs, history of drug abuse, psychological disorder, and uncooperative patients. The patients were divided into 2 groups each group included 20 patients:

Group I received 2% Lidocaine hydrochloride 17 ml with 1 ml normal saline.

Group II received 2% Lidocaine hydrochloride 17 ml +Neostigmine 500 µg in 1 ml normal saline.

A complete preanaesthetic evaluation was carried out, baseline pulse rate, blood pressure, ECG, SPO_2 were recorded. All patients were preloaded 15 minutes prior to epidural anesthesia with 500 ml ringers lactate solution. No premedication was given to the patients. The epidural procedure was done while the patients were in sitting position, under full aseptic technique and after skin infiltration with 2% plain Lidocaine, then epidural block was performed at level (L_2 – L_3) or (L_3 – L_4) interspaces with a touhy needle size 16 then epidural catheter was advanced 5 cm into the space, the test dose with separated syringe from main dose contain 3 ml 2% Lidocaine with 15 mcg epinephrine (1:200,000) was 1st administrated to exclude possible of occurrence of accidental intrathecal or intravenous injection and then followed after 3 minute interval by main dose. Patient's age, weight, height, and duration of surgery were recorded, patients were then observed for the following:

1. Time of drug administration (test dose, main dose).
2. Time of onset of sensory block at several level dermatomes (S_1, S_5, L_2, T_{10}, T_6).
3. Time of motor block.
4. Intraoperative vital parameters.

The sensory block was assessed by pin prick method at 2 minutes intervals for 20 minutes, using 21 gauge needles in cephalic to caudal fashion along the left anterior axillary line by a blinded observer. The onset of sensory block was defined as loss of sensation to a bilateral pin prick which was tested every 2 minutes at level of dermatomes mentioned above. Time of maximum cephalic spread was defined as time from onset of analgesia up to highest level of sensory analgesia achieved. The time of occurrence of motor block was assessed using bromage scale (Table 1) [8]. Surgery was permitted only when the block was adequate in density and spread an upper sensory level of T_6 and lower S_5 were considered to be appropriate. Any need to intravenous sedation or analgesia was recorded. Side effect such as nausea and vomiting, shivering were recorded during surgery. Fluid management was done according to requirements including fluid deficit, maintenance, and blood loss.

Table 1. Bromage Scale

No.	Grade of Motor Block	Degree of Motor Block
1	Full flexion of knee and foot	No Block
2	Just able to flex knee but full flexion of foot possible	Partial Block
3	Unable to flex knee but flexion of foot possible	Almost Complete Block
4	Unable to flex knee and foot	Complete Block

Results

With taking in consideration patients' age (years), weight (Kg), and height (cm), ASA status, surgical time and the distribution of the surgical procedures, demographic data were compared in both two groups; there was no significant difference between them.

Anesthetic characteristics of the two groups regarding the onset time of sensory block up to T_{10} dermatome was significantly more rapid in group II as shown in Table 2 and Fig. 1. Also the time to maximum cephalic spread to the level of T_6 was also significantly more rapid and intense in group II; there was no excessively higher block in either group.

Table 2. Sensory Block in Different Times

Level of Dermatome	Group	Mean±SD	*P* Value
S_5	I II	20.0±3.74 6.53±2.39	0.002*
S_1	I II	15.0±3.22 4.63±2.41	0.003*
L_2	I II	10.0±2.44 3.65±2.09	0.009*
T_{10}	I II	25.0±4.32 8.95±2.44	0.008*
T_6	I II	32.0±4.53 13.26±3.43	0.016*

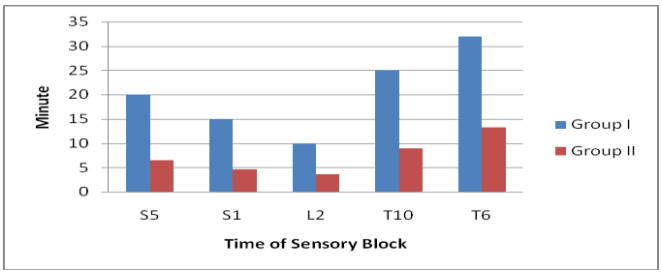

Fig. 2. Time of Sensory Block in Different Times

Regarding motor block, which was assessed by Bromage Scale, the motor block was significantly more intense and there was loss of motor function in group II as shown in table 3 and Fig. 2.

Bromage Scale	Group	Mean±SD	*P* value
Full flexion of the knee	I II	25.23±4.12 10.4±2.83	0.034*
Just able to flex knee but full flex foot	I II	30.2±6.4 13.11±5.52	0.020*
Unable to flex knee but full flex foot	I II	33.4±7.22 16.74±6.73	0.008*
Unable to flex knee and foot	I II	42.0±8.32 22.42±7.03	0.002*

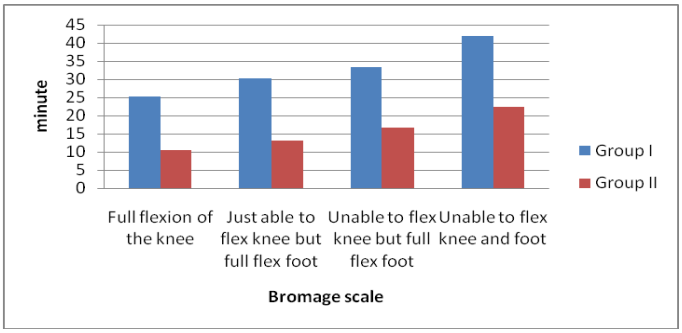

Fig. 2. Assessment of Motor Block in Different Times

The haemodynamic changes regarding arterial blood pressure and heart rate show no difference between both groups as demonstrated in the table 4 and 5.

Regarding associated side effects, there was no significant difference between both groups, in fact 4 patients complain from nausea and they have received IV medication (metoclopramide, dexamethasone, and ranitidine), 2 patients complain from vasovagal attack and they have received IV atropine. The incidence of hypotension was less the same in both groups, only 2 patients received 6mg IV ephedrine no

vomiting and no shivering was recorded in both groups (Table 6).

Table 4. Arterial Blood Pressure Monitoring in Different Times

Systolic Blood Pressure	Group I Mean±SD	Group II Mean±SD
Pre-induction	130.43±23.66	140.79±27.03
After 5 minutes	140.54±21.7	136.47±24.45
After 10 minutes	130.49±18.84	130.74±24.22
After 30 minutes	120.86±21.65	129.47±21.97
Diastolic blood pressure	**Group I**	**Group II**
Pre-induction	80.23±9.7	78.05±11.54
After 5 minutes	60.22±6.88	74.37±9.69
After 10 minutes	72.12±7.34	70.95±9.94
After 30 minutes	74.22±6.4	71.74±12.19

Table 5. Pulse Rate in Different Time Intervals

Pulse rate	Group I Mean±SD	Group II Mean±SD
Pre-induction	80.30±13.55	80.21±14.44
After 5 minutes	84.86±16.44	85.32±17.22
After 10 minutes	88.23±17.43	80.53±15.66
After 30 minutes	84.55±15.34	77.74±14.56

Table 6. Associated Side Effect

Complication	Group I		Group II	
	Count	%	Count	%
Hypotension	2	20	2	20
Vasovagal Attack/Bradycardia	0	0	2	20
Nausea	2	20	2	20
Vomiting	0	0	0	0
Shivering	0	0	0	0

Discussion

The result of our study shows that the addition of 500 µg Neostigmine as adjuncts with Lidocaine in Epidural anaesthesia reveals significant finding regarding accelerating the onset of sensory block and intensity of motor block.

The mechanism by which Neostigmine speed the onset in epidural anesthesia is not clear. As we know, alkalization of the local anesthetic solutions is known to shorten the onset time of sensory block [(5)]. The pH values of the 2% Lidocaine solutions used in this study, Lidocaine and Neostigmine, normal saline-Lidocaine solutions, were not different. Therefore, the pH changes cannot explain this result. Neostigmine is an anticholinesterase drug and several studies have demonstrated that the use of epidural Neostigmine is associated with less adverse effects and the proposed mechanism of analgesia is by drug spreading into cerebrospinal fluid (CSF) at the rate of 1/10th the epidural dose [(9-11)].

Other studies show the effect of using Neostigmine in intravenous regional anaesthesia (IVRA), the addition of Neostigmine in (IVRA) produced significantly reduced onset times of sensory and motor blocks while prolonging their recovery times. These findings are in agreement with those of Turan et al. [(12)]. However, McCartney et al [(13)] observed merely a reduced motor block onset time in their Neostigmine group. Prolongation of the sensory block may be related to the newly discovered acetylcholine-mediated sensory regulatory mechanism controlled by the motor system [(14)], and the prolonged motor block may be the result of the nicotinic agonistic effect of Neostigmine at the neuromuscular junction [(15,16)].

Lauretti et al, have proven that epidural Neostigmine in lignocaine produces dose independent analgesia [(11)].

Chittora et al in their study have concluded that epidural Neostigmine with lignocaine at a dose of 100 mg provides prolonged analgesia with lesser adverse [(17)]. We found much difference in the onset of anaesthesia between the two groups, which was not comparable to onset time recorded by Harjai et al who used 100 µg and 200 µg of Neostigmine and showed no much difference in onset of sensory block with control group in their study; mean time of onset was found among three groups (Mean sensory block in control group was 8.33 ± 0.48, 100 µg Neostigmine 8.50 ± 0.78 and in 200 µg Neostigmine 8.60 ± 0.77) .The average level of sensory block was around T8 [(18)].

In this result the time to maximum cephaled spread was definitely shortened in group II and it was statically significant, while in Kiran et al study [19], the time of maximum cephaled spread was also shortened but statically not significant. The intensity of motor block as shown in our study was significantly more potent in Group II, in comparison to other study, which was done by Chittora et al shows using Neostigmine induce more potent analgesia in epidural anesthesia [17]. The mechanism by which Neostigmine acts to speed the onset of sensory block is not clear; this may be due to synergistic effect between Lidocaine and Neostigmine, as Neostigmine being a quaternary amine, it does not cross blood-brain-barrier and by intrathecal (IT) route provides analgesia via M1 and M2 receptors in the spinal cord, inhibiting the breakdown of acetyl choline (ACh) [20], ACh induces analgesia by increasing cyclic guanidino-mono phosphate by generating nitric oxide [21], autoradiographic studies have shown muscarinic binding in substantia gelatinosa and to a lesser extent in lamina 2 and lamina 5 of dorsal gray matter of spinal cord [22]. Neostigmine also displays peripheral and supraspinal analgesic activity, however the dose necessary to achieve this seems to be higher [23].

Kirota et al reported that Lidocaine dose-dependently inhibited the cyclic adenosine monophosphate formation in Chinese hamster ovary cells [24]. Moreover, Li et al showed that Lidocaine inhibited both substance P binding and substance P–evoked increases in intracellular calcium [25]. Therefore, the combination of local anesthetics and Neostigmine may effectively inhibit multiple areas of neuronal excitability. The changes in vital parameters of both cardiovascular and respiratory system by different doses of neostigmine with lignocaine were studied by Altintas, Klamt and Minovsky their results correlate well with our studies, as heart rate, blood pressure and respiratory rate, remained stable [26,29]. The associated incidence of nausea and vomiting in our study was remarkably reduced about 2% in comparison to Kirota et al study [24] which was (5-10%) while it was the same as our study in comparison with the study of Kiran et al which was (2%) [19].

In conclusion, addition of Neostigmine 500 µg to 2% Lidocaine shortened the onset time of sensory block, rapid cephaled spread with more potent and more rapid onset motor block without significant increase in side effect.

We recommend to study a larger number of patients with longer duration of monitoring to evaluate the analgesic and sedative effect of Neostigmine as adjuncts in epidural anaesthesia through the use of the drug in different doses and to encourage the use of Neostigmine as adjuncts in epidural anaesthesia for its efficacy in speed the onset of sensory block and its potent motor block.

Acknowledgments

The authors are grateful to Dr. Haider F. Ghazi in the Department of Microbiology, Al-Nahrain College of Medicine for his help in statistical study, and to the nursing staff of Al-Yarmouk Teaching Hospital for invaluable assistance and finally thanks to Dr. Israa Al-Ani whose her comments improved the quality of this work.

Author contributions

Study conception and design by Dr. Sabah N. Al-Sa'ad and Dr. Zinah M. Mnati, acquisition of data by Dr. Tariq T. Atta and Dr. Zinah M. Mnati., and analysis and Interpretation of data by Dr. Zinah M. Mnati.

Conflict of interest

The authors declares no conflict of interest.

Funding

By Al-Yarmouk Teaching Hospital and Iraqi Council of Medical Specialization.

References

1. Marri SR. Adjuvant agents in regional anaesthesia. Anaesth & Inten Care Med. 2012; 13: 559-62.
2. Roelants F, Lavand'homme PM. Epidural neostigmine combined with sufentanil provides balanced and selective analgesia in early labor. Anesthesiology. 2004; 101: 439-44.

3. Roelants F, Rizzo M, Lavand'homme PM. The effect of epidural neostigmine combined with ropivacaine and sufentanil on neuraxial analgesia during labor. Anesth Analg. 2003; 96: 1161-6.
4. Roelants F, Lavand'homme PM, Mercier-Fuzier V. Epidural administration of neostigmine and clonidine to induce labor analgesia. Anesthesiology. 2005; 102: 1205-10.
5. Khangure N. Adjuvant; Agents in Neuraxial Blockade. Anaesthesia Tutorials of the Week 230. 2011. URL: http://www.frca.co.uk/Documents/230%20Neuraxial%20adjuvants.pdf. [accessed September 2013]
6. Morgan GJ, Mikhail MS, and Murray MJ. Clinical Anesthesiology. New York: Mc Graw Hill; 2013. p. 958-9.
7. Brown DL, Boezaart AP, Galway UA, et al. Atlas Regional Anesthesia. Philadelphia: Saunders Elsevier; 2010. p. 273-4.
8. Steven MY, Nicholas PH, Gary BS. Anaesthesia and Intensive Care A–Z. London: Churchill Livingstone; 2009. p. 498-9.
9. Batra YK, Arya VK, Mahajan R, et al. Dose response study of caudal neostigmine for postoperative analgesia in paedatric patients undergoing genitourinary surgery. Paediat Anaesth. 2003; 13: 515-21.
10. Turan A, Memis D, Basaran UN, et al. Caudal ropivicaine and neostigmine in paediatric surgery. Anesthesiology. 2003; 98: 719-22.
11. Lauretti GR, De OR, Reis MP. Study of three different doses of epidural neostigmine coadministered with lidocaine for postoperative analgesia. Anesthesiology. 1999; 90: 1534-8.
12. Turan A, Karamanlyoglu B, Memis D, et al. Intravenous regional anesthesia using prilocaine and neostigmine. Anesth Analg. 2002; 95: 1419-22.
13. McCartney CJ, Brill S, Rawson R, et al. No anesthetic or analgesic benefit of neostigmine 1 mg added to intravenous regional anesthesia with lidocaine 0.5% for hand surgery. Reg Anaesth Pain Med. 2003; 28: 414-7.
14. Chiou-Tan FY, Chiou GC. Contribution of circulating acetylcholine to sensory nerve conduction augmentation. Life Sci. 2000; 66: 1509-18.
15. Stoelting RK and Hillier SC. Pharmacology and physiology in anesthetic practice. Philadelphia: Lippincott Williams & Wilkins: 2006. p. 251-75.
16. Sethi D, Wason R. Intravenous regional anesthesia using lidocaine and neostigmine for upper limb surgery. J Clin Anesth. 2010; 22(5): 324-8.
17. Chittora SP, Ahmed F, Sharma R. A comparative analysis of neostigmine as an additive to lignocaine for postoperative analgesia in intrathecal and epidural anesthesia. Indian J Anaesth. 2003; 47(3): 185-89.
18. Harjai MM, Chandra GS, Bhatia VK, et al. A comparative study of two different doses of epidural neostigmine coadministered with lignocaine for post-operative analgesia and sedation. J Anaesthesia Clin Pharmacol. 2010; 26(4): 461-4.
19. Kiran MD, Sanikop CS, Koutuy PF. Randomized control trail of neostigmine and placebo as adjuvants to compound mixture of lignocaine and bupivicaine for epidural analgesia in lower abdominal surgeries. Indian J Anaesth. 2006; 50(6): 448-51.
20. Lauretti GR, Reis MP, Prado WA, et al. Dose response study of intrathecal morphine versus intrathecal neostigmine: their combination or placebo for postoperative analgesia in patients undergoing anterior and posterior vaginoplasty. Anesth Analg. 1996; 82: 1182-7.
21. Iwamoto ET, Marion L. Pharmacologic evidence that spinal muscarinic analgesia is mediated with an L1 arginine/nitric oxide/cyclic GMP cascade in rats. J Pharmacol Exp Ther. 1994; 271: 601-8.
22. Wansley JK, Lewis MS, Young WS, et al. Autoradiographic localization of muscarinic cholinergic receptors in rat brain stem. J Neurosci. 1981; 1: 176-91.
23. Lauretti GR, De OR, Perez MV, et al. Postoperative analgesia by intraarticular and epidural neostigmine following knee surgery. J Clin Anaesth. 2000; 12: 444-8.
24. Kirota K, Appadu BL, Grandy DK, et al. Effect of lidocaine on cAMP formation with recombinant opioid receptors expressed in Chinese hamster ovary cells. Anesthesiology. 1998; 89: A801.
25. Li YM, Wingrove DE, Too HP, et al, Local anesthetics inhibit substance P binding and evoked increases in intracellular Ca^{2+}. Anesthesiology. 1995; 82: 166-73.
26. De Rosa RC, De Robertis EM, Lanza AF, et al. Is the use of intrathecal neostigmine for postoperative analgesia worthwhile. Br J Anaesth. 1996; 76(2): A231.
27. Minovsky CH, Popava S, Milinich A, et al: Neostigimine methylsulphate as an additive in spinal and extradural anaesthesia : Comparative evaluation of analgesia duration and some side effects. Br J Anaesth .1996; 76(2): A229.
28. Altintas F, Bozkurt P, Utku T, et al. Significant sweating due to intrathecal neostigmine when used in adjunct to bupivacaine. Br J Anaesth. 1996; 76(2): A230.
29. Klamt JG, Slullitel A, Garcia IV, et al. Postoperative analgesic effect of intrathecal neostigmine and its influence on spinal anaesthesia. Anaesthesia. 1997; 52: 547-51.

Correspondence to Dr. Zinah M. Mnati
E-mail: zeena_majid@yahoo.com
Received 26th Sep. 2013: Accepted 16th Feb. 2014

Iraqi JMS

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

Early Laparoscopic Cholecystectomy in Acute Cholecystitis at Al-Kadhimiya Teaching Hospital

Osama M. Alabid *FRCS*, **Hassan A. Hassan** *FRCS*

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

Abstract

Background Despite the well-accepted success of laparoscopic cholecystectomy (LC) in the elective treatment of symptomatic gallstone, the safety and the efficacy of this technique has been subjected to some debate in the setting of acute cholecystitis (AC).

Objective To evaluate our institution's experience with early LC and to evaluate the safety and effectiveness of LC in the treatment of AC.

Methods Eighty nine patients were diagnosed as having AC based on the clinical, laboratory and ultrasound findings; 80 patients were divided randomly into two equal groups. Group 1 included 40 patients who had early LC for AC within one week from onset of the symptoms and group 2 included 40 patients who had late LC around 6 weeks from onset of symptoms as interval LC after conservative treatment.

Results No significant difference in the conversion rate (in early group 8 patients (20%) versus delayed group 6 patients (15%). Complication rate was insignificant (in early group 4 patients (10%) versus delayed group 3 patients (7.5%). The delayed group had a significantly shorter operative time (early group = 128±53.5 min versus delayed group = 107±50.1 min) and significantly shorter postoperative stay (early = 2.4±3.2 days versus delay = 1.4±1.4 days). The early group had a significantly shorter total hospital stay (early = 5.5±3.1 days versus delay = 8.5±4.5 days). The male gender had a significant higher conversion rate in both groups.

Conclusion Early LC can be performed safely in most patients with AC and it is considered as effective treatment, allows significantly shorter total hospital stay with no significant differences in conversion rate or complications compared with delayed LC, in the hands of a safe and well trained surgeon.

Keywords Early laparoscopic cholecystectomy, acute cholecystitis.

List of abbreviation: LC = laproscopic cholecystectomy, AC = acute cholecystitis, ACC = acute calculus cholecystitis, GB = gall bladder, BMI, body mass index

Introduction

Laparoscopic cholecystectomy (LC) became the gold standard treatment for symptomatic cholecystolithiasis, but the appropriate timing for LC in acute cholecystitis (AC) remains controversial [1,2]. Although a wide range of surgeons prefers the delayed policy of operation, however a considerable number of reports and randomized trials on the role of early LC in AC (within one week of onset of symptoms), have shown that it is a feasible and safe procedure, with shorter total hospital stay [3-5].

In spite of distorted biliary anatomy, inflammatory edema [6], adhesions and difficult dissection accompanying AC, which may increase complications and conversion rate, and led to consider AC as a relative or absolute contraindication to early LC [7], but with better experience, training and new technologies have

widened the range of AC management to include LC [8-11].

It was found in many studies that LC within 24, 48, and 72 hours from onset of AC, was associated with reduction in total hospital stay and operative time, without change in complications or conversion rate in comparison with delay LC, and avoid the risk of failed conservative treatment [12-14].

Many published studies showed that about (35-58%) of patients with acute calculus cholecystitis (ACC) were readmitted as emergencies, some with biliary pancreatitis, necessitating laboratory, X-ray investigation and high costs, in the conservative treatment period [15,16]. In addition to "patients suffering, loss of work hours and income, and the effect on the community as a whole". This philosophy of delayed treatment denies those patients presenting with AC from the advantages of early laparoscopic approach.

The aim of this study is to demonstrate the safety and efficacy of early LC in ACC at Al-Kadhimyia Teaching Hospital.

Methods

A prospective randomized clinical trial was conducted at the department of surgery at Al-Kadhimiya Teaching Hospital, Baghdad-Iraq, between January 2010 and February 2012. Eighty nine patients diagnosed as an acute cholecystitis were enrolled in this study.

The diagnosis of AC was based on the following diagnostic criteria. Right upper quadrant or epigastric acute abdominal pain, with tenderness under the right costal margin and localized peritoneal signs with or without fever (≥ 37.5 °C) and/or leukocytosis more than 10,000/mm³, ultrasonographic features suggestive of inflammation demonstrating gallstones, gallbladder (GB) wall thickness > 5 mm, edematous wall, GB distension, pericholecystic fluid collection and positive ultrasonographic Murphy's sign. The diagnosis of AC was finally confirmed by histopathological examination of the excised GB. These were inclusion criteria for our study.

Exclusion criteria were, age older than 70 year, no documented gallstones, those who had obstructive jaundice, biliary pancreatitis and those with comorbid diseases, which may need intensive care unit after the operation. By these criteria, 9 patients were excluded.

The remaining 80 patients were divided into two groups based on the length of time from onset of acute symptoms to surgical intervention. Forty patients had "Early" LC (group 1) within one week from onset of symptoms and 40 patients underwent "interval" LC (group 2) performed around 6 weeks from onset of symptoms.

Informed consent was obtained, and both groups admitted to the surgical ward were initially treated conservatively with medical treatment which included nil by mouth, intravenous fluid, parenteral third-generation cephalosporines and metronidazole, these agents were continued for at least 24 hours postoperatively in group 1. While in group 2, patients were discharged after improvement to arrange for interval LC after around 6 weeks. Postoperatively in both groups, the patients were advised to come for follow up after 7 days, 1 month and 3 months after discharge.

Standard four-trocar technique was employed for LC in both groups. Dissection of the related structures to the GB went smoothly in some cases because of tissue edema. In others, difficulties were encountered in the form of adhesions between omentum and GB, duodenum and GB, dissection of cystic duct and artery in Callot's triangle or the bed of GB. For that reason; modifications were used in some operations of early group to make exposure and dissection of GB easier, as aspiration of GB contents and the use of sharp grasper to retract the thick GB wall.

Statistical Analysis

Variables were compared using Student's *t* test and data were presented as mean ± standard deviation. Statistical analysis was performed using chi-square test, and Fisher's exact test

using SPSS version 10. Significant results were considered when the p value was less than 0.05.

Results

Eighty patients were enrolled in the present study. These patients were divided into two groups; Group 1 included 40 patients diagnosed as having ACC underwent early LC within 1 week from onset of symptoms. Group 2 included 40 patients underwent delayed LC around 6 week from onset of symptoms.

The demographics features of both groups were matched statistically in terms of age, gender and body mass index (BMI). Of the patients, 60 were females (75%) and 20 were males (25%) as shown in table 1.

Table 1. Demographic Features

Characteristics	LC within 1 week N = 40 mean ± SD	LC after 6 week N = 40 mean ± SD	*P Value*
Age (years)	44 ± 16	42 ± 14	0.78
Gender (F:M)	2.3 : 1	4: 1	0.13
BMI	27.9 ± 4.6	28.2 ± 6.9	0.85

LC = laparoscopic cholecystectomy, BMI =body mass index

A statistically significant difference was found between the two groups at the time prior to surgery ($P < 0.05$). Pain, temperature, and the WBC were more in group 1. Moreover the GB wall thickness, and pericholecystic fluid were prominent in group 1 (Table 2).

Table 2. Clinical features and US findings prior to surgery

Parameters		LC within 1 week (N = 40)		LC after 6 week (N = 40)		*P Value*
		No.	%	No.	%	
Upper abdominal pain		40	100	12	30	< 0.05
Fever (37.5 °C)		30	75	4	10	< 0.05
Murphy's sign		25	62.5	5	12.5	< 0.05
WBC > 10×10^9/L		31	77.5	11	27.5	< 0.05
Ultrasound results	Gallstones	40	100	40	100	0
	Thick-wall gallbladder	36	90	20	50	0.01
	Pericholecystic fluid	8	20	2	5	0.01
	Ultrasound Murphy's	25	62.5	5	12.5	< 0.05
History of DM		3	7.5	6	15	0.08
Prior abdominal surgery		16	40	14	35	0.53

LC = laparoscopic cholecystectomy, WBC = White blood cells, DM= diabetes mellitus

Sixty six (82.5%) out of 80 patients underwent LC while open cholecystectomy was done for the rest fourteen patients (17.5%) (8 of them in group 1 and 6 in group 2) without significant difference between the two groups. The mean operative time, including conversions, was 128 ± 53.5 minutes in group 1 and 107 ± 50.1 minutes in group 2 ($P = 0.008$). The mean postoperative stay was 2.4 ± 3.2 days in group 1 and 1.4 ± 1.4 days in group 2 ($P = 0.02$). The mean total hospital stay in group 1 was 5.5 ± 3.1

days compared with 9.5 ± 5.3 days in group 2 (*P* = 0.01) as shown in table 3.

Table 3. Outcome of laparoscopic cholecystectomy

Outcomes	LC within 1 week (N = 40) mean ± SD	LC after 6 week (N = 40) mean ± SD	*P Value*
Patients with complications (No., %)	4 (10%)	3 (7.5%)	0.25
Postoperative stay (days)	2.4 ± 3.2	1.4 ± 1.4	0.02
Total hospital stay (days)	5.5 ± 3.1	9.5 ± 5.3	0.01
Operative time (minutes)	128 ± 53.5	107 ± 50.1	0.008
Conversions (No., %)	8 (20%)	6 (15%)	0.29

LC = laparoscopic cholecystectomy

Adhesions were the commonest cause in group 2, while difficulty in verifying anatomy was the main cause in group 1 as a reason for conversion of the type of operation (Table 4).

Table 4. Reasons of conversion from laparoscopic cholecystectomy to open cholecystectomy

Reasons of conversion	LC within 1 week (N = 40)	LC after 6 week (N = 40)
Adhesion and chronic inflammation	0	6
Difficult anatomy	5	0
Necrotic gallbladder wall	2	0
Bleeding	1	0
Total	8	6

LC = laparoscopic cholecystectomy

Male gender significantly affects the conversion rate of the type of operation (42% in group 1 and 37.5% in group 2 ($P < 0.05$) as shown in table 5.

Table 5. Conversion rate: male versus female

Outcomes	LC within 1 week (N = 40) mean ± SD	LC after 6 week (N = 40) mean ± SD	*P Value*
Male	5 of 12 (42%)	3 of 8 (37.5%)	< 0.05
Female	3 of 28 (10.5%)	3 of 32 (9.5%)	< 0.05

LC = laparoscopic cholecystectomy

Four patients in group 1 and 3 patients in group 2 developed postoperative complication without significant difference between the two groups regarding complications. The complications were 2 cases with respiratory infection (one in each group) and 2 cases of wound infection in group 2, one case of retained common bile duct stone in group 2, one case of subhepatic collection in group 2, one case of liver bleeding in group 1. There was no mortality in this study (Table 6).

Table 6. Complications

Complication	LC within 1 week N = 40	LC after 6week N = 40
Wound infections	2	0
Chest infections	1	1
Retained CBD stone	0	1
Subhepatic collection	0	1
Liver bleeding	1	0
Total	4	3

LC = laparoscopic cholecystectomy, CBD = common bile duct

Discussion

Whether to do open or LC for AC was controversial and a debatable decision between surgeons. However with better training, experience and advanced technology LC rendered a common and preferred policy in the setting of AC [3,4,6-11].

This study aimed to evaluate early vs. late LC for AC, regarding conversion rate, operative time, complications, and total hospital stay. Patients in group 1 were operated within 7 days from the beginning of the attack, we found out that early LC in AC was associated with less total hospital stay, longer operative time, with no significant difference in morbidity or conversion rate, compared to delay LC in AC. There was no mortality in this study.

Al-Mulhim [12] and Madan et al [7] concluded that early LC (within 72 and 48 Hrs) respectively is a safe procedure in most patients, with short total hospital stay (5 days) similar to our result (5.5 days), although their conversion rate was (2.4%), complications (0%) and operative time (105 min), were better than ours (20%, 10%, 128 m) respectively, due to the fact that their operations were done within 72 Hr from onset of symptoms while in our study it was within 7 days. Delay patient presentation, busy elective laparoscopic operating room (as we don't have emergency laparoscopic theater), preoperative anesthetic assessment and other logistic facilities, all were preventing factors for us to perform LC in less than 72 hrs from the time of onset of the attack, which affected our results of LC in AC.

Stevens and colleagues [13], had even better results in total hospital stay (2 day), mean operative time (92 min), without increase in conversion rate (9%) as they operated only during the first 24 Hrs. However, Kolla et al [6] and Lau et al [14], had much higher conversion rate and complications than our study, even though their hospital stay and operative time were less, as shown in table 7.

Table 7. Comparison of our results with other studies

Study	Conversion rate		Mean operative time (min)		Morbidity		Mean hospital stay (days)	
	Early	Delayed	Early	Delayed	Early	Delayed	Early	Delayed
Al-Mulhim [12]	2(2.4%)	8(7%)	105	126	0	7(6%)	5	12.2
Stevens et al [13]	12(9%)	7(6%)	92	95	9(7%)	11(9%)	2	3
Madan et al [7]	0	29%	73	96	0	3	2.1	5.4
Kolla et al [6]	25%	25%	104	93	15%	20%	4.1	10.1
Our study	8(20%)	6(15%)	128	107	4(10%)	3(7.5%)	5.5	8.5

Overall, there was no significant difference in complications and conversion rate between the early and late group LC. Total hospital stay was reduced, despite longer single hospital stay in group 1, which confers socioeconomic and administrative advantages [17,18].

In the United States, the professional consensus is toward early LC. Failure of conservative treatment, recurrent symptoms, longer hospital stay, and greater overall cost, led to attend this policy [7,13]. This approach is also supported by an international consensus published as Tokyo Guideline [19,20], and preferred by a wide range of surgeons [7,9,20,21] except in United Kingdom, where 88% of surgeons still adopting the delay LC policy [22,23].

Moreover, several studies revealed that delay LC is associated with recurrent episodes in 36-58% of cases [24], multiple visits to emergency room, increase total hospital costs [15,16], with increase productive work time losses [25-28].

In conclusion, the outcome of this study showed that early LC is safe and effective treatment for acute calculus cholecystitis and is superior to delay LC in term of reduction in total hospital stay. The patients can undergo LC safely during initial admission without added risk of conversion or complications, but with longer operative time.

Acknowledgment

Our sincere gratitude to the staff of the department of surgery and the hospital directorate in Al-Kadhimiya Teaching Hospital for their great help and cooperation and great appreciation to our resident doctors for their big support in this work.

Author Contribution

The authors share the responsibility in preparing and completing this work.

Conflict of Interest

The authors declare no conflict of interest and any competitive intentions.

Funding

No third party or group participate in funding; the authors depend on their own.

References

1. Buanes T, Mjaland O. Complications in laparoscopic and open cholecystectomy: A prospective comparative trial. Surg Laparosc Endosc. 1996; 6: 266-72.
2. Papi CP, Catarci M, D'Ambrosio L, et al. Timing of cholecystectomy for acute calculus cholecystitis: a meta-analysis. Am J Gastroenterol. 2004; 99: 147-55.
3. Kiviluoto T, Siren J, Luukkonnen P, et al. Randomized trial of laparoscopic versus open cholecystectomy for acute and gangrenous cholecystitis. Lancet. 1998; 351: 321-5.
4. Ji HK, Jeong WK, In HJ, et al. Surgical outcomes of laparoscopic cholecystectomy for sever acute cholecystitis. J Gastrointest Surg. 2008; 12: 829-35
5. 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.
6. Kolla SB, Aggarwal S, Kumar A, et al. Early versus delayed laparoscopic cholecystectomy for acute cholecystitis: a prospective randomized trial. Surg Endosc. 2004; 18: 1323-7.
7. Madan AK, Aliabadi-Wahle S, Tesi D, et al. How early is early laparoscopic treatment of acute cholecystitis? Am J surg. 2002; 183: 232-6.
8. Chandler CF, Lane LS, Ferguson P, et al. Prospective evaluation of early versus delayed laparoscopic cholecystectomy for treatment of acute cholecystitis. Am Surg. 2000; 66: 896-900.
9. Pessaux P, Tuech JJ, Rouge C, et al. Laparoscopic cholecystectomy in acute cholecystitis: a prospective comparative study in patients with acute versus chronic cholecystitis. Sug Endosc. 2000; 14: 358-61.
10. Peng WK, Sheikh Z, Nixon SJ, et al. Role of laparoscopic cholecystectomy in the early management of acute gallbladder disease. Br J Surg. 2005; 92: 586-91.
11. Prakash K, Jacob G, Lekha V, et al. Laparoscopic cholecystectomy in acute cholecystitis. Surg Endosc. 2002; 16: 180-3.
12. Al-Mulhim AA. Timing of early laparoscopic cholecystectomy for acute cholecystitis. JSLS. 2008; 12: 282-7.
13. Stevens KA, Chi A, Lucas LC, et al. Immediate laparoscopic cholecystectomy for acute cholecystitis: no need to wait. Am J Surg. 2006; 192: 756-61.
14. Lau H, Lo CY, Patil NG, et al. Early versus delayed-interval laparoscopic cholecystectomy for acute cholecystitis. Surg Endosc. 2006; 20: 82-6.
15. Rosen M, Brody F, Ponsky J. predictive factors for conversion of laparoscopic cholecystectomy. Am J Surg. 2002; 184: 254-6.

16. Zisman A, Gold-Deutch R, Zisman E, et al. Is male gender a risk factor for conversion of laparoscopic into open cholecystectomy. Surg Endosc. 1996; 10: 892-5.
17. Serralta AS, Bueno JL, Planells MR, et al. Prospective evaluation of emergency versus delayed laparoscopic cholecystectomy for early cholecystitis. Surg Laparosc Endosc Percutan Tech. 2003; 13: 71-5.
18. Chau CH, Siu WT, Tang CN, et al. Laparoscopic cholecystectomy for acute cholecystitis: The evolving trend in an institution. Asian J Surg. 2006; 29(3): 120-4.
19. Yamashita Y, Takada T, Kawarada Y, et al. surgical treatment of patients with acute cholecystitis: Tokyo Guideline. J Hepatobiliary Pancreat Surg. 2007; 14: 91-7.
20. Lawrentschuk N, Hewitt PM, Pritchard MG. Elective laparoscopic cholecystectomy: implications for prolonged waiting time for surgery. Aust N Z J Surg. 2003; 73: 890-3.
21. Yamashita Y, Takada T, Kawarada Y, et al. surgical treatment of patients with acute cholecystitis: Tokyo Guideline. J Hepatobiliary Pancreat Surg. 2007; 14: 91-7.
22. Cameron IC, Chadwick C, Phillips J, et al. Management of acute cholecystitis in UK: time for a change. Postgrad Med J. 2004; 80: 292-5.
23. Tamim S, Alisdair Mac Donald, Peter S. Chong, et al. Early versus delayed laparoscopic cholecystectomy for acute cholecystitis: a meta-analysis of randomized clinical trials, Am J Surg. 2008; 195: 40-7.
24. Somasekar K, Shanker PJ, Foster ME, et al. Cost of waiting for gallbladder surgery. Postgrad Med J. 2002; 78: 668-70.
25. Johansson M, Thun A, Blomqvist RN, et al. Management of acute cholecystitis in the laparoscopic era: results of a prospective randomized clinical trial. J Gastrointest Surg. 2003; 7: 642-5.
26. Bender JS, Duncan MD, Freewick PD, et al. Increased laparoscopic experience dose not lead to improved results with acute cholecystitis Am J Surg. 2002; 184: 591-4.
27. Tan JT, Suyapto DR, Neo EL, et al. Prospective audit of laparoscopic cholecystectomy experience at a secondary referral center in South Australia. ANZ J Surg. 2006; 76: 335-8.
28. Li VKM, Yum JLK, Yeung YP. Optimal timing of elective laparoscopic cholecystectomy after acute cholangitis and subsequent clearance of choledocholithiasis. Am J Surg. 2010; 200: 483-8.

Correspondence to Dr. Osama Alabid
Phone: +964 781 182 2978
E-mail: oalabid@yahoo.com

Received 22nd Jul. 2013: Accepted 5th Jan. 2014

Iraqi JMS

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

Efficacy of Different Treatment Modalities on Spasticity Management of Spinal Cord Injury Using H-Reflex Study

Mohaimen A. Ridha[1] *MBChB MSc*, **Fakhir S. Al-Ani**[2] *MBChB PhD*

1Neurophysiology Unit, Al-Imamain Al-Khadhimain Medical City, Baghdad, Iraq, 2Dept. of Physiology, Faculty of Medicine, Muta'h University, Jordan

Abstract

Background Spasticity is one of the most frequently observed phenomena after a lesion of the upper motor neuron system. Treatment of spasticity should not be aimed at its complete removal but rather at improving function, easing care or alleviating pain.

Objective To evaluate the effectiveness of oral antispasticity drugs, transcutaneous electrical nerve stimulation and physical therapy alone on the management of spinal cord injury spasticity by using the H-reflex.

Methods Fifty nine patients with traumatic spinal cord injury suffering from spasticity divided into 5 groups (positive controls, group I who were subjected to a regular physical therapy program, group II who were taking the oral anti-spasticity drug (Baclofen) and were performing the same previous physical therapy program, group III who were subjected to transcutaneous electrical nerve stimulation therapy and performed the same previous physical therapy program, group IV who were taking the oral anti-spasticity drug (Tizanidine) and were subjected to the same previous physical therapy program and 31 normal volunteers were studied. Electrophysiologic study of H-reflex including H latency, H duration, H-reflex conduction velocity and H max/M max ratio.

Results Highly significant difference was noticed between the pre- and post-treatment assessments in group I and III in H max/M max ratio.

Conclusion Spasticity can be effectively treated but a multidisciplinary approach is required since it is unusual for a single intervention, such as oral medication or physiotherapy alone, to be the only modality needed.

Keywords H-reflex, Spasticity, Spinal cord injury.

List of Abbreviation: SCI = spinal cord injury, GABA = gamma aminobutyric acid, TENS = Transcutaneous electrical nerve stimulation, CMAP = compound muscle action potential.

Introduction

Spasticity may be defined as a motor disorder characterized by a velocity-dependent exaggeration of stretch reflexes, resulting from abnormal intraspinal processing of primary afferent input [(1)]. Spasticity has severe negative effects on motor performance and quality of life in patients with an upper motor neuron lesion [(3)]. More than 50% of individuals report spasticity secondary to spinal cord injury (SCI) [(2)] which may be due to loss of descending tonic or phasic excitatory and inhibitory inputs to the spinal motor apparatus, alterations in the segmental balance of excitatory and inhibitory control, denervation supersensitivity, and neuronal sprouting [(4)].

Spasticity management often requires multiple interventions such as physical therapy, oral or intrathecal antispasticity medications, local chemical neurolysis with phenol or alcohol, botulinum toxin injections and surgical interventions such as dorsal rhizotomies, nerve root resections, neurotomies and tenotomies [(5)].

Baclofen, a derivative of gamma aminobutyric acid (GABA), is widely used as the first line of pharmacological treatment for spasticity in people with SCI [(6, 7)]. Baclofen, also identified as

Lioresal, crosses the blood-brain barrier more readily than GABA itself and is believed to reduce spasticity by enhancing inhibitory influences on the spinal stretch reflex via increasing presynaptic inhibition [6].

The anti-spasticity effects of tizanidine are thought to be mediated by its α2- adrenergic agonistic properties. This pre-synaptic inhibition of the release of excitatory amino acids in the spinal cord results in an overall inhibitory effect on alpha motor neurons and a clinical reduction in motor reflexes [8].

Physical therapy is one part of the fight against spasticity. Physical treatment modalities that have been used in spastic hypertonia are superficial heat and cold, diathermies, electrical stimulation, implanted spinal stimulation and massage [9].

Transcutaneous electrical nerve stimulation (TENS) is a non invasive, readily applicable method that has few side effects, no drug interactions, no potential toxicity, can be applied by the patient and is less costly in the long term compared with drug treatment [10].

The Hoffmann's reflex (H-reflex) [11] is an electrically elicited spinal monosynaptic reflex, which was originally described by Hoffmann (1910). It is equivalent in many aspects to the monosynaptic reflex elicited by a mechanical tap to the tendon but the stimulus for the H-reflex bypasses the muscle spindle [12]. The H-reflex is believed to be a compound muscle action potential (CMAP) arising from an electrical afferent activation of a monosynaptic reflex arc [13]. The ratio of the peak-to-peak maximum H-reflex amplitude to maximum M-wave amplitude (H/M ratio) provides a measure of motor neuron pool activation and therefore excitability [14].

Methods

This study was performed on 59 spinal cord injured patients with a mean age ± SD = 33.96 ± 11.12 years and a mean height ± SD = 168 ± 6.91 cm with lower limb spasticity who were attending the outpatient clinic of Ibn Al-Quf hospital for spinal cord injuries or were admitted to the same hospital from the period of January to June 2012. All these patients were with traumatic SCI (above L2 segment) and problematic spasticity. Exclusion criteria included those with complications that may increase spasticity (e.g. pressure ulcer), those with systemic diseases that cause peripheral neuropathy (e.g. diabetes), orthopedic problems (e.g. hip dislocation), or neurological problems. All the patients were subjected to complete history taking and thorough clinical examination. Laboratory investigations, including hemoglobin, paced cell volume, fasting blood sugar and renal function tests were done for all the patients.

Additionally, 31 normal volunteers with a mean age ± SD= 38.96 ± 13.24 years and a mean height ± SD = 170.41 ± 5.73 cm were randomly selected and included in the present study. They had no history of any disabling diseases.

All patients were subjected also to the following physical therapy program, one session daily that included massage, passive, assisted active and active range of motion exercises, stretching exercises and gradually strengthening exercises.

The patients were divided into 5 groups considering that some patients were examined more than one time in a way that did not interfere with the results and as follows:

Positive controls: This group included (12) patients who were not taking any type of treatment or performing a regular previous physical therapy program as they are newly admitted to the hospital after stabilization of their injury.

Group I: Included (31) patients who were subjected a regular physical therapy program (massage, passive, assisted active and active range of motion exercises, stretching exercises and gradually strengthening exercises) which was performed as 1 session/day without any other type of treatment.

Group II: Included (30) patients who were taking oral Baclofen (the full therapeutic dose) and

were performing the same previous physical therapy program.

Group III: Included (33) patients who had one TENS session which was applied to the tibial nerve of the spastic lower limbs, each session lasted 10 minutes and was applied once before the test, in addition to the same previous physical therapy program. Zimmer Galva 5 therapies device with high voltage, Rectangular impulse 20 µs, 10 Hz surged vibration program was used for TENS therapy.

Group IV Included (13) patients who had taken Tizanidine 4 mg tablets for only one time 2 hours before performing the test in addition to the same previous physical program.

H-reflex measurements were performed with 4-channels electrodiagnostic apparatus (CMS6600A EMG/EP system). Measurements were made at room temperatures of 20-25 degrees centigrade, with the muscles at rest and the patients in the prone position and the feet suspended over the edge of the couch.

The active electrode was placed over the soleus muscle and the reference electrode was placed over the Achilles tendon. The ground electrode was placed over the calf between the stimulating site at the popliteal fossa and the active electrode. Stimulus pulses of long duration (1 msec) were used to preferentially activate large sensory fibers [15]. The latencies were measured from the stimulus onset to the beginning of the initial deflection of H-reflex; amplitude was measured from peak to peak.

The formula used to calculate H-reflex conduction velocity (HCV) is [19]:

HCV (m/sec) = (distance popliteal fossa to T11 x2) / (H-reflex latency – M latency – 1 msec).

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 16 for Windows [17]. All the results were expressed as mean ± SD. The students (*t*) test was used to evaluate the differences between any two groups. The probability limit (*P* value) of less than 0.05 was considered to be statistically significant for the results under the study. Correlation analysis was performed with Pearson correlation [16].

Results

In this study 31 healthy controls were included with a mean age ± SD = (38.96 ± 13.42 years) and a mean height ± SD = (170.41 ± 5.73 cm). Also, 59 spinal cord injured patients (who were suffering from spasticity, more in their lower limbs) were included in this study with a mean age ± SD = (33.96 ± 11.12years) and a mean height ± SD = (168 ± 6.91 cm). There was no significant difference ($P > 0.05$) regarding age and height between control subjects and patients. Physiological variation in various parameters of H reflex due to sex could not be assessed due to very small sample size of females. Hb, PCV, random blood sugar and renal function test was normal in all the patients. A significant correlation was noticed between the height of control subjects and both the H and M latency. Accordingly, a regression equation was used to predict the optimal H latency and optimal M latency for the patients and controls.

Table 1 shows that there was no significant difference between the control group and patients concerning the H and M latency, H duration, HCV and F wave latency after confirming that any treatment modality did not affect these parameters.

Table 1. Neurophysiologic parameters in SCI patients and controls

Parameter	Control group N = 31 Mean ± SD	SCI patients N = 59 Mean ± SD
H latency (msec)	32.19 ± 1.63	32.62 ± 1.35
M latency (msec)	6.25 ± 0.42	6.39 ± 0.35
H duration (msec)	15.73 ± 2.32	15.02 ± 1.17
HCV (m/s)	53.04 ± 1.84	52.48 ± 2.0
F latency (msec)	39.76 ± 4.73	38.45 ± 2.73

SCI = spinal cord injury

Table 2 shows highly significant improvement in the H/M ratio when comparing the positive controls to the other groups while the H/M ratio in the negative controls shows no significant

difference with group II and group IV and a significant difference with the other groups.

Table 2. H/M ratio in the different treatment groups in comparison with the positive and negative controls.

Group		H/M ratio Mean ± SD
Control group	+ve controls	72.94 ± 12.93
	-ve controls	35.54 ± 18.82**
Group I	Group I	50.94 ± 21.81
	+ve controls	72.94 ± 12.93**
	Group I	50.94 ± 21.81
	-ve controls	35.54 ± 18.82*
Group II	Group II	43.86 ± 17.06
	+ve controls	72.94 ± 12.93**
	Group II	43.86 ± 17.06
	-ve controls	35.54 ± 18.82
Group III	Group III	49.15 ± 21.68
	+ve controls	72.94 ± 12.93**
	Group III	49.15 ± 21.68
	-ve controls	35.54 ± 18.82*
Group IV	Group IV	42.06 ± 20.14
	+ve controls	72.94 ± 12.93**
	Group IV	42.06 ± 20.14
	-ve controls	35.54 ± 18.82

* $P < 0.05$, ** = $P < 0.001$

Discussion

Spasticity is one of the disabling symptoms in patients with upper motor neuron syndromes like spinal cord injury and brain injury [18]. Treatment of spasticity should not be focused on its removal but rather at improving function, easing care or alleviating pain [20].

Because the SCI in this study was traumatic, there will be no reason that the peripheral nerve conduction velocity (NCV) will be affected in those patients. As a result, H-reflex latency, duration, conduction velocity and F-wave latency were not statistically different from the values obtained from healthy controls as the H-reflex latency reflects the fastest conducting fibers, while its waveform reveals the functional status of the remaining slower conducting fibers expressed by the wave duration [13]. The HCV is particularly useful in evaluating spinal cord circuitry in a non-invasive manner as it evaluates the sensory and motor fibers at the same time [13].

H-reflex amplitude is considered to be the index of the activities in the spinal cord as a final common pathway and used to evaluate the effects of the upper spinal organs and the input from sensory systems [21]. The maximal M-wave (M max) amplitude indicates that all the α-motoneurons of the innervated muscles were fired. It may be used as a base to normalize H-reflex amplitude [22].

The heightened H/M ratio observed in the positive controls was similar to many other studies [16,23,24]. Some researchers [25] stated that the cause behind the increase of the motoneuron excitability in upper motor neuron lesion may be attributed to the loss of the supraspinal inhibitory control and similar impulses from interneurons. The results of group (I) was somewhat close to the results of other researchers [16] who showed significant improvement in H/M ratio in patients receiving a full program of physiotherapy only without other interventions for 6 weeks, but didn't reach significance in other parameters. The reduction in the H/M ratio after physiotherapy can be attributed to that the massage technique used in this study activates a wide spectrum of afferents, including both cutaneous and muscular mechanoreceptors, as mentioned in studies dealing with similar manual modalities [26,27]. The role of the cutaneous mechanoreceptors in the amplitude changes of the H-reflex in both neurologically healthy [28] and neurologically impaired persons [29] has been studied. However, the decrease in the H/M ratio noticed in this group may also be attributed to enhancement of muscle contraction by the massage technique and other rehabilitation procedures that led to increase the M amplitude in relation to H amplitude [30]. But, the H/M ratio of group (I) showed a significant difference when compared to the healthy controls value. This finding is in acceptance with the study of Vittorio who noticed that physiotherapy alone in spinal

cord injured patients is not sufficient in the management of focal spasticity [31].

In group (II), the reduction in H/M ratio of this group was close enough to the value of healthy group that there was no significant difference between the two groups ($P > 0.05$). Our results gained agreement with the findings of other researchers [16,10] that showed significant reduction as regards H/M ratio with non significant reduction as regards other electrophysiological parameters. The action of Baclofen, which is widely used as the first line of pharmacological treatment for spasticity in people with SCI, is via its crossing the blood-brain barrier more readily than GABA itself and is believed to reduce spasticity by enhancing inhibitory influences on the spinal stretch reflex via increasing presynaptic inhibition [6]. Additionally, Koella thought that Baclofen have some supraspinal activity in addition to its action at the spinal level to reduce muscle tone [33].

The results of group (III) revealed highly significant improvement in H/M ratio in comparison to the positive control subjects. Some researchers [10,34] found nearly similar findings in agreement with our study, they reported that there was significant improvement of the degree of spasticity both clinically and electrophysiologically (reduction H/M ratio) after application of TENS on the spastic lower. TENS is believed to activate sensory Ia afferent fibers switching on presynaptic inhibition mechanisms leading to reduction in spasticity [35]. Also, TENS has been reported to indirectly affect the sympathetic outflow from the spinal cord by stimulating peripheral afferent fibers which increase the blood flow in the muscles. Increased blood flow in the spastic muscle is reported to improve its efficiency, oxygen uptake, and waist product removal [32]. On the other hand, Improvement in H/M ratio that resulted from TENS program did not reach the level of healthy subjects that may be attributed to the short duration of TENS application used in this study (approximately 10 minutes for only one time) while it was longer and frequent in other studies.

Group (IV) study showed a highly significant reduction in the H/M ratio when compared to the positive control subjects and no significant difference when compared with the negative controls. This effect is consistent with a documented Tizanidine-mediated potentiation of presynaptic inhibition, suppression of flexor reflexes, as well as its direct action on α-motoneurons [36]. Its action arises from agonistic activity of the compound at noradrenergic α2 receptors; resulting in both direct impairment of excitatory amino acid release from spinal interneurons (presynaptic inhibition) and a concomitant inhibition of facilitatory caerulospinal pathways (Cerulospinal tract provides nonspecific activation of the motor neuron pool in anterior horn) that part of the antispastic action of tizanidine may be supraspinal in origin [37].

We conclude from this study that the H-reflex can provide information regarding neural function after spinal cord injury and the H/M ratio can be used as a good indicator for both spasticity assessment and response to treatment. Spasticity can be effectively treated but a multidisciplinary approach is required. TENS is effective, economic, non invasive and readily applicable method that has few side effects but it should only be used as a supplement to other treatment methods in the management of spasticity. Tizanidine hydrochloride is useful in the management of spasticity caused by SCI and can be used as a routine drug treatment although liver function tests should be periodically monitored. So, for better controlling of spasticity, we can use a combination of the three methods (Oral medication, TENS and physical therapy).

Acknowledgment

Many thank to my supervisor Prof. Dr. Fakhir S. Al-Ani for his support and assistance. My thanks extended to my colleagues at the physiology department- College of Medicine, Al-Nahrain University for their support.

Author Contribution

Prof. Dr. Fakhir S. Al-Ani suggests the study and co-wrote the manuscript and Dr. Mohaimen A. Ridha collected and analyzed the data and wrote the paper.

Conflict of Interest

Authors disclose no conflicts of Interest

Funding

The Research was supported by Ibn Al-Quf Hospital for Spinal Cord Injuries.

References

1. Young RR. Spasticity: a review. Neurology. 1994; 44(11 Suppl 9): S12-20.
2. Walter JS, Sacks J, Othman R, et al. A database of self-reported secondary medical problems among VA spinal cord injury patients: its role in clinical care and management. J Rehabil Res Dev. 2002; 39(1): 53-61.
3. Horney T, Kahn G, Jennifer H. Temporal facilitation of spastic stretch reflexes following human spinal cord injury. J Physiol. 2006; 571: 593-604.
4. Rizzo MA, Hadjimichael OC, Preiningerova J, et al. Prevalence and treatment of spasticity reported by multiple sclerosis patients. Mult Scler. 2004; 10(5): 589-95.
5. Jang SH, Ahn SH, Park SM. Alcohol neurolysis of tibial nerve motor branches to the gastrocnemius muscle to treat ankle spasticity in patients with hemiplegic stroke. Arch Phys Med Rehabil. 2004; 85: 506-8.
6. Kirshblum S. Treatment alternatives for spinal cord injury related spasticity. J Spinal Cord Med. 1999; 22: 199-217.
7. Taricco M, Pagliacci MC, Telaro E, et al. Pharmacological interventions for spasticity following spinal cord injury: results of a Cochrane systematic review. Eura Medicophys. 2006; 42(1): 5-15.
8. Gracies JM, Nance P, Elovic E, et al. Traditional pharmacological treatments for spasticity. Part II: General and regional treatments. Muscle Nerve. 1997; 6 (Suppl.): S92-120.
9. Gracies JM. Physical modalities other than stretch in spastic hypertonia. Phys Med Rehabil Clin N Am. 2001; 12: 769-92.
10. Aydin G, Tomruk S, Keles I. Transcutaneous electrical nerve stimulation versus baclofen in spasticity: Clinical and electrophysiological comparison. Am J Phys Med Rehabil. 2005; 84: 584-92.
11. Hoffmann P. Beitrag zur Kenntnis der menschlichen Reflexe mit besonderer Berucksichtigung der elektrischen Erscheinungen. Arch Anat Physiol. 1910; 1: 223-46.
12. Nadeem AS, El-Yassin DI, Al-Ani FS. The H-reflex parameters with age in normal Iraqi infants and children. J Fac Med Baghdad. 2000; 42: 342-54.
13. Kimura J. Electrodiagnosis in disease of nerve and muscle. Principles and practice. 3rd ed. New York: Oxford University Press; 2001. p. 178-214.
14. Aminoff MJ. Electrodiagnosis in clinical neurology. 5th ed. Elsevier, Churchil Living stone. 2005.
15. Burke D, Adams RW, Skuse NF. The effects of voluntary contraction on the H reflex of human limb muscles. Brain. 1989; 112: 417-33.
16. Seliem HA, Nagieb GS, Eliewa EA, et al. Efficacy of different modalities on spasticity management of spinal cord injury: clinical and electrophysiological study. Egypt Rheumatol Rehab. 2007; 34(3): 405-16.
17. Norusis M. SPSS 16.0 Guide to Data Analysis. 2nd ed. New Jersey: Upper Saddle River, Prentice Hall; 2008. p. 1-34.
18. Voerman GE, Gregoric M, Hemens HJ. Neurophysiologic methods for the assessment of spasticity: The Hoffman reflex, the tendon reflex, and the stretch reflex. Disability Rehabil. 2005; 27: 33-68.
19. Troni W. Analysis of conduction velocity in the H pathway: Part 1. Methodology and results in normal subjects. J Neurol Sci. 1981; 51: 223-33.
20. Jellinger KA: Spasticity Management: A Practical Multidisciplinary Guide. Eur J Neurol. 2007; 1: e50-e50.
21. Crone C, Nielsen J. Spinal mechanisms in man contributing to reciprocal inhibition during voluntary dorsiflexion of the foot. J Physiol. 1989; 416: 255-72.
22. Nakazawa K, Miyoshi T, Sekiguchi, H et al. Effects of loading and unloading of lower limb joints on the soleus H-reflex in standing humans. Clin Neurophysiol. 2004; 115: 1296-304.
23. Maupas E, Marque P, Roques CF, et al. Modulation of the transmission in group II heteronymous pathways by tizanidine in spastic hemiplegic patients. J Neurol Neurosurg Psychiat. 2004; 75:130-5.
24. Little JW, Halar EM. H-reflex changes following spinal cord injury. Arch Phys Med Rehabil. 1985; 66: 19-22.
25. Enola RM. Neuromechanical basis of kinesiology. 2nd ed. Champaign: Human Kinetics. 1994. p. 151-91.
26. Leone JA, Kukulka CG. Effects of tendon pressure on alpha motoneuron excitability in patients with stroke. Phys Ther. 1988; 68: 475-80.
27. Belanger AY, Morin S, Pepin P, et al. Manual muscle tapping decreases soleus H-reflex amplitude in control subjects. Physiotherapy Canada. 1989; 41: 192-6.
28. Wolf SL, Minkowitz JA. Topical anesthetics effects on the Achilles tendon and H-reflexes, I: able-bodied subjects. Arch Phys Med Rehabil. 1989; 70: 531-6.
29. Wolf SL, Minkowitz JA. Topical anesthetics effects on the Achilles tendon and H-reflexes, II: stroke patients. Arch Phys Med Rehabil. 1989; 70: 673-7.
30. Frigon A, Carroll TJ, Jones KE, et al. Ankle position and voluntary contraction alter maximal M waves in soleus and tibialis anterior. Muscle Nerve. 2007; 35: 756-66.

31. Vittorio A. Electrical stimulation for modulation of spasticity in hemiplegic and spinal cord injury subjects. Neuromodulation. 2001; 4: 85-92.
32. Abram SE, Asiddao CB, Reynolds AC. increased skin temperature during transcutaneous electrical stimulation. Anesth Analg. 1980; 59: 22-5.
33. Koella WP. Pharmacological aspects of spasticity with special reference to Lioresal. Postgrad Med J. 1972; 48: 13.
34. Joodaki MR, Olyaei GR, Bagheri H. The effects of electrical nerve stimulation of the lower extremity on H-reflex and F-wave parameters. Electromyogr Clin Neurophysiol. 2001; 41(1): 23-8.
35. Lauer RT, Stackouse C, Shewokis PA et al. Assessment of wavelet analysis of gait in children with typical development and cerebral palsy. J Biomech. 2005; 38: 1351-7.
36. Delwaide PJ, Pennisi G. Tizanidine and electrophysiologic analysis of spinal control mechanisms in humans with spasticity. Neurology. 1994; 44(11 suppl 9): S21-S27.
37. Palmeri A, Wiesendanger M. Concomitant depression of locus coeruleus neurons and of flexors reflexes by an α2 adrenergic agonist in rats: a possible mechanism for an α-mediated muscle relaxation. Neuroscience. 1990; 34: 177-87.

Correspondence to Dr. Mohaimen A. Ridha
E-mail: mohaimen79@yahoo.com
Received 18th Oct. 2012: Accepted 2nd Mar. 2014.

Iraqi JMS

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

Ghrelin and Insulin Resistance in a Sample of Iraqi Women with Polycystic Ovary Syndrome

Amal A. Hussein[1] ***BSc*, Rayah S. Baban**[2] *PhD*, **Alaa G. Hussein**[3] *FICMS*

[1]Central Lab., Al-Imamian Al-Kadhimiyain Medical City, [2]Dept. of Chemistry and Biochemistry, [3]Dept. of Pathology and Forensic Medicine, College of Medicine, Al-Nahrain University

Abstract

Background Polycystic ovary syndrome is associated with adiposity and metabolic changes predisposing to insulin resistance and diabetes mellitus. Ghrelin is an appetite-stimulating hormone, which acts through its receptor on the hypothalamus to regulate energy balance and thus plays a major role in the etiology of metabolic diseases.

Objective To investigate the relation between ghrelin hormone and insulin resistance in patients with polycystic ovary syndrome.

Methods Thirty nine women with polycystic ovary syndrome and 30 healthy controls were examined. Fasting ghrelin, insulin, glucose, lipid profile concentrations were determined. Insulin resistance indexes were calculated (HOMA-IR and QUICKI-IR indexes).

Results Serum ghrelin concentration was significantly lower in polycystic ovary syndrome patients than control subjects (235 ± 17.36 pg/ml *Vs* 489.7 ± 53.4 pg/ml). Insulin resistance and BMI were significantly higher in polycystic ovary syndrome than control group.

Conclusion Ghrelin hormone may be used as a new additional marker in the diagnosis of polycystic ovary syndrome. Hyperinsulinaemia and hyperleptinemia are associated features in polycystic ovary syndrome.

Keywords Ghrelin, Obesity, Polycystic ovarian syndrome (PCOS), Body mass index (BMI), Insulin resistance

List of Abbreviation: PCOS = polycystic ovary syndrome, DM = diabetes mellitus, HOMA-IR = Homeostatic Model Assessment of Insulin Resistance, TG = triglyceride, VLDL-C = very low density lipoprotein, HDL-C = high density lipoprotein, BMI = Body mass index, LH = luteinizing hormone, FSH = follicle stimulating hormone

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age with a reported prevalence up to 12% [(1)]. It is a complex multifactorial genetic disorder with dysregulated steroidogenesis [(2)]. The disease is characterized by chronic anovulation, functional hyper-androgenism and polycystic ovaries on ultrasound examination [(3)]. In women with PCOS, the presence of hyperinsulinaemia, dyslipidemia and/or hypertension is associated with obesity. The obesity and PCOS place this group of women at high risk of developing adverse metabolic profiles including insulin resistance [(4)]. Insulin resistance is a metabolic disorder caused by the impairment of insulin function in inducing glucose uptake and utilization [(5)]. As many as 70% of PCOS women are insulin resistant and 10% have diabetes mellitus (DM) [(6,7)].

Ghrelin is a peptide hormone consists of 28 amino acids; it is synthesized mainly in stomach [(8)]. Because circulating levels of ghrelin increases during fasting and decreases rapidly after a

meal, it is believed that ghrelin has a role in acute changes in energy balance and satiety [9]. It is also a pleiotropic hormone that can influence different metabolic functions such as inducing positive energy balance, increasing food intake, promoting enlargement of adipocytes and also releasing growth hormone [10], and so, it is a hormone that strictly related to obesity, insulin resistance and most probably to the reproductive function. Recently accumulating data suggest that ghrelin has central and peripheral effects on glucose regulation and insulin level [11].

As PCOS predisposes to obesity and metabolic changes such as insulin resistance, it may be assumed that ghrelin's function is connected with this syndrome [12,13]. In the other hand, adolescent obese polycystic ovarian syndrome (which is characterized by insulin resistance) patients had lower ghrelin level compared with lean subjects and ghrelin was negatively correlated with Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) [14]. The present study was undertaken to investigate the relation between insulin resistance and ghrelin hormone in a sample of Iraqi women with polycystic ovary syndrome.

Methods

A case control study was carried out from November 2012 till June 2013. A total of 69 women aged between (18-45) years were attending from Al-Imamian Al-Khadhmiyian Medical City and Higher Institute for Infertility and Assisted Reproductive Techniques in Baghdad.

Thirty nine out of 69 women were patients diagnosed by their physicians as polycystic ovary syndrome compared with thirty apparently healthy women who shared as control. Patients and control were with a comparable age.

All disorders that can results in menstrual irregularity and hyperandrogenism, including adrenal or ovarian tumors, thyroid dysfunction, congenital adrenal hyperplasia, hyper-prolactinaemia (caused by pituitary diseases), acromegaly, Cushing syndrome, and other causes of infertility besides PCOS were excluded from the study.

A fasting serum sample was used to determine, glucose, lipid profile including total cholesterol, triglyceride (TG), VLDL-C, and HDL-C [measured by the precipitation of chylomicrons] done using colorimetric enzymatic method using Biomaghreb, Sa, France kit.[15] LDL-C was calculated if TG < 400 mg/dl by the formula of Friedewald *et al.* [16] Body mass index (BMI) was calculated by dividing study subjects weight (Kg) on their height (m^2).

Serum ghrelin concentration was measured using an Enzyme Linked immunosorbant Assay (ELISA) technique; Human ghrelin (ghr) ELISA Kit (DRG Instruments GmbH, Germany). Expected normal concentrations are between (346-719 pg/ml). Serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured by using enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) (Biomerieux, Sa, France) with reference ranges (1.5-8.0) mIU/ml and (3.9-12.0) mIU/ml respectively.

Ethical approval and patient permission were obtained from the local ethics committee to conduct this study.

Regarding Insulin resistance (IR), HOMA-IR index (homeostasis model assessment of insulin resistance) [17] of the PCOS patients was calculated from fasting glucose and insulin levels as the following equation: Fasting serum insulin (mU/l) × fasting plasma glucose (mg/dl) /405. While QUICKI -index index (Quantitative Insulin Sensitivity Check) was used for measurement of insulin sensitivity from the following equation: 1 / (log F. Insulin+log FBG) [18].

Statistical analysis

Data were statistically analyzed by SPSS version 16. All data were presented as a mean ± SE. Statistical differences between data of patients and control groups were determined according to student *t*-test. Correlation between the variables was performed by Pearson's correlation coefficient. *P* values were significant if < 0.05.

Results

Sixty nine women aged between (18-45) years were divided into 2 groups: Patient's group: includes 39 with PCOS and control group includes 30 apparently normal healthy women. This study was design to compare between the level of ghrelin and other biochemical parameters in addition to insulin resistance in PCOS patients and healthy women.

Regarding anthropometric parameters (age and BMI), no significance was found between patients and control ages (P = 0.391), while a highly significant differences was found between their BMI (P < 0.001) (Table 1).

The higher percentage (33.33%) was found among overweight PCOS women with BMI (25-29.9 Kg/m^2) compared with (46.67%) healthy normal women at the same BMI interval, while the lowest percentage was found among those with normal BMI (7.69 %) compared with (40.0 %) healthy subjects at the same BMI interval. In contrast, the lowest percentages regarding healthy subjects were found among obese and very obese BMI with 6.67% for both (Table 2).

Table 1. Comparison between patients and control anthropometric parameters

Anthropometric parameter	Patients N = 39	Controls N = 30	P value
Age (years)	30.60 ± 1.40	32.18 ± 1.19	NS
BMI (Kg/m^2)	31.83 ± 0.82	26.21 ± 0.82	< 0.001

BMI= Body mass index

Table 2 Comparison between patients and control BMI intervals

BMI	Patients N = 39		Control N = 30		Total		*P* value
	No.	%	No.	%	No.	%	
Normal (< 25 Kg/m^2)	3	7.69	12	40.00	15	21.74	0.001*
Over weight (25-29.9 Kg/m^2)	13	33.33	14	46.67	27	39.13	
Obese (30-34.9 Kg/m^2)	11	28.21	2	6.67	13	18.84	
Very obese (35-39.9 Kg/m^2)	10	25.64	2	6. 67	12	17.39	
Morbid obesity (> 40 Kg/m^2)	2	5.13	0	0.00	2	2.90	
Total	30	100	30	100	69	100	
Mean BMI (kg/m^2)± SE	31.83±0.82		26.21±0.82		29.38±0.67		<0.001†

Chi-square test, †Independent sample t-test, SE= Standard error, BMI= Body mass index

In table 3, serum total cholesterol and triglyceride concentrations and atherogenic index (AI) in PCOS group were found significantly higher than in healthy controls with P value = 0.011, 0.004, 0.002 respectively. No significant difference was found between patients and control FBG level in this study.

PCOS LH, TES, and insulin hormone levels were significantly higher *vs.* control with P = 0.008, 0.035, and < 0.001 respectively as shown in table 4, while patients ghrelin level and fasting glucose/insulin ratio were found significantly decreased compared with the control with P < 0.001.

PCOS HOMA-IR was significantly higher compared *vs.* healthy control (5.77±0.69 *vs.* 2.20±0.29; P < 0.001), while their QUICKI -index was significantly lower than found in control women with (0.31± 0.00 *vs.* 0.35 ± 0.00 and P < 0.001) as noticed in table 5.

No significant correlations were found between patient's ghrelin and all biochemical parameters except with HDL-C with P = 0.02 (Table 6), while

their insulin hormone was correlated significantly with IR index ($P < 0.0001$).

Table 3. Comparison between biochemical parameters of patients and healthy control subjects

Parameters	Controls N = 30 Mean ± SE	Patients N = 39 Mean ± SE	P value
FBG (mg/dl)	91.73 ± 7.81	94.05 ± 1.94	0.748
Cholesterol (mg/dl)	157.40 ± 4.58	175.97 ± 5.18	0.011
Triglyceride (mg/dl)	75.23 ± 5.11	100.38 ± 6.81	0.004
HDL-C (mg/dl)	56.70 ± 1.98	53.28 ± 2.11	0.254
VLDL-C (mg/dl)	16.78 ± 1.80	27.13 ± 4.63	0.065
LDL-C(mg/dl)	85.96 ± 3.93	98.91 ± 6.56	0.096
AI	1.55 ± 0.10	2.12 ± 0.15	0.002

FBG= Fasting blood glucose, HDL-C=High density lipoprotein, VLDL-C= Very low density lipoprotein, LDL-C=Low density lipoprotein, AI =Atherogenic index.

Table 4. Comparison between patients and healthy control hormones

Parameters	Control Group N = 3 Mean±SE	Patients Group N = 39 Mean±SE	*P* value
LH (mIU/ml)	4.56 ± 0.52	9.35 ± 1.64	0.008
FSH (mIU/ml)	5.22 ± 0.39	4.59 ± 0.36	0.255
TES (ng/ml)	0.30 ± 0.03	0.94 ± 0.29	0.035
Insulin (µU/ml)	9.32 ± 0.38	25.03 ± 3.09	< 0.001
Ghrelin (pg/ml)	489.70 ± 53.41	235.10 ± 17.36	< 0.001
FBG/Finslin	9.93 ± 0.61	5.38 ± 0.45	< 0.001
FIns×FBG	890.21 ± 115.58	2334.90 ± 280.52	< 0.001

LH= Luteinizing hormone, FSH= Follicle-stimulating hormone, TES = Testosterone hormone, FBG/Finslin= Fasting blood glucose/Fasting insulin ratio, FIns×FBG= Fasting insulin× Fasting blood glucose.

Table 5. Insulin resistance indexes (HOMA-IR index) in the patients and control groups

Insulin resistances indexes	Patients		control		Total		P value
	No.	%	No.	%	No.	%	
Resistant (> 3.5)	24	61.54	0	3.33	24	34.78	
No (< 3.5)	15	38.46	30	96.67	45	65.21	< 0.001
Total	39	100	30	100	69	100	
Mean HOMA- index	5.77±0.69		2.20±0.29		4.21±3.83		< 0.001
Mean QUICKI- index	0.31±0.00		0.35±0.00		-------		< 0.001

HOMA-IR index= Homeostasis model assessment, QUICKI-IR index= Quantitative Insulin Sensitivity Check

Discussion

PCOS is the most common endocrine disorder in women of reproductive age [19]. Obesity by itself represents an unfavorable metabolic state. A high percentage of PCOS patients is indeed overweight or obese (20-85%) [20]. Most of PCOS patients in this study were overweighed (33.33%), and (28.21%) were obese, and lesser

were very obese with (25.64%). These results are in agreement with other data comparing women with PCOS and age-matched controls; the women with PCOS demonstrated a lower proportion of BMI<25 kg/m^2 and higher proportion of BMI > 30 kg/m^2 and 40 kg/m^2 [21].

Also, Moran *et al*. 2010 reported that the severity of obesity, and especially of abdominal fat disposition, seems in proportion to the severity of the clinical and endocrinological manifestations [22].

Table 6. Correlation between patient's insulin and ghrelin hormone levels with some biochemical parameters

Parameters	Correlation parameters	Insulin (µU/ml)	Ghrelin (pg/ml)
FBG (mg/dl)	r	-0.085	0.004
	P	0.609	0.981
Cholesterol (mg/dl)	r	-0.044	-0.109
	P	0.792	0.509
Triglyceride (mg/dl)	r	-0.372*	-0.045
	P	0.020	0.786
HDL-C (mg/dl)	r	-0.065	0.371*
	P	0.694	0.020
VLDL-C (mg/dl)	r	-0.227	-0.103
	P	0.165	0.533
LDL-C (mg/dl)	r	0.144	-0.159
	P	0.382	0.334
AI	r	-0.014	-0.130
	P	0.932	0.429
LH (mIU/ml)	r	-0.195	0.166
	P	0.234	0.312
FSH (mIU/ml)	r	-0.053	-0.147
	P	0.750	0.373
TES (ng/ml)	r	-0.207	-0.120
	P	0.212	0.472
Insulin resistance	r	0.983**	-0.012
	P	0.000	0.943

* $P < 0.05$, ** = $P < 0.01$, LH = Luteinizing hormone, FSH = Follicle stimulating hormone, TES: Testosterone hormone, FBG = Fasting blood glucose, HDL-C = High density lipoprotein, VLDL-C = Very low density lipoprotein, LDL-C = low density lipoprotein, AI = Atherogenic index.

In addition to that, PCOS patients with normal BMI are also found to present with adverse metabolic profiles [23] in this study with BMI < 25 kg/m^2. However, not all obese females have PCOS and not all PCOS patients are obese (Escober and Morreate, 2005) [24].

Obesity is one of the clinical characteristics of the PCOS along with oligomenorrhea, hirsutism, and infertility and it results from a chronic imbalance between energy intake and energy expenditure [25].

Although several studies have shown that women with excess body weight are more likely to have fertility problems [26], and many researchers believed that obesity is more prevalent in women suffering from PCOS [27]. Results of present study showed BMI of PCOS were ranging from normal to over obese.

The cause of obesity in the PCOS patients remains unknown; however, it is thought that obesity may play a pathogenetic role in the development of the syndrome in susceptible individuals [28]. In 2001 in Iraq, Azziz *et al* reported that PCOS patients have a higher body weight than their counterparts. They suggested that this result may due to the different nutritional habits in Iraq than other countries [29]. Their findings were similar to Carmina *et al.,* 2003 [30].

PCOS includes metabolic abnormalities like changes in reproductive hormones, glucose metabolism and lipid profile changes [20]. Levels of total cholesterol, LDL-C, VLDL-C, triglyceride and atherogenic index (AI) were significantly increase in PCOS patients sera more than control (table 3), while the HDL-C was significantly decrease. This result was agreed with Moran *et al.,* 2010 whom concluded it may potentially cause cardiovascular disease (CVD) [12].

A high percentage of patients with PCOS have abnormal lipid profiles including increased total cholesterol, triglyceride, and LDL-C, whereas HDL-C levels are decreased. Many retrospective studies found significantly increased risk of hypertension and cardiovascular disease (CVD) in women with irregular cycles and the current estimated risk for CVD is 4-11 fold increased in PCOS [31].

Regarding LH and FSH hormones level in this study, PCOS patients LH level was increased significantly more than controls unlike FSH, which was not significant. In addition to that, LH / FSH ratio of cases study was about (2.1). Their result agreed with Asmathulla *et al*., 2013 and Ketel *et al.*, 2009 whom reported that androgen and LH concentrations were increased in both normal weight and obese women suffering from PCOS, while FSH was slightly lower in the normal weight women with PCOS as compared to the normal weight controls. In their study, LH/FSH ratio of 33 patients (56.89%) was above 2 and in 25 (43.1%) cases the ratio was less than 2 [31,32].

Fasting BG, F. insulin, HOMA-IR index and Quicki-IR index were used to evaluate the status of insulin resistance in the two study groups.

Women with PCOS have a high incidence of insulin resistance, a HOMA–IR value > 3.5 probably reflects severe IR, [33], and the QUICKI index is useful for measuring insulin sensitivity, which is the inverse of insulin resistance [34]. In the present study, the incidence was 61.54% (by HOMA-IR method) (Table 5). Although there was no significant difference in fasting glucose levels between control and PCOS women {the entire PCOS patients participant in the present study had normal glucose levels of mean $\pm$SE (94.05$\pm$1.94)}, significantly higher fasting insulin levels were found in the PCOS women. This indicates that the normal fasting glucose value is due to the effect of compensatory hyperinsulinaemia, *i.e*. increased insulin secretion to overcome the insulin resistance [35]. This can be caused by a post –binding defect in signal transduction in women with PCOS. The defect results in a selective insulin activity in the target organs, which causes impaired cellular glucose uptake [36].

Regarding ghrelin, PCOS patients have lower level compared to controls in this study. Mitkov *et al.,* 2008 and Shiva et al*.,* 2012 have showed similar results [37,38]. In studies conducted by Kamal *et al.* 2010, and Glintborg *et al.,* 2006, serum ghrelin concentration was found lower in the PCOS group than in healthy controls [39,40]. Despite these results, Wasko *et al.,* 2004 have reported elevated levels of plasma ghrelin in PCOS patients compared to healthy controls [41]. This discrepancy of results may be explained by confounding factors, such as body weight, fat mass, age, hormonal status, and severity of disease. The reduced fasting ghrelin levels and the relatively smaller increase in ghrelin after weight loss suggest a greater suppression of appetite in obesity and a reduced increase in appetite in weight loss. Moreover, it have been now demonstrated that subjects with PCOS are significantly hungrier and less acutely satiated after a test meal. These observations suggest that subjects with PCOS have impaired defenses against overeating and may not have as strong a drive for meal termination as non-PCOS subjects, the reason for these observed differences in

ghrelin between subjects with and without PCOS are unclear. Similar findings to our study, Schofl *et al.* 2002 showed that ghrelin level did not correlate with BMI [42]. On the other hand, in 2011, Daghestani *et al.* showed a significant inverse relationship between ghrelin and BMI in both PCOS and healthy subjects [43].

From the no significance relation between PCOS ghrelin and most of their biochemical parameters, it can suggest that these biochemical characteristic of PCOS are not affected by ghrelin, and this finding is similar to Pagotto *et al*., 2002 results. They have reported the same findings [44]. Caminos *et al* 2003 [45] have concluded that the ghrelin receptor is found not only in the CNS but also in the ovarian tissues, suggesting a possible reproductive function. Moreover, the capability of ghrelin to alter stimulated testosterone secretion in -*vitro* has been documented [46]. In previous studies there were reports of no significant association [48] and an inverse association have found between serum levels of ghrelin and testosterone [43].

Also, the present results is similar to previous studies that found decreased ghrelin levels in insulin resistant PCOS patients compared with healthy controls matched controls [40,41].

Despite the high circulating level of insulin in PCOS of this study, there was not a significant correlation between their FBG and fasting ghrelin concentrations. This may be due to unmatched BMI among PCOS group. Although many observations support interactions between ghrelin, insulin, and carbohydrate metabolism in PCOS patients, the exact nature of these interactions is not yet clarified, and conflicting results have been reported. *In -vivo* infusion of ghrelin acutely increases serum glucose levels and decreases insulin secretion [42]. Because ghrelin is expressed in pancreatic islet-cells, it may exert direct inhibitory actions on insulin release [44]. Other *in- vivo* and *in- vitro* data suggests a stimulatory action of ghrelin on insulin release [45].

In comparison to matched controls, PCOS women had greater serum insulin levels, confirming previous reports. Low ghrelin levels were associated with increased insulin levels and increased diabetes risk, thus suggesting ghrelin to be an independent risk factor of type-2 diabetes [46].

Orio *et al.* found higher fasting insulin levels in PCOS patients compared with controls but similar ghrelin levels [36], thus supporting a minor importance of insulin for ghrelin secretion. Also Orio *et al.* and Villa 2011 demonstrated significantly inverse correlations between ghrelin and BMI in PCOS, whereas correlations between insulin and ghrelin were non-significant [36,47].

In conclusion, ghrelin hormone in PCOS patients was significantly lower than healthy controls, so it may be used as a new additional marker in PCOS diagnosis. Also, hyperinsulinaemia and hyperleptinemia are associated features in PCOS, and BMI of PCOS patients was ranging from normal to morbid obesity.

Acknowledgment

This research paper is made possible through the help and support of my supervisors for their significant advisors and encouragement, advices on grammar and organization of the manuscript.

Author Contribution

Study conception and design by Dr. Alaa Ghani and Dr. Rayah Baban, acquisition of data by Dr. Thaer Wali and analysis and Interpretation of data by Dr. Rayah Baban.

Conflict of Interest

The authors declare no conflict of interest.

Funding

No founding was for this research.

References

1. March WA, Moore VM, Willson KJ, et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Rebred. 2010; 25: 544-51.
2. Setji TL, Brown AJ. Polycystic ovary syndrome: diagnosis and treatment. *Am J Med*, 2007; 120:128-32.

3. Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004; 19: 41-7.

4. Barber TM, Wass JA, McCarthy MI, et al. Metabolic characteristics of women with polycystic ovaries and oligomenorrhea but normal androgen levels: implications for the management of polycystic ovary syndrome. Clin Endrocrinol (Oxf). 2007; 66: 513-7.

5. Lebovitz HE. Insulin resistance: definition and consequences. Exper Clin Endocrinol Diab. 2001; 109(suppl. 2): S135-S148.

6. Freeman R, Pollack R, Rosenbloom E. Assessing impaired glucose tolerance and insulin resistance in Polycystic Ovarian Syndrome with a muffin test: Alternative to glucose tolerance test. Endocr Pract, 2010; 16: 810-17.

7. Farrell K, Antoni MH. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. Fertil Steril. 2010; 94: 1565-74.

8. Bennett NR, Boyne MS, Cooper RS, et al. Impact of adiponectin and ghrelin on incident glucose intolerance and on weight change. Clin Endocrinol. 2009; 70: 408-14.

9. Longo KA, Govek EK, Nolan A, et al. Pharmacologic Inhibition of Ghrelin Receptor Signaling Is Insulin Sparing and Promotes Insulin Sensitivity. J Pharmacol Exper Therap. 2011; 339: 115-24.

10. Wells T. Ghrelin – Defender of fat. Prog Lipid Res. 2009; 48: 257-74.

11. Andrews ZB, Liu ZW, Walllingford N, et al. UCP2 mediates ghrelin/'s action on NPY/AgRP neurons by lowering free radicals. Nature. 2008; 454: 846-51.

12. Waśko R, Komarowska H, Warenik-Szymankiewicz A. Elevated ghrelin plasma levels in patients with polycystic ovary syndrome. Horm Metab Res. 2004; 36: 170-3.

13. Skommer J, Katulski K, Poręba E, et al. Ghrelin expression in women with polycystic ovary syndrome–a preliminary study. Eur J Gynaecol Oncol. 2005; 26: 553-6.

14. Ozgen IT, Aydin M, Guven A, et al. Characteristics of Polycystic Ovarian Syndrome and Relationship with Ghrelin in Adolescents. J Pediat Adolesc Gynecol. 2010; 23: 285-9.

15. Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004; 19: 41-7.

16. Barber TM, Wass JA, McCarthy MI, et al. Metabolic characteristics of women with polycystic ovaries and oligoamenorrhoea but normal androgen levels: implications for the management of polycystic ovary syndrome. Clin Endrocrinol (Oxf). 2007; 66: 513-7.

17. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28: 412-9.

18. Katz A, Nambi SS, Mather K, et al. Quantitative Insulin Sensitivity Check Index: A Simple, Accurate Method for Assessing Insulin Sensitivity In Humans. J Clin Endocrinol Metab. 2000; 85: 2402-10.

19. Farrell K, Antoni MH. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. Fertil Steril. 2010; 94: 1565-74.

20. Lebovitz HE. Insulin resistance: definition and consequences. Exper Clin Endocrinol Diab. 2001; 109(suppl. 2): S135-S148.

21. Sakata I, Sakai T. Ghrelin cells in the gastrointestinal tract. Int J Pept 2010; 2010: 1-7.

22. Moran LJ, Misso M, WIld RA, et al. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: A systematic review and meta-analysis. Hum Reprod Update. 2010; 16: 347-63.

23. Skommer J, Katulski K, Poręba E, et al. Ghrelin expression in women with polycystic ovary syndrome–a preliminary study. Eur J Gynaecol Oncol. 2005; 26: 553-6.

24. Rifai N, Warnick R. Lipids, lipoproteins, apolipoproteins and other cardiovascular risk factors. In: Carl AB, Edward RA, David EB (eds). Tietz textbook of clinical chemistry and molecular diagnosis. 4th ed. St. Louis, USA: Elsevier Saunders; 2006. p. 918-22.

25. Moran L and Teede H. Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. Hum Reprod Update. 2009; 15: 477-88.

26. Law GD, Maclehose R, Longnecker M. Obesity and time to pregnancy. Hum Reprod. 2007; 22: 414-20.

27. Diamanti-Kandarakis E, Papavassiliou AG, Kandarakis SA, et al. Pathophysiology and types of dyslipidemia in PCOS. Trends Endocrinol Metab. 2007; 18: 280-5.

28. Ronald S, Beth Y, Arthur F. Danforths obstetrics and gynecology. 9th ed. Philadelphia: Lippincott Williams & Wilikins; 2003. p. 663-683.

29. Azziz R, Ehrmann D, Legro RS, et al. PCOS/Troglitazone Study Group Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab. 2001; 86(4): 1626-32.

30. Carmina E, Legro RS, Stamets K, et al. Difference in body weight between American and Italian women with polycystic ovary syndrome: influence of the diet. Hum Reprod. 2003; 18(11): 2289-93.

31. Asmathulla S, Rupa Vani K, Kripa S, et al. Insulin resistance and its relation to inflammatory status and

serum lipids among young women with PCOS. Int J Reprod Contracept Obstet Gynecol. 2013; 2(3): 325-9.

32. Ketel IJ, Stehouwer CD, Serné EH, et al. Obese but not normal-weight women with polycystic ovary syndrome are characterized by metabolic and microvascular insulin resistance. Obstet Gynecol Surv, 2009; 93(9): 3365-72.

33. Legro RS, Castracane VD, Kauffman RP. Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. Obstet Gynecol Surv 2004; 59(2): 141-54.

34. Katz A, Nambi SS, Mather K, et al. Quantitative insulin-sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000; 85: 2402-10.

35. Pauli JM, Raja-Khan N, Wu X, et al. Current perspectives of insulin resistance and polycystic ovary syndrome. Diabet Med. 2011; 28: 1445-54.

36. Orio FJr, Lucidi P, Palomba S, et al. Circulating ghrelin concentrations in the polycyctic ovary syndrome. J Clin Endocrinol Metab. 2003; 88(2): 942-5.

37. Shiva H, Bahram PG, Laya F. Serum leptin and ghrelin levels in women with polycystic ovary syndrome: correlation with anthropometric, metabolic, and endocrine parameters. Int J Fertil Steril. 2012; 6(2): 117-26.

38. Mitkov M, Pehlivanov B, Orbetzova M. Serum ghrelin level - in women with polycystic ovary syndrome and its relationship with endocrine and metabolic parameters. Gynecol Endocrinol. 2008; 24(11): 625-30.

39. Kamal M, Mohi A, Fawzy M, et al. Fasting plasma ghrelin in women with and without PCOS. MEFS J. 2010; 15(2): 91-4.

40. Glintborg D, Andersen M, Hagen C, et al. Evaluation of metabolic risk markers in polycystic ovary syndrome (PCOS). Adiponectin, ghrelin, leptin and body composition in hirsute PCOS patients controls. Eur J Endocrinol. 2006; 155(2): 337-45.

41. Waśko R, Komarowska H, Warenik-Szymankiewicz A, et al. Elevated ghrelin plasma levels in patientswith polycystic ovary syndrome. Horm Metab Res. 2004; 36(3): 170-3.

42. Schöfl C, Horn R, Schill T, et al. Circulating ghrelin levels in patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2002, 87(10): 4607-10.

43. Daghestani MH, Daghestani MH, El-Mazny A. Circulating ghrelin levels and the polycystic ovary syndrome: correlation with the clinical, hormonal and metabolic features. Eur J Obstet Gynecol Reprod Biol. 2011; 155(1): 65-8.

44. Pagotto U, Gambineri A, Vicennati V, et al. Plasma ghrelin, obesity, and the polycystic ovary syndrome: correlation with insulin resistance and androgen levels. J Clin Endocrinol Metab. 2002; 87(12): 5625-9.

45. Caminos JE, Tena-Sempere M, Gaytán F, et al. Expression of ghrelin in the cyclic and pregnant rat ovary. Endocrinology. 2003; 144(4): 1594-602.

46. Tena-Sempere M, Barreiro ML, González LC, et al. Novel expression and functional role of ghrelin in rat testis. Endocrinology. 2002; 143(2): 711-25.

47. Villa J, RE P. Adipose tissue dysfunction in polycystic ovary syndrome. Curr Diab Rep. 2011; 11: 179-84.

Correspondence to Dr. Rayah S. Baban
P.O.Box 70036
E- mail: r_baban@hotmail.com
Received 23rd Oct. 2013: Accepted 17th Feb. 2014

Iraqi JMS

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

Continuous Darkness Induces Changes in the Urinary Space of the Rat's Kidney

Samia A. Eleiwe *PhD*

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

Abstract

Background Melatonin known as "the light of night" secreted from the pineal gland and implicated in a number of cyclical bodily performance and circadian rhythmic activities in humans. The kidney is the main organ regulating water-electrolyte homeostasis in the body adjusted to a daily rhythm.

Objective To study the effect of rising periods of continuous darkness onthe urinary space of adult male rat's kidney.

Methods Eight groups of adult Wister albino rats, each of 4 rats were kept in absolute 24 hours darkness for successively rising 4 periods. Group II, III, IV and V were situated in continuous darkness for two, four, six and eight weeks in succession. Group I^a, Group I^b, Group I^c and Group I^d were the control groups for group II, III, IV and V, respectively. All rats were dissected under general anesthesia at the end of experiment and the right kidney was removed, weighed and arranged for morphometric as well as anatomical and histological studies.

Result No imperative structural effects were noticed with the short and medium periods, but with long periods significant effects were noticed, which were in ratio to the length of darkness.

Conclusion The continuous darkness has structural influence on the glomerular urinary space in the kidney of adult male rats according to the length of exposure.

Key words Darkness, melatonin, kidney and urinary space.

Introduction

All organisms are subjected to the Earth's rotation around the sun with its periodicity of night darkness and day light, with cyclic changes in the length of the daily light and dark and, also, with seasons and climatic changes. Hence, a range of biological activities oscillate inside the organism including: blood and fluid homeostasis, physiological functions, biochemical factors and even behavior. If an event within the biological system returns at roughly regular intervals, it is known as a biological rhythm. These rhythms affect a range of activities, such as the migration behavior (birds, fish), sleep-wake cycle, seasonal change in weight, reproductive cycles etc. The prime rhythms in life are daily rhythms e.g., heart and respiratory rate, blood pressure, rest-activity, body temperature, synthesis and secretion of many hormones, renal plasma flow, glomerular filtration rate, electrolyte concentrations in urine . . etc. Revision on chronobiological side of physiological functions had been carried out essentially on humans and laboratory animals [1-4].

Melatonin is called "the light of night", since it is secreted from the pineal gland mostly at night. It is concerned with the circadian rhythm and in a number of so many cyclical activities in humans. Melatonin is completely involved in signalling the time of day and time of year to all physical

tissues, so, it is considered to be the body's chronological pacemaker [5-7].

Quiroz *et al* had recognized that; melatonin administration ameliorates the course of chronic renal failure, in rats having renal mass reduction. But they suggested that further studies are necessary to describe the potential usefulness of this treatment in the other animal models and in the patients with chronic renal disease [8].

Kidney is the main organ, which regulates the water-electrolyte homeostasis in the body. It is responsible for uphold the total volume of water and its distribution in exacting water spaces, for the electrolyte contents of systemic fluids and also for preserving the acid-base balance. These functions are done, normally, by the plasma filtration process in the renal glomeruli, and the filtrate enters, soon, after passing through the filtration slits, to Bowman's space or known as the urinary space, or the capsular space, which presents just between the visceral and parietal layers of Bowman's capsule. The practice of filtration is regulated to what is called an activity-rest rhythm. These diurnal changes are manipulated by a twenty four hour cycle of activity, of the hormones involved in the regulation of the renal activity. Characteristic of the prospect knowledge of these rhythms is precious for clinical, pharmacological and practical purposes, as well as studies on their physical act [1,8-10].

Objective: To study the effect of rising periods of continuous darkness on the urinary space of adult male rat's kidney.

Methods

This project was achieved in the course of the period from the morning of 4th of March, till the morning of 29th of April of 2013. Thirty two of male Wister albino rats, of ten weeks age were acquired. They were placed in a controlled animal room, specialized for the post graduated researches, at the Department of Anatomy, Histology and Embryology of College of Medicine, Al-Mustansiriyah University, Baghdad, Iraq. These rats were divided into eight groups, each hold four rats. Group I^a, group I^b, group $I^c$and group I^d were the control of group II, III, IV, and V, respectively. The entire of the 4 control groups were put on controlled 12:12 light – dark cycle.

Group II, III, IV, and V were kept in more or less exactly uninterrupted darkness, for a period of 2, 4, 6 and 8 weeks correspondingly. All of the 8 groups were set in a restricted room temperature of 22 ± 2°C, and were located separately in wire meshed stainless steel special cages, fed prohibited food and the tap water was offered for drinking *ad libitum*.

At end the last day of 1st couple of weeks, rats of group II with their control rats (GroupI^a),were dissected with the use of diethyl ether anesthesia. The whole right kidney was in use, fixed in Boun's solution right away and processed through for histopathological study by aid of light microscopy, using serial paraffin sections of 4-6 µm thickness, then, stained with haematoxylin and eosin [11-12]. At the equivalent approach the rats of group III were dissected, just at the end of the 4th weeks also with its control group (Group I^b). The rats of group IV with its own control rats (Group I^c) were managed at the equal way, of the previous groups, at end of the 6th week, and rats belong to group V with their own control group (Group I^d), were maneuvered, at the end of the 8th week.

Anatomical, histopathological as well as biochemical examinations were prepared. The morphometric measurement of the urinary spaces were made by a light microscope, with digital camera, to select the images of the studied section, then, the images of the prepared slides were managed and analyzed, by means of the computerized program named Image J, so that the urinary space width was estimated for any proposed diminution [13]. Morphological description, also, were done to evaluate any probable histopathological changes, might be come to mind. Biochemical analysis was done, as well, by measuring some selected electrolytes and urea level in the serum, to assess the degree of physiological disturbance, if present, in the renal filtration.

Biostatical analysis was prepared to assess the significance of results by analysis of variance, using student-t-test [14].

Results

The data collected from the revision of every variable group, whether for the histometrical, biochemical or morphological examination might be highlighted as the followings:

In group II: the renal tissues samples, showed no any clear deformity, when they were studied, by the light microscope, whenever they were compared (as usually carried out in any research) with its own control group (Fig. 1 & 2 and table 1).

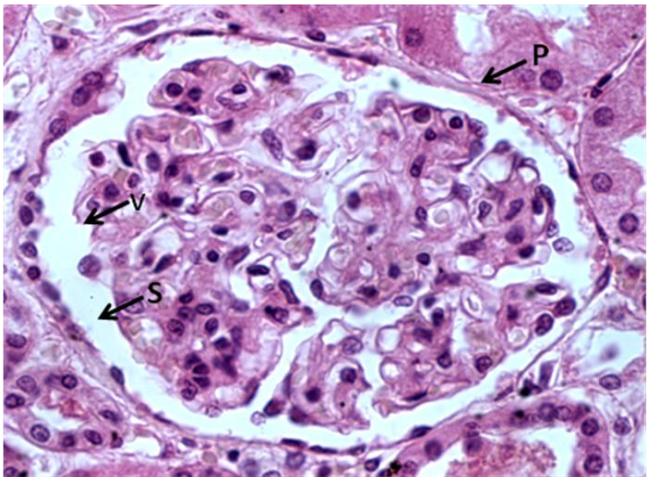

Fig. 1. Renal corpuscle of control adult male rat, P: parietal layer, V: visceral layer & S: urinary space. X400, H & E.

In group III: In the same slide; the renal tissue assessment revealed some fields with a significant decrease in the average width of the Bowman's space of the renal corpuscle, whilst other fields illustrated more or less the same picture as that seen at the examination of its control, apart from some scattered dilated blood vessels (Table 1).

In group IV: The renal tissue study naked the significant decrease in the average width of the Bowman's space of the renal corpuscle, plus, on some corpuscles this space was diminished, to an extent, that it was hardly distinguished, with more dilated blood vessels seen (Fig. 3 and table 1).

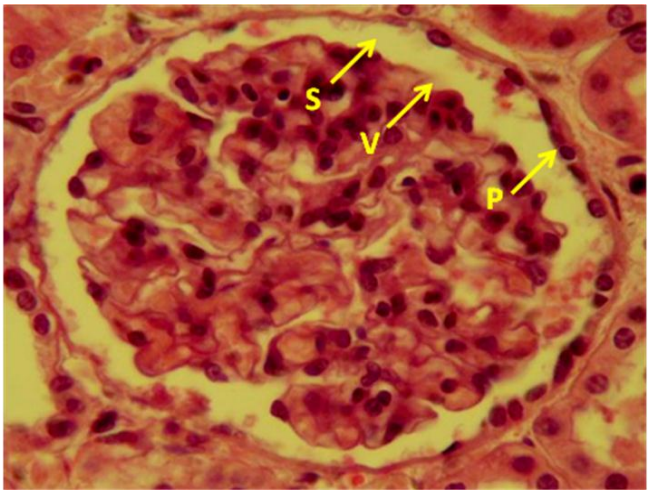

Fig. 2. Renal corpuscle of adult male rat of group II: No obvious changes as that of the control. P: parietal layer, V: visceral layer & S: urinary space. X400, H & E.

Table 1. The effect of continuous darkness on average width ofthe corpuscular urinary space in male rat.

Time of continuous darkness	% Decrease in urinary space of renal corpuscle
Two weeks	0.66±1.43
Four weeks	32.44±15.20*
Six weeks	61.12±18.1**
Eight weeks	94.89±12.7***

* = *P*< 0.01), ** = *P*< 0.005, *** = *P*< 0.001.

In group V: The renal corpuscles were viewed with even more shrink in the average width of the Bowman's space, than that of previous group, and on most of the corpuscles this space was diminished, to a point, that it was approximately obliterated (Fig. 4 and table 1). Dilated blood vessels seemed to be greatly more abundant in this group, with many areas of local hemorrhage.

Biochemical changes were shown in Table 2. Which illustrated non-significant effects, on short dark periods, whereas, it has injurious outcome with the longer periods of darkness.

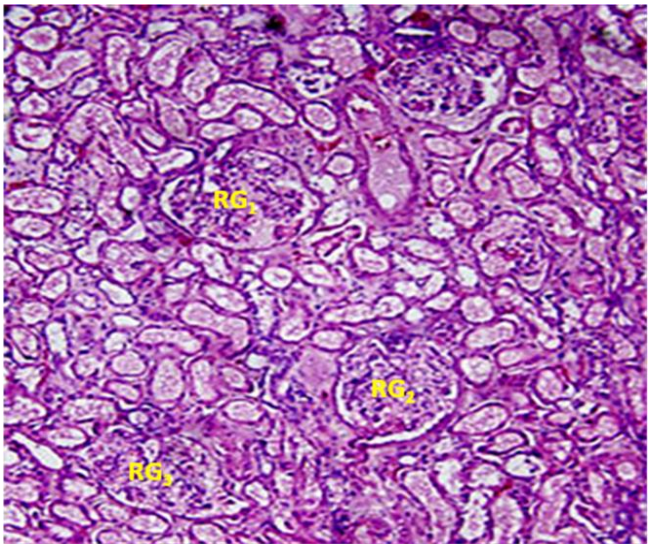

Fig. 3. Renal tissue of adult male rat of group IV showing gloeruli: partial obliteration (RG1); normal width (RG2); complete space obliteration (RG3): X 100, H & E.

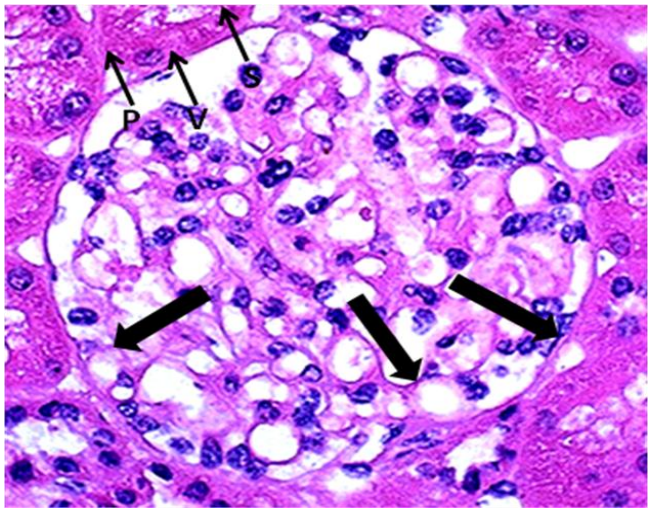

Fig. 4. Renal corpuscle of adult male rat of group V: P = parietal layer, V = visceral layer & S = urinary space. Urinary space was almost obliterated (Black arrows). X400, H & E.

Discussion

Melatonin is the well celebrated pineal neuro-hormone that released to the circulation, primarily at night. It is made from the amino acid, tryptophan, via the serotonin within the pineal gland [5-7]. Even though, many decades of investigations concerning its role on the tissue histology, physiology, and biochemistry had been performed, but, it is still a context of vast interest to investigate more and more its function, hence, it was selected to be involved at this study [15-17].

The kidney was chosen in the instant study because, indeed, it is one of the most vital bodily organs. It has a former role in the all of body homeostasis, controlling the electrolyte concentrations, acid-base balance, extracellular fluid volume, as well as it plays a crucial role in the blood pressure regulation. It eliminates a wide variety of waste products. It secretes a group of hormones, such as erythropoietin. It also secretes the enzyme rennin and Calcitriol which is activated form of vitamin D. All of the variety of kidney's functions are finished, simply, by the mechanisms of re-absorption, filtration and secretion [18-22], hence the filtration site was tried to be investigated here.

The anatomical, histological and biochemical changes seen in the ongoing study, which were induced by putting in continuous darkness, might be discussed by the fact; that melatonin is able, relatively, to reach all bodily tissues and cells, inducing its action, via the meticulous melatonin receptors, which present, nearly, in all of the body tissues [23-26]. These changes were in ratio with the length time exposure to darkness; because melatonin has a dose – dependent natural achievement [27-29], so no factual injurious effect was occurred, to the group put on 2 weeks of continuous darkness, still with the longer dark exposure; there was an apparent renal harm. These consequences could be confer by the fact that; melatonin is known to have no harmful effect, on little doses, whereas, it has injurious outcome, whenever it is supplied on prominent levels [17,27-29].

The time-route of 30 days, for rats, is regarded as a long period of treatment, according to Peltier *et al.* [30]. Hence, it caused no any detectible harmful histological nor functional effects on short period of darkness, and those findings were equivalent with the findings of Hoyos *et al* [31], when he planned that; melatonin, in its normal pharmacological level, do not affect the serum biochemical parameters

including overall serum proteins, creatinin and urea. Thus, in the ongoing study, it did not affect the renal function; yet, it slightly decreased the uric acid, which was regarded as a pleasant effect for sure.

When group II and III were assessed, the products were quite different, and significant histological changes were noticed on corpuscle space, presented by the obvious narrowing of the corpuscular urinary space with concomitant biochemical changes as well.

Table 2. The effect of continuous darkness on some renal biochemical profiles of male rat

Time of continuous darkness	Sugar mg/100ml	Urea mg/100ml	Ceatinine mg/100ml	K MEq/L	TSP g/100ml	Albumin g/100ml
Control Group I[a]	87.9± 0.9	23.8±1.3	0.33±0.04	4.1±0.1	7.4±0.6	3.22±0.1
2 wk	89.1±1.4	22.9±1.9	0.32±0.07	4.0±0.3	7.5±0.8	3.21±0.9
Control Group I[b]	88.8±1.9	22.6±2.1	0.35±0.06	3.9±0.7	7.5±0.02	3.31±0.2
4 wk	90.3±2.1	24.7±2.3	0.47±0.09*	4.6±0.9	7.3±0.5	3.25±0.8
Control Group I[c]	89.1±2.4	23.6±2.8	0.36±0.1	4.0±0.7	7.5±0.4	3.20±0.6
6 wk	112.8±1.7*	41.9±1.8*	0.89±0.05**	4.9±0.6*	6.7±0.3*	2.93±0.2**
Control Group I[d]	88.3±2.2	22.9±2.6	0.32±0.3	4.1±0.5	7.3±0.6	3.24±0.6
8 wk	119.8±1.6**	67.9±1.6**	1.23±0.8**	5.8±0.4***	6.1±0.2**	2.07±0.4***

* = $P< 0.04$, ** = $P< 0.03$, *** = $P< 0.001$).

Those unwanted changes whether histological or functional might be caused by the harmful damaging effect of melatonin wherever it is used in a large dosage [27-29], because melatonin can reach almost all body tissues and cells, applying its action through its receptors [23-26], so it perhaps had damaged any of the corpuscular constituents such as the endothelium of the glomerular capillaries, glomerular basement membrane, mesangealcomponants or the podocytes; leading to resultant glomerular filtration abnormalities, since a damage to any of these components whether functional or structural could contribute to the filtration disturbance [18,19,21]. Although the study of the blood vessels is not our main goal in the instant research, but it was observed there for interest; the dilatation of blood vessels, could be attributed to the fact which documented the melatonin, to have a vasodilation action [32-34]. Uncertainties and doubts seemed to be still surrounding the role of melatonin, in human biology and pathophysiology and future researches might be needed.

In conclusion: The continuous darkness has structural as well as functional influence on the glomerular urinary space in the kidney of adult male rats in accordance with the length of darkness exposure.

Acknowledgement

Great thanks to my supervisors for their significant advice and encouragement.

Conflict of Interest

The author declares no conflict of interest.

Funding

No specific grant from any funding agency in the public, commercial or not-for-profit sector.

References

1. Skotnicka E, Muszczyñski Z, Dudzinska W, et al. A review of the renal system and diurnal variations of renal activity in livestock. Irish Veter J. 2007; 60(3): 161-8.
2. Koopman MG, Koomen GC, Krediet RT, et al. Circadian rhythm of glomerular filtration rate in normal individuals. Clin Sci J. 1989; 77:105-11.
3. Refinetti R, Menaker M. Review: The circadian rhythm of body temperature. Physiol Behav.1992; 51:613-7.
4. Kostoglou-Athanassiou I. Therapeutic Applications of Melatonin. J Therap Adv Endocrinol Metab. 2013; 4(1):13-24.
5. Grivas TB, SavvidouOD. Melatonin the "light of night" in human biology and adolescent idiopathic scoliosis. Scoliosis. 2007; 2:6.doi: 10.1186/1748-7161-2-6

6. Waldhauser F, Vierhapper H, Pirich K. Abnormal circadian melatonin secretion in night-shift workers. N Engl J Med. 1986, 315:1614.
7. Goes E. Sleep, Melatonin and the Temperatures of your Skin and Core Anatomy & physiology, May 2, 2012. Read more at Suite101: Sleep, Melatonin and the Temperatures of your Skin and Core | Suite101.comhttp://suite101.com/article/sleep-melatonin-and-the-temperatures-of-your-skin-and-core-a407088#ixzz238m7mu4f.
8. Quiroz Y, Ferrebuz A, Romero F, et al. Melatonin ameliorates oxidative stress, inflammation, proteinuria, and progression of renal damage in rats with renal mass reduction. Am J Physiol-Renal Physiol. 2008; 294:F336-F344.
9. Stow LR, Gumz ML. The circadian clock in the kidney. J Am Soc Nephrol. 2011; 22(4):598-604.
10. Firsov D, Bonny O. Circadian regulation of renal function. Kidney Int. 2010; 78(7):640-5.
11. Baker FJ, Silverton RE, Pallister CJ. Baker and Silverton´s Introduction to medical laboratory technology.7th ed. UK: Arnold publisher Comp.; 1998. p. 182-242.
12. Bancroft JD, Stevens A. Theory and practice of histological techniques. Edinburgh: Churchill Livingstone; 1987. p. 482-502.
13. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to Image J: 25 years of image analysis. Nature Meth. 2012; 9:671-5.
14. Daniel WW. Biostatistics: A Foundation for analysis in the health sciences. 9thed. NY: John Wiley; 2008. p. 273-95.
15. McMullan CJ, Schernhammer ES, Rimm EB, et al. Melatonin Secretion and the Incidence of Type 2 Diabetes. J Am Med Assoc. 2013; 309(13):1388-96.
16. Jiménez-Aranda A, Fernández-Vázquez G, Campos D, et al. Melatonin induces browning of inguinal white adipose tissue in Zucker diabetic fatty rats. J Pineal Res. 2013; 55(4):416-23.
17. Exposing yourself to light at night shuts down your melatonin and raises your cancer risk. March 19, 2013 | 103,829 views Visit the Mercola Video Library.
18. Stevens LA, Coresh J, Greene T, et al. Assessing kidney function-measured and estimated glomerular filtration rate. New Engl J Med. 2006; 354 (23): 2473-83.
19. Rule AD, Larson TS, Bergstralh EJ, et al. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. Ann Int Med. 2004; 141(12):929-37.
20. Stevens A, Lowe J. Human Histology, 2nd ed. Mosby, Mirror International Publishers Limited. 1997; p. 85, 117-135, 285.
21. National Kidney Foundation. "K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification". Am J Kid Dis. 2002; 39(2Suppl 1):S1-266.
22. Ruth R. A Historical introduction to Gray's anatomy. In: Standring S (ed). Gray's Anatomy: The anatomical basis of clinical practice. 39th ed. Edinburgh: Elsevier Churchill Livingston; 2005. p. 4.
23. Dubocovich ML. Melatonin receptors: are there multiple subtypes? Trends Pharmacol Sci. 1995; 16:50-6.
24. Morgan PJ, Barrett P, Howell HE, et al. Melatonin receptors: localization, molecular pharmacology and physiological significance. Neurochem Int. 1994; 24:101-46.
25. Sugden D, Davidson K, Hough KA, et al. Melatonin, melatonin receptors and melanophores: a moving story. Pigment Cell Res. 2004; 17(5):454-60.
26. Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, et al. Melatonin membrane receptors in peripheral tissues: Distribution and functions. Mol Cell Endocrinol. 2012; 351:152-66.
27. Anisimov VN, Alimova IN, Baturin DA, et al. Dose-dependent effect of melatonin on life span and spontaneous tumor incidence in female SHR mice. Exp Gerontol. 2003; 38(4): 449-61.
28. Bahadori MH, Ramazani M, Asghari-Nohadani Z. Melatonin dose- dependent effect on oocyte maturation capacity, in vitro fertilization and blastocyst development in mouse. Kowsar Med J. 2011; 16(2):67-71.
29. Succu S, Berlinguer F, Pasciu V, et al. Melatonin protects ram spermatozoa from cryopreservation injuries in a dose-dependent manner. J Pineal Res. 2011; 50(3):310-18.
30. Peltier MR, Robinson G Sharp DC. Effect of melatonin implants in pony mares 2. Long-term effects. Theriogenology.1998; 49(6):1125-42.
31. Hoyos M, Guerrero JM, Perez-Cano R, et al. Serum chlesterol and lipid peroxidation are decreased by melatonin in diet induced hypercholesterolemic rats. J Pineal Res. 2000; 28(3):150-5.
32. Stow LR, Richards J, Cheng KY, et al. The circadian protein period 1 contributes to blood pressure control and coordinately regulates renal sodium transport genes.Hypertension.2012; 59(6): 1151-6.
33. Simko F, Pechanova O. Potential role of melatonin and chronotherapy among the new trends in hypertension treatment. J Pineal Res. 2009; 47:127-33.
34. Masana MI, Doolen S, Ersahin C, et al. MT_2 melatonin receptors are present and functional in rat caudal artery. J Pharmacol Exp.2002; 302(3):1295-302.

E-mail: samia_a_eleiwe@yahoo.com
Mobile: + 964 07901237244-07704260292
Received 3rd Feb. 2013: Accepted 19th Feb. 19th Feb. 2014

Iraqi JMS

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

Assessment of Risk Factors for Postsplenectomy Pulmonary Hypertension

Waseem F. Al Tameemi[1] *CABMS, FIBMS*, **Maan M.A. Hamid**[2] CABS, FIBMS, **Haider N. Dawood**[3] *CABS, FIBMS*

[1]Dept. of Medicine, College of Medicine, Al-Nahrain University, [2]Dept. of Surgery, [3]Dept. of Medicine, Al-Imamian Al-Kadhimiyain Medical City, Baghdad, Iraq

Abstract

Background Splenectomy has been associated with several long-term complications; pulmonary arterial hypertension has gained special attention. It seems that the absence of a spleen, rather than underlying condition for which the splenectomy was performed, is the primary cause of this condition.

Objectives Assessing the risk factors for development of pulmonary hypertension in different indications of splenectomy

Method Fifty postsplenectomy patients were included and transthoracic echocardiographic study looking for right ventricle size; ejection fraction and pulmonary artery pressure were performed for each patient in addition to complete blood count.

Results The patients' mean age was 32.5±1.8 years. The mean duration after splenectomy was 5.2±0.34 years with a range of 1-10 years**.** Hemoglobinopathies in different types formed 54% (27/50) of these indications, while non hematological indications were reported in 7 cases (14%). Pulmonary arterial hypertension was reported in 22% of patients with mean pressure 30.10±1.18 mmHg. It is positively correlated with right ventricular size. The highest risk of pulmonary arterial hypertension was reported with splenectomy due to hemolytic diseases in comparison with other indication despite persistence of similar risk in non hemolytic indication but of no statistical significance. The more severe degree of anemia has negative correlation with pulmonary arterial hypertension as well as high WBC count unlike thrombocytosis.

Conclusion Whatever the underlying indications of splenectomy, the risk of pulmonary hypertension exists, which may not related only to thrombocytosis but also for anemia and leucocytosis and it needs long duration follow up to be diagnosed.

Key words Splenectomy, pulmonary hypertension.

List of Abbreviation: PAH = pulmonary arterial hypertension, CTEPH = chronic thromboembolic pulmonary hypertension, TTE = Transthoracic echocardiographic, RV = right ventricle, EF = ejection fraction, PAP = pulmonary artery pressure, ITP = immune thrombocytopenic purpura.

Introduction

Splenectomy is not free from variable important long-term complications. Pulmonary arterial hypertension (PAH) is an important vascular complications [1,2] in addition to risk of deep venous thrombosis [3,4], and atherothrombosis [5]. A specific subcategory of PAH is restricted to patients with hemoglobinopathies and/or splenectomy [6].

Idiopathic pulmonary hypertension or chronic thromboembolic pulmonary hypertension (CTEPH) are reported in cases of thalassemia and sickle cell disease [7,8]. The reported prevalence of splenectomy in patients with PAH ranged

from 8.6% to 11.5% compared with 0% to 0.6% in the other groups (patients with other forms of pulmonary disease) [1].

The underlying indication of splenectmoy is one of the factors that play a role in development of postsplenectomy PAH, but it is definitely not the sole factor in this process. The subsequent hyposplenism state is an important issue regardless the presence of ongoing hemolysis or not [1,7,8].

Splenectomy in thalassemic patients will increase the frequency of PAH over the general population [9,10] and similar risk is reported in sickle cell anemia patients (who had auto-spelnectomy) [11].

Many cases were reported as they developing PAH after splenectomy for variable underlying diseases like hereditary stomatocytosis, hereditary spherocytosis, myeloid metaplasia, paroxysmal nocturnal hemoglobinuria, and unstable hemoglobinopathies [12-19].

The aim of this study is to assess the risk factors for development of pulmonary hypertension in different indications of splenectomy.

Methods

A cross sectional study had been conducted on 50 postsplenectomy patients (with different indications and for different durations). They were met at hematology outpatient clinic at Al-Imammain Al-Kadhimain Medical City during their routine follow up over the period between April 2011 and Dec. 2012. For every patient complete history and examination were performed including indication of spelenctomy, duration since spelenctomy, and complications.

Adult patients with sickle cell disease were also included in this study as they have state of hyposplenism secondary to autosplenectomy as it is proven by ultrasonagraphy, but its duration was estimated crudely (since the age of adolescence). In addition to those having splenectomy for combined hemoglobinopathy (these had been referred as other hematologic indications).

Each patient had informed about the enrollment in this study according to declaration of Helsinki. The study was following the local scientific research ethical committee guidelines.

A complete blood count was requested for all patients; in addition to transthoracic echo-cardiographic (TTE) study (using Philips C9) was performed looking for right ventricle (RV) size, ejection fraction (EF) and pulmonary artery pressure (PAP) according to Bernoulli's equation. The normal RV diastolic dimension is11-28 cm^2 and normal EF must be >55%[20]. PAH defined by a mean PAP >25 mm Hg at rest or> 30 mm Hg during exercise [20,21].

Statistical analysis using SPSS program and Microsoft excel program. T test, ANOVA and Spearman's rank correlation study were used considering a *P* value < 0.05 as significant difference.

Results

Total number of patients were 50, [60% were females (30/50))] their ages range from 16-58 years with a mean of (32.5±1.8 years). The mean duration after splenectomy was 5.2±0.34 years with a range of 1-10 years

The most frequent indications for spelenctomy were hemoglobinopathies in different types that form 54% (27/50), while non hematological indications were reported in 7 cases (14%) as demonstrated in (Table 1).

Table 1: Indications of splenectomy

Indications	No. (%)
Different hematological indications	11 (22)
Thalassemia major	10 (20)
Thalassemia intermedia	6 (12)
Immune hemolytic anemia	8 (16)
Immune thrombocytopenic purpura	5 (10)
Hereditary spherocytosis	3 (6)
Surgical indications	7 (14)
Total	50 (100)

Laboratory parameters for patients define that the hemoglobin level varied between 4.00-13.00 g/dl with a mean of 9.93±0.3, the mean platelet count was 480.62±32.4 x 10^3/ml with a range between 150.0 - 886.0 x10^3/ml (Table 2).

Table 2: Hematologic characteristics of the patient group

Laboratory Characteristic	Mean±SE	Range
Hemoglobin (g/dl)	9.93±0.31	4.00-13.00
Platelet x10^3 (/ml)	480.62±32.49	150.00-886.00
WBC x10^3 (/ml)	8.868±0.58	4.00-20.00

Pulmonary hypertension was reported in 22% of patients with mean pressure 30.10±1.18 mmHg through a range between 15-50 mmHg (Table 3).

Table 3: transthoracic Echocardiographic characteristics of the patient group

Echocardiography Characteristic	Mean±SE	Range
RV size (cm^2)	24.68±0.6	14.00-38.00
EF (%)	62.12±0.81	50.00-75.00
PAP pressure (mmHg)	30.10±1.18	15.00-50.00

RV = right ventricle, EF= left ventricle ejection fraction, PAP= pulmonary artery pressure

RV enlargement was documented in 30% of cases (15/50) (Fig. 1).

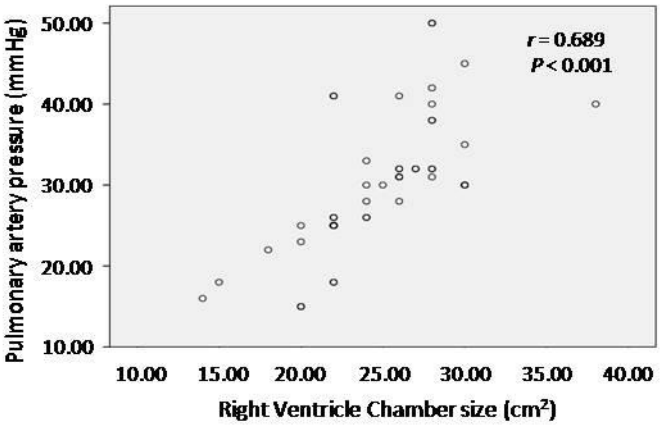

Fig. 1. Distribution of right ventricle size in relation to pulmonary artery pressure

Pulmonary hypertension is positively correlated with RV size in significant value (r = 0.689, P <0.001) but has negative correlation with EF (r = -0.485, P < 0.001).

There are significant differences between genders concerning mean PAP pressure (P = 0.039). Mean PAP pressure in male patients is 33.05±1.98 mmHg while it is 28.13±1.35 mmHg in females with statistically significant difference (P = 0.039)

The highest risk of PAH was reported with splenectomy due to hemolytic diseases (hemoglobinopathies especially thalassemia major and intermedia) in comparison with other indications for splenectomy with statistical significance difference (p = 0.003) (Fig. 2).

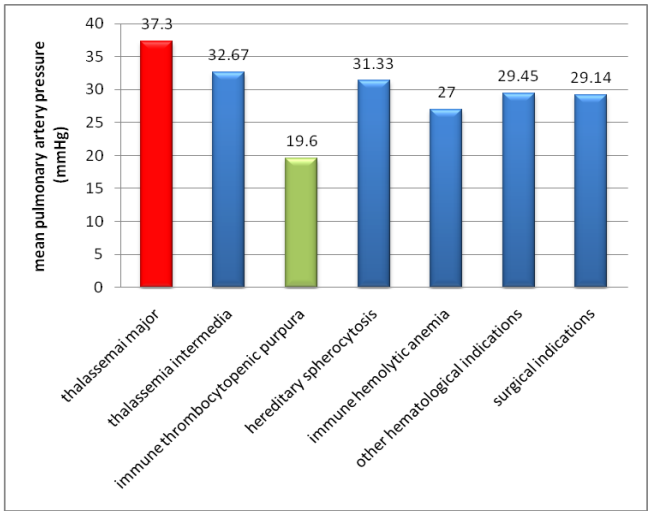

Fig. 2. Comparison of mean PAP among different indications of splenectomy

However; a surgically indicted splenectomy was also associated with risk of pulmonary hypertension but in non significant association (P = 0.065). ITP patients did not show such a risk as their mean PAP is 19.6±1.4 mmHg.

The correlation between PAP and other hematologic parameters were evaluated and It reveals that post splenectomy duration is positively correlated but in non significant value with development of PAH (r = 0.22, P = 0.11) (Fig. 3) and similarly concerning the patient age. While, it is found that the more severe degree of anemia has negative correlation which is of statistical significance with development of PAH (r = -0.314, P = 0.026,) as well as WBC count (r = 0.330, P = 0.019,) unlike thrombocytosis (r = 0.053, P = 0.715).

Discussion

When a patient performed splenectomy, PAH would be a consistent risk consequence

especially in cases of underlying haemolytic anaemia [22].

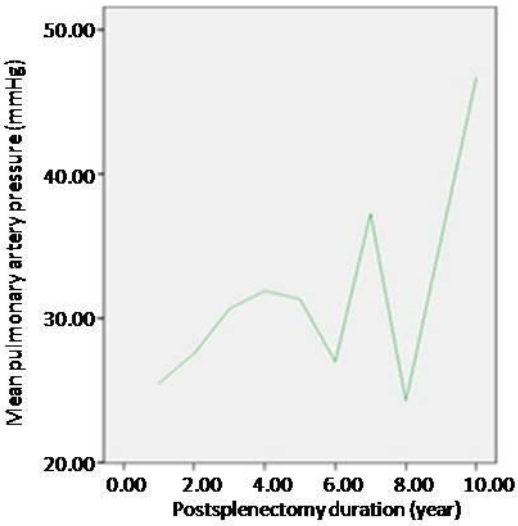

Fig. 3. Relationship of post splenectomy duration and mean pulmonary artery pressure

It is found in over 60% of the patients with thalassaemia [23,24] and while it is reported in patient with sickle cell disease as 32% [25]. The question that is raised, does it follow other indications of splenectomy?

In this study different indications were included, PAH is reported more frequently (22%) than what is reported by Hoeper et al (11.5%) [7]. It can be well understood that this difference may be due to limitation of transthorasic echocardiography in diagnosis of pulmonary hypertension and other variables like the inter operator & intra operator variation [26].

One complicating factor in many of these cases, which is the consideration of splenectomy as a treatment line and therefore it, is difficult to differentiate between the roles of splenectomy per se or the effect of the underlying haemolytic disease in development of PAH.

Hemoglobinpathies, in this study, form the major component of the patients group (54%) which is already known as risk factor for PAH (*P* = 0.003), (a process that may started even before splenectomy) in agreement with report of Aessopos et al [27] who found that 59% of patients with thalassaemia intermedia who had had splenectomy developed thromboembolic pulmonary hypertension and similarly other author conclusion [28].

Despite all these limitations , non hematological indications were forming around 14% of the studied cases which also showed increasing risk of PAH but in non significant manner (*P* = 0.065).

The simplest explanation of the other authors who have studied this problem is that, following splenectomy, there is both thrombocytosis and also increased numbers of damaged circulating red cells which will activate these platelets leading to in situ thrombosis [29,30], but it couldn't be approved in this study as thrombocytosis didn't show any significant correlation with such a risk (*r* = 0.053, *P* = 0.715). In the contrary, the severity of anemia and higher WBC count showed significant negative and positive correlation (*r* = -0.314, *P* = 0.026,) and (*r* =0.330, *P* = 0.019,) respectively.

This may indicate that different factors other than hypercoagulability will play a role like impact of anemia, leucocytosis on blood flow dynamics or endothelial dysfunction [31,32]. Peacock stated that "is not simply one of increased coagulability due to loss of the splenic filter but one of abnormal endothelial surface resulting in *in situ* thrombosis or another factor " [33].

The exact mechanism by which pulmonary hypertension develops after splenectomy remains unclear. The pathophysiological mechanisms have been proposed as": (i) thromboembolic occlusion of the pulmonary vasculature; (ii) an increase in the production of reactive oxygen species; and (iii) the depletion of nitric oxide by free hemoglobin released by damaged red cells leading to pulmonary vasoconstriction" [24].

Some were recognized the presence of megakaryocytes in the lungs, and therefore they postulated their contribution in PAH in these states [24]. Vascular endothelial growth factor, platelet-derived growth factor and transforming growth factor-b will be released there as fiberogenic mediators from these trapped platelets inside capillary beds (regardless the

thrombocyte count in circulation) in a phenomenon called 'pathological emperipolesis' leading to increase in pulmonary artery pressure [34,35].

The mean PAP pressure in those performed splenectomy for other than chronic hematologic disease was 29.14±2.32 mmHg that is consider as evidence for pulmonary hypertension and this was also reported by Jaix et al [1] in his paper at Thorax journal where only four of the 22 patients who developed CTEPH after splenectomy had a haemolytic disorder, and in most of the others the spleen had been removed for trauma which had demonstrated.

Post splenectomy duration is positively correlated but in non significant value with risk of PAH (r = 0.22, P = 0.11) which is noticed by Jaix et al [1] who demonstrated long duration (up to 35yr) required for this complication to be developed that suggest the pulmonary hypertension is a very slow process or that some additional factor developed which resulted in a prothrombotic state, perhaps a change in endothelial function or a change in red cell characteristics. Therefore, any case that needs splenectomy still may have this risk of PAH even for other than hemolytic or hematologic disorders [33]. This consequence may be related erythrocyte membrane alteration and subsequent activation of coagulation cascade due to the loss of spleen filter function [36]. In addition to the fact that in case of trauma, there is an associated thromboembolic complications even if not documented immediately after the surgery [1].

In conclusion, whatever the underlyng indication of splenectomy, the risk of pulmonary hypertension exist, which may not related only to thrombocytosis but also for anemia and leucocytosis and it needs long duration follow up to be excluded. Transthoracic echocardiography can help in this follow up by demonstration of right ventricle size.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Dr. Waseem F. Al Tameemi is the caring physician and hematologist for group of patient; Dr. Maan M. A. Hamid is the surgeon who performed splenectomy and Dr. Haider N. Dawood is the physician who record echocardiography.

Funding

The work not funded by any mean.

References

1. Jais X, Ioos V, Jardim C, et al. Splenectomy and chronic thromboembolic pulmonary hypertension. Thorax 2005; 60:1031-1034.
2. Bonderman D, Skoro-Sajer N, Jakowitsch J, et al. Predictors of outcome in chronic thromboembolic pulmonary hypertension. Circulation 2007; 115:2153-2158.
3. Romano F, Caprotti R, Conti M, et al. Thrombosis of the splenoportal axis after splenectomy. Langenbecks Arch Surg 2006; 391:483-488.
4. Ikeda M, Sekimoto M, Takiguchi S, et al. High incidence of thrombosis of the portal venous system after laparoscopic splenectomy: A prospective study with contrast-enhanced CT scan. Ann Surg 2005; 241:208-216.
5. Schilling RF. Spherocytosis, splenectomy, strokes, and heart attacks. Lancet 1997; 350:1677-1678.
6. Simonneau G, Galie N, Rubin LJ, et al. Clinical classification of pulmonary hypertension. J Am Coll Cardiol 2004; 43(suppl 12):5S-12S.
7. Hoeper MM, Niedermeyer J, Hoffmeyer F, et al. Pulmonary hypertension after splenectomy? Ann Intern Med 1999; 130(6):506-509.
8. Bonderman D, Jakowitsch J, Adlbrecht C, et al. Medical conditions increasing the risk of chronic thromboembolic pulmonary hypertension. Thromb Haemost 2005; 93(3):512-516.
9. Singer ST, Kuypers FA, Styles L, et al. Pulmonary hypertension in thalassemia: association with platelet activation and hypercoagulable state. Am J Hematol 2006; 81(9):670-675.
10. Phrommintikul A, Sukonthasarn A, Kanjanavanit R, et al. Splenectomy: a strong risk factor for pulmonary hypertension in patients with thalassaemia. Heart 2006; 92(10):1467-1472.
11. Gladwin MT, Sachdev V, Jison ML, et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med 2004; 350(9):886-895.
12. Steward GW, Amess JAL, Eber SW, et al. Thrombo-embolic disease after splenectomy for hereditary stomatocytosis. Br J Haematol 1996; 93:303-10.

13. Yoshimoto A, Fujimura M, Nakao S. Pulmonary hypertension after splenectomy in hereditary stomatocytosis. Am J Med Sci. 2005; 330(4):195-197.
14. Hayag-Barin JE, Smith RE, Tucker FCJr. Hereditary spherocytosis, thrombocytosis, and chronic pulmonary emboli: a case report and review of the literature. Am J Hematol 1998; 57(1):82-84.
15. Jardine DL, Laing AD. Delayed pulmonary hypertension following splenectomy for congenital spherocytosis. Intern Med J 2004; 34(4):214-216.
16. Smedema JP, Louw VJ. Pulmonary arterial hypertension after splenectomy for hereditary spherocytosis. Cardiovasc J Afr 2007; 18(2):84-89.
17. Marvin KS, Spellberg RD. Pulmonary hypertension secondary to thrombocytosis in a patient with myeloid metaplasia. Chest 1993; 103(2):642-644.
18. Heller PG, Grinberg AR, Lencioni M, et al. Pulmonary hypertension in paroxysmal nocturnal hemoglobinuria Chest 1992; 102(2):642–643.
19. Lode HN, Krings G, Schulze-Neick I, et al. Pulmonary hypertension in a case of Hb-Mainz hemolytic anemia. J Pediatr Hematol Oncol 2007; 29(3):173-177.
20. Lang RM, Bierig M, Devereux RB, et al. *Recommendations for chamber quantification.* Eur J Echocardiogr 2006; 7(2):79-108.
21. McGoon M, Gutterman D, Steen V, et al. Screening, early detection, and diagnosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest 2004; 126(suppl):14S-34S.
22. Farber HW, Loscalzo J. Pulmonary arterial hypertension. N Engl J Med 2004; 351: 1655-65.
23. Du ZD, Roguin N, Milgram E, et al. Pulmonaryhypertension in patients with thalassemia major. Am Heart J 1997; 134:532-7.
24. Thachil J. The enigma of pulmonary hypertension after splenectomy—does the megakaryocyte provide a clue? Q J Med 2009; 102:743–745.
25. Gladwin M, Sachdev V, Jison M, et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med 2004; 350:886-5.
26. Sciomer S, Badagliacca R, Fedele F. Pulmonary hypertension: echocardiographic assessment. Ital Heart J 2005; 6 (10): 840-845
27. Aessopos A, Farmakis D, Karagiorga M, et al. Cardiac involvement in thalassemia intermedia: amulticenter study. Blood 2001; 97:3411-6.
28. Phrommintikul A, Sukonthasarn A, Kanjanavanit R, et al. Splenectomy: A strong risk factor for pulmonary hypertension in patients with thalassaemia. Heart 2006; 92:1467-1472.
29. Boxer MA, Braun J, Ellman L. Thromboembolic risk of postsplenectomy thrombocytosis. Arch Surg 1978; 113:808-9.
30. Visudhiphan S, Ketsa-Ard K, Piankijagun A, et al. Blood coagulation and platelet profiles in persistent post splenectomy thrombocytosis. The relationship to thromboembolism. Biomed Pharmacother 1985;39: 264-71.
31. Howard LSGE, Watson GMJ, Wharton J, et al. Supplementation of iron in pulmonary hypertension: Rationale and design of a phase II clinical trial in idiopathic pulmonary arterial hypertension. Pulm Circ 2013; 3(1): 100-107
32. Crary SE, Buchanan GR. Vascular complications after splenectomy for hematologic disorders. Blood 2009; 114(14): 2861-2868.
33. Peacock AJ. Pulmonary hypertension after splenectomy: a consequence of loss of the splenic filter or is there something more? Thorax 2005; 60: 983-984.
34. Zucker-Franklin D, Philipp CS. Platelet production in the pulmonary capillary bed: new ultrastructural evidence for an old concept. Am J Pathol 2000; 157:69-74.
35. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. J Clin Oncol 2005; 23: 8520-30.
36. Kuypers FA, Yuan J, Lewis RA, et al. Membrane phospholipid asymmetry in human thalassemia. Blood 1998; 91:3044-51.

Correspondence to Dr. Waseem F. Al-Tameemi
E-mail: drwaseem72@hotmail.com
Received 2nd Jan. 2014: Accepted 11th Mar. 2014

Iraqi JMS

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

Effects of Atorvastatin and Melatonin on Glycemic Control and Lipid Profile in Type 2 Diabetic Patients

Haitham M. Kadhim[1] *PhD*, **Faruk H. Aljawad**[1] *PhD*, **Hashim M. Hashim**[2] *MRCP*

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

Abstract

Background Dyslipidemia is a modifiable cardiovascular disease risk factor that remains largely uncontrolled in patients with type 2 diabetes mellitus. Administration of melatonin may improve tissue responses to insulin and increase the efficacy of drugs which act through this pathway like Sulfonylurea.

Objective To investigate the effectiveness of Atorvastatin and melatonin that possess antioxidant and/or hypolipidemic effects on the changes that occur in patients with type 2 diabetes mellitus due to uncontrolled glycemic status.

Methods Forty one diabetic patients (26 female and 15 male) with an age 35-60 years and disease duration of 5-10 years were studied. Patients allocated to 3 groups, first group was treated with Placebo (starch 50mg; n=13), second group was treated with (Atorvastatin 20mg/day; n=14), while third group was treated with (Melatonin 10mg/day; n=14), in addition to the already given oral hypoglycemic agent (glibenclamide) and dietary control for 12 weeks. Biochemical parameter (baseline, 6 and 12 weeks later) including and lipid profile tests were done.

Results Atorvastatin and melatonin administration significantly increases fasting serum glucose and glycated hemoglobin levels, with significant decrease in cholesterol, triglycerides, and low density lipoprotein. However, the effects on these parameters were variable between the studied groups.

Conclusion The administration of Atorvastatin may induce hyperglycemia despite of its hypolipidemic effect, while melatonin could improve both glycemic control and lipid profile in patients with type 2 diabetes mellitus.

Key words Atorvastatin; melatonin; glycemic control; type 2 diabetes mellitus.

List of Abbreviation: T2 DM = type 2 diabetes mellitus, TC = total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, TG = triglyceride, FSG = fasting serum glucose, HbA_1c = glycated hemoglobin, CVD = cardiovascular disease, OS = oxidative stress, ROS = reactive oxygen species, Glut 4 = glucose transporter 4.

Introduction

Type 2 diabetes mellitus (T2 DM) is characterized by defective insulin secretion in pancreatic β-cells in response to glucose and by deficiencies in the action of insulin on its target tissues. Hyperglycemia increases the risk of microvascular complications [(1)], while dyslipidemia is a major risk factor for macrovascular complications in patients with type 2 diabetes [(2)]. Elevated low-density lipoprotein cholesterol (LDL-C) is a major risk factor for cardiovascular disease (CVD) [(3)]. As such, management of LDL-C is the primary goal of therapy for diabetic dyslipidemia [(4)]. As the prevalence of type 2 diabetes increases in the United States, prevention of CVD is becoming an increasingly urgent public health concern, requiring aggressive management of the entire lipid profile [(5)].

Evidence that oxidative stress (OS) is present in diabetes originates from the frequent

observation that both reactive oxygen species (ROS) and antioxidants are increased. The later is logically rather seen in early stage of diabetes and should be interpreted as a tentative compensation of cells against increasing OS [6].

The use of melatonin for medicinal purposes has become something of an 'alternative medicine' fad, although there are few properly controlled trials of its efficacy [7]. Melatonin administration decreased the amount of thiobarbituric acid-reactive substances, increased glutathione levels and superoxide dismutase activity [8].

Atorvastatin is structural analogue of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A). It is most effective in reducing LDL. Other effects include decreased oxidative stress and vascular inflammation with increased stability of atherosclerotic lesions. It has become standard practice to initiate reductase inhibitor therapy immediately after myocardial infarction, irrespective of lipid levels [9].

The objective of the study was to investigate the effectiveness of atorvastatin and melatonin that possesses antioxidant and/or hypolipidemic effects on the changes that occur in patients with type 2 DM due to uncontrolled glycemic status.

Methods

This study was carried out on forty one (41) patients (26 females and 15 males) with T2 DM who attend the Specialized Center for Endocrinology and Diabetes - AL-Risafa Directorate of Health - Baghdad were enrolled from October 2011 to April 2013.

Inclusion criteria: Patients with T2 DM and hyperlipidemia of both sexes on sulfonylurea (glibenclamide), with age range 35-60 years (46.76 ± 7.89), and have disease duration of 5-10 years.

Exclusion criteria: They should not have other associated chronic diseases like liver and kidney disorders and cardiovascular complications. Patients who are pregnant and breast feeding are excluded. They should not be on insulin therapy or other antidiabetic drugs, or on antioxidant drugs like aspirin, and any associated drugs should be considered. They should not taking other hypolipidemic agent; anti-inflammatory or non steroidal anti-inflammatory drugs.

Patients treated previously with full maximum dose of sulfonylurea (glibenclamide) (15 mg/day) and kept on dietary control, (but with poor glycemic control as evidenced by abnormal values of fasting plasma glucose; glycated hemoglobin (HbA_1c); and dyslipidemia; those patients are carefully evaluated while they are on their already established treatment program for DM control) for 2 weeks before randomization into three groups:

Group I: includes 13 patients treated with placebo (starch 50 mg) in capsule dosage form in addition to the already given oral hypoglycemic agent (glibenclamide) and dietary control, for 12 weeks.

Group II: includes 14 patients treated with atorvastatin 20 mg given as single daily doses in a tablet dosage form, in addition to the already given oral hypoglycemic agent (glibenclamide) and dietary control for 12 weeks; and

Group III: includes 14 patients treated with melatonin 10mg hard gelatin capsule once daily (10 mg/day) in addition to the already given oral hypoglycemic agent (glibenclamide) and dietary control for 12 weeks.

After 12 hours fasting, blood samples were collected from all subjects by venepunture (10 ml), before starting drug treatment (as base line samples) and then after 6 weeks and 12 weeks of treatment to follow the changes in the studied parameters. Blood samples were divided into two tubes, one heparinized tube (1 ml of whole blood used for HbA_1c determination) and the other part was transferred into a plane tube to collect serum after centrifugation at 3000 rpm for 10 min at 4 ^{o}C.

Fasting serum glucose level (FSG) was determined using a readymade kit for this purpose, according to the method of Barham and Trendoer [10]; Glycated Hemoglobin (HbA_1c) was evaluated using the VARIANT HbA_1c program utilizes the principles of ion exchange high performance liquid chromatography [11].

Serum total cholesterol (TC) was estimated according to the method of Richmond [12]; serum triglyceride (TG) levels were determined according to the method of Fossati and Prencipe [13]; serum high density lipoprotein cholesterol (HDL-C) levels were estimated according to the method of Burstein [14] and serum low density lipoprotein cholesterol (LDL-C) was calculated by using this formula:

LDL-C = Total cholesterol – (TG/2.2) – (HDL-C)[15].

All Results were expressed in mmol/L except of HbA_1c in percent. Paired *t*-test and ANOVA were used to examine the degree of significance, and a value of $P < 0.05$ was considered significant.

Results

The data presented in table 1 clearly showed that in comparison with value at baseline in the same group after 12 weeks of treatment, no significant difference in FSG of placebo and atorvastatin with a high significant decrease in FSG of melatonin group was recorded. In comparison with a placebo-treated group at corresponding duration, after 6 weeks of treatment there is a significant increase in FSG of atorvastatin group. After 12 weeks of treatment, no significant difference in FSG of atorvastatin with a high significant decrease in melatonin group were recorded (Table 1).

In comparison with value at baseline in the same group after 6 weeks of treatment there is significant decrease in S. HbA1c of Placebo group, a high significant decrease in HbA_1c of melatonin group and significant increase in atorvastatin group.

After 12 weeks of treatment, no significant difference in S. HbA1c of placebo and atorvastatin groups, and a high significant decrease in HbA_1c of melatonin group recorded. In comparison with a placebo-treated group at corresponding duration, after 12 weeks of treatment, no significant difference in HbA_1c of atorvastatin group while a significant decrease in HbA_1c of melatonin group were seen (Table 1).

Table 1. Comparison of fasting serum glucose and glycated hemoglobin at different duration of treatment in each group

Group	Fasting serum sugar (mmol/l)			Glycated hemoglobin (%)		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
Group I N = 13	11.68±3.64	10.62±2.94*	11.62±2.79	8.35±1.93	8.6±1.94	8.15±1.61
Group II N = 14	11.92±3.22	14.26±4.8*a	13.27±2.88	8.19±1.24	8.83±1.39*	8.57±1.52
Group III N = 14	10.39±2.42	9.15±2.78*	7.64±1.83**b	8.36±1.13	7.76±1.03**	6.91±1.15**a

Comparison is with baseline value within the same group (* = $P < 0.05$), ** = $P < 0.001$), a = significant difference ($P < 0.05$) between drug group and placebo group at corresponding duration, b = $P < 0.001$ between drug group and placebo group at corresponding duration.

Concerning the effect of drugs treatment on lipid profile in comparison with value at baseline in the same group after 12 weeks of treatment, there was no significant difference in TC, TG and HDL-C with a significant increase in LDL levels of placebo group. While a high significant decrease in TC and TG was found in atorvastatin and melatonin groups. No significant difference in HDL-C of atorvastatin group and a high significant increase in HDL-C of melatonin group

were recorded. While a high significant decrease in LDL-C of atorvastatin and melatonin groups were found.

In comparison with a placebo-treated group at corresponding duration, after 12 weeks of treatment there was a significant decrease in TC was found in melatonin group with a highly significant decrease in TC of atorvastatin group were found (Table 2). No significant difference in TG of atorvastatin and melatonin groups and a significant increase in HDL-C of atorvastatin and melatonin groups. There was no significant difference in LDL-C of melatonin group and a significant decrease in LDL-C of atorvastatin group was found (Table 2).

Table 2. Comparison of lipid profile at different duration of treatment in each group

Lipid profile		Group I N = 13	Group II N = 14	Group III N = 14
Cholestrol (mmol/l)	Baseline	5.77±1.25	6.79±1.77	6.19±0.82
	6 Weeks	5.68±0.89	5.84±1.39**	5.79±0.77**
	12 Weeks	6.08±0.95	4.65±1.01**b	4.94±0.81**a
Triglyceride (mmol/l)	Baseline	1.92±0.83	2.55±1.53	2.84±1.07a
	6 Weeks	2.07±1.2	1.76±1.04*	2.34±0.86**
	12 Weeks	2.12±0.86	1.5±0.83*	1.98±0.81**
High-density lipoprotein (mmol/l)	Baseline	0.99±0.3	1.39±0.25a	0.97±0.11
	6 Weeks	1.16±0.21*	1.38±0.24a	1.08±0.1**
	12 Weeks	1.04±0.22	1.39±0.29a	1.24±0.09**a
Low-density lipoprotein (mmol/l)	Baseline	2.72±1.02	2.96±1.39	3.97±0.57b
	6 Weeks	2.51±1.01*	2.38±0.56	3.8±0.58b
	12 Weeks	2.94±0.96*	2.12±0.82**a	3.29±0.43**

Comparison is with baseline value within the same group (* = $P < 0.05$, ** = $P < 0.001$), a = $P < 0.05$ between drug group and placebo group at corresponding duration, b = $P < 0.001$ between drug group and placebo group at corresponding duration.

Discussion

Atorvastatin has been reported in some cases to disrupt glycemic control in patients with T2 DM [16]. The mechanism by which atorvastatin disrupts glycemic control remains unknown; however, atorvastatin was shown to inhibit adipocyte maturation and glucose transporter 4 (Glut 4) expression by blocking isoprenoid biosynthesis, thus impairing glucose tolerance [17]. These statements agreed with the results obtained in the present study which shown an increment in the FSG and HbA_1c levels after 12 week of treatment with atorvastatin (Table 1). There is evidence for a diabetes-preventing effect of melatonin, whereas pinealectomy increases the risk of diabetes [18]. Likewise, further data demonstrated that melatonin directly influences both glucose metabolism and insulin secretion from the *β*-cell of the pancreas [19].

In this respect, the data presented in this study are consistent with those indicated previously (Table 1), in which the administration of 10 mg per day of melatonin after 12 weeks significantly ($P < 0.001$) decreases FSG and HbA_1c levels. The presumed action of melatonin as a regulator of β-cells responsiveness to glucose may be exerted by both direct and indirect mechanisms. It has been reported that melatonin receptors are present in the pancreas [20].

Atorvastatin competitively inhibit this enzyme resulting in decreasing *de novo* cholesterol synthesis, and increasing expression of LDL receptors on hepatocytes. This increases LDL-C uptake by the hepatocytes, resulting in decreasing the amount of LDL-cholesterol in the

blood [21]. Like other statins, atorvastatin also reduces blood levels of TGs and slightly increases levels of HDL-C [22]. The present results in table 2 were compatible with the studies mentioned above about the effects of atorvastatin on serum levels of cholesterol; triglycerides and LDL.

As shown in table (2), treatment with 10 mg melatonin significantly reduces total cholesterol, triglyceride, and LDL-C plasma levels. These data are compatible with those reported by (Sudhakumari *et al*, 2001) [23] who showed that plasma melatonin levels were inversely related to the levels of lipids. In conclusion, administration of melatonin in therapeutic doses significantly improves the impaired lipid profile in dyslipidemia states associated with diabetes mellitus [24].

Acknowledgment

We would like to thank the College of Medicine, Al-Nahrain University and the Specialized Center for Diabetes and Endocrinology, Baghdad, Iraq for supporting this project.

Author contribution

Dr. Haitham M. Kadhim collected the cases and interpretation of results and discussion; Professor Dr. Faruk H. Aljawad interpreted the results; and Professor Dr. Hashim M. Hashim suggested the statistical analysis and clinical assessment throughout the study.

Conflict of interest

There is no conflict of interest**.**

Funding

Personal funding with contribution of all authors.

References

1. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000; 321: 405-12.
2. Farmer JA. Diabetic dyslipidemia and atherosclerosis: evidence fromclinical trials. Curr Diab Rep. 2008; 8: 71-7.
3. Turner RC, Millns H, Neil HA, et al. Risk factors for coronary artery disease in non-insulindependent diabetes mellitus: United Kingdom Prospective DiabetesStudy (UKPDS 23). BMJ. 1998; 316: 823-8.
4. Brunzell JD, Davidson M, Furberg CD, et al. Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. Diabetes Care. 2008; 31: 811-22.
5. Goff DC Jr, Gerstein HC, Ginsberg HN, et al. Prevention of cardiovascular disease in persons with type 2 diabetes mellitus: current knowledge and rationale for the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. Am J Cardiol. 2007; 99:4i-20i.
6. Zobali F, Avci A, Canbolat O, et al. Effects of vitamin A and insulin on the antioxidant state of diabetic rat heart: a comparison study with combination treatment. Cell Biochem Funct. 2002; 20: 75-80.
7. Rang HP, Dale MM, Ritter JM, et al. Rang and dales pharmacology, 6th ed.: Churchill Livingstone, 2011; p 503.
8. Pandi-Perumal SR, Srinivasan V, Maestroni GJM, et al. Melatonin, Nature's most versatile biological signal? FEBSJ, 2006; 273:2813–2838.
9. Bertrum KG. Basic and clinical pharmacology, 9th ed. McGraw-Hill, 2010; p.p. 627
10. Barham D, Trendoer P. An improved color reagent from the determination of blood glucose by the oxidative system. Analyst. 1972; 97: 142-5.
11. Mayer TK, Freedman ZR. Protein Glycosylation in diabetes mellitus: A review of laboratory measurements and of their clinical utility. Clin Chem Acta. 1983; 127: 147-84.
12. Richmand W. Proceedings in the development of an enzymatic technique for the assay of cholesterol in biological fluids. Clin Sci Mol Med. 1974; 46: 6-7.
13. Fassati P, Principe L. Measurement of serum triglyceride colorimetrically with an enzyme that produce H_2O_2. Clin Chem. 1982; 28(10): 2077-80.
14. Burstein M, Scholink HR, Morfin R. Measurement of HDL-C in the plasma with a sensitive colorimetric method. J Lipid Res. 1970; 19: 583.
15. Burtis CA, Ashwood ER. Tietz, Textbook of clinical chemistry, 3rd ed.: WB Sawanders Comp. 1999. p. 837-46, 766-73, 921-2.
16. Yamakawa T, Takano T, Tanaka S, et al. Influence of putavastatin on glucose tolerance in patients with type 2 diabetes mellitus. J Atheroscler Thromb. 2008; 15: 269-75.
17. Nakata M, Nagasaka S, Kusaka I, et al. Effects of statins on the adipocyte maturation and expression of glucose transporter 4 (SLC2A4): Implications in glycemic control. Diabetologia. 2006; 49: 1881-92.
18. Conti A, Maestroni GJ. Melatonin rhythms in mice: role in autoimmune and lymphoproliferative diseases. Ann NY Acad Sci. 1998; 840: 395-410.

19. Peschke E, Mühlbauer E, Musshoff U, et al. Receptor (MT (1)) mediated influence of melatonin on cAMP concentration and insulin secretion of rat insulinoma cells INS-1. J Pineal Res. 2002; 33: 63-71.
20. Williams LM, Hannah LT, Adam CL, et al. Melatonin receptors in red deer fetuses. J Reprod Fertil. 1997; 110: 145-51.
21. Nissen S, Sipahi I, Libby P, et al. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA. 2006; 295(13): 1556-65.
22. Fox LP, Merk HF, Bickers DR. Dermatological pharmacology: In: Brunton LL, Lazo JS, Parker KL (eds). Goodman & Gilman's: the pharmacological basis of therapeutics. 11th ed. McGraw-Hill; 2008. p. 1679-706.
23. Sudhakumari CC, Halder C, Senthikumaran B. Seasonal changes in adrenal and gonadal activity in the quail, Perdiculaasiatica: Involvement of the pineal gland. Comp Biochem Physiol Biochem Mol Biol. 2001; 128(4): 793-804.
24. Ismail SH. Dose dependent hypolipidemic effects of melatonin in dyslipidemia associated with diabetes, University of Baghdad, PhD thesis. 2004.

Correspondence to Dr. Haitham M. Kadhim
E-mail: haitham7424@yahoo.com
Tel. +964 7901442943.

Received 28th Oct. 2013. Accepted 15th Mar. 2014

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 Effect of Finasteride on Bleeding During Transurethral Resection of the Prostate

Firas S. Al-Qurayshe[1] *MBChB*, **Adib M. Alkazzaz**[1] *FICMS*, **Usama S. Al-Nasiry**[2] *FRCS*

[1]Dept. of Urology, Al-Imamain Al-Kadhimain Medical City, [2]Iraqi Commission for Medical Specialization, Baghdad, Iraq

Abstract

Background Finasteride is a selective 5-α reductase enzyme inhibitor that blocks the conversion of testosterone to dihydrotestosterone, down regulates prostatic angiogenesis and blood flow and promotes apoptosis of prostatic cells. It significantly decreases suburethral prostatic microvessel density in patients with benign prostatic hyperplasia, which may explain its efficacy for decreasing benign prostatic hyperplasia -associated bleeding. Transurtheral resection of the prostate is considered the gold standard operation for symptomatic benign prostatic hyperplasia. It is characterized by immediate treatment success with long-lasting improvement of symptoms.

Objective To study the effect of finasteride on bleeding during transurethral resection of the prostate.

Methods Thirty eight patients with benign prostatic hyperplasia underwent transurethral resection of the prostate. Patients were randomly assigned into two groups, the finasteride group (18 patients) and the control group (20 patients). The intra-operative bleeding was measured.

Results Statistically less significant bleeding during transurethral resection of the prostate in patients who were placed on 10 mg finasteride for 6 weeks prior to the surgery.

Conclusion Finasteride given daily for 6 weeks prior to transurethral resection of the prostate reduces time of surgery, bleeding, and irrigation fluid requirements.

Keywords Hematuria, Finasteride, Benign prostatic hyperalgesia.

List of Abbreviations: B.I.D = Twice daily, BPH = Benign prostatic hyperplasia, DRE = Digital rectal exam, 5-ARIs = Five-alpha reductase inhibitors, Hb = Hemoglobin, KUB = Plain x-ray of the kidney, ureter, bladder, MSU = Midstream urine, PSA = Prostate specific antigen, US = Ultrasonography, TURP = Transurethral resection

Introduction

For most of the 20th century from 1909, when Hugh Hampton Young performed his first cold-cut prostatic punch operation, until the late 1990s, when effective medical therapy, and newer less invasive technologies for prostatic obstruction were developed, transurethral resection of the prostate (TURP) remained the most common surgical option for benign prostatic hyperplasia worldwide. Despite the minimally invasive nature of TURP, bleeding remains the most common complication of the procedure [(1)]. Historically, transfusion rates during TURP were reported to be as high as 20% [(2)]. Improvements in resectoscopes, optics, anesthesia, and energy sources have caused the transfusion rate to fall, but hemorrhage remains a common complication. A recent multi-institutional study reported transfusion rates of up to 2.9% after TURP [(3)]. Other studies have shown the transfusion rate to be below 2% at major surgical centers [(4)].

Patients with large prostates, concurrent urinary tract infections, or indwelling urinary catheters have traditionally been at greater risk of TURP related bleeding [(5)].

Finasteride is a selective 5α-reductase inhibitor for type 2 isoenzyme. The aim of this study is to assess the effect of finasteride on bleeding during TUR-P.

Methods

The study was conducted between April 2010 to October 2011, in the department of urology, Al-Imamain Al-Kadhemain Medical City, Baghdad. Thirty eight patients with prostatic enlargement greater than 60 cm^3 were included in the current study. Patients with vesicle stone, bladder tumor, bleeding tendency, renal impairment, and uncontrolled hypertension were excluded from the study. Patients already taking finasteride, on anticoagulant drugs or elevated prostatic specific antigen (PSA) were also excluded from the study.

All of the included patients underwent full pre-operative work-up, including digital rectal exam (DRE), PSA, coagulation tests, plain x-ray of the kidney, ureter and urinary bladder (KUB), sonography of the abdomen and pelvis, urine analysis, and mid-stream urine for culture and sensitivity.

Eighteen patients were given 10 mg (5 mg BID) finasteride daily for 6 weeks prior to TUR-P (The Finasteride group), while 20 patients underwent TUR-P without taking finasteride (The control group).

The operation was done by the same surgeon, using unipolar Storz resectoscope, and distilled water as the irrigate fluid. In each TUR-P operation the following parameters were evaluated, actual duration of resection, amount of irrigation fluid used, hemoglobin (Hb) in a sample of the irrigation fluid, and Hb of the patient.

To calculate the amount of intra-operative blood loss, we use the following formula:

$$\text{Blood loss (ml)} = \frac{\text{Hb level in irrigate fluid (gm/dl)} \times \text{total volume of irrigate fluid (ml)}}{\text{Hb of the patient (gm/dl)}}$$ [6]

Statistically, the T-test is used to measure the P value of the evaluated parameters.

Results

A total of 38 patients with prostatic enlargement underwent TUR-P. They were assigned into two groups.

The mean age of Finasteride group was 70.5 years (range 62 to 80), the mean prostate size was 77.1 cm^3 (range 64 to 89), the mean duration of resection was 44.1 minutes (range 33 to 58), the mean irrigation fluid used was 11.4 liter (range 9 to 14), and the mean intra-operative blood loss was 261.5 ml (range 210 to 352).

On the other hand, for the 20 patients of the control group, the mean age was 68.2 years (range 58 to 79), the mean prostate size 76.6 cm^3 (range 67 to 85cm^3), the mean duration of resection was 65.7 minute (range 55 to 72min.), the mean intra-operative blood loss was 461.8 ml (range 343.3 to 612.4ml), and the mean irrigation fluid used was 16.1 liter (range 12 to 19 L).

These results showed a difference in the mean duration of resection between both groups which was 21.6 minutes. In addition, the difference in the mean intra-operative blood loss was 200 ml and the difference in the intra-operative irrigation fluid used was 4.7 liter (Table 1).

Using the T-test to measure the P value, comparative statistical analysis of these results showed a significant difference in the duration of resection, the intra-operative blood loss and the intra-operative irrigation fluid used, between the two groups of the study ($P < 0.0005$) as seen in table 1.

Discussion

Hematuria associated with BPH is probably related to increased vascularity in the prostate,

which is associated with friable prostatic tissue (either primary or regrowth after prostatectomy). Marshall and Narayan first reported the beneficial effects of hormonal therapy on prostatic bleeding, which they proposed a result of suppressed angiogenesis secondary to androgen deprivation [7].

Table 1. Differences in the comparative parameters between TURP+Finastride and control groups

Parameter	Control group	TURP + Finastride
Resection duration (min)	65.7	44.1*
IO blood loss (ml)	461.8	261.8*
IO irrigate fluid (L)	16.1	11.4*

IO = intraoperative, * *P* = <0.0005

While this initial observation was made to patients with prostate cancer, Puncher and Miller subsequently described a similar association of finasteride with BPH associated gross hematuria [8]. Hochberg et al provided one of the first histological proofs for decreased suburethral microvessel density in finasteride treated prostates. Given that BPH associated hematuria has been correlated with suburethral prostatic microvessel density, so it can be postulated that the efficacy of finasteride for decreasing BPH associated prostatic bleeding is mediated through suppression of microvessel density [9]. Donohue et al has also shown in his trial that finasteride reduces prostatic vascularity rapidly within 2 weeks [10].

The results of the current study show that the finasteride group patients showed less bleeding, during TUR-P, than the control group. This finding is in agreement with the studies conducted by Hagerty et al [11], Donohue et al [12] and Ozdal et al [13]. As bleeding is reduced, visibility during resection improves, thus speed of resection accelerates, and the amount of fluid irrigate used for surgery is reduced.

In conclusion, finasteride given prior to TURP as a combination therapy is found to reduce time of surgery, intraoperative blood loss and irrigation fluid requirement.

Limitations

This study is unique in Iraq; however, the relatively small number of patients is one limitation. The conclusions reached herein have to be checked with the American Urological Association and European Association of Urology guidelines.

Acknowledgement

We thank Professor Dr. Zaid Al-Medfaee from the department of physiology, college of medicine, Baghdad University for performing the statistical analysis.

Author contributions

Professor Usama Al-Nasiry planned the study design and setting, Dr. Adib did the operative work and the writing of the manuscript and Dr. Firas collected the clinical data and assisted in the operative work.

Declaration of interest

None of the authors has conflict of interest of any kind and with whomever.

Funding

The current research was self-funded. The authors have no financial support to declare.

References

1. Ahyai SA, Gilling P, Kaplan SA, et al. Meta-analysis of functional outcomes and complications following transurethral procedures for lower urinary tract symptoms resulting from benign prostatic enlargement. Eur Urol. 2010; 58: 384-97.
2. Mebust WK, Holtgrewe HL, Cockett AT, et al. Transurethral prostatectomy: immediate and postoperative complications. Cooperative study of 13 participating institutions evaluating 3,885 patients. J Urol. 2002; 167: 999-1003.
3. Wendt-Nordahl G, Bucher B, Hacker A, et al. Improvement in mortality and morbidity in transurethral resection of the prostate over 17 years in a single center. J Endourol. 2007; 21: 1081-7.
4. Reich O, Gratzke C, Bachmann A, et al. Morbidity, mortality and early outcome of transurethral resection of the prostate: a prospective multicenter evaluation

of 10,654 patients. J Urol. 2008; 180: 246-49.
5. Uchida T, Ohori M, Soh S, et al. Factors influencing morbidity in patients undergoing transurethral resection of the prostate. Urology. 1999; 53: 98-105
6. Elmalik EM, Ibrahim AL, Gahli AM, et al. Risk factors in prostectomy bleeding. Eur Urol. 2000; 37: 199-204.
7. Marshall S, Narayan P. Treatment of prostatic bleeding: suppression of angiogenesis by androgen deprivation. J Urol. 1993; 149: 1553-4
8. Miller MI, Puchner PJ. Effects of finasteride on hematuria associated with benign prostatic hyperplasia. Urology. 1998; 51: 237-40.
9. Hochberg DA, Basillote JB, Armenakas NA, et al Decreased suburethral prostatic microvessel density in Finasteride treated prostates. J Urol. 2002; 167: 1731-3.
10. Donohue JF, Hayne D, Kamik U, et al. Randomized, placebo- controlled trial showing that finasteride reduces prostatic vascularity rapidly within 2 weeks. BJU Int. 2005; 96: 1319-22.
11. Hagerty JA, Ginsberg PC, Harmon JD, et al. Pretreatment with finasteride decreases perioperative bleeding associated with transurethral resection of the prostate. Urology. 2000; 55: 684-9.
12. Donohue JF, Sharma H, Abraham R, et al. Transurethral prostate resection and bleeding: a randomized, placebo controlled trial of role of finasteride for decreasing operative blood loss. J Urol. 2002; 168:2024-2026.
13. Ozdal OL, Ozden C, Benli K, et al. Effects of short term finasteride therapy on preoperative bleeding in patients who were candidates for TUR-P. Prostate cancer prostatic Dis. 2005; 8(3): 215-8.

Correspondence to Dr. Adib M. Alkazzaz
E-mail: alkazzaztrd@hotmail.com
Received 3rd Sep. 2013: Accepted 26th Mar. 2014.

Iraqi JMS

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

Assessment of Complete Blood Count in Patients with Coronary Artery Disease

Qais A. AL-Oqaily *MSc*

Dept. of Pathology & Forensic Medicine, College of Medicine Al-Nahrain University, Baghdad, Iraq.

Abstract

Background Several studies have demonstrated the role of hematological parameters like hemoglobin (Hb), white blood cells (WBC) count and platelet count in the assessment of patients with coronary artery disease; some studies suggest an additional role of platelet indices in the prognosis of patients with coronary artery disease.

Objective To assess complete blood count and platelet indices in patients with coronary artery disease.

Methods Blood sample of 42 newly diagnosed ischemic heart disease patients including 20 patients with myocardial infarction (MI) and 22 patients with unstable angina were studied. The control group included 15 individuals with no history of heart disease and a normal electrocardiogram. Clinical and laboratory information regarding age, sex, packed cell volume, WBC count, platelets count, platelet distribution width and platelet volume were obtained.

Results The frequency of diabetes and smoking was higher in patients with coronary heart disease in comparison to the control group ($P < 0.05$), while the frequency of hypertension was not significntly different. Significant difference in WBC count and Hb level of patients with coronary heart disease ($P < 0.05$), while no significant difference in the platelet count, platelet distribution width and mean platelet volume was found. In patients with unstable angina there was a significant difference in the Hb level ($P < 0.05$) while no significant difference in WBC count, platelet count, platelet distribution width and mean platelet volume was observed. In patients with myocardial infarction, there was a significant difference in the Hb level and the WBC count ($P < 0.05$), whereas no significant difference in platelet count, platelet distribution width and mean platelet volume was found.

Conclusion Hemoglobin level was significantly lower in patients with coronary artery disease while the mean level of WBC count was significantly higher than that of the control group. There was no significant difference in platelet count and platelet indices between those two groups. In patients with unstable angina the WBC count was not statistically different from that of the control group.

Key words Coronary artery disease, Hb, WBC, PDW, MPV.

List of Abbreviation: CHD = Coronary Heart Disease, MI = Myocardial Infarction, PCV = Packed Cell Volume, WBC = White Blood Cells, PDW = Platelet Distribution Width, MPV = Mean Platelet volume, EDTA = Ethylene Diamine Tetra Acetic acid

Introduction

Coronary heart disease (CHD) is the most common form of heart disease and is the single most important cause of premature death in Europe. By 2020, it is estimated that it will be the major cause of death in all regions of the world. Disease of the coronary arteries is almost always due to atheroma and its complications, particularly thrombosis [1].

Angina pectoris is the symptom complex caused by transient myocardial ischemia; it may occur whenever there is an imbalance between myocardial oxygen supply and demand. Unstable

angina is a clinical syndrome that is characterized by new-onset or rapidly worsening angina, angina on minimal exertion or angina at rest. The condition shares common pathophysiological mechanisms with acute myocardial infarction and the term 'acute coronary syndrome' is used to describe these disorders collectively [1]. Myocardial infarction (MI) is due to the formation of occlusive thrombus at the site of rupture or erosion of an atheromatous plaque in a coronary artery [1].

Ischemic heart disease is mainly caused by atherosclerosis and its complications. Platelets and their activity have an important role in initiation of atherosclerotic lesions and coronary thrombus formation. Larger platelets are enzymatically and metabolically more active and have a higher potential thrombotic ability as compared with smaller platelets [2].

Inflammation is a key feature of atherosclerosis and its clinical manifestations. The leukocyte count is a marker of inflammation that is widely available in clinical practice. WBC counts are a predictor of coronary heart disease mortality independent of the effects of smoking and other traditional cardiovascular disease risk factors [3].

The objective of this study was to assess variations in complete blood count and platelet indices in patients with coronary artery disease compared to healthy individuals.

Methods

This study is a case-control study, which was designed to include 42 cases with ischemic heart disease; 20 patients had MI and 22 had unstable angina. The diagnosis was based on history and characteristic electrocardiographic changes. The third group comprised 15 aged and sex matched healthy controls with no history of heart disease and a normal electrocardiogram.

Medical history was taken for patients including history of hypertension and/or diabetes mellitus in addition to drug history and smoking.

This study was conducted from August 2013 to October 2013 on the blood sample of 42 newly diagnosed ischemic heart disease patients including 20 patients with MI and 22 patients with unstable angina, in addition to 15 control individuals with no history of heart disease and a normal electrocardiogram. The cases were collected from the Coronary Care Unit of Al-Kadhimiya Teaching Hospital. Clinical and laboratory information regarding age, sex, packed cell volume (PCV), white blood cells (WBC) count, platelets count, platelet distribution width (PDW) and mean platelet volume (MPV), were obtained. Two milliliters of k3 ethylenediamine tetra acetic acid (EDTA) blood was obtained from each patient and a full blood count was performed within 2 to 4 hours using the Sysmex blood analyser.

Statistical analysis

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Data were presented as mean ± SD or as number and percent. One way ANOVA and LSD tests were used to compare means of more than two groups while t-test was used to compare means of two groups. Chi-square test was used to compare frequency. *P*-value less than 0.05 was considered significant.

Results

In this study, thirty three of the patients with coronary heart disease were males (78.6%) and 9 of them were females (21.4%) with a male to female ratio of 3.6:1; on the other hand, 9 of 15 control cases were males (60%), while 6 were females (40%) with a male to female ratio of 1.6:1. There was no significant difference in gender of patients with coronary artery disease and control group.

The age range of patients with coronary heart disease was between 33-76 years with a mean of 58.8 ± 11 (mean ± SD) years. By dividing the patients` age according to 10-year intervals, the largest number of patients (13 out of 42) fall in the age group 50-59. Regarding the control group, the age range was between 14-38 years and the mean was 24.7 ± 7 (mean ± SD) years, with the largest number of cases falling in the 20-29 years age group. This study showed that there was a significant difference ($P < 0.05$) in age of patients with coronary artery disease and the control group.

When evaluating the risk factors for coronary artery disease, this study showed that the frequency of diabetes and of smoking was higher in patients with coronary heart disease in comparison to the control group ($P < 0.05$), while the frequency of hypertention was not significntly different in patients with coronary artery disease in comparison to the control group (Table 1).

Table 1. Age, gender, and frequency of various risk factors in patients with coronary artery disease

Parameter	Coronary artery disease (N = 42)		Control (N = 15)		*P* value
	No.	%	No.	%	
M/F	33/9		9/6		0.185
Age (yrs)	58.83	11.47	24.73	7.39	0.000
Hypertension	17	40.48	2	13.33	0.056
Diabetes	22	52.38	1	6.67	0.002
Smoking	10	23.81	0	0	0.049

In the current study, thirteen patients with unstable angina were males while nine patients were females. On the other hand all patients with myocardial infarction were males. There was a significant difference in gender of patients with unstable angina as compared to myocardial infarction in which there was male predominance, $P = 0.001$. This study revealed that smoking, hypertention and diabetes millutes have no significant difference in frequency between unstable angina and myocardial infarction (Table 2).

Table 2. Age, gender, and frequency of various risk factors in patients with unstable angina and myocardial infarction

Parameter	Unstable angina		Myocardial Infarction		Total		*P* value
	No.	%	No.	%	No.	%	
M/F	13/9		20/0		33/9		0.001
Age (yrs)	59±11.48		58.65±11.74		58.83±11.46		0.923
Hypertension	8	36.36	9	45	17	40.48	0.569
Diabetes	12	54.55	10	50	22	52.38	0.768
Smoking	4	18.18	6	30	10	23.81	0.477

Regarding the hematological parameters, there was a significant difference in WBC count and hemoglobin (Hb) level of patients with coronary heart disease in comparison with that in the control group ($P < 0.05$). No significant difference in the platelet count, platelet distribution width and mean platelet volume was found between patients with coronary heart disease and the control group (Table 3). Regarding patients with unstable angina there was no significant difference in WBC count, platelet count, platelet distribution width and mean platelet volume between the unstable angina group and the control group ($P > 0.05$); on the other hand there was a significant difference ($P < 0.05$) in the Hb level (Table 4).

The mean age of patients complaining from unstable angina was 59±11.4 (mean ± SD) years and the mean age of patients complainig from myocardial infarction was 58.6±11.7 (mean ± SD) years and the mean age of the control group was 24.73±7.3 (mean ± SD) years. This study showed that patients with unstable angina and MI were

significantly ($P < 0.001$) older than the control group (Tables 4 and 5).

In patients with myocardial infarction, there was no significant difference in platelet count, platelet distribution width and mean platelet volume between the myocardial infarction group and the control group ($P > 0.05$), whereas there was a significant difference ($P < 0.05$) in the Hb level and the WBC count (Table 5).

Table 3. Hematological parameters in the coronary artery diseased group and the control group

Parameter	Coronary artery disease (N = 42)	Control (N = 15)	*P* value
	Mean±SD	Mean±SD	
Hb (g/dl)	13.50 ± 2.39	15.53 ± 1.95	0.005
WBC (x10^9)	11.77 ± 4.55	7.65 ± 1.33	0.000
Platelet (x10^9)	230.98 ± 69.41	223.33 ± 40.19	0.690
PDW (%)	14.36 ± 3.2	13.68 ± 1.52	0.435
MPV (fl)	10.50 ± 1.66	10.57 ± 0.53	0.826

Table 4. Comparison of age and hematological parameters between unstable angina group and the control group

Parameter	Unstable Angina (N = 22)	Control (N = 15)	*P* value
	Mean±SD	Mean±SD	
Age (yrs)	59.0 ± 11.48	24.73 ± 7.39	< 0.001
Hb (g/dl)	13.39 ± 1.89	15.53 ± 1.95	0.008
WBC (x10^9)	9.79 ± 3.89	7.65 ± 1.33	0.079
Platelet (x10^9)	220.64 ± 77.74	223.33 ± 40.19	0.899
PDW (%)	15.13 ± 3.71	13.68 ± 1.52	0.127
MPV (fl)	10.82 ± 1.78	10.57 ± 0.53	0.604

Table 5. Comparison of age and hematological parameters between myocardial infarction group and the control group

Parameter	Myocardial Infarction (N = 20)	Control (N = 15)	*P* Value
	Mean±SD	Mean±SD	
Age (yrs)	58.65 ± 11.74	24.73 ± 7.39	< 0.001
Hb (g/dl)	13.61 ± 2.89	15.53 ± 1.95	0.018
WBC (x10^9)	13.96 ± 4.28	7.65 ± 1.33	< 0.001
Platelet (x10^9)	242.35 ± 58.78	223.33 ± 40.19	0.382
PDW (%)	13.51 ± 2.34	13.68 ± 1.52	0.856
MPV (fl)	10.16 ± 1.48	10.57 ± 0.53	0.406

Discussion

In this study, 78.6% of the patients with coronary heart disease were males and 21.4% of them were females with a male to female ratio of 3.6:1, which is in line with Debra *et al* [4] and Michaels *et al* [5] who stated that at any given

age the prevalence of coronary artery disease is higher in men than in women. Moreover, there was a significant difference between unstable angina and myocardial infarction in terms of gender, where females complaining from myocardial infarction were significantly lower than those complaining from unstable angina; this may support Shehab *et al* [6] who found gender differences in acute coronary syndrome.

The current study showed that there was an increased incidence of developing coronary artery disease with older age, which agrees with Michaels *et al* [5].

When evaluating the risk factors for coronary artery disease, this study showed that diabetes and smoking were more frequently found among patients with coronary heart disease in comparison to the control group and thus may have an important effect in the development of coronary heart disease, which is in concordance with Michaels *et al* [5] and Véronique *et al* [7] studies. However, the frequency of hypertension in patients with coronary artery disease was not significantly different from that of control group. This disagrees with many studies including Michaels *et al* [5] and Véronique *et al* [7] studies. This difference may be attributed to the small sample size.

Regarding WBC count, there was a significant increase in the WBC count in patients with coronary heart disease compared to control group, this finding is in agreement with Madjid *et al* [8], Hoffman *et al* [9], and Lee *et al* [10] who found that patients with elevated white blood cell counts are at higher risk of developing acute myocardial infarction and acute coronary events.

Regarding the Hb level, there was a significant decrease in the Hb in patients with coronary heart disease compared to the control group. This finding agrees with Asimacopoulos *et al* [11] who found that anemia can be manifested as angina due to decreased oxygen carrying capacity to the heart.

This study showed that there was no significant difference in the platelet count, platelet distribution width and mean platelet volume between patients with coronary heart disease and the control group. This finding does not agree with Khandekar *et al* [12] who found that platelet distribution width and mean platelet volume are significantly raised in patients with acute myocardial infarction and unstable angina, however Beyan *et al* [13], Stokol *et al* [14], and George [15] stated that mean platelet volume is not a reliable index with the use of EDTA in complete blood count, because the EDTA causes time dependent variation in the platelet size indicating that the platelet volume will increase by 30% within 5 minutes of exposure to EDTA; so they stated that MPV measurements should be standardized before depending on it in the diagnosis.

In conclusion, hemoglobin level was significantly lower in patients with coronary artery disease while the mean level of WBC count was significantly higher than that of the control group. There was no significant difference in platelet count and platelet indices between those two groups. In patients with unstable angina the WBC count was not statistically different from that of the control group.

Acknowledgments

Great thanks to the staff of Pathology Department in the college of Medicine /Al-Nahrain University and Coronary Care Unit in Al-Imamain Al-Khadhimain Medical City.

Conflict of interest

There is no conflict of interest.

Funding

Al-Imamain Al-Khadhimain Medical City funded this research.

References

1. Bloomfield P, Bradbury A, Grubb NR, et al. Cardiovascular disease. In: Colledge NR, Walker BR, Ralston SH (eds). Davidson's clinical and practice of medicine, 20th ed. Edinburgh. Churchill Livingston. 2007. pp: 581-9.
2. Ranjith MP, Divya R, Mehta VK, et al. Significance of platelet volume indices and platelet count in ischemic heart disease. J Clin Pathol. 2009; 62(9): 830-3.

3. Brown DW, Giles WH, Croft JB. White blood cell count: An independent predictor of coronary heart disease mortality among a natural cohort. J Clin Epidemiol. 2001; 54(3): 316-22.
4. Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: A 26-year follow-up of the Framingham population. Am Heart J. 1986; 111(2): 383-90.
5. Michaels AD. Coronary heart disease. In: Goldman L and Schafer AI (eds). Cecil essentials of medicine, 8th ed. Philadelphia: Saunders Elsevier; 2010. p. 96.
6. Shehab A, Yasin J, Hashim MJ, et al. Gender differences in acute coronary syndrome in Arab Emirati women—implications for clinical management. Angiology. 2013; 64(1): 9-14.
7. Véronique LR, Alan SG, Donald ML, et al. Heart disease and stroke statistics. Circulation. 2011; 123: e18-e209.
8. Madjid M, Awan I, Willerson JT, et al. Leukocyte count and coronary heart disease : Implications for risk assessment. J Am Coll Cardiol. 2004; 44(10): 1945–56.
9. Hoffman M, Blum A, Baruch R, et al. Leukocytes and coronary heart disease. Atherosclerosis. 2004; 172(1): 1-6.
10. Lee CD, Folsom AR, Nieto FJ, et al. White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and white men and women. Atherosclerosis risk in communities study. Am J Epidemiol. 2001; 154(8): 758-64.
11. Asimacopoulos PJ, Groves MD, Fischer DK, et al. Pernicious anemia manifesting as angina pectoris. South Med J. 1994; 87(6): 671-2.
12. Khandekar MM, Khurana AS, Deshmukh SD, et al. Platelet volume indices in patients with coronary artery disease and acute myocardial infarction: an Indian scenario: J Clin Pathol. 2006; 59(2): 146-9.
13. Beyan C. Mean platelet volume for distinguishing between inherited thrombocytopenias and immune thrombocytopenia. Br J Haematol. 2013; 163: 404-20.
14. Stokol T, Erb HN. A comparison of platelet parameters in EDTA- and citrate-anticoagulated blood in dogs. Veter Clin Pathol. 2008; 36(2): 148-54.
15. George TI. Automated hematology instrumentation: up to date. Jul 24, 2012. Available from: http://www.uptodate.com. Accessed October 2013.

E-Mail: gaisalogaily@yahoo.com
Received 14th Oct. 2013: Accepted 27th Oct. 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

Renal Lower Pole Ratio as a Predictor of Lower Pole Stone Clearance after extracorporeal shock wave lithotripsy

Adil H. Al-Soufi *FICMS*, **Raghib J. Hameed** *FICMS*, **Saif AM. Abdul-Hameed** *CABMS*

Dept. of Urology, Medical City Complex, Baghdad, Iraq

Abstract

Background The Lower pole anatomy (apart from other factors: stone size, shock wave energy) is an important determinant of success after extracorporeal shockwave lithotripsy.

Objectives In this study, we aimed to determine if there is a significant relationship between lower pole ratio (infundibular length: infundibular width) on preoperative excretory urograms and stone fragment clearances after shockwave lithotripsy.

Methods A total of 60 patients with isolated lower pole stones were prospectively included in the study. Anatomical factors, such as infundibular length and width were measured and the lower pole ratio was calculated on pretreatment excretory urogram. Stone fragment clearance was assessed on periodic follow up visits (1-8weeks) with a plain abdominal X-ray for kidney, ureter and bladder.

Results The overall eight-week stone-free rate was 56.66%. Mean stone size ± SD was 11.383 ± 5 mm, mean infundibular length was 11.95 ± 6.52 mm, mean infundibular width was 4.25 ± 1.66 mm and mean lower pole ratio was 3.2 ± 2.4. Stone free status after shockwave lithotripsy was significantly related to infundibular length and width as well as to lower pole ratio. Infundibular length less than 25 mm, width greater than 4 mm and lower pole ratio less than 3.5 were noted to have an improved eight week stone-free rate.

Conclusion Lower pole anatomy is an important predetermining factor for lower pole stone clearance after shockwave lithotripsy. The present study suggests that a lower pole ratio of less than 3.5, which considers both infundibular length and width, is a promising and easily applicable predictor for stone-free status.

Key words Lower pole ratio, extracorporeal shockwave lithotripsy, stone clearance.

List of Abbreviation: SWL = shock wave lithotripsy, ESWL = extracorporeal shock wave lithotripsy, PCNL = percutaneous nephrolithotomy, KUB = kidney, ureter and bladder, CPH = calyceal pelvic height.

Introduction

Replacement of open surgery with minimally invasive techniques for treating stones in the renal tract has greatly reduced patients' morbidity and mortality and the period of hospitalization and convalescence. Extracorporeal shockwave lithotripsy does not require anesthesia and requires little analgesia so that treatment can be given on an outpatient basis, and there is no wound to heal [(1)].

Beginning in 1969 and funded by the German Ministry of Defense, Dornier began a study of the effects of shockwaves on tissue [(2)].

In 1972, on the basis of preliminary studies performed by Dornier Medical Systems [(3)] the development of the Dornier lithotripter progressed through several prototypes, ultimately culminating in February 1980 with the first treatment of a human by shock wave lithotripsy (SWL). The production and distribution of the Dornier HM3 lithotripter

began in late 1983, and SWL was approved by the U.S. Food and Drug Administration in 1984. Since Dornier's pioneering work, numerous other companies have demonstrated that shockwaves capable of stone fragmentation may be generated by electromagnetic induction, micro explosions, focused lasers, and piezoelectric crystals. To date, more than 3000 lithotripters of all types have been placed worldwide, and more than 1 million patients are treated annually with SWL [3].

Generally, extracorporeal shock wave lithotripsy (ESWL) is characterized by a low complication rate and only by a few absolute contraindications [4].

An accurate estimation of the individual's probability of stone clearance may be essential for proper treatment selection to determine who will experience maximum benefit from ESWL. Therefore, the identification of prognostic factors compromising the clinical outcome of ESWL-treated calculi might be crucial to opt for the most appropriate maneuver [5].

The likelihood of fragmentation with ESWL depends on stone size and location, anatomy of renal collecting system, degree of obesity, and stone composition. ESWL is most effective for stones < 2 cm in diameter, in favorable anatomical locations. It is less effective for stones > 2 cm diameter, in lower-pole stones, in a calyceal diverticulum (poor drainage), and those composed of cystine or calcium oxalate monohydrate (very hard). Lower stone-free rates as compared with open surgery or percutaneous nephrolithotomy (PCNL) are accepted because of the minimal morbidity of ESWL [5].

Calculi situated in the lower calices represent a particular problem. First of all, a large number of renal calculi originate in the lower calices, and, obviously, the clearance rate of these stones appears to be reduced compared to other locations [6].

Secondly, ESWL fragments even from other parts of the kidney are recovered in favor of the lower calyces. This issue may be mainly attributed to the gravity. Moreover, the geometrical features of the lower calyx anatomy are also supposed to hamper the clearance. Prognostic factors such as the angle, length, or tightness of the infundibulum were analyzed in detail [6].

ESWL is a sophisticated procedure and demands skill. Knowledge about the characteristic features of the lithotripter is essential. Lithotripters vary in the source of shock wave generation, and later generation devices use smaller focal zones, allowing higher peak point pressures [7].

As any surgical procedure, ESWL yields a better clinical outcome when it is performed by an experienced user familiar with the device. Thus, urology training programs are recommended to focus carefully on ESWL because it is the least invasive of the common modalities for definitive stone treatment [7]. In general, the clearance rate of renal calculi varies, ranging from 45% to 95% [8].

The outcome of stone clearance after ESWL is strongly related to stone disintegration and clearance of the fragments [9]. Stone disintegration is affected by several factors, including stone factors (burden, number, composition), patient factors (obesity, body habitus), operator`s experience, and machine factor (type of lithotripter, shock wave number, shock wave energy) [10-12].

In addition, the clearance rate of stone fragments is influenced by stone location and the patterns of intrarenal collecting system drainage and urinary transport [13-14].

Hence, in 1992, Sampaio and Aragao studied the correlation of lower pole collecting system anatomy and ESWL from cadavers [15]. Lingeman et al demonstrated that the clearance rate of stone fragments was worse over the lower calyces than over the middle or upper calyces [13]. Accordingly, after the measurement of lower calyceal anatomy in excretory urography (EU) initially demonstrated by Elbahnasy et al many authors raised different viewpoints about the measurement of the lower calyceal anatomy [16].

Plain-film radiography of the kidneys, ureters and bladder may be sufficient to document the size and location of radiopaque urinary calculi.

Stones that contain calcium, such as calcium oxalate and calcium phosphate stones are easiest to detect by radiography [17]. Less radiopaque calculi, such as pure uric acid stones and stones composed mainly of cystine or magnesium ammonium-phosphate, may be difficult, if not impossible, to detect on plain-film radiographs. Excretory urograms have been considered the standard imaging modality for urinary tract calculi. The excretory urograms provides useful information about the stone (size, location, radiodensity) and its environment (calyceal anatomy, degree of obstruction), as well as the contralateral renal unit (function, anomalies). Excretory urogram is widely available, and its interpretation is well standardized [17] (Fig. 1).

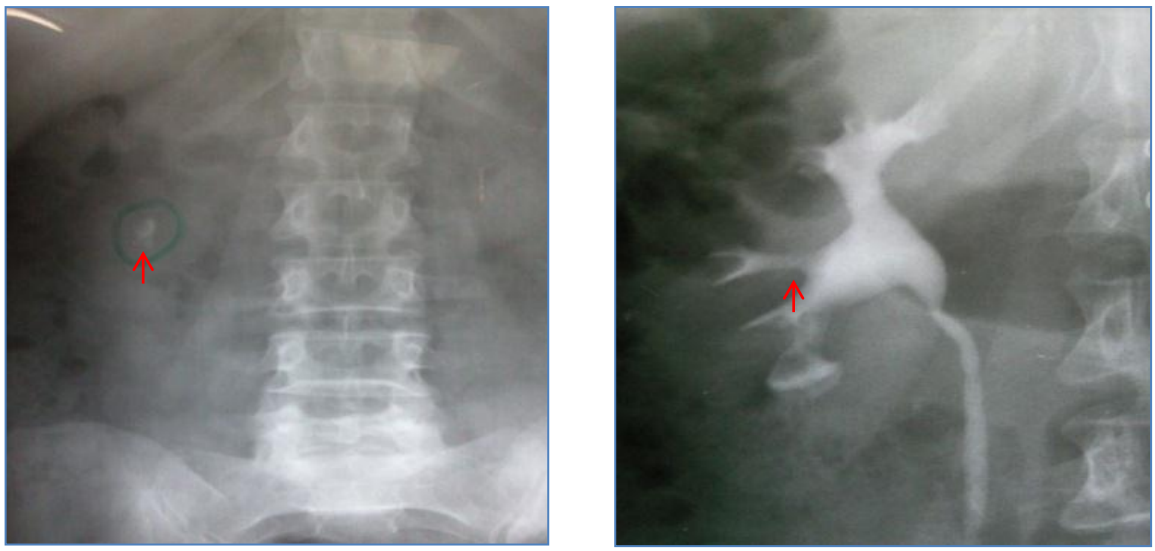

Fig. 1. X-ray (kidney, ureter and bladder) and excretory urogram showing lower pole stone.

Methods

In the time period between September 2008 and September 2011, a total of 80 patients, referred from the urology outpatient clinic in the Surgical Specialties Hospital, underwent ESWL for a solitary radio opaque lower pole renal stone detected on (excretory urograms) performed in the radiology department of the same hospital.

Exclusion criteria included stones greater than 20 mm, a documented pyelonephritis, previous renal surgery in two patients and renal anomalies (duplex kidney) in two patients. Sixteen patients defaulted follow-up or X-ray.

A total of 60 patients (34 male and 26 female) between 21 and 71 years old who underwent ESWL with Storze SLX FII machine were included in the study. Stone length was measured as the maximum diameter of the stone on the plain X-ray (KUB film). Pre-ESWL excretory urograms (5 minutes and 10 minutes films) were used to determine the lower pole infundibular length and width as previously described (Fig. 2).

The lower pole ratio (infundibular length: width) was then calculated. Lower pole infundibular length is the distance in mm from the most distal point at the bottom of the calyx to the midpoint at the lower lip of the renal pelvis. Lower pole infundibular width was measured at the narrowest point along the infundibular axis in mm [16] (Fig. 3).

Success of ESWL was determined by the stone-free status after 8 weeks. The patients were scheduled for follow up visits in outpatient clinic at (1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 6^{th}, and 8^{th} week) post primary treatment. Routine abdominal X-rays of the KUB film were employed for follow up. Any stone fragment detectable on kidney, ureter and bladder abdominal X-rays (KUB films), regardless of size, persisting after 8 weeks was defined as a

residual stone and they were managed with another treatment line.

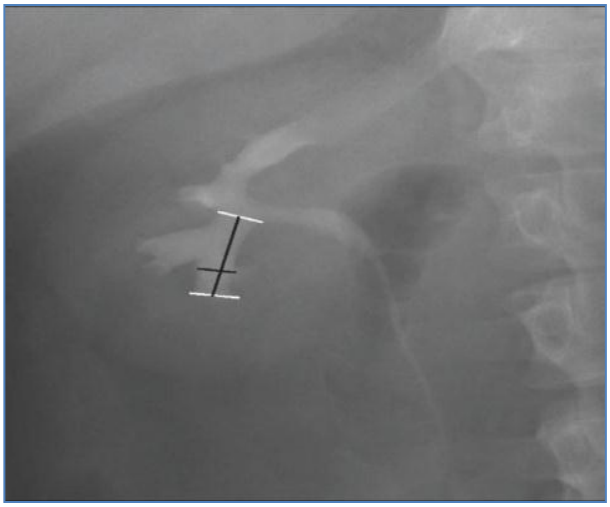

Fig. 2. Measuring lower pole infundibular length in 10 min. Excretory urogram (EU) film

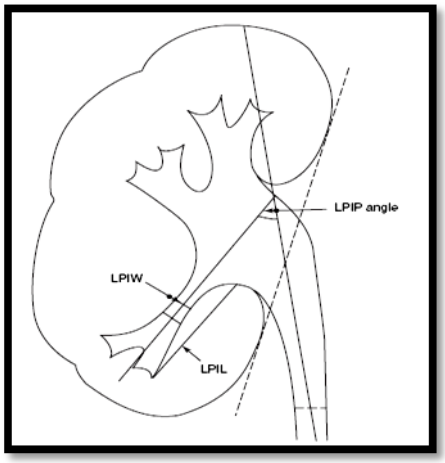

Fig. 3. Measurement of lower pole infundibular length, width and infundibulopelvic angle. LPIP angle, lower pole infundibulo-pelvic angle: LPIL. lower pole infundibular length: LPIW. lower pole infundibular width

Results

All 60 solitary stones were located in the lower pole, with a mean stone diameter of (11.38 ± 4.5 mm) for a patients group with a mean age of (46.31 ± 13.08 years). Of the 50 patients (28 males and 22 females), 34 were stone-free eight weeks after treatment for an overall stone clearance rate of 56.66%. There was no significant difference in the ages, sex distribution and mean stone sizes in the stone-free and residual stone group.

Significant difference between the stone-free and the residual stone groups were noted in the follow up period and the number of shock waves required, since the residual stone group were more reluctant to treatment (Table 1).

Table 1. Patient's characteristics and ESWL data

Parameter	Patients group			
	Stone free		Residual Stone	
	mean ± SD	range	mean ± SD	range
Age (years)	43.41 ± 15.42	21-71	50.12 ± 10.15	32-65
Number of shocks	3412 ± 1270	1500 - 6000	5058 ± 1614.5**	3000 - 7000
Stone Diameter (mm)	10.47 ± 4.33	6-20	12.58 ± 4.52	8-20
Follow Up Period (weeks)	1.56 ± 1	1-4	5.62 ± 2.2*	1-8

* = $P < 0.001$, ** $P = 0.00004$

The male:female ratio = 21:13 for stone free group and 17:9 for the residual stone group. The mean infundibular length was 11.95 ± 6.52mm, mean infundibular width was 4.25 ± 1.66 mm and the mean lower pole ratio was 3.2 ± 2.4.

Univariate analysis revealed that infundibular length, width and lower pole ratios were significant predictors for stone clearance (Table 2).

Table 2. Univariat analysis of anatomical factors of Patient's groups predicting 8-weeks stone-free status

Parameters	Patients Group		*P* value
	Stone free	Residual Stone	
Mean Infundibular length (mm)	9.53 ± 5.21	15.12 ± 6.81	0.00065*
Mean Infundibular width (mm)	4.82 ± 1.59	3.42 ± 1.50	0.00017*
Mean Lower pole ratio	1.96 ± 1.06	4.87 ± 2.96	0.00001*

We evaluated the effect of lower pole anatomy on stone clearance based on the criteria described by Elbahasy *et al* [16] and Madbouly *et al* [18]. We found that eight-weeks stone-free rates improved significantly in infundibular width of more than 4 mm (*P* = 0.0442) and that infundibular width of ≤ 4 mm predicted stone persistence and failure of ESWL (*P* = 0.0001) as noticed in Table 3.

Table 3. Infundibular width (mm) in Patient's groups

Infundibular width (mm)		Patients Group			*P* Value
		Stone free	Residual Stone	Total	
≤ 4	**No.**	13	22	35	
	% within infundibular	37.1	62.9	100	0.055
	% from Total	21.7	36.7	58.3	0.11
> 4	**No.**	21	4	25	
	% within infundibular	84.0	16.0	100	0.000006
	% from Total	35.0	6.7	41.7	0.00033
Total	**No.**	34	26	60	
	% within infundibular	56.7	43.3	100	
P value within patient group		0.0442	0.0001		

Most of patients in this study, 93.3%, had infundibular lengths of less than 25 mm. Infundibular length of less than 25 mm predicted successful treatment (*P* = 0.0001), while patients with infundibular length of ≥ 25 mm all had failed treatment with ESWL (Table 4).

Table 4. Infundibular length (mm) in Patient's groups

Infundibular length (mm)		Patients Group			*P Value*
		Stone free	Residual Stone	Total	
< 25	**No.**	34	22	56	
	% within infundibular	60.7	39.3	100	0.038
	% from Total	56.7	36.7	93.3	0.044
≥ 25	**No.**	0	4	4	
	% within infundibular	0.0	100	100	
	% from Total	0.0	6.7	6.7	
Total	**No.**	34	26	60	
	% within infundibular	56.7	43.3	100	
P value within patient group		0.0001			

Forty-two patients (70%) had lower pole ratio of 3.5 or less compared to 18 (30%) with lower pole ratio > 3.5 and this ratio was associated with better stone clearance ($P < 0.0002$) as seen in (Table 5).

Table 5. Lower pole ratio in patient's groups

L.P Ratio	Patients Group				Total		*P* value
	Stone free		Residual Stone				
	No.	%	No.	%	No.	%	
≤ 3.5	34	100	8	31	42	70	0.0002
> 3.5	0	0	18	69	18	30	
Total	34	100	26	100	60	100	

* = $P < 0.05$.

Discussion

Though ESWL is widely used in the treatment of renal stones, its efficacy in clearing lower pole stones has been questionable. In this study, the eight-week stone-free rate of 56.66% is comparable to other centers, which reported stone-free rates between 25 and 85% [19].

In a meta-analysis of the management lower pole stone, Lingeman *et al* [14] suggested that stone-free rate was significantly affected by stone size, dropping from 74% in lower pole stones less than 10 mm to 56.3% and 32.6% in stone size between 10 and 20 mm and more than 20 mm, respectively.

Most authors agreed that lower pole stone greater than 20 mm should be treated with percutaneous removal, but controversy arises when it comes to the primary management of lower pole stones between 10 and 20 mm in size. It is for this reason we decided to limit the present study stone size to less than 20 mm, with the majority of the stones between 10 and 20 mm [15].

The reason for the dismal result for lower pole stone clearance is due to fragment retention rather than failure of stone disintegration. Sampaio and Aragao first described the importance of inferior pole collecting system in ESWL in 1992 [15]. Subsequently, Elbahnasy *et al* [16] established the role of lower pole spatial anatomy, namely the infundibular length, width and angle in predicting the success of ESWL.

Other important anatomical factors affecting stone clearance after ESWL included calyceal pelvic height (CPH) by Tuckey *et al* [20] renal morphology by Madbouly *et al* [18] and number of minor calyces by Sumino *et al*.[21]

We excluded patients with abnormal renal morphology either congenital or acquired from infection or renal surgery. We did not consider the measurement of CPH as it may vary with respiration or postural changing of the kidney.[21]

In the present study, we used the method of Elbahnasy *et al*. for the measurement of lower pole spatial anatomy [16]. The mean infundibular length in our patients was found to be shorter than that described by Elbahasy *et al* [16] and Madbouly *et al* [18] (11.95 ± 6.52 mm *vs.* 29.9 mm and 36.4 mm, respectively). This difference may be due to the different study population or different imaging films used in excretory urograms. Unfortunately no other Asian study is available for reference. However, the mean infundibular width was comparable using the lower pole anatomical predictors proposed by Elbahasy *et al* [16]. In this study lower pole eight-weeks stone-free rates were significantly better in patients with infundibular widths of more than 4 mm and infundibular lengths of less than 25 mm. Univariate analyses confirmed that both infundibular length and width were important predictors for stone-free rate and that infundibular width (using the mean infundibular width) was a stronger predictor. The mean

infundibular length was significantly shorter and the infundibular width significantly wider in the stone-free group.

An interesting article by Knoll *et al* [22] showed a high interpersonal variation in the measurement of the lower pole anatomy. In the present study, we measured the parameters twice on two separate contrast-filled films by two urologists. The average of the measurements was taken for the final analysis. Most of the measurements were within the 10 percent margin. However, this is an important consideration for the measurement of the parameters and further prospective studies will need to be done to improve the reproducibility of these measurements.

Anatomical measurements of the lower pole were all derived from intravenous urograms, which neglected the 3-D anatomy of the lower pole, especially the lower pole infundibular opening. However, it is time consuming and expensive to employ CT scans with 3-D reconstruction to obtain 3-D measurements. We measured the narrowest point along the infundibular axis as the infundibular width and it correlated well with the success of shockwave lithotripsy for lower pole stone. We propose the use of a lower pole ratio for predicting the success of ESWL, as it considers both these important factors. Patients with a lower pole ratio of 3.5 or less had a significantly better stone clearance rate when compared to those with a ratio >3.5 (*P* value=0.0002) making it a better candidate for predicting eight-weeks stone-free status compared to infundibular length or width alone.

In conclusion, lower pole spatial anatomy, namely the infundibular length and width, has a significant role in the stone-free status after ESWL. Classifications of an infundibular width > 4 mm and an infundibular length ≤ 25 mm had an impact on the eight-week stone-free rate. A lower pole ratio of 3.5 seems to be a promising predictor, as it considers both anatomical factors. This will be especially helpful in deciding the first-line treatment for lower pole stones measuring between 10 and 20 mm in maximum diameter.

Acknowledgments

We would like to thank the shock wave lithotripsy unit staff and our colleagues at the urology department at the Surgical Specialties Hospital for their tremendous help in presenting this humble effort.

Author contribution

Dr. Adil Al Soufi and Dr. Raghib Jassam were diagnosed and collected the cases and Dr. Saif Al Haideri follows the patients during their treatment course and statistically interpreting the data.

Conflict of Interest

The author declares no conflict of interest.

Funding

None.

References

1. Wickham JEA. Minimally invasive surgery, treatment of urinary stones. BMJ. 1993; 307: 1414-7.
2. Matlaga BR, Lingeman JE. Campbell-Walash urology 10th ed. Chap. 48, Surgical Management of Upper Urinary Tract Calculi, 2010; p.p 1324-60.
3. Chaussy C, Fuchs G. Extracorporeal lithotripsy in the treatment of renal lithiasis. 5 years' experience. J Urol (Paris). 1986; 92: 339-43.
4. Skolarikos A, Alivizatos G, de la Rosette J. Extracorporeal shock wave lithotripsy 25 years later: complications and their prevention. Eur Urol. 2006; 50: 981-90.
5. Albala DM, Gomella LG, Allen F. Oxford American Handbook of Urology, Chap. 8, 1st ed., 2011; p.p 372-73.
6. Tiselius HG, Ackermann D, Alken P, et al. Working Party on Lithiasis. Guidelines on urolithiasis. Eur Urol. 2001; 40: 362-71.
7. Augustin H. Prediction of stone-free rate after ESWL. Eur Urol. 2007; 52: 318-20.
8. Clayman RV, McClennan BL, Garvin TJ, et al. Lithostar: an electromagnetic acoustic shock wave unit for extracorporeal lithotripsy. J Endourol. 1989; 3: 307-10.
9. Ruggera L, Beltrami P, Ballario R, et al. Impact of anatomical pyelocalyceal topography in the treatment of renal lower calyces stones with extracorporeal shock wave lithotripsy. Int J Urol. 2005; 12: 525-32.

10. Gerber R, Studer UE, Danuser H. Is newer always better? A comparative study of 3 lithotriptor generations. J Urol. 2005; 173: 2013-6.
11. Graber SF, Danuser H, Hochreiter WW, et al. A prospective randomized trial comparing 2 lithotriptors for stone disintegration and induced renal trauma. J Urol. 2003; 169: 54-7.
12. Portis AJ, Yan Y, Pattaras JG, et al. Matched pair analysis of shock wave lithotripsy effectiveness for comparison of lithotripters, J Urol. 2003; 169: 58-62.
13. Schulz E, Hengst E, Brundig P, et al. Disturbed urinary transport in the pelvi-calyceal system in calcium-oxalate stone patients. Urol Res. 1987; 15: 109-13.
14. Lingeman JE, Siegel YI, Steele B, et al. Management of lower pole nephrolithiasis: a critical analysis. J Urol. 1994; 151: 663-7.
15. Sampaio FJB, Aragao AHM. Inferior pole collecting system anatomy: it`s probable role in extracorporeal shock wave lithotripsy. J Urol. 1992; 147: 322-4.
16. Elbahnasy AM, Clayman RV, Shalhav AL, et al. Lower-pole caliceal stone clearance after shockwave lithotripsy, percutaneous nephrostolithotomy, and flexible ureteroscopy: impact of radiographic spatial anatomy. J Endourol. 1998; 12: 113-9.
17. Portis AJ, Sundram CP. Diagnosis and Initial Management of Kidney Stones. Am Fam Physician. 2001; 63(7): 1329-34.
18. Madbouly K, Sheir KZ, Elsobky E. Impact of lower pole renal anatomy on stone clearance after shock wave lithotripsy: Fact or Fiction? J Urol. 2001; 165: 1415-18.
19. Chen RN, Streem SB. Extracorporeal shock wave lithotripsy for lower pole calculi: long-term radiographic and clinical outcome. J Urol. 1996; 156: 1572-5.
20. Tuckey J, Devasia A, Murthy L. Is there a simpler method for predicting lower pole stone clearance after shock wave lithotripsy than measuring infundibulopelvic angle? J Endourol. 2000; 14: 475-8.
21. Sumino Y, Mimata H, Tasaki Y. Predictors of lower pole renal stone clearance after extracorporeal shock wave lithotripsy. J Urol. 2002; 168: 1344-7.
22. Knoll T, Musial A, Trojan L. Measurement of renal anatomy for prediction of lower pole caliceal stone clearance: reproducibility of different parameters. J Endourol. 2003; 17: 447-51.

Correspondence to Dr. Saif AM. Abdul-Hameed
E-mail: saifal_haideri@yahoo.com
Mobile: + 964 7705352630
Received 21st Jan.2014: Accepted 25th Mar. 2014

المجلد الثاني عشر، العدد الاول، 1435 هـ، 2014م

المجلة العراقية للعلوم الطبية

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

رقم الإيداع في دار الكتب و الوثائق ببغداد 709 لسنة 2000