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Iraqi Journal of Medical Sciences

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

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

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.
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- Manuscripts must be accompanied by a covering paper signed by all authors that the paper has not been published in and will not be submitted to any other journal if accepted in IJMS.
- The page should contain (a) title of the manuscript, (b) names of each author (first name, middle initial and family name) including highest academic degree, (c) official academic and/or clinical title and affiliation (d) name and address of the institution where the work was done (e) name and address (E-mail if available) of the author to whom correspondence should be sent.

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

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

Examples

1. Standard Journal Article: use et al when the number of authors exceeds 6.
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.
3. Chapter in book: Phillips SJ, and Whisnant JP. Hypertension and strock. In: Laragh JH, and Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

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

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

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Toward More Objective Teaching

Hikmat AR Hatim *FRCS*

Head of National Accreditation Committee Of Medical Colleges in Iraq

Lectures

The lecture may be the oldest method used to transferring information in Medical education. There are however serious questions regarding the effectiveness of the traditional lecture approach so academic doctors often are not trained in giving effective lectures.

Lecture together with small group teaching and bedside teaching formed the traditional picture of the Medical School. There are many calls to move away from the traditional lecture to interactive computer learning systems that allow students access to information when and where they need it Skill there is and will continue to be, a need for educators' who are prepared to deliver lectures.

Lecture are not effective for demonstrating practical skill or detailed procedures nor for having students apply ideas, solve problems or clarify values but lectures can be used to present and organized information, promote understanding of concepts and ideas and create interest in a subject.

Characteristics of the lecture method

Lecture in medical education often have a poor reputation and even claim that the lecture format for large classes is outdated and ineffective

Lecture is frequently a one – way process unaccompanied by discussion, questioning or immediate practice which makes it a poor teaching method. So why do we lecture? Most educators how to teach based on their experience as students. This (teach as I was taught)

Most educators lecture how to teach based on their experiences as student this (teach as I was taught approach. Tends to perpetrate the lecture as a passive one – way method of Transferring information.

The lack of faculty training in presenting effective lecture rather than the method itself may be the greatest weakness of the lecture.

Appropriate lecture is

- Disseminating information quickly to a large audience
- Presenting new information before using other media or activities, i.e. a brief Lecture
- Providing an over view of a topic
- Arousing interest in a topic
- The Lecture will be not appropriate when Presenting complex, detailed or obstruct information.
- Dealing with information concerning feeling and attitudes.
- Training in psycho motor – skill.
- Teaching high – level cognitive skill (e.g synthesis and evaluation)

Planning Lecture

Lecture should be planned. The lecture must:

1. Establish the purpose of the lecture

2. Consider the logistics of Lecture
3. Plan a variety of approaches, i.e., use questioning , small group activates
4. Prepare a set of lecture notes

Relevance and applications

The material presented should be shown to be relevant to the course and to material presented. The relevance of the contents should be made obvious to the student and any key points should be emphasized.

Purpose of the lecture

The primary purpose of the lecture is to transfer information from the instructor to the student. Before developing the content of the lecture it is a good idea to clearly state the purpose of the lecture. The purpose should describe in general terms what the student will learn during the lecture. The objective of the lecture should be precise and measurable and what the student will learn by attending the lecture.

Lecture components

- Use of opening summary
- At the beginning of the lecture, present major points and conclusion to help student organize their listening.
- Presses key terms
- Offer examples – provide real - life illustration of the Idea in the lecture.
- Use analogies; if possible make a comparison between contort of the lecture and knowledge the students already have.
- Use visual backups – to enabled student to see as well as to hear what is being said.

The key it as effective lecture style is to break down the lecture into its component parts and use of variety of approaches within each component.

Duration

Studies have shown that student attention deteriorates after 45 mints.

Presentation techniques

Presentation skill

The lecturer is performer who has to entertain the audience.

- Where to stand?
- are you near the microphone
- Are you obstructing the audience view or the screen
- Are you going to sit or be able to a walk around

How to speak

1. It should be clearly stated at the beginning whether the lecturer intends student to take note or not.
2. Taking down every word should be discouraged.

a. If the role of the lecture is to point a general overview then only the occasional word need be written down and the pace and style of lecture can be quicker and more colloquial.

3. It is very bad and fatal to read a lecture if student concentration is to be maintained but some notes will help to keep the main points in view and are invaluable to fall back on if interrupted or diverted.
4. Be sure you are audible at the back.
5. Variations in pitch and speed make a lecture more enjoying.
6. An occasional appropriate joke or reference to a current social event which may help to break up the style of your lecture and recapture student attention.
7. Allow student to catch up if they are being expected to take detailed notes.
8. Visual aids should be cued into notes so that they are not forgotten or brought in at the wrong time.

Keeping in contact with the audience

- Maintain eye contact with the audience but not with particular student.
- Do not speak to the walls or the screen.
- It is possible to walk around the lecture hall and speak from different points
- Beware of dimming the hall light completely as you will lose eye contact with audience and encourage sleep.

- Communicate on a personal level the lecturer should attempt to relate to the student during the lecture.
- Avoid the use of slang or repetitive words, phrases or gestures that may become distracting with extended use.
- Ask a number of questions and Encourage student to ask questions.
- Provide positive feedback when students ask questions, use students names as often as possible.
- Mule smooth transitions between parts of the lecture which might include:
    1. A brief overview of the next topic
    2. A review of the agenda between topics.
    3. A change of media
    4. An interim summary before a new topic
    5. An activity – case study or problem solving activity

Aids

Any additional material presented in a lecture must be of good quality

Slides and pictures

Slides and pictures can be used to illustrate clinical features, radiographs, results of investigations, etc… Attention should be paid to the size of the words.

Hand out

May be given at the beginning of the lecture. To give an outline of the content.

Over head projector

Care must be paid to the amount, size and clarity of writing, it should not be

- Too small
- Too crowded
- Too scribbled

Computerized presentation

Power point presentation is highly recommended and encouraged as the best suitable way of delivering a proper lecture.

It can project all above including, pictures and procedures it is important, however that technical assistance is available and that breakdown can be dealt with promptly.

The lecturer must have.

Practiced to become familiar with the computer program used.

Wed site

Copies of handout, illustration, power point, slides and other recourses martial can subsequently be made available at a website for student

Smeary

With planning and effective presentation techniques, the lecture can be a highly effective and interactive method for transferring knowledge to student and in modern curriculum a lecture is only one of several ways of imparting factual information.

Its role in the context of the entire course most be made clear. As well as presenting core material; lectures can also be used to give overview of the course.

The lecture should be cleanly structured, have defined aims and objectives and be accompanied by explicit lesson plan.

An evaluation of lecture using a video recording or an observer can assess the lecture. In assessing the quality of the presentation and improving the lecture skill.

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Foxp3-Infiltrating Cellular Expression in Rheumatic Mitral Valve Tissue Lesions

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Abstract

Background The Foxp3 gene is exclusive. It found in nTregs and correlates with the suppressive activity of these cells.

Objective To detect Foxp3 expression in infiltrating cells in the rheumatic mitral valve tissue lesions and its correlation with the extent of histopathological abnormalities when considered naturally occurring CD4+CD25+ regulatory T cells (nTregs) as the main expressers for this protein.

Methods Rheumatic mitral valve surgical fragments were taken from a total of 48 Iraqi patients with chronic rheumatic heart disease underwent mitral valve replacement in Ibn Al-Bitar Hospital for Cardiac Surgery-Iraq-Bagdad from October 2006-September 2007. Formalin-fixed paraffin-embedded mitral valve tissue sections were prepared. Foxp3-expressing cells were detected by using immunohistochemical staining technique, and histopathological picture was visualized by using hematoxylin and eosin staining.

Results Our results showed that there were no significant association between Foxp3 expression and the history of acute rheumatic fever (negative or positive), and/or the frequency of attacks (single or multiple) among all groups under study ($p > 0.05$). Also, we found a significant negative correlation between the percentage of Foxp3 strong positive cells and the extent of histopathological abnormalities.

Conclusions There was a negative correlation between Foxp3 strong positive expression and the extent of histopathological abnormalities which reinforce the immunosuppressive role of nTregs against the inflammatory and autoimmune reactions in chronic rheumatic heart disease.

Key words Foxp3, CD4+CD25+ regulatory T cells, mitral valve

Introduction

The Foxp3 gene encodes the protein Scurfin, a member of the forkhead \winged-helix family of transcriptional regulators and is highly conserved in humans [(1)]. It is exclusively found in naturally occurring CD4+CD25+ regulatory T cells (nTregs) and correlates with the suppressive activity of these cells. In mice, Foxp3 is almost exclusively expressed intracellulary in nTregs, whereas in humans, its expression is also detected in extrathymically generated regulatory T cells [(2)].

Foxp3 has been shown to be important for nTregs function [(3)]. There is also evidence suggest that Foxp3 may be important for the induction of nTregs [(4)], in that the introduction of the Foxp3 gene into CD4+CD25- cells resulted in the generation of an anergic\suppressor phenotype similar to regulatory T cells [(5)] .These cells were able to suppress T cell activation in vitro.

Unlike CD25, cytotoxic T lymphocyte associated antigen-4 (CTLA-4), glucocorticoid-induced tumor necrosis factor receptor-related gene

(GITR), and lymphocyte activation gene-3 (LAG-3) markers proposed to define the Treg cell population, Foxp3 is not upregulated in recently activated CD4+CD25- T cells. Only a subset of the Foxp3-expressing cells upregulated CD25 expression and acquired suppressor function after 2 weeks in vivo. More aggressive autoimmune syndrome resulting from genetic deficiency in Foxp3 as compared to that resulting from CD25+ T cell depletion [(6)]. Analogous mutation of human Foxp3 was found to be responsible for immunodysregulation, polyendopathy, enteropathy, X-linked diseases (IPEX) [(1-8)]. In both mouse and human, the Foxp3 mutation is responsible for the most aggressive autoimmune diseases that resulted in early lethality. More importantly, targeted mutation of Foxp3 in hematopoietic cells is both necessary and sufficient to ablate nTregs development. Analysis studies of the immunological basis of autoimmune diseases associated with Foxp3 mutation observed a very substantial reduction in thymic cellularity. The reduction was caused by a decrease in the proliferation of immature thymocytes that lake both CD4 and CD8 co-receptors, thus autoimmune diseases associated with Foxp3 mutation require both T cell-intrinsic defects that result in defective development of regulatory T cells and T cell-extrinsic defects in thymocyte production [(9)]. Thus, the aim of this study was to detect Foxp3 expression in infiltrating cells in the rheumatic mitral valve tissue lesions and its correlation with the extent of histopathological abnormalities.

Methods

This study was conducted from October 2006 to September 2007. Rheumatic mitral valve surgical fragments were taken from 48 patients with chronic rheumatic heart disease underwent mitral valve replacement surgery in Ibn Al-Bitar Hospital for Cardiac Surgery.

All patients were divided according to the positive or negative history of rheumatic fever (PHORF and NHORF), PHORF patients were subdivided according to the frequency of rheumatic fever, and according to the period of medical treatment into single attack under continuous medication (SA^{UCM}), single attack without continuous medication (SA^{WCM}), high risk under continuous medication (HR^{UCM}), and high risk without continuous medication (HR^{WCM}). Negative controls for mitral valve tissue samples were taken from 20 cadavers, their cause of death was not related to the acute or chronic rheumatic heart disease, infective endocarditis or any other heart disease, and their age and sex were matched with chronic rheumatic heart disease patients. Paraffin embedded mitral valve tissue sections (5µm thickness) were prepared using positive charge slides (Fisher Scientific, USA).

Hematoxylin and eosin staining was performed on mitral tissue sections for each patient. Mouse anti- Human Foxp3 protein (Serotic, UK) were used to detect Foxp3 expression in mitral valve infiltrating mononuclear cells using immunohistochemical staining technique.

Statistical Analysis

Foxp3 signals were evaluated by counting the number of positive cells which adhere to the valvular endothelium and the total number of infiltrating cells in 5 to 50 microscopic fields to measuring the percentage of positive cells which was calculated according to the following equilibrium:

The percentage of positive cells = the number of positive cells / the number of total cells X 100

The immunohistochemistry signals of Foxp3 expression on infiltrating cells demonstrating on the mitral valve tissue sections were considered +, (< 10%); ++, (10 to 50%); and +++, (> 50%) as positive cells [(10)]. All statistical analysis was performed with the statistical package for social sciences (SPSS 10.01) and also Excell 2003. A p value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

Hematoxylin and eosin stained tissue sections of rheumatic mitral stenosis appeared different

degrees of fibrosis, inflammatory cellular infiltrates, neovascularization and mineralization (Figure 1).

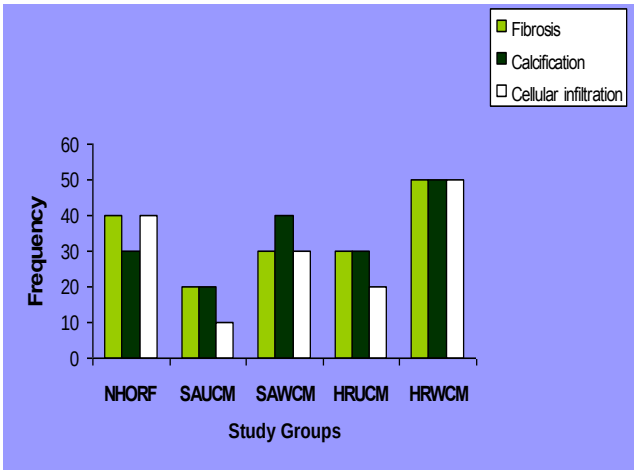

Figure 1: The degree of fibrosis, calcification, and cellular infiltration among different study groups

The clinical features were graduated in severity among all patients due to the continuous inflammatory processes against the heart, as a result, highly significant differences were shown in histopathological features among different study groups when compare between them. In general high risk patients were display very large degree of histopathological abnormalities (HPA) (fibrosis, calcification, and cellular infiltration) which reflects the more severity of disease than other groups, and there was a highly significant difference between the degree of CI in the patients of HR and SA- groups ($p < 0.01$). Medical care groups (SA^{UCM} and HRUCM) were exhibited no or very low-grade of the inflammatory response and subsequently low heart lesion, fibrosis and calcification when compared with patients of intermittent medical therapy (SA^{WCM} and HR^{WCM}).

There was no significant difference in the severity of disease between negative history and SA^{WCM} groups except the increasing in the cellular infiltration in the negative history population with subclinical symptoms which leads to more heart damage. Immunohistochemical staining for Foxp3 was detected as a brown color precipitated in the nuclei of mitral valve infiltrating mononuclear cells (Figure 2), Foxp3 positive cells percentage is shown in (Table 1).

Table 1: Mean percentage of Foxp3 positive cells from the total number of mitral infiltrating mononuclear cells among study population groups

Group type		Patients		ICsC	Foxp3 positive cells		Total Foxp3 value
		No.	(%)		No.	Mean%± SD	
NHORF		14	20.59	33.36	16.71	48.8±12.675	++
PHORF	SA^{UCM}	5	7.35	25.6	6.6	25.48±9.068	++
	SA^{WCM}	18	26.47	36.89	23.67	61.47±13.127	+++
	HR^{UCM}	4	5.88	27.25	9.5	31.32±20.966	+++
	HR^{WCM}	7	10.29	51.71	41.71	80.34±4.563	+++

$\chi^2 = 8.547$, $p > 0.05$; SD = Slandered Deviation; Total value = + (< 10) %, ++ (10-50) %, +++ (≥ 50) %. ICsC = Infiltrating cells count; NHORF = negative history of rheumatic fever; PHORF = positive history of rheumatic fever; SA^{UCM} =single attack under continuous medication; SA^{WCM} =single attack without continuous medication; HR^{UCM} = high risk under continuous medication; HR^{WCM} =high risk without continuous medication.

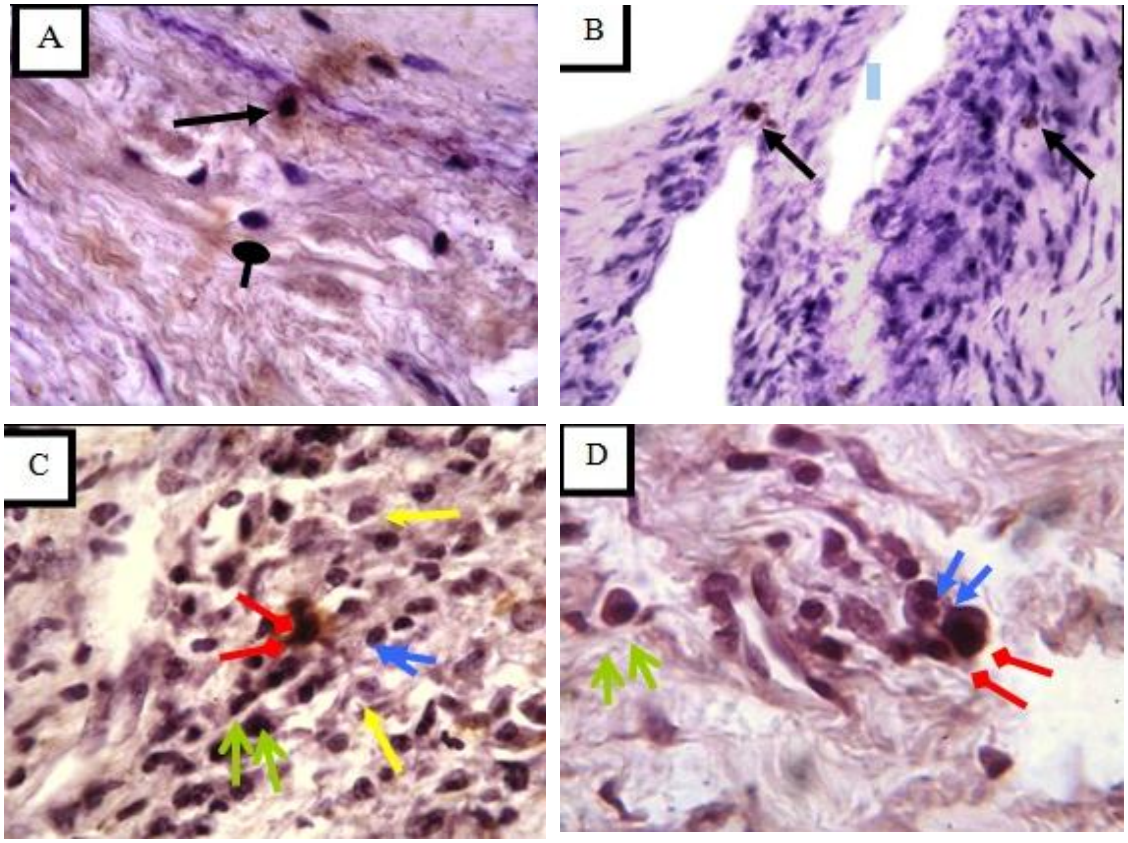

Figure 2: Immunohistochemical staining for human Foxp3 transcriptional factor in the rheumatic mitral tissue sections. (A&B) show Foxp3 positive cells (10-50%) (++). Pin point long arrows indicate Foxp3 positive cells whereas circled short arrows indicate negative cells. (C&D), show Foxp3 positive cells (> 50%) (+++) Foxp3 positive cells infiltrates the myocardium and illustrate various degrees of Foxp3 immunoreactivity, weak positive signals are indicated by blue arrows, positive signals are indicated by green arrows, red arrow indicate strong Foxp3 positive cells staining, and yellow arrows indicate negative Foxp3 signals. Microscopic magnification power: X400 for B; X1000 for A, C, and D.

In general, Foxp3 expression was high with some exceptions. When very high Foxp3 positive cells was shown in the mitral valve of SA^{WCM} (61.47%), and HR^{WCM} patients (80.34%), very low number of Foxp3 positive cells was infiltrate the mitral valve of patients under continuous medication (SA^{UCM} 25.48%, HR^{UCM} 31.32%) but, intermediate to high Foxp3 expression level was found in the patients with negative history (48.8%). Chi-analysis (Table 1) showed no significant statistically association between Foxp3 expression and the history of ARF (negative or positive), and/or the frequency of attacks (single or multiple) among all studied groups (χ^2 = 8.547, p > 0.05). However, HR^{UCM} group included 2 patients who displayed high Foxp3 positive cells, the first patient recorded 51.51% positive Foxp3 from the total number of intralesional mitral valve infiltrating mononuclear cells, and 46.87% of Foxp3 positive cells were found in the second patient. Negative Foxp3 expression was not recorded in any of chronic rheumatic heart disease patients. We now known that the presence of nTregs at the inflammatory sites will reverse the aggressive activity of CD4+ T cells by a certain pathway of suppression, but according to these results, there was no correlation between the high predominance of Foxp3 positive cells in all groups and the extent of histopathological abnormalities. However, non of cases with lower Foxp3 expression

showed severe histopathological abnormalities and not all cases with lower histopathological abnormalities had large number of Foxp3 positive cells except one SA^{UCM} patient who exhibited (30.43%) positive Foxp3 expression and at the same time showed low fibrosis and calcification with very low number of mitral valve infiltrating mononuclear cells (23 cell/field). According to the degree of Foxp3 immunostaining (saturation of pigment), we were found different immunostained intensities are recorded as negative, weak positive, positive, and strong positive (Table 2).

Table 2: The degree of the Foxp3 immunostaining signals in mitral infilterating mononuclear cells in different study groups

Group type		MeanFoxp3 positive cells (No.)	Signal immunostaining				Total
			Negative	Weak +ve	Positive	Strong +ve	
NHORF		17	0 (0.00%)	5(29.41%)	8(47.06%)	4(23.53%)	17(100%)
PHORF	SA^{UCM}	7	0 (0.00%)	0 (0.00%)	4(57.14%)	3(42.86%)	7 (100%)
	SA^{WCM}	24	0 (0.00%)	6 (25%)	16(66.67%)	2 (8.33%)	24(100%)
	HR^{UCM}	10	0 (0.00%)	3 (30%)	4 (40%)	3 (30%)	10(100%)
	HR^{WCM}	42	0 (0.00%)	7(16.67%)	34(80.95%)	1(2.38%)	42(100%)
Control		0	20(100%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	20(100%)

NHORF = negative history of rheumatic fever; PHORF = positive history of rheumatic fever; SA^{UCM} =single attack under continuous medication; SA^{WCM} =single attack without continuous medication; HR^{UCM} = high risk under continuous medication; HR^{WCM} =high risk without continuous medication.

Discussion

Foxp3 which was known as the master regulator of CD4+CD25+ nTreg cells and considered the more specific marker for the identification of these cells. In this study we found that this transcriptional factor was highly expressed by the mitral valve infiltrating mononuclear cells and (48.8%) from the total infiltrating mononuclear cells were positive for Foxp3 protein in the negative history patients, (25.48%) in SA^{UCM}, (61.45%) in SA^{WCM}, (31.32%) in HR^{UCM}, and (80.34%) in HR^{WCM} groups. These values give completely different picture than the expected one when considered that the CD4+CD25+ nTreg cells (which were found in 5-10% of the normal the peripheral blood) are the target of Foxp3 immunoreactivity. Early reports suggested that proportion of human CD4+ T cells always express Foxp3+ when activated, and that these cells then become phenotypically and functionally indistinguishable from nTregs[11]. In human, Foxp3 expression is readily induced in majority of both human CD4+CD25+Foxp3- and CD8+CD25+Foxp3- T cells by the activation via the T cell receptor (TCR), whereas Foxp3 expression is not induced in mouse CD25-T cells under similar activating conditions. The expression of Foxp3 after TCR stimulation of human CD25- T cells can approach that of the resting CD4+CD25+Foxp3+ population. These studies may explain the high predominance of Foxp3 expression by infiltrating mononuclear cells in the rheumatic mitral valve lesions since of course, all CD4+ T cells which infiltrate the autoimmue heart lesion are activated cells, but now there is evidence that Foxp3+ T effector cells does not suppress the proliferation or cytokine production of Foxp3- CD4+ T cells or

other immune cells which usually suppressed by naturally occurring Treg cells [(12)]. In other words, the induced Foxp3+ population is neither anergic nor suppressive in vitro, and the expression of Foxp3 appears to be transient, declining to baseline amounts with prolonged culture. Thus, the high percentage of Foxp3 positive cells may be as a result from the presence of both CD4+CD25+ nTregs and activated effector CD4+ T cells. Therefore, in this case, false positive result was obtained here may explain why no significant correlation was found between the positive expression of Foxp3 and the extent of histopathological abnormalities. Also this study found that the lower severity of the histopathological picture was highly significant associated with the high numbers of strong positive Foxp3 expression, and these results reinforce the immunosuppressive function of CD4+CD25+ regulatory T cells. Whereas, the mean percentage of positive /weak-positive Foxp3 expression was found to be positively correlated with the extent degree of calcification ($p < 0.01$), fibrosis and cellular infiltration ($p > 0.05$).

Our findings revealed that Foxp3-CD4+CD25+ regulatory T cells play an important role in controlling the autoimmune process against the heart in rheumatic heart disease, and this may open new future ways for using nTreg cells in immunotherapy.

Acknowledgement

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Hypomagnesemia and Obesity in Relation to Insulin Resistance and Glycemic Control in Type 2 Diabetic Patients

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Abstract

Background Obesity and diabetes mellitus are the most common health problems with both macro- and micro-vascular complications and consequences of end organ damage. The alteration in trace elements could have deleterious effects on the health of the diabetic patients. Magnesium (Mg) is an important factor for enzymes involved in carbohydrate metabolism and good evidence suggests the presence of an important role for hypomagnesaemia in insulin resistance and metabolic control.

Objective To evaluate the relation of hypomagnesaemia with insulin resistance (IR), and glycemic control in obese and non obese diabetic people.

Methods The study included 65 patients with type 2 diabetes mellitus (Type 2 DM) who were all on oral hypoglycemic drugs only. They were divided according to their body mass index (BMI) and the presence or absence urinary protein, into three groups. The results were compared with those of another 52 normal controls grouped on the same bases.

Fasting venous blood specimens were aspirated for the measurement of glycated hemoglobin (HbA1c) by A1c variant reader, glucose, urea, creatinine, protein and albumin by routine enzymatic chemical and colorimetric methods, insulin by immuno-enzymometric assay and magnesium by atomic absorption spectrophotometer, while Mg ions and Quicki test (for IR) were estimated by calculations. Morning urine specimens from each subject were examined for the presence of protein by dip Stick.

Results As compared with the healthy controls the study reveals a significant reduction in Quicki test (increased IR) and low serum Mg^{2+} in all diabetic patients, with the presence of a significant positive correlation between the two parameters. Serum Mg^{2+} was significantly lower in the normal weight non proteinuric diabetics than the normal weight controls. In diabetic patients the presence of proteinuria caused a further reduction in serum Mg^{2+}. Glycated hemoglobin (HbA1c) negatively correlated with total serum Mg^{2+} in all diabetics and their controls.

Conclusions Insulin resistance and poor glycemic control are important events associating hypomagnesaemia in type 2 DM. Proteinuria is an additional factor which may aggravate hypomagnesaemia, which involves both ionized and total Mg to the same degree.

Key words type 2 diabetes mellitus, magnesium, Insulin resistance, HbA1C, proteinuria

Introduction

Obesity is the most common cause of insulin resistance (IR) which does not necessarily lead to diabetes [(1,2)].

Magnesium (Mg) is an important factor for enzymes involved in carbohydrate metabolism. A strong relationship between Mg^{2+} and insulin action has been reported in adults [(3)], where low serum and intracellular where low serum and intracellular Mg^{2+} concentrations were found to be associated with IR, impaired glucose tolerance and increased insulin secretion with increased risk of Type 2 DM [(4)].

The present study deals with phenomenon of hypomagnesaemia and its relation to insulin

resistance in obesity and diabetes mellitus with special reference to the effect of proteinuria.

Methods

A- Patients:

Sixty five type 2 diabetics with age range of 19-53 years attending the National Diabetes Centre (NDC) during the periods from June to September 2008 were enrolled in the study. They were all on oral hypoglycemic drugs only.

They were divided according to their body mass index (BMI) and urinary protein into 3 groups:

1. Normal weight diabetics (BMI < 25) with no proteinuria (N-NPU) included 31 patients.
2. Over weight - obese diabetics (BMI > 25) with no proteinuria (O-NPU) included 24 patients.
3. Over weight - obese diabetics (BMI > 25) with proteinuria (O–PU) included 10 patients.

B- Controls:

Comprised 52 healthy subjects of matching age (20 -50 years) and sex were included in the study and grouped as in the patient group into:

1. Normal weight controls (NC) included 26 patients.
2. Overweight - obese controls (OC) included 26 patients.

C- Blood specimens:

Ten milliliters (10 ml) of venous blood were aspirated from each subject involved in the study after an overnight fast. Two mls were added to an EDTA tube for the measurement of glycated hemoglobin (HbA1c) and the rest was used for the measurement of other study parameters.

D- Methods:

Serum glucose, urea and creatinine were measured by routine enzymatic method, total protein by Biuret method [5], insulin by immunoenzymometric assay, HbA1c by ion exchange high performance liquid chromatography using Bio-Rad Variant HbA1C program, and total Mg^{2+} by Atomic Absorption Spectrophotometer after dilution by Lanthinium chloride (1:20), while serum Mg ions were calculated from the chart of (Willis & Sunderman 1952) [6]. Insulin resistance (IR) was estimated by Quicki test (the reverse index of IR) through the following formula:

Quicki test = 1/ [log (FI) + log (FG) 7.

Where FI = fasting insulin, and FG = fasting glucose.

Results

Table 1 shows significantly higher serum glucose in the diabetics as compared with their controls, significantly higher serum insulin in the diabetics than their controls and in the overweight-obese than the normal weight diabetics.

Quicki test (the reverse index of IR) shows a significant reduction in the diabetics being lower in the overweight-obese than the normal weight diabetics.

For serum total Mg and Mg^{2+}, there was a significant reduction in the diabetics as compared with their controls, with no significant change in the ratio of Mg^{2+}/ Mg.

Serum protein was significantly lower in the proteinuric diabetic group as compared with others.

Serum total Mg shows clearly a significant positive correlation with Quicki test, and a significant negative correlation with HbA1C in both control and diabetic groups (Figures 1 and 2).

Table 1: Serum fasting glucose(FG), insulin, glycated hemoglobin(HbA1c), QUICKI test, total Mg, Mg^{2+}, Mg^{2+}/ Mg ratio and total protein in type 2 diabetics (T2DM) and their healthy controls

Blood tests	Controls, n=52		T2DM , n=65		
	NC n=26	OC n=26	O- NPU n = 31	N -NPU N= 24	O - PU N = 10
FG mg / dL	80.7±18.89	96.8±9.73	184.84±75.4**	237.6±96.64**	188.3±38.9**
Insulin (µmol/L)	5.73±1.73	8.6±1.53	19.46±13.94**	9.195±9.73**	14.85±11.4**
QUICKI test	0.382±0.029	0.343±0.009	0.297±0.033**	0.328±0.047**	0.307±0.038*
HbA1c %	4.71±0.75	5.45±0.59	8.43±2.29**	8.43±2.29**	10.97±2.93**
Total Mg mmo/l	0.901±0.092	0.79±0.093	0.56±0.11*	0.666±0.112*	0.501±0.106**
Mg^{2+} (mmo/l)	0.602±0.064	0.54±0.093	0.38±0.09	0.451±0.073	0.352±0.084*
Mg^{2+}/Mg ratio	0.672±0.074	0.693±0.094	0.68±0.09	0.68±0.057	0.7±0.047
TP (g/dl)	6.72±0.42	6.45±0.45	6.44±0.46	6.36±0.45	5.56±0.53*

NC: Normal weight controls, OC: Overweight – obese controls, N- NPU: Normal weight non proteinuric diabetics, O-NPU: Over weight – Obese non proteinuric diabetics, O-PU = Over weight - obese proteinuric diabetics, TP: total protein, * $p < 0.05$, ** $p < 0.01$

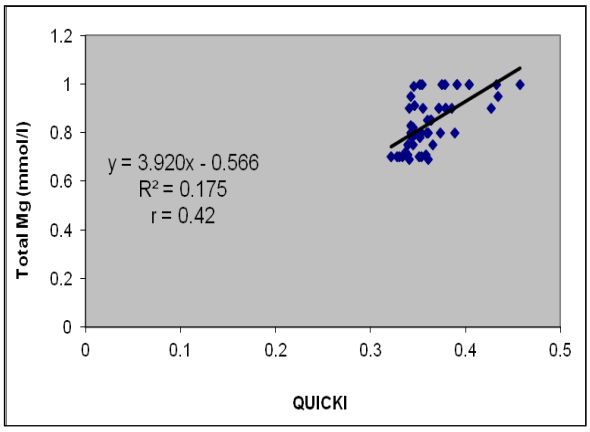

Figure 1a. Correlation between serum total Mg and QUICKI test in control group (n=52), r = 0.42, $p < 0.001$

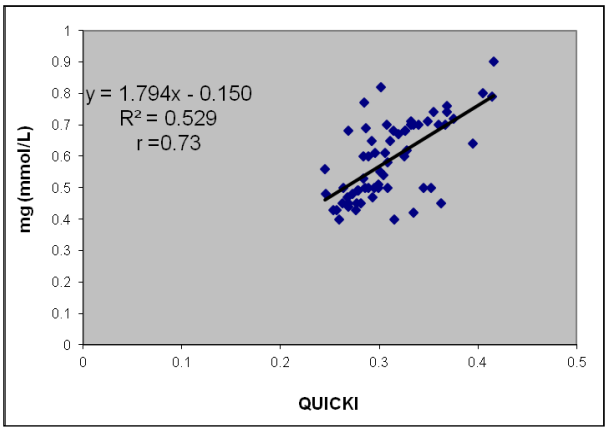

Figure 1b. Correlation between serum total Mg and QUICKI test in the diabetic group (n=65) r= 0.73, $p < 0.001$

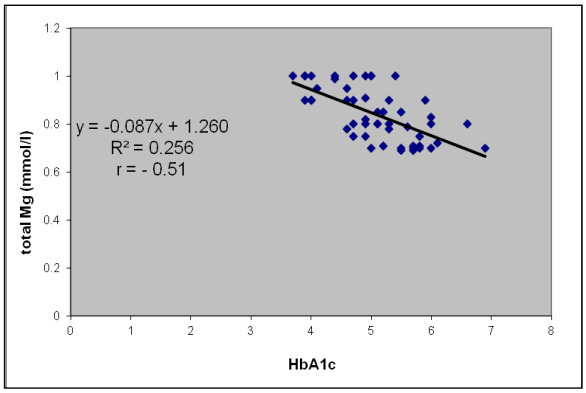

Figure 2a. Correlation between serum total Mg and QUICKI test in control group (n = 52) r= 0.51, $p < 0.001$

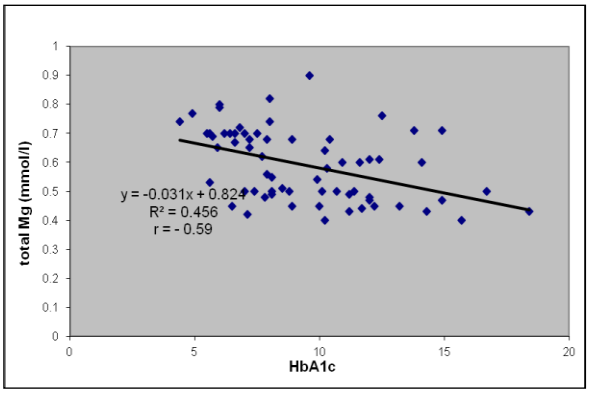

Figure 2: (b) Correlation between serum total Mg^{2+} and HbA1c in diabetic group (n=65), r = 0, 59, $p < 0.001$

Discussion

In the present results the significant increase in IR (presented by the decrease in QUICKI test) with increasing BMI of the obese Type 2DM patients when compared with their matching control groups agrees with previous reports [1,8].

Perhaps the common cause of insulin resistance is an excessive amount of fat in the body [1]. In obese person, however, too much fatty acid is released and tissues become overloaded with fat. Thus obese people acquire excess fat in their tissues, particularly muscle and liver as well as in adipose tissues. This excess tissue fat leads to IR [9].

Increased fatty acid concentration has also been implicated to increase the rate of their oxidation resulting in an increase in the production of acetyl-COA in the mitochondria and this, in turn, will inhibit pyruvate dehydrogenase, the rate limiting enzyme of glucose oxidation, and so interferes with glucose utilization in addition to the suppressive effect of fatty acids on pancreatic cell insulin secretion [10,11].

Hypomagnesaemia in patients with diabetes may result from poor oral intake, poor gastrointestinal absorption and enhanced renal magnesium excretion [12,13].

Reduced tubular reabsorption may be another cause, because insulin has been implicated in enhancing Mg reabsorption at the thick ascending loop (TAL), insulin deficiency or resistance in the diabetic state can promote Mg wasting at this nephron segment [14 ,15] .

In addition to the above causes various metabolic disturbances that are associated with diabetes also have been previously suggested to promote urinary Mg excretion [16].

Metabolic acidosis, In addition to its role in increasing serum ionized Mg concentration and, hence, ultra filterable Mg load for renal excretion, has been also suggested to enhance protonation of the Mg channel in the distal convoluted tubules and subsequent inhibition of cellular Mg uptake [16,17]. The common use of diuretics, and their type, among patients with diabetes also may contribute to magnesuria and its degree [18].

Finally, the more common use of antibiotics and antifungal agents such as amino glycosides and amphotericin in patients with diabetes may also contribute to renal Mg wasting ([19].

The interrelation of serum Mg and diabetes has been reported by many previous studies. Some showed that Caucasian men with serum Mg (0.58 mmol/L) had a two fold increase in incidence of T2DM compared to those with Mg concentration (0.78 mmol/L) [20]. The mechanism by which Mg deficiency may lead to IR has not been fully elucidated; however, Mg is a cofactor for multiple enzymes involved in carbohydrate metabolism [21].

The presence of a significant positive correlation between QUICKI test and total serum Mg may be secondary to a decrease in dietary intake of Mg [22]. However this low Mg may be associated with hyperinsulinemia, decreased insulin mediated glucose disposal and metabolic syndrome [23, 24].

The association of low Mg^{2+} with IR in the present T2DM patients confirms previous study [25], who suggested that a loss in Mg^{2+} and Hypomagnesaemia would contribute to the cardiovascular disease. This study seems to be in agreement with this (ionic hypothesis) and further confirmed that an alteration in Mg metabolism play a key pathophysiological role in the metabolic syndrome or IR [22,26] but was in contrast with others who showed that obesity was not associated with hypomagnesaemia [24].

Other reports showed that oral Mg supplementation had improved insulin sensitivity and metabolic control in T2DM [27].

Moreover the present study shows a negative association between Mg^{2+}/Mg ratio and serum total protein in the control and T2DM groups. One of the reports [28] showed that an increased serum protein will lead to reduced

serum Mg^{2+} and cause low Mg^{2+}/Mg ratio as seen in this study.

As concerning the negative correlation of serum Mg^{2+} with HbA1c in this study, HbA1c was found to decrease Mg^{2+} when it was above 7% and, hence, will lead to a decrease in Mg^{2+}/Mg ratio. This result is in agreement with a report suggested that putative role of increased urinary Mg loss may be linked to a prolonged hyperglycemic control state [(29)], and also in agreement with others who showed the benefits of oral supplementation of Mg as an adjuvant therapy for fasting glucose and HbA1c [(27)]. Moreover, the group of T2DM with proteinuria who had an increased Mg loss could be invoked to explain the low concentration of serum Mg^{2+} observed in patients with HbA1c greater than 7% in this case, consequently hypomagnesaemia was claimed to associate with coronary heart disease [(30)], or to promote a decline in renal function of the diabetics [(28)], which makes it an important factor to be considered in various metabolic studies.

At last it could be concluded that changes in total and ionized Mg in diabetics associate insulin resistance and glycemic control, while proteinuria is an additional factor which aggregates or worsen Mg loss and hypomagnesaemia.

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Open Versus Closed Methods in Treatment of Pilonidal Sinus Disease

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Abstract

Background Pilonidal sinus disease can be managed surgically either by excision & primary closure, or by excision and leaving the wound to heal by secondary intention. This study is designed to show the difference between these two methods.

Objective To assess the difference between excision and primary closure versus excision and healing by secondary intention in treatment of pilonidal sinus disease.

Methods Between January 2005 and January 2009, 60 median aged patients with Pilonidal sinus disease were studied in Al-Kadhimyia Teaching Hospital, Baghdad, Iraq; 30 cases were operated by excision and primary closure (group I); the remaining 30 cases were operated by excision and healing by secondary intention, without closure (group II). The principle outcome measures recorded were duration of hospital stay, operative time, duration of complete healing, wound infection and recurrence rate. Satisfaction and comfort of patient was monitored by using visual analogue scale during first five days post-operatively. Data were statistically analyzed by using SPSS & Chi square.

Results A total of 60 patients were divided into 2 groups, 30 patients operated by using primary closure (group I), and 30 patients operated without closure (group II). Age range of patients was from (16 - 37 yrs). Duration of hospital stay and duration of complete healing was longer in patients of group II than those of group I ($p \leq 0.001$). Operative time in group I is more than that in group II ($p \leq 0.001$). Pain scores were lower in group I than group II ($p = 0.004$). The frequency of wound infection and recurrence rate after one year follow up were more in group I than group II, this was statistically of little significance.

Conclusions Excision and primary closure is recommended as a preferred procedure in the management of chronic sacral PNS disease. It has the advantages of short hospital stay, early wound healing, rapid return to work.

Key words pilonidal sinus, primary closure, secondary intention

Introduction

Sacral pilonidal sinus (PNS) disease is an acquired condition, usually seen in young adults manifest by midline pits in the natal cleft and associated with hair. The underlying pathophysiologic feature is enlarged hair follicles due to midline vacuum and pulling forces; when plugged with hair or keratin, the follicle rupture leading to a foreign body reaction within the presacral subcutaneous tissue and subsequent acute and chronic abscess [(1)].

Sacrococcygeal pilonidal disease is a common condition causing discomfort that may interfere with education or employment, sometime for prolonged period [(2)]. It is most common in the third decade of life its incidence peaks between 16-20 years of age. Men are affected three to four times more commonly than women [(3)].

The management of pilonidal sinus disease is variable, debatable, and problematic [(4,5)]. A number of surgical treatment options exist:

simple incision and drainage, lying open, marsupialization, excision and primary closure or rhomboid excision and Limberg flap procedure [6,7,8,9].

Primary wound closure versus wound healing by secondary intention, after excision of sacral PNS, are the two principal surgical options for a chronic pilonidal sinus treatment [10].

Methods

Sixty patients with sacral pilonidal sinus disease were studied prospectively from January 2005 till January 2009 in Al-Kadhmyia Teaching Hospital. Each case was followed up for at least one year. Those cases were uncomplicated and have no other associated diseases and were approved the study.

Operative technique

Thirty cases were operated by excision and primary closure (group I); the remaining thirty cases were operated by excision and healing by secondary intention, without closure (group II).

In group one, after probing the sinus with canula and injection of methylene blue dye, ellipse incision is made around the sinus excising the whole tract down to the sacral fascia as shown in figure 1.

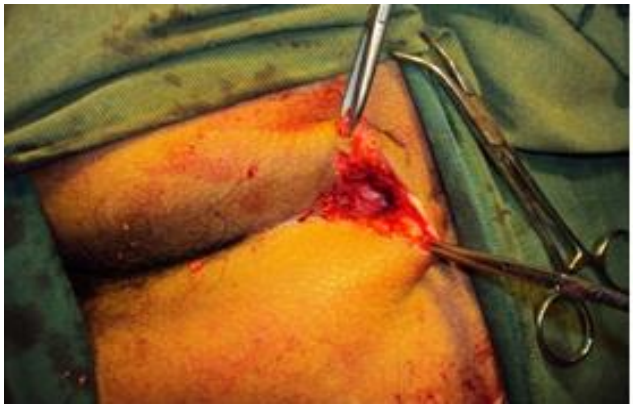

Figure 1. Excision of sacral PNS

After full hemostasis of the elliptical defect, three to four deep nylon stitches, depending on the size of the defect, are applied through full thickness of the defect including the presacral fascia, as seen in figure 2.

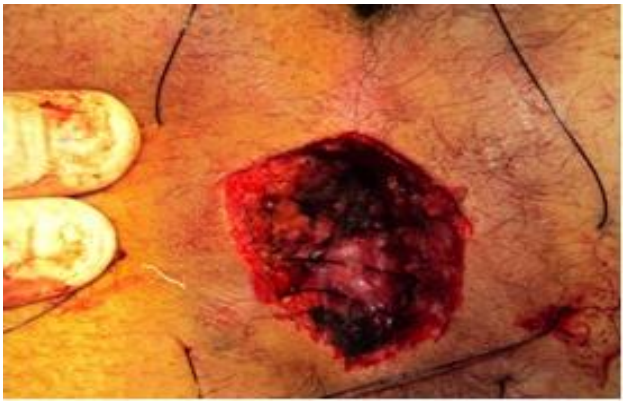

Figure 2. Applying nylon stitches

Holding these sutures untied, the skin edges of the defect are closed by mattress silk suture, and then a pack of gauze is applied between the nylon sutures which then are tied over the pack as seen in figures 3, 4 and 5.

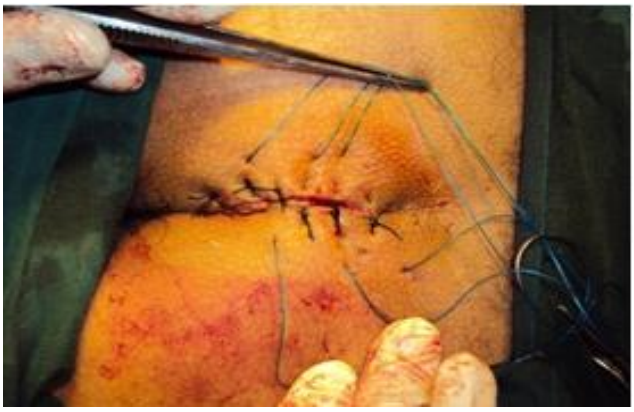

Figure 3. Closing skin

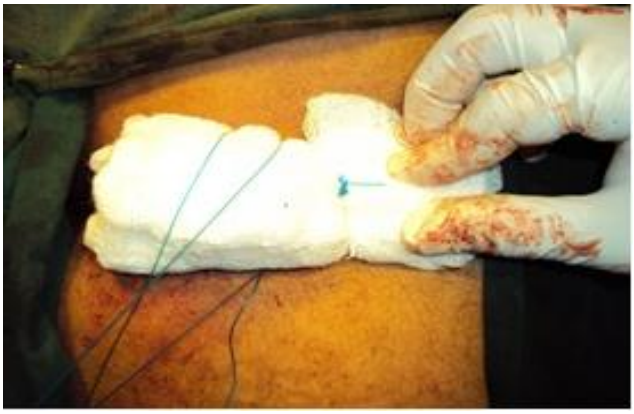

Figure 4. Applying pack

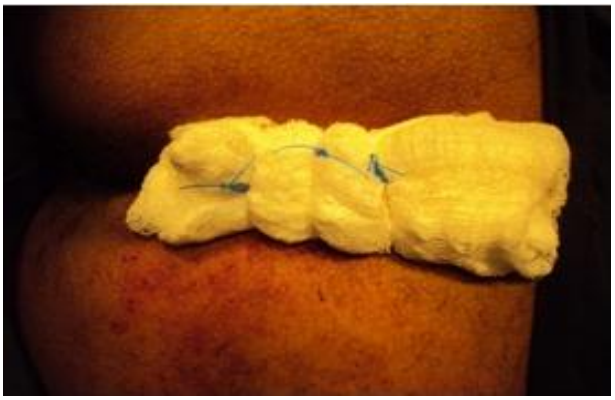

Figure 5. Tying nylon suture

The nylon and silk suture are removed after ten days from surgery. Figure 6 showed healed scars after sacral PNS closed method surgery.

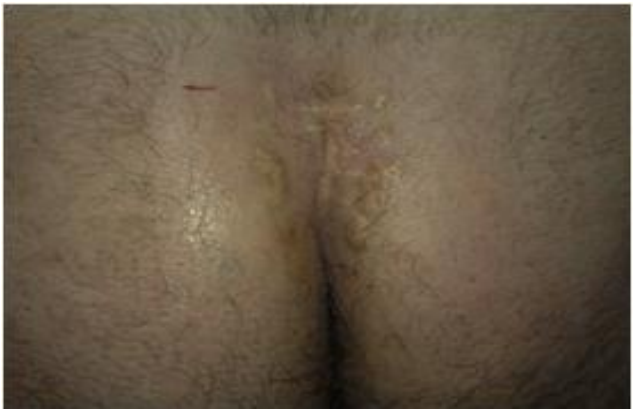

Figure 6. Healed sacral PNS by closed method

In group two, after identification of the sinus tract with methylene blue dye, full excision of the sinus down to the presacral fascia, hemeostasis is ensured and pack of gauze is inserted in the elliptical defect with daily dressing till time of healing.

Follow up

To evaluate satisfaction and comfort of patient, a questionnaire including postoperative visual analog scale (VAS), time to sitting on toilet without pain, and time to walking without pain were recorded. Patients were asked to complete a 10-cm long VAS for their health status before and after surgery that ranged from 0 for "very bad" to 10 for "very good." The scale was constructed with numeration, thus allowing patients to mark a point along the scale that best represented their health status at that time.

Statistical analysis

All data were collected and analyzed by using SPSS. Statistical analysis was performed using Chi-squared test to compare discrete variables and two tailed paired Student's t-test to compare continuous variables between groups. $p < 0.05$ was considered statistically significant for all tests.

Results

The study involved sixty median aged patients complaining of sacral PNS (ranging from 16 to 37 years), as shown in table 1.

Table 1: Age distribution in two groups of patients

Age	Mean±SD	SE	Sig. (2-tailed)
Group 1	24.867±5.056	0.923	≥ 0.05
Group 2	24.800±5.378	0.982	

Those patients were admitted to Al-Kadhmyia Teaching Hospital, Baghdad, Iraq from January 2005 till January 2009. Thirty patients were treated by closed method or primary closure, (group one) and the other thirty patients were treated by open method or healing by secondary intention (group two). Symptoms of sacral PNS before surgery in both groups of patients had the same duration of time, as shown in table 2.

Table 2: Duration of preoperative symptoms in both groups of patients

Duration of pre operative symptoms	Mean±SD	SE	Sig. (2-tailed)
Group 1	1.310±0.723	0.132	≥ 0.05
Group 2	1.330±0.648	0.118	

Duration of hospital stay is more in patients of group 2 than those of group 1, as shown in table 3 and figure 7.

Table 3: Duration of hospital stay of both groups of patients

Duration of hospital stay	Mean±SD	SE	Sig. (2-tailed)
Group 1 Group 2	1.467±0.419 2.267±0.338	0.076 0.062	≤ 0.001

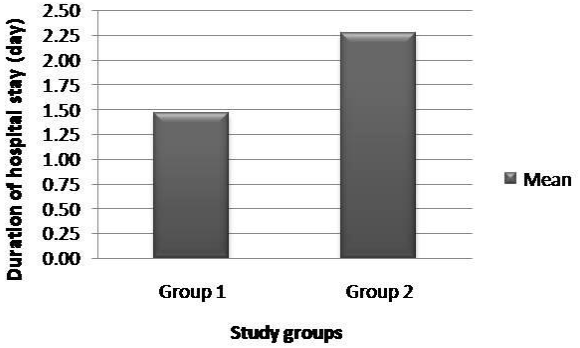

Figure 7: Duration of hospital stay

Complete healing consumes more time in patient of group 2 than those of group 1, as shown in table 4 and figure 8.

Table 4: Duration of complete healing in both groups of patients

Duration of complete healing	Mean±SD	SE	Sig. (2-tailed)
Group 1 Group 2	18.533±2.488 27.600±4.248	0.454 0.775	≤ 0.001

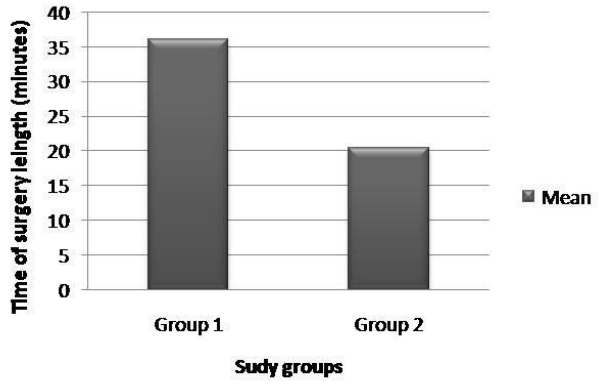

Figure 8: Duration of complete healing

Surgery consumes more time in patients treated by primary closure (first group) than those treated by simple excision and secondary intention (second group), as shown in table 5 and figure 9.

Table 5: Operative time in both groups of patients

Time of surgery length	Mean±SD	SE	Sig. (2-tailed)
Group 1 Group 2	36.138±3.623 20.500±2.418	0.673 0.441	≤ 0.001

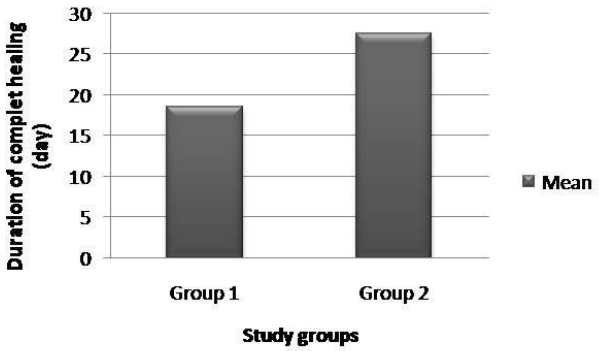

Figure 9: Operative time

The clinical outcome can be compared in two groups of patients and as shown in table 6. Differences in frequency of Wound infection and recurrence rate after one year follow up in both groups of patients can be compared as shown in table 7.

Table 6: Clinical Outcome in two groups of patients

Clinical Outcome	Group 1	Group 2	Sig. (2-tailed)
Post-operative VAS* during first five days	5.1	7.5	0.004
Duration of hospital stay	1.467	2.267	< 0.001
Duration of complete healing	18.533	27.600	< 0.001
Age	24.867	24.800	0.961
Duration of pre-operative symptoms	1.310	1.330	0.911
Operative time	36.138	20.500	< 0.001

*VAS: visual analogue scale for pain (from 0-10)

Table 7: Comparison between the frequency of Wound infection and recurrence rate after one year follow up in two groups of patients

parameter		Group I	Group II	Significance
		Count (Percentage)	Count (Percentage)	
Wound infection	negative	27 (90%)	29 (97%)	0.301
	positive	3 (10%)	1 (3%)	
Recurrence after one year follow up	negative	25 (83%)	27 (90%	0.448
	positive	5 (17%)	3 (10%)	

Discussion

Pilonidal sinus is a disease that occurs in sacrococcygeal area, it is a blind track lined by granulation tissue that leads to a cystic cavity. It's an acquired condition that usually seen in young adults of working age. In this study, both groups of patients had near & equal age group range [6].

On the other hand, there were no significant changes between two groups of patients participating in this study regarding the duration of pre-operative symptoms of pilonidal disease [1]. This fact is well explained in table 2.

Regarding the duration of hospitalization, table 3 and figure7 showed that patients in group 1 had significant ($p \leq 0.001$) shorter hospital stay than those of group 2, the same result was obtained from table 4 and figure 8 that deals with the duration of complete healing in both groups of patients. This can be explained by the fact that the healing process took longer time in simple excision and healing by secondary intention than in primary closure that provides an earlier wound healing and reduced hospital stay [3,11]. Most patients return to work in 3 to 4 weeks [4,8,13].

The operative time for treatment of patients in group one by primary closure was longer than that recorded for group two of patients treated by open method (secondary intention), as shown in table 5 and figure 9 (with p value ≤ 0.001). This can be explained by the fact that wound closure needs additional time over that needed for dissection [2, 12, 13].

In table 6, a comparison was made between the clinical outcomes of both groups of patients of the study. By monitoring VAS (visual analogue scale) which is a scale for pain from 0-10 during first five days after surgery, we noticed lower pain scores in group 2 than in group 1. The reason behind this is that simple excision and healing by secondary intention would cause more patient discomfort, more outpatient attendance for many painful dressings [9,14]. Although, the difference between two groups of patients regarding VAS was not significant ($p = 0.004$), this can be explained by the fact that excision and primary

closure technique causes restriction of activity because of tissue tension [5], and that most complaints by patients after surgery were caused by wound tenderness [7,10,15].

Table 7 showed a comparison between the frequency of wound infection and recurrence rate after one year follow up in two groups of patients. We did not encounter any significant difference in the wound infection rate (10% vs. 3%) of both groups. The recurrence rate in closed and open methods was 17% and 10% respectively, which was statistically of little significance [2,4,5].

From the above, we can conclude that excision and primary closure is recommended as a preferred procedure in the management of chronic uncomplicated sacral PNS disease. It has the advantages of short hospital stay, early wound healing, rapid return to work. Although the frequency of wound infection and recurrence rate were more in closed method, but the difference was of little significance.

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Subclinical Hypothyroidism and Female Infertility

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Abstract

Background Thyroid disorders are amongst the commonest endocrine disorders in women of childbearing age. Population-based infertility data of women with subclinical hypothyroidism are not available.

Objective Assess the role of subclinical hypothyroidism in female infertility.

Methods A prospective clinical study of 40 infertile women with subclinical hypothyroidism treated with thyroxine after exclusion of the basic causes of infertility in Wasit Governorate/Iraq from June 2006 until June 2009 (3 years).

Result 24 of the women (60%) were complaining of primary infertility with infertility period ranged from 1-6 years and 16 (40%) with secondary infertility with infertility period range from 1-5 years. Pretreatment mean TSH level was 7.6 mIU/L which normalized after treatment to a mean level 1.9 mIU/L. Conception was recorded in 14 (35%) women during the period of the study however, only 11 pregnants succeeded to continue pregnancy resulting in a live birth rate of 27.5%.

Conclusions Our results support the role of subclinical hypothyroidism as a predisposing factor for female infertility that should not be forgotten.

Key words subclinical hypothyroidism, female infertility, thyroxine supplementation

Introduction

Subclinical hypothyroidism is defined as a serum TSH concentration above the statistically defined upper limit of the reference range when serum free T4 (FT4) concentration is within its reference range [(1)].

Population-based infertility data of women with subclinical hypothyroidism are not available. Hyperprolactinemia due to increased hypothalamic thyroxin-releasing hormone (TRH) secretion was suggested 17 years ago as a cause of infertility in hypothyroidism. However recent epidemiological and clinical observations of a large number of patients demonstrating that hypothyroidism is associated with minimal changes in the serum prolactin concentrations [(2)].

There are experimental data of both stimulatory and inhibitory effects of thyroid hormones on mammalian granulosa cell gonadotropin-induced steroidogenesis. These controversial effects of thyroid hormones may be due to the different responsiveness to T3 of granulose cells isolated from the follicles at different stages of antral development, with small and medium follicles displaying a higher number of T3 binding sites than large antral follicles [(3)]. As obtained from patients undergoing therapeutic abortions at 7-8 weeks gestation, T4 and T3 in first trimester placentas were amplifiers of differentiated trophoblast function. In addition, data from clinical studies have demonstrated that thyroid hormone replacement therapy increased the success rate of ovulation induction by clomiphene citrate in women with subclinical hypothyroidism. Taken together,

hypothyroidism may, even at an early stage, have an important impact on conception [4]. Once pregnancy has occurred, thyroid hormones contribute to the stability of the fetoplacental unit, protecting from early loss of the conceptus [5]. For patients with TSH levels higher than 10 mIU/L, no controversy exists, and treatment is recommended. For patients with TSH levels between 5 and 10 mIU/L observation or treatment is recommended on an individual basis. Symptomatic patients and patients with fertility problems, pregnant women and women contemplating pregnancy should receive treatment [6].

Methods

From June 2006 until June 2009 (3 years), women with primary or secondary infertility were evaluated prospectively for subclinical hypothyroidism after exclusion of other basic causes of infertility in AL-Karama Teaching Hospital in Wasit Governorate/Iraq. The patients were assessed for symptoms and signs of hypothyroidism, including fatigue lethargy, diminished sweating, dry skin, cold intolerance, dry hair, weight gain, constipation, hoarseness, paresthesia, menstrual alterations, and muscle pain; the thyroid gland was examined carefully. Patients were informed about the rationale and investigation schedule and gave informed written consent before entering the study. The sera from those women were assayed for thyroid functions tests at first visit using highly sensitive miniVIDAS technique in private laboratory in Wasit. Before making treatment decision and as guidelines recommend repeating the serum TSH and measuring FT4 within 2 to 12 weeks, depending on the clinical setting, to exclude transient forms of hypothyroidism and those with subclinical (mildly underactive) thyroid with TSH levels of 4.5-10 mIU/L and normal FT4 included in the study [7,8]. Exclusion criteria include any women with history of thyroid disease or receiving treatment for previous thyroid problem and those with clinically overt hypothyroidism or when TSH level > 10 mIU/L [8]. Again any women not completed the treatment with one year of follow up were excluded from the study.

Forty women were included in this study and were assigned to receive oral thyroxine 50 microgram administrated daily before breakfast for 3 months, with the aim of restoring serum TSH to the reference range, and invited every 3 months until one year or conception. If pregnancy occurred they were invited every 4 weeks until 12th week, then every 3 months until delivery and TSH test was performed at every visit to allow the adjustment of thyroxine therapy.

Rates of pregnancy, abortion and live birth were recorded, and our results were compared with the other studies.

Results

Forty infertile women were evaluated in this study with the diagnosis of subclinical hypothyroidism, their age ranged from 19-42 years (mean 31.5 years), Table 1.

Table 1. The distribution of infertile patients according to age

Age (years)	Primary infertility	Secondary infertility	Total No. (%)
<20	2	-	2(5%)
21-30	8	8	16(40%)
31-40	12	6	18(45%)
>40	2	2	4(10%)
Total	24	16	40(100%)

24 of them (60%) were complaining of primary infertility with infertility period ranged from 1-6 years (mean 3 years) and 16 (40%) with secondary infertility with infertility period range from 1-5 years (mean 2.6), Table 2.

Table 2. The distribution of infertile patients according to infertility period (years)

Infertility period (years)	Primary infertility	Secondary infertility	Total No. (%)
1-2	6	2	8(20%)
2-3	4	4	8(20%)
3-4	6	4	10(25%)
4-5	2	2	4(10%)
>5	6	4	10(25%)
Total	24	16	40(100%)

The TSH level before starting treatment ranged from 5.6-10.0 mIU/L, mean (7.6 mIU/L) which normalized after treatment to a mean level 1.9 mIU/L, Table 3.

Table 3. The distribution of infertile patients according to TSH level in mIU/L

TSH (mIU/L)	Primary infertility	Secondary infertility	Total No. (%)
5-<6	4	1	5(12.5%)
6-<7	8	2	10(25%)
7-<8	4	2	6(15%)
8-<9	2	4	6(15%)
9-10	6	7	13(32.5%)
Total	24	16	40(100%)

Conception was recorded in 14 (35%) women while on thyroid replacement, 6 of them were with primary infertility and 8 were with secondary infertility, however, only 11 pregnancies were succeeded to continue. Pregnancy resulted in a live birth rate of 27.5 %, abortion rate of 7.5%, Table 4.

Table 4. Rates of pregnancy, abortion and live birth rates

Rates	Primary infertility No. %	Secondary infertility No. %	Total No. (%)
Pregnancy	6 (15%)	8 (20%)	14 (35%)
Abortion	1 (2.5%)	2 (5%)	3 (7.5%)
Live birth	5 (12.5%)	6 (15%)	11(27.5%)

Discussion

Forty infertile women enrolled in a prospective clinical study to investigate the biochemical diagnosis of subclinical hypothyroidism as a possible infertility factor. Each one was followed for not less than one year, received thyroxin treatment and then assessed for pregnancy, abortion and live birth rates. Their age ranged from 19-42 years (mean 31.5 years),which was close to the age in the study of Sampath et al 2007 [9], where the average age of females with subclinical hypothyroidism was 30.8 years, 5.4 years less than females with overt hypothyroidism.

In our study the overall conception rate was 35% and abortion rate was 7.5 % while the live birth rate was 27.5%, in the study of Raber et al 2003 [10]; they reported 37% pregnancy rate and 9% abortion rate and they found that abortion was associated with higher TSH level and all occur in the first trimester which was higher than our abortion rate, while the higher pregnancy rate in their study might be attributed to the larger number of patients in their study (283) and longer period of follow up (5 years) and the diagnostic and therapeutic approach by detecting Subclinical hypothyroidism depending on TRH- stimulated TSH response, Furthermore a new study of Abalovich et al 2007 [11] reported a higher incidence of Subclinical hypothyroidism in infertile group depending on the use of TRH stimulation test where it was useful in detecting subclinical hypothyroidism in 12.7% and reported over all pregnancy rate of 44.1 % Our result was still higher than other earlier studies [12,13] who reported lower conception rates (0-24%) than that observed in the present study. Abortion or parturition rates were not available in those studies.

In the study of Akhter et al 2006 [14] from Bangladesh, they reported a prevalence of subclinical hypothyroidism of 6.5% and 15%, in primary and secondary infertility respectively with a mean TSH level higher in secondary infertility (3.6±3.7 mIU/L) than primary infertility (2.3±2.7 mIU/L), while in our study

the TSH level range was from 5.6-10.0 mIU/L and a mean of 7.6 mIU/L, which normalized after treatment to a mean level of 1.9 mIU/L. This might be a contributory factor in improving the present pregnancy rate; as never achieving basal TSH less than 2.5 mIU/L resulted in a lower conception rate [10]. The recent Egyptian study of Rahman et al in 2010 [15], reported a significantly lower mean TSH level after treatment (1.1±0.3 muIU/ml) and reduced miscarriage rate to 9% while the delivery rate, were significantly improved to a 35% in those females with subclinical hypothyroidism treated with thyroxin before IVF cycle [15].

Data on the natural history of infertility in untreated subclinical hypothyroidism are limited to one retrospective large study of infertile women (no. = 857) suggested that infertile women with untreated subclinical hypothyroidism do not conceive at all [16]. While in the cohort of Raber et al in 2003 [10], although females were not left untreated, at the time of pregnancy more than 25% of them were still sub clinically hypothyroid suggesting that conception is still possible in a state of mild thyroid failure [10]. Increasing evidence derived from experimental and clinical studies suggest that the hypothalamic-pituitary-thyroid axis (HPT) and the hypothalamic-pituitary-ovarian axis (HPO) are physiologically related and act together as a unified system in a number of pathological conditions. The suggestion that specific thyroid hormone receptors at the ovarian level might regulate reproductive function, as well as the suggested influence of estrogens at the higher levels of the HPT axis, seems to integrate the reciprocal relationship of these two major endocrine axes also occur, but it is rare [17]. It is well known that hypothyroidism impairs reproductive function in both humans and experimental animals. However, the mechanism of this dysfunction has not been completely established. In several species irregular estrous cycles were also detected. They showed a decrease in the number of primordial, antral and Graafian follicles, disturbed folliculogenesis and absence of corpora lutea when hypothyroidism was induced since birth. In women, hypothyroidism is associated with delay in the onset of puberty, anovulation, amenorrhea or hypermenorrhea, menstrual irregularity, infertility and increased frequency of spontaneous abortions. It was suggested that these alterations may be caused by a decrease in LH secretion. LH frequency and pulsatility, having a luteolytic effect and causing inhibition of folliculogenesis, estrogen synthesis, and ovulation [18].

Finally our results support the role of subclinical hypothyroidism as a factor in infertile women that should not be forgotten.

From the preliminary data we could recommend:

1. Screening for subclinical hypothyroidism in women with reproductive failure.
2. Thyroxin supplementation to achieve clinical pregnancies in infertile female with subclinical hypothyroidism.
3. Further multicentric studies with control trail to support the results of the present study.

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Proton Beam Radiation Targeted Nucleotides with Negligible Effect on Interferon

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Abstract

Background There is increasing evidence that localized irradiation of the tumor may also modify the tumor microenvironment and generate inflammatory cytokines.

Objective This study is aimed to clarify the effect of proton beam radiation on the interferon (IFN-α**,** IFN-β, IFN-γ) and nucleotide.

Methods The Microsoft "The Stopping and Range of Ions in Matter (TRIM-SRIM)" version 1998, and 2003 was used. A model of targeting certain interferon (IFN-α, IFN-β, IFN-γ) as well as the nucleotide pair was created. Each target was subjected to proton radiation of hydrogen [H], helium [He], or carbon [C] at different range of energy seeking for the Bragg's peak**.**

Result The results showed that the cross sections IFN-α, IFN-β, IFN-γ and nucleotide targeted by proton therapy were 0.9776, 0.8317, 0.8297 and 0.7305 [keV/(μg/cm^2)] for hydrogen ion, and 2.3354, 2.3414, 2.3377, 2.0842[keV/(μg/cm^2)] for helium ion, and 8.3032, 8.3198, 8.3109, 7.5394 [keV/(μg/cm^2)] for carbon ion respectively.

Conclusions It concludes that targeting of well precise located tumor with proton beam radiation therapy resulted in nucleotide damage of cancer cell without affecting the immune system in term of interferon surveillance.

Key words Proton, Nucleotide, Interferon

Introduction

Interferons (INFs) are glycoprotein belong to cytokines that released in response to the presence of virus, bacteria, parasites or tumor cells. They activate natural killer cells and macrophages and they increase recognition of infective or tumor cell to T lymphocytes. IFN- γ has pleiotropic effects in the tumor microenvironment, including the inhibition of cell proliferation and angiogenesis [(1)]. They reversed the signal defect in T lymphocytes in patients with melanoma and the synthetic INF-α_{2b} is useful as an adjuvant therapy for high risk melanoma [(1)]. Because abnormally low levels of INF-γ are produced by tumor cells and local T lymphocyte in the glioma, it is a promising adjunct to other immunotherapeutic modalities in the treatment of brain tumors [(2)]. Radiation is an important treatment for the local control of cancer based on its ability to directly kill tumor cells.

However, there is increasing evidence that localized irradiation of the tumor may also modify the tumor microenvironment and generate inflammatory cytokines, which can increase the robustness of the immune response [(4,7)]. Radiotherapy has been demonstrated to cause inflammation, a potentially beneficial state in which IFN-γ is undoubtedly involved as well as it created a tumor microenvironment conducive for T cell infiltration and tumor cell target recognition [(6)]. Interferon-α potentiated the cytotoxicity of X-ray radiation [(8)]. *In vitro* model the production of INF-γ by cells is suppressed by ultraviolet A1 radiation and thereby the immune system is suppressed [(3)]. Recently proton radiation gets

access in management of cancer as a preferable therapeutic modality because fewer harmful adverse reactions, more direct impact on the tumor and increased tumor control. Its effect on the hemopoietic system was generally less pronounced compared to gamma rays and X-rays [9]. Proton radiation was significantly modified the pattern of gene expression in T lymphocytes and highly dependent upon total dose and it may enhance their responsiveness at low dose radiation [10]. This study is aimed to explore the effect of proton radiation on the immune system using the hydrogen, helium or carbon as proton source and interferon as the target in Trim-Srim model.

Methods

This study was carried on in Department of Physiology/Medical Physics, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq. The Microsoft "The Stopping and Range of Ions in Matter (SRIM)" version 1998, and 2003 was used. A model of targeting certain interferon (IFN-α, IFN-β, IFN-γ) as well as the nucleotide pair was created. Each target was subjected to proton radiation of hydrogen [H], helium [He], or carbon [C] at different range of energy seeking for the Bragg's peak. The characteristics of the proton sources and the targets showed in table 1. The stopping power is given (-dE/dx) by applying Bethe-Bloch formula where (– dE) is the energy increment lost in infinitesimal material thickness (dx).

-dE⁄dx = $(2\pi e^4 / m_\circ v^2)$ NB

$$B=Z\left[\ln \frac{m_o v^2 T}{2I^2(1-\beta^2)} - (ln2)\left(2\sqrt{1-\beta^2} - 1 + \beta^2\right) + 1 - \beta^2 + \frac{1}{8}\left(1-\sqrt{1-\beta^2}\right)^2\right]$$

Where $\beta = \frac{v}{c}$, *T* is a constant factor

The total stopping power for electron can be given as a combination of collisional (elastic collision with atomic electrons) and radiative (inelastic collision with nucleus) types of interaction:

[dE/dx]total = [dE/dx] collision+[dE/dx]radiative

The stopping power (S) is given by:

N.S = - (dE/dx)

The quantity of S (keV/μ) is referred to specific energy loss

E: charged particle kinetic energy

-dE: the energy increment lost in infinitesimal material thickness (dx)

N: is number of atom /volume

The specific energy loss is expressed by Bethe-Bloch formula

For heavy charged particle:

$$-\frac{dE}{dx} = \frac{4\pi e^4 z^2}{m_o v^2} NB$$

Where

$$B = Z\left[\ln \frac{2m_o v^2}{I} - \ln\left(1 - \frac{v^2}{c^2}\right) - \frac{v^2}{c^2}\right] - S/2$$

With the following definitions:

v velocity of the charged particle

Z charge of the charged particle

N number density of absorber atoms

Z atomic number of absorber atoms

m electron rest mass

e electron charge

I A parameter, treated as experimentally determined, representing average excitation and ionization potential

B is known as the stopping number (atomic number scaled for stopping)

S is the density correction

Bethe-Bloch formula for electrons:

For heavy particles, orbital electron interactions are only considered since the probability of nuclear interaction resulting in energy loss is much smaller.

$$-\left(\frac{dE}{dx}\right)_r = \frac{NTZ(Z+1)e^4}{137 m_0^2 c^4}\left(4\,ln\frac{2T}{m_0 c^2} - \frac{4}{3}\right)$$

The percent of the energy loss goes to emitted rays is expressed by:

$$(\frac{dE}{dx})_r / (\frac{dE}{dx})\text{total} = EZ/1000$$

Where E is in MeV, where Z is the atomic number of the absorber.

The range of a charged particle can be derived from stopping power formula:

$$R = \int_E^0 dx(cm) = \int_E^0 \frac{dE}{dE} dx = -\int_0^E \frac{1}{dE/dx} dE = \int_0^E \frac{dE}{S}$$

The summal distance elements as kinetic energy goes from *E* down to 0 is the total distance along the incident direction, or the range.

The quantity of stopping power (KeV/(μg/cm^2) is referred to specific energy loss per cross section of targeting molecule. Microsoft Excel 2003 was used for calculations and figures plotting.

Results

Table 2 shows that higher energy is required to achieve the Bragg's peak (-dE/dx) as the atomic number of projected ion is increased. The effect of proton originated from hydrogen source on the INF-α and INF-β is similar in targeting distance but differs in Bragg's peak as well as the targeting cross section (Table2, Figure 1). The Bragg's peak of proton targeting nucleotide is far away than those of interferons with lesser effect on the cross section of nucleotide (Table 2, Figure 1). The results obtained with proton of helium or carbon sources are similar in pattern but not in magnitude to that obtained with hydrogen source in targeting the interferons or nucleotide (Table 2, Figure 1). The cross section of INF-γ targeted by proton of whatever sources (hydrogen, helium or carbon) is less affected than INF-α and INF-β and its targeted depth is more INF-α and INF-β by 100-400 Angstrom. The cross section of nucleotide targeted by proton is less than those observed with interferon despite of higher Bragg's peak and longer projected distance for different sources of proton (Table 2, Figure 1). The spread out cross section of INF-γ targeted by protons in terms of longitudinal and lateral struggling is higher than corresponding INF-α and INF-β (Table 3). Moreover, the spread out effect of proton targeting nucleotide is higher than interferons by 1.3 for all ion sources (Table 3).

Table 1. The constituents of the targets

	IFN-α	IFN-β	IFN-γ	Nucleotide
Density (g/cm^3)	0.98010	0.98276	0.97573	1.1165
Atomic percent (Mass percent)				
C	31.82 (53.69)	32.18 (54.45)	31.55 (53.32)	29.62 (38.41)
H	50.02 (7.08)	49.92 (7.09)	50.01 (7.09)	35.83 (3.90)
N	8.39 (16.52)	8.72 (17.21)	8.87 (17.48)	18.51 (28.0)
O	9.43 (21.21)	8.93 (20.13)	9.32 (20.98)	14.81 (25.58)
S	0.33 (1.5)	0.25 (1.12)	0.25 (1.12)	-
P	-	-	-	1.25 (4.12)

C (carbon), H (hydrogen), N (nitrogen), O (oxygen), S (sulfur), P (phosphate)

Table 2. Effect of proton originated from different ions sources on the interferon and nucleotide

Ion	Target	Energy (KeV)	-dE/dx (KeV/μ)	Depth (μm)	Cross section keV/(μg/cm^2)
H	IFN-α	90	81.27	1.39	0.9776
	IFN-β	90	81.74	1.39	0.8317
	IFN-γ	90	80.96	1.40	0.8297
	Nucleotide	100	81.56	1.52	0.7305
He	IFN-α	550	228.9	3.47	2.3354
	IFN-β	550	230.1	3.45	2.3414
	IFN-γ	550	228.1	3.49	2.3377
	Nucleotide	600	232.7	3.70	2.0842
C	IFN-α	2400	813.8	4.33	8.3032
	IFN-β	2400	817.6	4.30	8.3198
	IFN-γ	2400	810.9	4.34	8.3109
	Nucleotide	2800	842.4	4.77	7.5394

Table 3. The lateral and radial struggle of proton of each target at Bragg's peak

Ion	Target	Longitudinal (μm)	Lateral (μm)	Cross section of damage beyond the target (μm^2)
H	IFN-α	0.1106	0.1514	0.01674
	IFN-β	0.1094	0.1498	0.01638
	IFN-γ	0.1110	0.1520	0.01687
	Nucleotide	0.1271	0.1732	0.02201
He	IFN-α	0.2051	0.2689	0.05515
	IFN-β	0.2029	0.2661	0.05399
	IFN-γ	0.2058	0.2698	0.05552
	Nucleotide	0.2304	0.3020	0.06958
C	IFN-α	0.1879	0.2397	0.04503
	IFN-β	0.1862	0.2373	0.04418
	IFN-γ	0.1885	0.2404	0.04531
	Nucleotide	0.2114	0.2729	0.05769

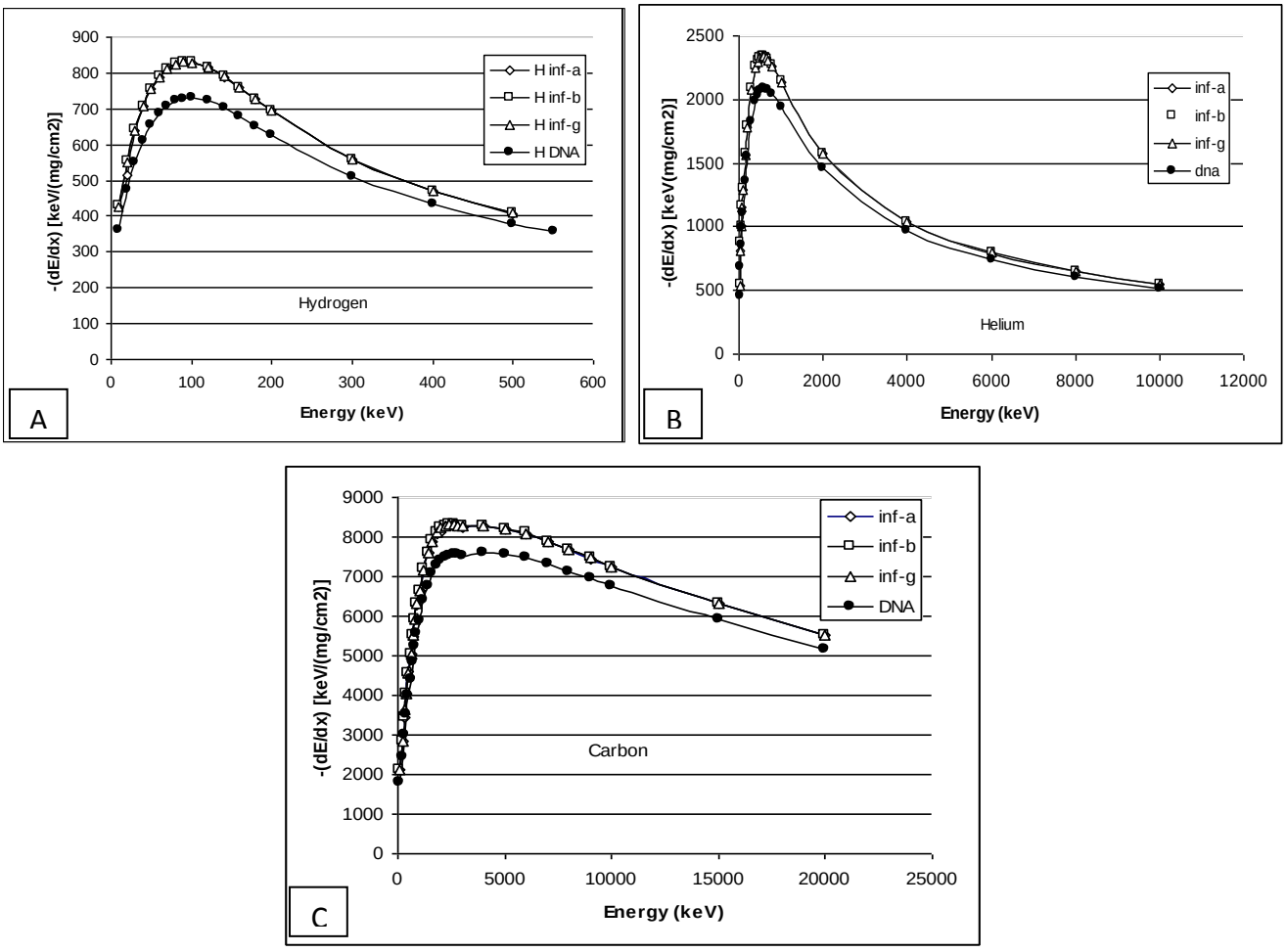

Figure 1. Bragg's peak deposited in different molecules; INF-α, INF-β, INF-γ and DNA targeted by hydrogen [A], helium [B] or carbon [C].

Discussion

The results showed that the Bragg's peak of proton (the maximum energy loss) of nucleotide is differed from that of interferon which means that proton beam radiation targeting the nucleotide will not affect the interferon and thereby not interferes with immune system. Moreover, the spread out effect of proton against the nucleotide at the Bragg's peak was higher by 1.3 fold of interferon at their Bragg's peak which indicated that proton showed selective effect against nucleotide.

Khvostunov et al (2010) found that whole cell nucleus as a function of proton energy shows a distinct peak at 550 keV using biophysical modeling of radiation effects induced by exposure of V79 cells which is approximated to that obtained with helium in this study [(11)]. In vivo, proton beam was found to be more cytotoxic to A549 lung adenocarcinoma cell than gamma radiation [(12)]. Previous studies showed that proton radiation exerts minimal effect on immune system as showed in this study.

The cell death in the splenic white pulp of irradiated whole body ICR mice with proton was lower compared with gamma radiation in spite of an increase damaged DNA [(13)]. Moreover, there is an evidence of using interferon, which is not targeted by proton in this study, in cutaneous melanoma patients to prevent metastasis and recommended to use interferon following proton radiation in patients with high risk of metastasis [(14)].

This study adds more information that endogenous interferon was not affected by proton when the later targeted the nucleotide which means that the immune system is free from the effect of proton radiation as it happens with conventional X-ray radiation [15]. It concludes that targeting of well precise located tumor with proton beam radiation therapy resulted in nucleotide damage of cancer cell without affecting the immune system in term of interferon surveillance.

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Evaluation of Hormonal Effects on Peripheral Blood Lymphocyte Apoptosis in Normal Menstruating Females

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Abstract

Background Apoptosis is a physiological type of cell death plays an important role in the regulation and maintenance of cell populations in tissues upon physiological and some pathological conditions.

Objective To evaluate the hormonal status and peripheral blood lymphocyte apoptosis in normal menstruating female during normal menstrual cycle.

Methods 50 healthy menstruating females with regular cycles were studied. Two samples of peripheral blood were aspirated; the first during the ovulation day and the second at the first day of the menstrual cycle. Lymphocyte separation was done; by Trypan blue exclusion test, and the morphological features of lymphocyte apoptosis by the DNA binding dye (Hoechst stains). Hormonal assessments of FSH, LH, Estradiol, and progesterone were also done.

Results Lymphocyte apoptosis in the first day of cycle was (9.31%±1.7) using Trypan blue exclusion test and (9.76%±1.36) using Hoechst stain while at the day of ovulation the percentages were (2.33%±0.7) and (1.38%±0.84) respectively with P value < 0.00001. Serum level of hormones were FSH (5.76±1.77; 9.14±3.34), LH(3.65±1.4; 28.35±18.94), Estradiol (40.05±14.73; 206.38±70.3) and Progesterone (0.35±0.13; 1.41±0.98) in the first day of the menstrual cycle and during ovulation respectively which showed a highly significant difference with P value <0.00001.

Conclusion The increment in the lymphocyte apoptosis in the first day of the menstrual cycle compared with ovulation day is mostly due to low ovarian steroid hormones and gonadotrophins. This might confirm the hypothesis that FSH, LH, estrogen and progesterone act as survival hormones for different tissues including peripheral blood lymphocytes.

Key words Lymphocyte apoptosis, FSH, LH, estrogen, progesterone, Hoechst stains

Introduction

Apoptosis is a physiological type of cell death that plays a key role in normal development and is critical for cellular homeostasis including immune cellular homeostasis and homeostasis in a variety of tissues [(1)]. The maintenance of tissue homeostasis is finely tuned between cell proliferation and programmed cell death (apoptosis). The maintenance of this balance is crucial to any multicellular organism. Too much proliferation leads to hyperplasia and to anatomical and physiological problems that are associated with it. The worst-case is a total loss of homeostatic control and development of cancer. If apoptosis supersedes proliferation, the result is a reduction of the tissue mass. If the process of apoptotic cell death is abnormal, it eventually reaches a point where physiological function is no longer possible [(2)]. Apoptosis represents a normal function to eliminate excess, old, injured or dysfunctional cells. Many evidences suggest that apoptosis helps to maintain cellular homeostasis during the menstrual cycle by eliminating senescent cells from the functional layer of the uterine endometrium during the late secretory and menstrual phase of the cycle [(3)].

Apoptosis has a key role in the regulation of the T-cell repertoire, both in the development of T-cells in the thymus, and in the elimination of activated T-cells in the periphery. Circulating T cells become active that is, they proliferate and produce proteins that promote inflammation when their receptors bind tightly to foreign antigens. Such activity is valuable when an infectious agent is still present, but when the infection is gone, the cells must die. Otherwise they might accumulate, giving rise to chronic inflammation and possibly to autoimmunity [4, 5].

Apoptosis of unwanted cells is induced by different mechanisms among which is by deprivation of survival factors as cytokines or interleukines and hormones as sex hormones which depend on Fas- Fas ligand interaction [4].

Hormones such as estradiol regulate function directly related to cellular event such as apoptosis. There are accumulating evidences suggesting that steroid hormones regulate apoptosis in hormone responsive tissues. This depends on hormone for survival and proliferation extends to neoplasm arising from these tissues. These reports also show that one mechanism by which estrogens may affect apoptosis is through the increased expression of bcl-2 gene, a member of a family of apoptosis regulating proteins whose expression has been shown to suppress apoptosis. These studies provide evidence that estrogens may play a role in both tumor genesis and drug resistance through suppression of apoptosis. From these studies, it was found that estrogen withdrawal induces apoptosis [6, 7].

Recent studies showed that estrogens may function as endocrine disrupters both in wildlife and humans, leading to developmental defects, disease and, potentially, cancer. The potential exists that these compounds, acting through the ER (Estrogen Receptors), can affect the apoptotic pathways of estrogen-responsive cells. With mounting evidence for the role of estrogen in the regulation of apoptosis, different studies had shown that estrogen can inhibit tumor necrosis factor (TNF)-induced apoptosis [7].

Studies had shown that estrogen may modulate the immune actions of leptin. Leptin induces the release of pro-inflammatory cytokines, including TNF-alpha, from human Peripheral Blood Mononuclear Cells (PBMCs). Estrogen can inhibit the signaling and immune actions of leptin. This could explain the sexual dimorphism observed in the immune response [8].

In vitro studies showed that estrogen decreases apoptosis of peripheral blood mononuclear cells from women with normal menstrual cycles and decreases TNF-alpha production in SLE patients but not in normal subjects[9].

Aim of the study

To evaluate the hormonal status, and peripheral blood lymphocyte apoptosis in normal menstruating females during normal menstrual cycle.

Methods

This study included 50 healthy menstruating females with regular cycles, their age ranged between 20 to 35 years with a mean of (23.96±3.31). Two blood samples were aspirated from each volunteer; the first was taken at first day of the menstrual cycle and the second at the day of ovulation.

7 ml of blood were taken and divided in 2 parts; 2ml was mixed with EDTA as anticoagulant for lymphocytes separation and study. The remaining 5ml of blood were examined for hormonal assessment using VIDAS assay kits for FSH, LH, Estradiol, and Progesterone hormones using mini VIDAS apparatus.

Trypan blue exclusion test was used to determine cell count and cell viability by using Heamocytometer Neubauer counting chamber and light microscope according to a method described by Doyle and Griffiths, 2000[10].

Hoechst stain (DNA binding) was used to study cell morphology using fluorescent microscope

using a method described by Harley and Prescott, 1996 [11].

The data were presented as mean ± standard deviation. Paired T-test was used to compare between ovulation day and the first day of the menstrual cycle. Difference was considered significant statistically when P< 0.05.

Results

The hormonal estimation including Estradiol, Progesterone, FSH, and LH in venous blood of fifty normal healthy female during ovulation day and the first day of the menstrual cycle were listed in table 1.

Table 1: The values of hormones during ovulation day and the first day of the menstrual cycle

Hormones	Ovulation day Mean± SD	First day of menstrual cycle Mean± SD	t test p value
Estradiol E2 (pg/ml)	206.38±70.3	40.05±14.73	<0.00001
Progesterone (ng/ml)	1.41±0.98	0.35±0.13	<0.00001
FSH (miu/ml)	9.14±3.34	5.76±1.77	<0.00001
LH (miu/ml)	28.35±18.94	3.65±1.4	<0.00001

While table 2 shows the percentage of apoptotic lymphocytes stained by Hoechst stain and also Trypan blue exclusion test for lymphocytes viability at the ovulation day and at the first day of the menstrual cycle.

Table 2: The percentage of apoptotic lymphocytes stained by Hoechst stain and Trypan blue exclusion test at the ovulation day and at the first day of the menstrual cycle.

Dead cells percentage	Ovulation day Mean± SD	First day of menstrual cycle Mean± SD	t test p value
Trypan blue	2.33±0.7	9.31±1.17	P<0.00001
Hoechst stain	1.38±0.84	9.76±1.36	P<0.00001

Figure 1 shows trypan blue exclusion test of peripheral blood lymphocyte in normal menstruating females, were the dead cells stained blue while normal cells exclude the dye.

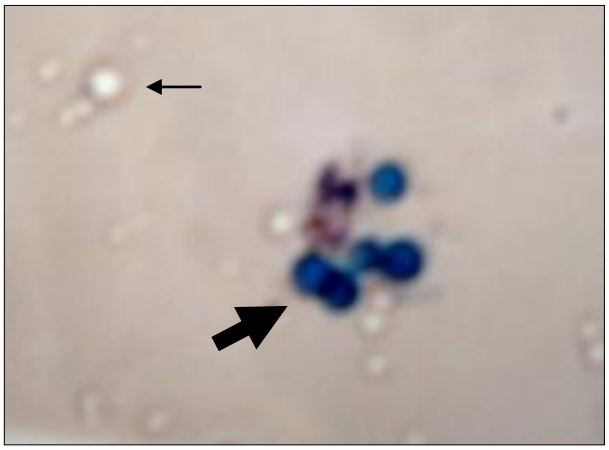

Figure 1:Trypan blue exclusion test ;thin arrow normal lymphocyte , thick arrow dead cell stained blue.

The morphological changes of lymphocyte apoptosis was studied by the DNA binding dye (Hoechst stain) which included: membrane blebbing, kidney shaped nucleus, lobulation of nucleus and lastly distruction of cell into apoptotic bodies. As shown in figure 2.

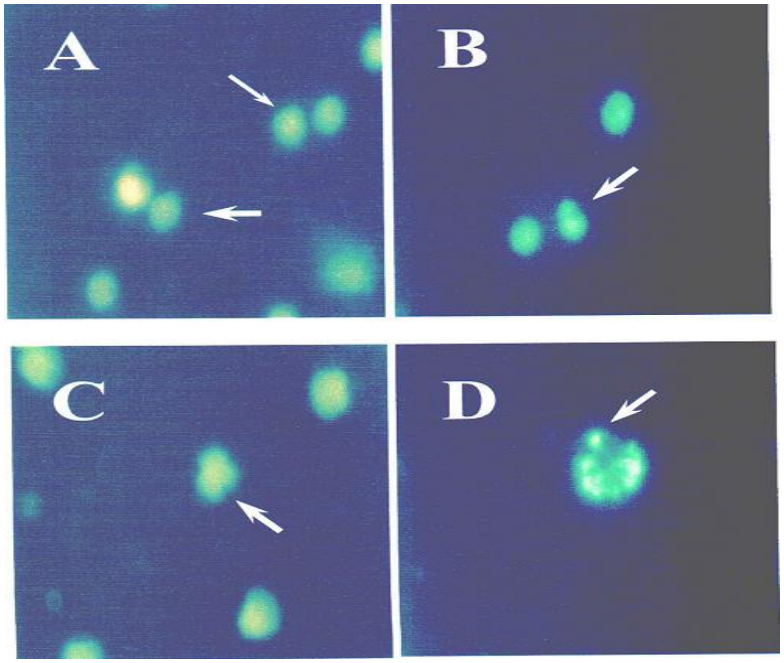

Figure 2: Fluorescent staining (1000X magnification) of peripheral blood lymphocytes of normal menstruating female by Hoechst stain (DNA binding stain) shows: *A*. normal lymphocytes. *B*. kidney shaped nucleus. *C*. lobulated nucleus. *D*. destruction of cell into apoptotic bodies.

Discussion

Female menstrual cycle is normally associated with many hormonal and physiological changes not only in the reproductive system but throughout the whole body. This cycle sometimes associated with pain, hyperpyrexia, psychological changes, and many other sign and symptoms that felt by some females during that period.

In this study peripheral blood lymphocyte apoptosis was studied in normal menstruating females. The results showed that the percentage of apoptotic lymphocytes were found to be significantly higher with values of (9.31 % ± 1.17) by Trypan blue exclusion test and (9.76 % ±1.36) by Hoechst stain during the first day of the menstrual cycle than during ovulation with values of (2.33 % ± 0.7) by Trypan blue exclusion test and (1.38 % ± 0.84) by Hoechst stain. This was with the association of significantly higher estradiol (E2) level during ovulation with a value of (206.38 ± 70.3) than during the first day of the menstrual cycle with a value of (40.05 ± 14.73). The decreased estrogen level during the first day of the menstrual cycle may have played a role in increasing lymphocyte apoptosis. These findings were similar to what was found by Yuan and Giudice in 1997[12], Kaipia and Hsueh in 1997[13] they claimed that the process of apoptosis is hormonally controlled process and that estrogens inhibit this process.

The results simulate those of Conlon and Raff in 1999[14]; they found that hormones act as survival factors to escape cell death and that failure to supply adequate levels of survival factors lead to activation of apoptosis.

This can explain the low lymphocyte apoptosis percentage during ovulation. This can also explain the high lymphocyte apoptosis percentage during the first day of the menstrual cycle; this result was found by Burow and coworkers in 1999[7] that hormones withdrawal induces apoptosis.

In the endometrium, estradiol and progesterone are the main regulators of the cyclical transformations and prevent cell death. This is responsible for the cyclical shedding during menstruation [14]. In this study estrogen and progesterone were significantly higher during ovulation than during the first day of the menstrual cycle and they may have played a role in reducing lymphocyte apoptosis percentage while the reverse was true during the first day of the menstrual cycle as both hormones were of a lower value with a highly significant increase in peripheral blood lymphocyte apoptosis.

Gosden and Spears in 1997[16] had found that progesterone emerges as a major survival factor in the reproductive system; this was obvious during ovulation with reduced lymphocyte apoptosis percentage by both Trypan blue exclusion test and Hoechst stain.

Results of this study also showed that the level of FSH in peripheral blood in the first day of the menstrual cycle was significantly lower than during ovulation (5.76 ± 1.77; 9.14 ± 3.34) respectively. The peripheral blood lymphocyte apoptosis was significantly higher in the first day of the menstrual cycle than during ovulation (9.31 % ± 1.17, 2.33% ± 0.7) respectively by Trypan blue exclusion test and (9.76% ± 1.36, 1.38% ± 0.84) respectively by Hoechst stain, this may explain the change in FSH and may reflect the increase in lymphocyte apoptosis in the first day of the menstrual cycle.

In this study, it was found that both estrogen and progesterone were lower in the first day of the menstrual cycle than during ovulation (40.05 ± 14.73; 206.38 ± 70.3); (0.35 ± 0.13; 1.41 ± 0.98) respectively with increased lymphocyte apoptosis due to decreased hormones levels reflecting a relation between estrogen, progesterone and lymphocyte apoptosis, this simulate the results of Lü and coworkers in 2002[17], they found that T cells may have sex hormones receptors. Among human T cells, estrogen receptors are present only in T cells of the $CD8^+$ suppressor/cytotoxic subset. Further, the effect of estrogen and progesterone on rhesus monkey B-cell physiology in vitro is mediated indirectly through $CD8^+$ T cells. Thus, it is likely that B-cell immunity in women is regulated by $CD8^+$ T cells under the influence of ovarian steroid hormones.

Changes at the cellular level *in vivo* and in cell culture *in vitro* showed that sex steroids have a major importance in over all pathogenesis of immune disorders. It is also mentioned that normal females have greatest changes of immune function during menstrual cycle. These changes involve changes in the immunoglobulines and changes in the cytokines and chemokines (down regulation of T helper-2 cells) [6, 18].

Lü and coworkers in 2003[18] found also that endogenous ovarian steroids regulate the immunoglobulin-secreting cell (ISC) frequency and this may explain why women are more resistant to viral infections and tend to have more immune-mediated diseases than men do. They found that Immunoglobulin A (IgA)-secreting cells (IgA-ISC) were fourfold more frequent than IgG-ISC in peripheral blood mononuclear cells (PBMC) and ISC frequency in PBMC was highest during the periovulatory stage of the menstrual cycle. While Poznansky and coworkers in 2002[19] found that LH, FSH, estrogens, androgens, progesterone, and thyroid hormones all decline during infection.

Another study by DA-SILVA in 1999 [20] showed that sex hormones have direct immunological effects that impact a clear gender dimorphism on the immune system. Globally, estrogens depress T cell-dependent immune function and diseases, but enhance antibody production and aggravate B cell-dependent diseases.

These findings might explain the difference between the strength and nature of immune responses between women and men. Hormonal and cellular immune responses in females are stronger than those in males. Immunoglobulin M (IgM), but not IgG, levels and CD4/CD8 T-cell ratios are significantly higher in the blood of women than in that of

men. Women also develop autoimmune diseases at a much higher rate than men do. These observations clearly demonstrate a role for ovarian steroid hormones in mediation of the immune system [21].

After viral exposure females are more likely to develop a T helper- 1 -type response. When T helper-2 responses predominate such as lymphocytic choriomeningitis virus infections, females have a more severe disease. Clearly, ovarian sex hormones affect the nature and effectiveness of antiviral immunity [22] [23].

Conclusion

From this study, we concluded that lymphocytes apoptosis increased in the first day of menstruation compared with the day of ovulation which is mostly due to the changes in the ovarian steroid hormones and gonadotrophins.

Our study also concluded that FSH, estrogen, and progesterone are survival hormones (anti apoptotic) and that they reduce peripheral blood lymphocyte apoptosis.

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Serum Copper, Zinc and Oxidative Stress in Patients with Psoriasis

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Abstract

Background Psoriasis is a chronic inflammatory skin disease characterized by well-demarcated erythema and scaly plaques. The pathogenesis of psoriasis still remains unclear. An increased reactive oxygen species (ROS) and insufficient antioxidant activity associated with the pathogenesis of psoriasis lesions.

Objective To evaluate the link between oxidative stress parameters and some trace elements like zinc (Zn) and copper (Cu) ions with the pathogenesis of psoriasis disease.

Method Fifty patients with psoriasis were included in this study, 32 patients with localized psoriasis, and 18 with general psoriasis, another fifty healthy controls were included in this study. We measured serum malondialdehyde (MDA), super oxide dismutase (SOD), vitamins E and A, and Zn and Cu in patients and control subjects.

Results Serum MDA in total psoriasis patients (1.8±0.2 nmol/ml) was significantly higher than those of control (0.6±0.19 nmol/ml; p<0.001). The SOD activity (9.1±1.0 U/ml) in serum of total psoriasis patients was significantly lower than that of controls (10.8±0.3 U/ml). serum vitamin A and E patients (56.8±3, 9.0±1.0 µg/ml respectively) were significantly lower than control (59.0±1.3, 9.7±0.9 µg/ml correspondingly p<0.0001), for trace elements the level of Zn in serum patients (79.0±9.1µg/ml) was significantly lower than control (83.3±5.8 µg/ml), while for Cu statistically significant higher levels were noted in patients (111.8±14.2 µg/ml) as compared with control (106.0±9.0 µg/ml).

Conclusion Our results suggest that lipid peroxidation of cellular membrane of keratiocytes by free radicals and decreased antioxidants may associate the pathogenesis of psoriasis lesion. In addition, there was a possible benefit of an enriched diet or of a supplement of vitamins A, E and Zn in treatment of psoriasis diseases.

Key words Psoriasis, Zinc, Copper, Oxidative Stress, malondialdehyde, super oxide dismutase

Introduction

Psoriasis is a common inflammatory and proliferative skin disease of unclear etiology and is known to affect about 2–4.8% of the global population [(1)]. Typical psoriatic lesions are erythomatous papules which form plaques characterized by sharp borders and increased scaling [(2)]. Recently, oxidative stress has been implicated in the etiopathology of psoriasis [(3, 5)]. Significant abnormalities of antioxidant mechanisms have been demonstrated in the blood and plaques of psoriatic patients [(6)]. An insufficient antioxidant system, together with increased levels of reactive oxygen species (ROS) has been suggested to be important in the pathogenesis of this disease [(2)].

ROS can react with all macromolecules, such as lipids, proteins, nucleic acids, and carbohydrates, particularly polyun-saturated fatty acids on cell membrane. After the beginning of an initial reaction with ROS, a continuing chain reaction is started and cell injury and ultimately cell death occurs [7].

Oxidative stress can result from deficiency of trace elements such as zinc Zn, copper Cu and selenium Se.

Trace elements and their compounds have been used since ancient times for their therapeutic as well as cosmetic effects on the skin [8,9]. The unique process of keratinization and melanin formation is enzyme-dependent and therefore could be influenced by trace element deficiencies or excesses as trace elements are involved in enzymatic activities and immunologic reactions [10]. Studies have also shown that essential trace elements like iron (Fe), copper (Cu), chromium (Cr), and vanadium (V) undergo redox cycling and have physiological significance, while nonessential toxic elements like cadmium (Cd), mercury (Hg), nickel (Ni) and lead (Pb), deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species (ROS) like superoxide ion, hydrogen peroxide, and hydroxyl radical [11].

There is no comprehensive study on the levels of trace elements, oxidants and antioxidants defence mechanism and their inter-element relationships in psoriasis. In the present study, we have analyzed the levels of 2 elements in serum samples of localized and generalized psoriasis patients and also measured malondialdehyde (MDA) and the status of antioxidant enzymes such as super oxide dismutase (SOD), and, non-enzymatic antioxidants like vitamins E and A.

Methods

A case-control study was conducted in the department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University. It included 50 cases, 32 were with localized plaque psoriasis (LPP), and 18 patients with generalized plaque psoriasis (GPP) who were attending the department of Dermatology, Al-Khadymia Teaching Hospital, and another 50 age- and sex-matched healthy individuals were included as controls.

Patients and controls with diabetes mellitus, thyroid disease, autoimmune disorder or concomitant dermatological diseases, or had taken systemic or topical treatment within three months before the present study, or had a history of smoking or alcoholism or taking drugs for any other reason or taking antioxidant or vitamins were excluded.

All blood specimens were taken after 10-12 hours fast in the morning between 9- 11 hours. The blood from forearm vein was collected in a plain tube and allowed to clot at room temperature for 30 minutes and centrifuged for 15minuts at 3000rpm (755xg). The serum was divided into proper aliquots and frozen at -20Ċ until used for measuring of Zn, Cu, MDA, SOD, vitamins A, and E.

All the precautions were taken in accordance with the Clinical and Laboratory Standards Institute criteria [12] to eliminate metal contamination while collecting and storing the samples

Serum Zn and Cu concentrations were estimated in the samples using flame atomic absorption spectrophotometry (AAS). The samples were diluted (1:10). Standards (prepared in deionized water) were run in the range of 10–40 µg/dl for Cu and Zn.

Serum MDA levels were determined by the method of Draper and Hadley [13] based on the reaction of MDA with thiobarbituric acid (TBA) at 70°C. In the TBA test reaction, MDA and TBA react to form a pink colour with maximum absorption at 532 nm.

For SOD activity determination we used (RANSOD kit, from Randox. The principle of the determination of xanthine by xanthine oxidase, and reduction of iodophenyl-nitrophenol-phenyltetrazolium (I.N.T) by the H_2O_2 produced.

Serum vitamins A and E were evaluated by high performance liquid chromatography (HPLC), in

brief for vitamin A serum was first deproteinized by 15% 5-sulphosalicylic acid, mixed and centrifuged, the sample was diluted and analyzed by HPLC system using (column C-18). The mobile phase was acetonitrile (100%) at a flow rate of 1ml/min and wavelength of 290 nm, while for vitamin E the mobile phase used was absolute ethanol-water (95:5 v/v) at a flow rate of 1ml/min and the wavelength of 229 nm.

Statistical analysis

All data were given as mean ± standard deviation (SD). Statistica version-6 for windows was used for statistical analysis. Levels of Zn, Cu, MDA, SOD, vitamin A and vitamin E in sera of patients and control subjects were compared by paired student's *t*-test. The differences were considered to be significant when the *p* value was less than 0.05.

Results

The study included 50 patients with psoriasis (29 women and 21 men) of age varied from 17-53 years, and 53 controls (27 women and 23 men) with age of between 17-53 years (Table 1).

Table1: MDA, SOD, VE, VA, Zn, and Cu in total psoriasis patients and normal control subjects

	No.	AGE	MDA (nmol/ml)	SOD (U/ml)	VA (ug/ml)	VE (ug/ml)	Zn(ug/dl)	Cu (ug/dl)
Control	50	34.20±10.7	O.6±0.19	10.8±0.3	59.0±1.3	9.7±0.9	83.3±5.8	106.0±9.0
Psoriasis	50	33.8±10.8	1.8±0.2	9.1±1.0	56.8±3.0	9.0±1.0	79.0±9.1	111.8±14.2
P value		0.85	0.001	0.000	0.0000	0.000	0.006	0.01

Table 2 show the mean values of total lipid peroxidation (MDA), plasma antioxidants (SOD enzymes, Vit. A, and Vit. E), and the levels of trace elements (Zn and Cu), in both patients and controls.

When compared with the control group, total psoriasis patients showed significantly higher values for MDA ($p<0.001$), and significantly lower values for SOD ($p<0.0001$), Vit. A ($p<0.0001$), and Vit. E ($p<0.0001$). Furthermore, trace elements Zn level show a significantly lower value ($p<0.0001$) in serum of psoriasis groups compared to the control, while for Cu, patients group showed significantly higher concentration ($p<0.01$) as compared with the control group.

Table2: MDA, SOD, VE, VA, Zn, and Cu in LP, and GP patients and normal control subjects.

	AGE	MDA (nmol/ml	SOD (u/ml)	VA (µg/ml)	VE (µg/ml)	Zn (µg/dl)	Cu (µg/dl)
Control (n=50)	34.2O±10.7	0.6±0.19	10.8±0.3	59.0±1.3	9.7±0.9	83.3±5.8	106.0±9.0
LPP (n=32)	34.7±11.3	1.8±0.1 a***P=0.00	9.0±1.0 a***P=0.000	56.5±2.8 a***P=0.000	9.1±1.1 a**P=0.01	78.1±8.6 a**P=0.001	110.8±14.6 aP=0.07
GPP (n=18)	32.3±9.9	1.8±0.2 a**P=0.00 bP=0.60	9.4±1.1 a**P=0.000 bP=0.15	57.2±3.3 a**P=0.00 bP=0.46	8.7±0.9 a***P=0.000 bP=0.25	80.6±10.0 aP=0.17 bP=0.36	113.6±13.7 a**P=0.01 bP=0.51

LPP = localized plaque psoriasis, GPP = generalized plaque psoriasis

[a]t-teat: comparison of the LP, GP groups with control

[b]t-teat: comparison of the lP patients with GP groups.

We have also compared the values according to the type of psoriasis (LPP and GPP); we found no significant differences for all values studied.

Discussion

Oxidative stress is now considered to be important in the pathogenesis of psoriasis [4,6]. Under normal physiological conditions, free radical–induced oxidative stress is combated by a complex antioxidant defence system. In humans there are two main defence systems against oxidative stress, the first involves mineral–dependent enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH–Px), the second defence system consists of non–enzymatic substances. Albumin, uric acid, and ascorbic acid account for over 85% of the total antioxidant capacity in human plasma [14]. This predominance is due largely to their high concentrations relative to those of other antioxidants in blood, e.g. α–tocopherol, β–carotene, and bilirubin. Although individual antioxidants play a specific role in the antioxidant defence system, the above antioxidant molecules may act cooperatively *in vivo* to provide synergistic protection against oxidative damage [15]. Measuring the levels of specific antioxidant molecules can yield valuable information, and low levels of such antioxidants provide suggestive, but not definitive evidence of oxidative stress.

The oxidative destruction of polyunsaturated fatty acids (PUFAs) of phospholipids, known as lipid peroxidation, can be in fact considered as a hallmark of oxidative stress. MDA an end product of lipid peroxidation induced by ROS is well correlated with the degree of lipid peroxidation[16]. There are different reports about MDA level in the literature. Yildirim et al [4] found no significant difference in the serum MDA levels between controls and psoriasis groups, while they found statistically significant increased tissue levels of MDA in psoriasis group, but Petronila et al [17] reported that the level of thiobarbituric acid (TBA) in plasma was significantly higher in patients with psoriasis than in controls, also Arpita et al [18] found that the MDA levels were significantly higher in psoriasis patients as compared with normal volunteers; furthermore Vijaykumar et al [19] found a positive correlation between increased MDA, and NO in sera of psoriasis patients with severity of psoriasis. In our study, the significantly higher levels of serum MDA support previous finding and indicate that lipid peroxidation may have a role in the pathogenesis of psoriasis.

Superoxide dismutase (SOD) is a group of metalloenzymes that protects cells from the toxic effects of superoxide radicals are produced as endogen. We found significantly lower levels of serum SOD activity in patients with psoriasis compared to healthy controls and this is in agreement with Yildirim et al [4] They found significant decreased levels of erythrocyte SOD and GP activities were noted in psoriatic subjects, and with Vijaykumar et al [19] there study showed that the serum SOD activity was observed to be decreased significantly from mild to moderate and from moderate to severe psoriasis patients. Superoxide and hydroxyl radicals are the most important radicals in lipid peroxidation. Decreased SOD activity could be responsible for the increase of superoxide radicals, which may explain the increased level of MDA [6].

There is an association between the antioxidant enzymes such as glutathione peroxidase and superoxide dismutase and trace elements including selenium, zinc, copper, and manganese [20]. A deficit of those elements may result in the decrease of antioxidant enzyme activity and the increases of oxidative stress induce cell damage.

We measured copper and zinc in order to illuminate the possible role of trace metals in the pathogenesis of psoriasis. This approach appears reasonable because copper and zinc are known to be among the constituents of the skin and to play essential roles in maintenance of its function in association with the enzyme systems activated by trace metals [21].

Essentiality of zinc is related mainly to its

function as the metal moiety of important enzymes. Zinc is considered as an antioxidant because the extracellular enzyme superoxide dismutase is zinc- dependent, it plays a vital role in the protection against free radical damage. Trace elements including zinc catalyze the rearrangement of dopachrome to form 5,6 - dihydroxy indole – 2 carboxylic acid (DICA) in the process of melanogenesis[22].

It was reported in literatures that both normal and psoriasis skin show no significant differences in Zn [21, 23, 24] while Bhatnagar et al [28] found in their study on active and remissive phases of psoriasis an increased in serum Zn level, our results indicated that Zn concentrations in psoriasis groups show a decreasing trend and are consistent with other studies [9, 25-27]. A reduction in blood Zn concentration with increasing surface area involvement in psoriatic may be due to Zn depletion secondary to loss of Zn through exfoliation [29]. An alternative possibility is that disturbance in the blood Zn status might actually be resulting in greater surface area involvement [9].

Our results show increased concentration of serum Cu in psoriasis groups. This is in accordance with few studies where elevated levels of serum Cu have been reported in psoriasis and other skin diseases [25, 26, 30]. Increased Cu may be attributable to inflammation associated with the disease.

We also evaluated some natural liposoluble antioxidants vitamins, namely vitamins A and E. These vitamins, being supplied by diet, may allow the control of their plasma levels by an enriched diet or even by a therapeutic vitamin supplementation [31, 32]. Early studies on the relationship between a vitamin A and E deficient diet and disturbances in epidermal growth and differentiation suggest the importance of those compounds for the maintenance of skin homeostasis [26]. It is known that moderate and severe inflammatory reactions lead to a decrease in the blood retinol and beta-carotene content, whereas lipid peroxidation increases in inflammatory reactions [20-35]. In this study vitamin A and vitamin E were significantly lower in patients with psoriasis than in healthy controls and this is in agreement with Petronila et al [17] they found decreased vitamins A and E levels in (inactive and active) psoriasis patients as compared with the control group. Our observed low vitamin A and E in the psoriasis patients might be explained by the inflammatory conditions since the Psoriasis is a chronic and recurrent inflammatory skin disease.

In conclusion, we observed that there is impairment in the antioxidant system in psoriasis, leading to free-radical mediated damage to skin cells. Our finding revealed that this oxidative stress is not a localized phenomenon but a generalized process and may be one of the reasons for the progressive nature of the disease. In view of these findings, the levels of MDA increased in patients with psoriasis by peroxidation whereas serum SOD and vitamins A and E and Zn element decreased. Antioxidant treatments such as vitamins A and E and Zn in patients with psoriasis may be of use in treatment of psoriasis in two respects: a- to decrease the inflammation in skin tissues by virtue of the inactivating effect of free radicals and b- to confirm stability on cell membranes by a positive effect on membrane stabilization and repair.

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Medico-Legal Study of Fatal Flame Burn Victims in Sulaimani Province

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Abstract

Background Fatal flame burn injuries remain one of the most common causes of fatalities referred to Al-Sulaimania Medico-Legal Institute in spite of many recent advances in care and management. They occupied number one of all cases which were managed medico-legally.

Objective To study, evaluate and determine the causes of death in victims of flame burn injuries.

Method The study was conducted on 221 cadavers of flame burn injuries referred to the medico-legal institute in Sulaimania province during the period between 8th of May 2008 and 7th of May 2009. Complete classical autopsy was performed on each case as well as proper laboratory investigation (CO and renal function tests).

Results Fatal flame burn injuries constituted (28%) of the total number of medico-legal mortalities during the period of the study. The highest percentage of flame burn deaths occurred during the 1st and 2nd day of burn which was related to the state of shock and primary toxemia in (46.08%) of the cases. Late deaths were due to septicemia, cumulative effects of the early cause and miscellaneous causes.

Conclusion Flame burn injuries were the most common police cases managed medico legally. The vast majority of victims died within the first ten days. Staphylococcus aureus was the most common organism isolated from wounds and blood of victims.

Key words Autopsy, flame burn, Al-Sulaimania.

Introduction

Flame burn remains one of the most dangerous and devastating injury in spite of many recent advances in care and management [(1)]. In physical terms, it involves the transfer of an excessive amount of heat energy to the body by many physical modes [(2)]. Recently wound sepsis is considered a major factor of burn death[(3, 4)]. The second common cause of immediate death was inhalation of toxic fumes and gases [(5)]. Shock is the most post-burn concern either neurogenic or of progressive hypovolemic type [(4)]. Blunt trauma may associate burning resulting in death [(6)]. Curling type of duodenal ulcer may perforate with high mortality rate [(7)]. Moors et al revealed male to female ratio of (2:1) [(8)], while Chen-Fm in his study, showed ratio of (5:1) [(9)]. Al-Qaissi stated that more than (50%) of burn deaths were due to state of shock and primary toxemia [(10)]. Feket in his study found different states of renal impairment in postmortem vitrous humor urea and creatinine [(11)].

Methods

Autopsy of 221 cadavers of flame burn victims including those who died as inpatients were examined in Al-Sulaimani Medico-Legal Institute from 8th of May 2008 to the 7th of May 2009.

Complete medical information was obtained from medical files for those who were admitted to hospital prior to death including their lab investigations.

Complete external examination of each cadaver was done in order to re-assess the extent, percentages and degree of burn, with microscopical examination of the wound and any associating injuries.

Complete autopsy examination was done for each case with gross examination of all organs. Investigation were done including heart blood samples for carbon monoxide (CO) level determination using visible spectrophotometer and vitreous humour samples withdrawn from the outer canthus of the eye using disposable syringes G21 to determine the level of urea and Creatinine. Statistical analysis of the results was done using the SPSS version 11.

Results

During one year of work, a total of 221 flame burn cases referred to Sulaimani Medico-legal institute were included in this study. They represented (28%) of the total cases referred for autopsy which was 791 during the period 8th of May 2008 till 7th of May 2009.

Age and sex distribution were shown in the table 1. Age group 21-30 years showed the highest number of victims among male while female highest age group death was 11-20 years.

Table 1. Age and Sex Distributions among flame burn victims.

Age/ Sex	0-10 years	11-20 years	21-30 years	31-40 years	41-50 years	51-60 years	61-70 years	71-80 years	Total
Male	4	5	9	3	4	2	3	0	30
Female	10	87	62	15	7	5	2	3	191
Total	14	92	71	18	11	7	5	3	221
%	6.3	41.6	32.1	8.1	5	3.1	2.3	1.4	100

Table 2 shows the highest survival number was within the first ten days and the number then decreased with increasing survival period.

Table 2. Duration of Survival following flame burns

Survival days	Number	(%)
0-10	192	86.87
11-20	13	5.88
21-30	7	3.16
31-40	4	1.8
41-50	5	2.26
Total	221	100

Most common bacterial organisms found in burn wounds and blood cultures among those admitted to hospital prior to death were Staphylococcus aureus representing 70.4%, pseudomonas aeruginosa representing 48.1% and Klebsiella representing 37% of all patient cases as it is shown in table 3.

Table 3. Bacteriologic wound isolates from 108 admitted burn victims and Microbiologic blood isolates from 60 admitted burn victims

Micro-organism	Wound isolates	No.%	blood isolates	No.%
S. Aureus	+	76(70.4%)	+	40(66.7%)
P. Aeruginosa	+	52(48.1%)	+	28(46.7%)
Klebsiella	+	40(37%)	+	12(20%)
Proteus	+	32(29.6%)	+	5(8.3%)
E. Coli	+	8(7.4%)	+	32(53.3%)
S. Albus	+	5(4.6%)	-	
Enterobacter	+	4(3.7%)	-	
Strepto.	+	3(2.8%)	-	
C.albicans	-	-	+	4(6.7%)

Urea was less than 30 mg/100 cc in 85 cases (38.5%), while it was 100mg /100cc and more in 14 cases. Creatinine level was higher than normal limits in nearly more than 50% of cases as shown in table (4).

Table 4. Post-mortem values of vitreous humor Creatinine and urea in 221 flame burn victims

Creatinine	No. (%)	Urea	No. (%)
		<30	85 (38.5)
<1	109 (49.3)	40	46 (20.8)
2	70 (31.8)	50	27 (12.2)
3	26 (11.8)	60	24 (10.8)
4	13 (5.9)	70	13 (5.9)
5	2 (0.9)	80	6 (2.7)
>6	1 (0.4)	90	6 (2.7)
		>100	14 (6.4)
Total	221 (100)	Total	221 (100)

Carbon monoxide reached the lethal level in 3 cases as shown in table (5) which is 50%-60% saturation in healthy subjects.

Table 5. Postmortem blood carboxyhemoglobin saturation

CO Hb Saturation	No. (%)
0- 9%	89 (40.27)
10- 40%	128 (57.91)
41- 60%	4 (1.82)
Total %	221 (100)

Discussion

In this prospective study, the number of flame burn victims was 221 cases and this does not reflect the real size of thermal deaths at particular time in the area in which they became the unique source of cases including Sulaimani, because of excluded cases like scalding, and electrical burns. The number of victims in this study represented 28% of all cases managed medico-legally during the duration of study. Highest incidence rates of burns were found in this study among teenager and young aged groups.

The explanation for higher incidence among these age group is related to the most active stage of life .The highest age group of flame burn victims in our study was 21- 30 year which is less than the age group in other study done by Chen Yu Lin [10]. Female: male ratio in our study was 6.4:1 which is an inverse result to other study done by Moors, et al and Chen-FM [8,9]. This inversion in our result is du to the source of our cases being domestic and not industrial where females in our society almost always deal with all domestic activities. The death peak during the first and second day of burn were related to the state of shock and primary toxemia in 90\221 cases (46.8%) and this is explained to a fact that all of them presented with extensive burns (more than 80% of body surface area) with systemic complications.

Many studies showed collateral finding for the predominance of staphylococcus aureus and pseudomonas aeruginosa in wound and eschar cultures [8]. Blood cultures showed predominance of pseudomonas aeruginosa and E.coli. The existence of E.coli is a strong indication of translocation of the sepsis from the gut or urinary system, but the result was with lower incidence in a study held by Artz [12]. This is related to lack of proper burn victim's isolation during the course of treatment in the admitted hospital which is very essential to prevent cross infection from other burned patients, suboptimal treatment, and presense of resistant types of microorganisms.

Post mortem vitreous humor urea and Creatinine values usually reflect the state of re-hydration and renal impairment which might be related to inappropriate management and follow up, and this is similar to the result of a study held by Feket [11]. Carbon monoxide poisoning proved in three cases to be a main cause of death at scene.

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Expression of Fas and FasL in Trophoblastic Tissue of Women with Spontaneous and Induced Abortion Using *insitu* Hybridization Technique

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Abstract

Background Apoptosis of trophoblastic cells may play a role in the pathogenesis of abortion, and one mechanism of apoptosis is Fas receptor and legand system.

Objective Estimate the level of Fas and FasL in trophoblastic tissue of aborted women.

Methods In this study, 25 women with spontaneous abortion and 5 women with induced abortion were included from attendants of Gynecology department at Al-Kadhimiya Teaching Hospital in Baghdad. In-situ hybridization (ISH) tests were done to detect the level of expression of Fas and FasL in trophoblastic tissue.

Results The highest percentage of expression of Fas and FasL was found in the trophoblastic tissue of females with spontaneous abortion (34.36% for Fas and 31.86% for FasL), while the expression of them was low in induced abortion group (9.2% and 9.4%, respectively).

Conclusion The data strengthen the possibility that the apoptosis of trophoblastic cells via enhanced expression of Fas-FasL system is an important mechanism of spontaneous abortion.

Key words Miscarriage, Trophblast, Apoptosis, Fas, FasL, ISH.

Introduction

Miscarriage, the loss of pregnancy before 20 weeks, is a devastating event with both physical and emotional components. The common causes of miscarriage are genetic error, abnormal hormonal levels (especially if progesterone levels are low), polycystic ovarian syndrome, structural problems, blood incompatibility, clotting disorders, environmental factors and infections which affect fetal development and in some cases result in miscarriage [(1)].

A number of studies have suggested that apoptosis plays a role in the normal development, remodeling, and aging of the placenta [(2,3)].

Analysis of apoptosis mechanism that leads to abortion may provide a new insight into pathogenesis of abortion. Since previous studies indicate that the apoptosis of placental could be as a result of immune responses, which could be mediated by Th-1 response, and most probably via enhance expression of Fas-FasL [(4)].

Apoptotic messages from outside the cell (extrinsic inducers), whereas apoptotic messages from inside the cell (intrinsic inducers) are a response to stress, such as nutrient deprivation or DNA damage. Both extrinsic and intrinsic

pathways have in common the activation of central effectors of apoptosis, a group of cysteine proteases called caspases, which carry out the cleaving of both structural and functional elements of the cell, resulting in the morphological changes of apoptosis [5].

One mechanism of inducing apoptosis is activation of the CD95 receptor and ligand system [6]. The CD95 molecule (synonyms: APO-1, Fas) is a type I transmembrane receptor which belongs to the nerve growth factor/TNF receptor superfamily [7,8]. CD95 is constitutively expressed in a wide range of normal human tissues. Moreover, expression of CD95 can be induced in various conditions [9]. CD95 ligand (CD95L) is a transmembrane protein which belongs to the TNF family [7]. CD95 ligand is expressed in various human cells and tissues, such as activated T lymphocytes, lung, liver or kidney [10,11]. Binding of CD95L to the extracellular domain of CD95 induces trimerization of the receptor then, the intracellular domain of CD95 recruits via an adaptor Fas associated death domain (FADD) the cytoplasmic caspase-8 [12].

In this study a try was made to investigate the level of expression of trophoblastic Fas and FasL (using in-situ hybridization method) as an indicator of apoptotic process in trophoblastic cells in abortion.

Methods

Twenty five pregnant female patients with spontaneous abortion, their age range from 18 to 44 years were included in this prospective study. They were attendants of Obstetrics and Gynecology department at Al-Kadhimyia Teaching Hospital in Baghdad, from 5th April 2007 to 30th September 2007. All were admitted to the hospital for evacuation. Another five females were also included, they were admitted for elective termination of pregnancy (induced abortion) due to maternal cardiac disease and they were considered as a control group.

From each patient and control included in this study, trophoblastic tissue was collected from the evacuated retained pieces during the procedure of curettage and placed in 10% formaldehyde. Paraffin embedded blocks were prepared and were sectioned, one section stained with haematoxyline and eosin, and only sections that contained trophoplastic tissue were included in this study.

In-situ hybridization technique using biotinylated long cDNA probe (318 bp for human Fas-gene detection and 250 bp for human Fas-L gene detection) together with Maxim's ISH detection kit was used.

Paraffin embedded sections of trophoblastic tissue were cut into 5 µm thickness, placed on positively charged slides and placed in a 65ºC hot air oven overnight, and cleared in two changes of xylene for 5 minutes each, rehydrated in two changes of absolute ethyl alcohol for 2 minutes each, then in fresh 95% ethyl alcohol for further 2 minutes. Slides then were placed in deionized water for 5 minutes then drained and blotted gently.

Deproteinization of the tissue was done by placing protienase K solution onto the tissue section at 37ºC for 15 minutes. 10-20 µL of the working (biotinylated probe) DNA probe/hybridization solution was placed onto the tissue sections, and then slides were placed in the oven at 70ºC for 10 minutes to denature the secondary structure of RNA.

In the next morning one to two drops of RNase A were placed onto the tissue sections and incubated at 37ºC for 30 minutes, and then the slides were washed in 1X proteinblock. One to two drops of the (biotinylated antibiotin Abs) were placed onto the tissue sections and incubated at 37ºC for 1 hr, then 1-2 drops of conjugate (red) (streptavidin-AP) were added for 20 minutes at 37ºC, and then 1-2 drops of the substrat, was placed onto the tissue section and incubated at room temperature for 15-20 minutes, and then the sections were

counterstained with neuclear fast red (NFR) stain for 30 seconds.

Dehydration of the sections was carried out by sequential dipping of the slides once in 95% ethanol, twice in 100% ethanol and then once in xylene. One to two drops of DPX were placed on xylene-wet sections, and the sections were quickly covered with coverslips and left overnight to dry. The slides were examined under light microscope.

A scoring system that includes an evaluation of the staining percentage of stained cells was employed for the expression of Fas & FasL, as following: Negative if < 5% of the cells were positively stained. Positive if ≥ 5% were positively stained.

Results

Among the two groups (spontaneous and induced abortion), the highest percentage of Fas were found in the spontaneous abortion group, while the lowest percentage were found in the induced abortion group, and the highest percentage of FasL were found in the spontaneous abortion group, while the lowest percentage were found in the induced abortion group, as shown in the table 1.

There is significant difference (p=0.016) in the mean value of Fas between spontaneous abortion group and induced abortion group, and there is significant difference (p=0.019) in the mean value of FasL between spontaneous abortion group and induced abortion group. There is high positive correlation (0.809) between Fas and FasL expression in trophoblastic tissue and it is highly significant statistically (p<0.001)

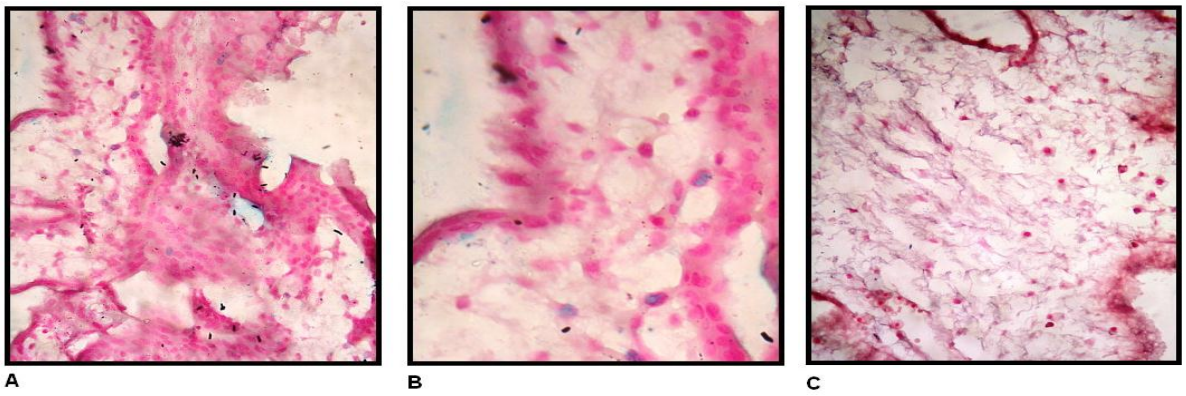

Figure 1: *In-situ* hybridization for human Fas in trophoblastic tissue sections. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red (pink). A: Positive case, magnification power (100X), B: Positive case, magnification power (400X) C: Negative case, magnification power (100X).

Table 1: Fas and FasL expression in spontaneous and induced abortion cases.

Abortion type	Fas			FasL		
	Mean+SE	N	p-value	Mean+SE	N	p-value
Spontaneous abortion	34.36±3.18	25	0.016	31.86±2.91	25	0.019
Induced abortion	9.20±1.56	5		9.40±1.03	5	

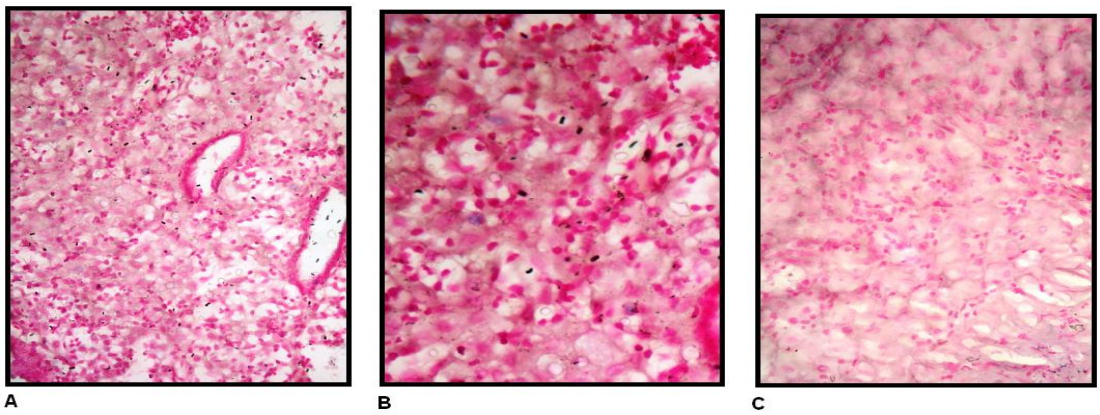

Figure 2: *In-situ* hybridization for human FasL in trophoblastic tissue sections. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red (pink). A: Positive case, magnification power (100X), B: Positive case, magnification power (400X) C: Negative case, magnification power (100X).

Discussion

Our results shed light on the delicate balance between immunoprotection and trophoblast death. The finding is that Fas ligation on trophoblastic cells induces proinflammatory cytokine production (which is IL-8 in our study), resulting in neutrophil chemo attraction agrees with the findings of a study done by David and co-workers, they investigated Fas-induced cytokine responses of normal human blood monocytes and monocyte-derived macrophages. Their principal finding was that Fas ligation on these cells induces predominantly proinflammatory cytokine production, resulting in potent neutrophil chemoattractant bioactivity. This represents the first evidence that Fas ligation activates human monocyte and macrophage proinflammatory cytokine responses. These observations are especially significant because they run counter to the prevailing notion that Fas ligation on phagocytes and Fas-induced phagocyte apoptosis result in predominantly anti-inflammatory effects that contribute mainly to the resolution of inflammation [13].

Our study demonstrate that Th-1 proinflammatory cytokine (IL-8) promotes Fas expression thereby increasing the sensitivity of trophoblast cells to Fas-mediated apoptosis and the enhanced sensitization to Fas results in trophoblast autocrine-induced apoptosis once FasL binds to and activates the Fas receptor, and this agrees totally with a study done by Sarit and co-workers, they demonstrate that Th-1 proinflammatory cytokines promote Fas expression, whereas Th-2 anti-inflammatory cytokines inhibit the expression of Fas, thereby decreasing the sensitivity of trophoblast cells to Fas-mediated apoptosis. The enhanced sensitization to Fas results in trophoblast autocrine-induced apoptosis once FasL binds to and activates the Fas receptor; they demonstrate that cytokines influence trophoblast sensitivity to apoptosis by regulating the expression and function of the Fas/FasL system [14].

We found that trophoblastic apoptosis occurred by the FasL/Fas pathway and suggest that FasL expressed on the trophoblasts and activated maternal lymphocytes can induce trophoblast Fas-mediated apoptosis and the increased Fas expression on trophoblasts may make them more sensitive to Fas-mediated apoptosis, this agrees with a study done by Dhruv and co-workers, they found that trophoblast apoptosis occurred by the FasL/Fas pathway, their findings suggest that FasL expressed on the trophoblasts

and activated maternal lymphocytes can induce trophoblast Fas-mediated apoptosis by autocrine or paracrine interactions.

In addition, the increased Fas expression on trophoblasts may make them more sensitive to Fas-mediated apoptosis. The increase in trophoblast apoptosis associated with chorioamnionitis provides support for their hypothesis that immune cells in placenta regulate trophoblast apoptosis via cytokines at the fetomaternal interface. One possible reason for the increase in trophoblast apoptosis associated with chorioamnionitis could be the changes taking place in cytokine composition in the placental microenvironment, by the activation of the placental and decidual immune cells, resulting in the release of inflammatory mediators , specifically cytokines and chemokines, into the placental microenvironment [15, 16].

This release of inflammatory mediators results in dense neutrophil and lymphocytic infiltration in the chorion, amnion, and placental villi. In addition to the immune cells, trophoblasts also produce cytokines. The proinflammatory cytokines are cytotoxic to trophoblasts, resulting in their apoptosis [17]. So we suggest that a possible mechanism for the increased trophoblast apoptosis associated with infection could be the activation of the FasL/Fas pathway of apoptosis. This is obvious from the results of increased expression of Fas/FasL in our research. These data have implications for understanding the mechanism of abortion and may help in preventing abortion if the baby normal.

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Immunohistochemical Study of Some Chemokines Receptors in Atopic Epidermis: Before and After Treatment with Topical Tacrolimus–steroid Therapy

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Abstract

Background Tacrolimus is an immunosuppressive agent used topically, it has been found to be effective in treating moderate to severe atopic dermatitis without causing the atrophy that might occur with prolonged use of topical corticosteroids. There is a lack of studies on the effect of tacrolimus and steroid Therapy on CCR3 and CCR5 in atopic dermatitis patients.

Objective To assess expression of some chemokine receptors in the epidermis of atopic skin (chronic lesions) and to evaluate any differences in the degree and pattern of epidermal expression before and after topical tacrolimus or steroid therapy.

Methods Twenty five cases of atopic dermatitis before and after treatment by tacrolimus ointment and topical steroids were evaluated immunohistochemically for the epidermal expression pattern and intensity of some chemokine receptors namely CCR3 and CCR5 before and after treatment.

Result CCR5 and CCR3 positive epidermal cells seem to be produced in situ in higher amount before treatment compared with that after treatment. Although these cells are predominantly CCR5+.

Conclusions Enhanced expression of CCR3 and CCR5 on the surface of epidermal keratinocytes may be significant for the determination of atopic reactivity in general and also observed differences in frequencies of these activation markers before and after treatment by topical steroids-tacrolimus therapy.

Key words Atopic dermatitis, CCR3, CCR5, Immunohistochemistry, Tacrolimus

Introduction

Atopic dermatitis (AD) is a common pruritic disease that occurs primarily in infancy and childhood [(1)]. AD is characterized by itching, with patients having an individual or family history of atopic diseases in their background. Barrier dysfunction, immunological dysfunctions (type 1 and type 4 allergy) [(2)], genetic disorders and psychological factors contribute to the pathogenesis of AD. It is a chronically relapsing eczematous skin disease resulting from complex interactions between genetic and environmental factors [(3)].

In spite of controversies as regards the exact pathophysiology of eczematous lesion (and the exact type of immune reaction), three main types of cells have been confirmed to play the major role in the evolution of characteristic pathology of AD namely T- lymphocytes, Antigen Presenting Cells (APCs) and keratinocytes [(4)].

The role played by these effector cells is orchestrated by a growing list of cytokines (or chemokines), adhesion molecules and other mediators that control trafficking and action of the inflammatory cells and subsequently determine the nature, extent and duration of the inflammatory reaction [(5)].

There are many mechanisms involved in the pathogenesis of AD such as type 1 allergy [(6)], type4 allergy and barrier dysfunctions.

Moreover, in recent years, a considerable body of evidence has implicated T cells as having a major role in the pathogenesis of AD [(7)]. Increased numbers of T helper cells with a Th2-type cytokine profile are present, especially in the initial phase of skin inflammation[(8)], whereas both Th2-type cytokines Th1- type cytokines are up-regulated in chronic lesions.

Human Th1 and Th2 cells express distinct chemokine receptors (generated under the influence of IL-12 and IL-4 respectively) and are differentially recruited in response to chemokines [(9)]. Th2 cells were shown to express CCR3 preferentially and selectively migrate in response to eotaxin.CCR4 and CCR8 were shown to be expressed on Th2 cells. In contrast, CXCR3 and CCR5 were shown to be expressed on Th1 cells.

Topical tacrolimus and pimecrolimus have been developed as new non-steroidal immunomodulators [(10)]. Tacrolimus ointment 0.1% approved for use in adults; a frequently observed side effect with topical calcineurin inhibitors is a transient burning sensation of the skin. Importantly, treatment with topical calcineurin inhibitors is not associated with skin atrophy, thus they are particularly useful for the treatment of areas such as the face and intertriginous regions. So the new immunomodulators clear the rash with few side effects than do older steroids [(11)].

Methods

Twenty five patients with chronic atopic dermatitis (AD) and attended the private clinic in Baghdad during the period extended from May 2008 to September 2008, were randomly selected to participate in the study. Skin biopsy was taken from patients before tacrolimus or steroid therapy and after one month of therapy.

The diagnosis of atopic dermatitis was based on the criteria described by Hanifin and Rajika [(12)].

To investigate whether the patients were in allergic status and apart from suggestive clinical data, blood samples from all subjects were tested for total serum IgE titer and eosinophil count before and after treatment. Enzyme linked Immunosorbant Essay (ELISA) was used for the measurement of the total IgE in sera of the studied groups. Anti-Human IgE peroxidase conjugate IgG antibody was used for this purpose. The procedure of ELISA is making according to the Hunter et al 1986[(13)]. The results were expressed in IU/Ml and by cut of value were expressed as positive or negative.

These twenty patients return back to the private clinic after two weeks to one month of treatment with tacrolimus or topical and systemic steroids. The patients that re-analyzed after treatment were presented with same size and degree of severity of lesions for each patient was treated by topical steroids or topical tacrolimus. A second specimen (blood and biopsy) were taken from them.

Ten biopsies were taken before treatment (topical steroid or topical tacrolimus) and ten biopsies were taken from the same patients and the same sites after one month of treatment. After cleaning, local anaesthetic is infiltrated, an excisional biopsy is planned after considering the local anatomy. The ellipse to be excised is drawn on the skin using a marker pen. The ellipse is freed from surrounding skin, secured at one end with a skin hook and removed from the underlying fat, usually using the scalpel blade and preserved immediately in 10% formalin and subsequently embedded in paraffin blocks. Four micron sections were prepared for subsequent immunohistochemical staining.

The procedure of immunohistochemistry as following:

1. Slide baking; the slides were placed in a vertical position in incubator at 37 °C overnight then the slides were placed in a vertical position in a drying oven (hot air oven) at 65 °C for one hour.
2. Deparaffinizing the tissue sections: the slides were immersed sequentially in the following solutions at room temperature for the indicated times:

Xylene for 5 minutes, Absolute ethanol for 5 minutes, 95% ethanol for 5 min, 70% ethanol for 5 min, 50% ethanol for 5 min. and distilled water for 5 min.

3. After draining and carefully blotting around the specimen to remove any remaining liquid, the slides were placed in the humid chamber then 100 µl of protein – blocking reagent were applied onto the tissue to cover the whole specimen then incubated at room temperature for 15 minutes. Then the slides were rinsed gently with distilled water then drained and blotted as before.

4. Hundred µl of the diluted primary antibody were applied onto the tissue after the slides were placed in the humid chamber then incubated at 37 °C for 1 hour. After that, the slides were rinsed with (1X) rinse buffer for a minimum of 15 seconds then the slides were drained and blotted as before.

5. 100 µl of the diluted conjugate secondary antibodies were applied onto the tissue after the slides were placed in the humid chamber then incubated at 37 °C for 10 minutes. After that, the slides were rinsed with (1X) rinse buffer for a minimum of 15 seconds then the slides were drained and blotted as before.

6. 100 µl of DAB solution were applied to the in a dark place for 10 minutes at room temperature. The slide were washed in distilled water for 5 minutes and then drained and blotted gently.

7. The tissue was stained by 100 µl of counter (Haematoxyline) stain which was placed onto the tissue and incubated for 30 seconds at room temperature. Slides were drained gently.

8. Slides were washed in distilled water then drained and cleaned gently.

9. A drop of mounting medium (DPX) was placed onto the tissue section and then quickly covered with a cover slip and left to dry.

10. Slides were examined by light microscope at 40 X magnification. Immunostaining was scoring.

Table 1. Primary Ab and secondary Ab working dilution

Primary Ab	manufecture	Source	Working dilution	Secondary Ab	Manufacture	Source	Working dilution
Anti-human CCR3	USA Biological	Rabbit	6-32 µg/ml	Anti-Rabbit IgG	USA Biological	SHEEP	1/50-1/100
Anti-human CCR5	USA biological	Mouse	10 µg/ml	Anti-mouse IgG	USA biological	Goat	1/50

The drugs used in our study are topical corticosteroids (beta– methasone valerate), tacrolimus ointment and newer macrolide antibiotics (oral azithromycin). The details of each drug were shown in Table 2.

Table 2. The details of drugs are used in our study

Drug Name	Trade name	Name of company	Rout of administration	Duration of use	Dose concentration
Tacrolimus ointement	Talimus ointment	Ajanta pharma	Topical	2 weeks-	0.1% w/w
Betamethason ointment	Betnovate ointment	Glaxo Smith	Topical	2 weeks-	0.1%
Azithromycin	Zithroiv	Riva pharma	Oral, single dose/daily	2 weeks	250 mg

Statistical analysis

Statistical analysis was performed with the SPSS 15.01 statistical package for social sciences and also Excell 2003. Data analysis was done using paired sample t-test for tables with pre treatment and post treatment data means, independent sample t- test if we have two different groups. *P* value of ≤ 0.05 was used as the level of significance. Descriptive statistics for the clinical and laboratory results were formulated as mean and standard deviation (SD) and standard error (SE).

Cut-off value was measured by calculation the upper limit of the 99% confidence interval, which calculated by the calculation of the mean of the (OD-values) of standard readings (M) and the stander deviation (SD) and the stander error (SE). Cut-off value = M + 2.57(SD × SE).

Pearson correlation was done to explore possible association between markers involved in the study. (Al-Murrani, 2000) [13].

Slides were examined by light microscope at 40X – magnification power equipped with Image Analysis Computer System, the dark brown (homogenous) staining identified positive labeled cells. A total of 100 cells were counted to determine the percentage of reactivity of each of the tested monoclonal Abs. The percentage of positive cells calculated as following: percentage of positive cells = (No. of positive cells/ total No. of cells × 100%). Four sections per specimen have been examined; the first two sections for CCR3 marker and second two sections for CCR5 marker.

Results:

Table 3 demonstrates the clinical data of the material of the study and of the biopsied lesions.

Table 3. Clinical data of patients

	Total patients	Pre treament	Post treatment	Clinical response (topical steroid)	Clinical Response (topical tacrolimus)
Number	20	20	20	7/10	9/10
Age (year)	6-45 (27.08±11.37)	6-45 (27.08±11.37)	6-45 (27.08±11.37)	--------	--------
Site of biopsy	Arm/ (4) Leg /(10) Back/(2) Foot /(4)	Arm/ (2) Leg /(5) Back/(1) Foot/(2)	Arm /(2) Leg/(5) Back/(1) Foot/(2)	----------	---------

Table 4 shows results of estimation and comparison of eosinophil count in which a highly significant difference recorded among AD group at time of diagnosis when compared with those after treatment and was elevated in most AD patient correlating roughly with the disease severity.

Also there was significant difference between pre-treatment group and post-treatment group to evaluate the disease activity.

Table 4. Result of eosinophil count

Count		Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
Eosinophil count (Percentage in blood)	pre treatment	273.256	120.687	18.405	0.000**
	post treatment	197.674	72.336	11.031	

(No. = 20)// **=statistical highly significant difference (p≤0.001).

The determination of total IgE in the serum was performed by using sandwich ELISA for all subjects and the results in Table 5 show that AD patient's serum contains significant higher level at time of diagnosis when compared with that of post-treatment group. Furthermore, there was highly significant difference between pre-treatment group and post-treatment group.

Table 5. Result of total serum IgE

	Serum IgE		Sig. (1-sided)
	Negative	Positive	
Pre treatment	18	32	0.000
Post treatment	34	9	

Immunohistochemical examination revealed significantly increased immunoreactivity for CCR-5 (Mean value= 49.500±18.922) in lesional epidermis of compared to CCR3 (Mean value= 26.5±7.8) at time of diagnosis. When evaluated separately, CCR-3, showed statistically significant difference ($p \leq 0.05$) in lesional epidermis of pre-treatment group compared to post treatment group but in lesser degree than that of CCR5+ expression (marker of Th1).
For CCR-5, statistically highly significant difference between pre and post-treatment groups ($p \leq 0.001$). (See Table 6 and Figure 1).

Table 6. Mean percentage of immunohistochemical expression of CCR3 and CCR5 pre and post-treatment.

Immunohistochemistry		Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
Immunohistochemical expression of CCR-3	pre treatment	26.500	7.835	2.478	0.007*
	post treatment	17.500	7.906	2.500	
Immunohistochemical expression of CCR-5	pre treatment	49.500	18.922	5.984	0.000**
	post treatment	27.500	16.874	5.336	

*statistical significant difference ($p \leq 0.05$) **statistical highly significant difference ($p \leq 0.001$).

One month after treatment with steroid-tacrolimus therapy, the skin lesions regressed. Table 7 showed that there was no significant difference could be found between the effects of both drugs on expression of CCR-3 and CCR-5 in skin lesions before and after treatment. Also see Figure 1 and 3.

Table 7. Effect of steroid-tacrolimus on expression of CCR-3 and CCR-5 on lesional epidermal cells

Immunohistochemical expression CCR-3	Steroid Tacrolimus	10 10	24.000 29.000	8.216 7.416	3.674 3.317	0.342
Immunohistochemical expression CCR-5	Steroid Tacrolimus	10 10	49.000 50.000	18.507 21.506	8.276 9.618	0.939

As Table 8 and Figure 1 show the expression of CCR-3 and CCR-5 on lesional epidermal cells significantly correlated with each symptoms, which was the sum of three individual skin aspects (itching, skin dryness, skin condition) ($p < 0.05$).

Table 8. Relationships between different parameters and total VAS score; significant correlation, ($p < 0.05$)

		VAS (itching + skin condition)
Immunohistochemical expression CCR-5	pretreatment Post treatment	$p < 0.001$ (**) $p < 0.001$ (**)
Immunohistochemical expression CCR-3	pretreatment	$p < 0.05$ (*)

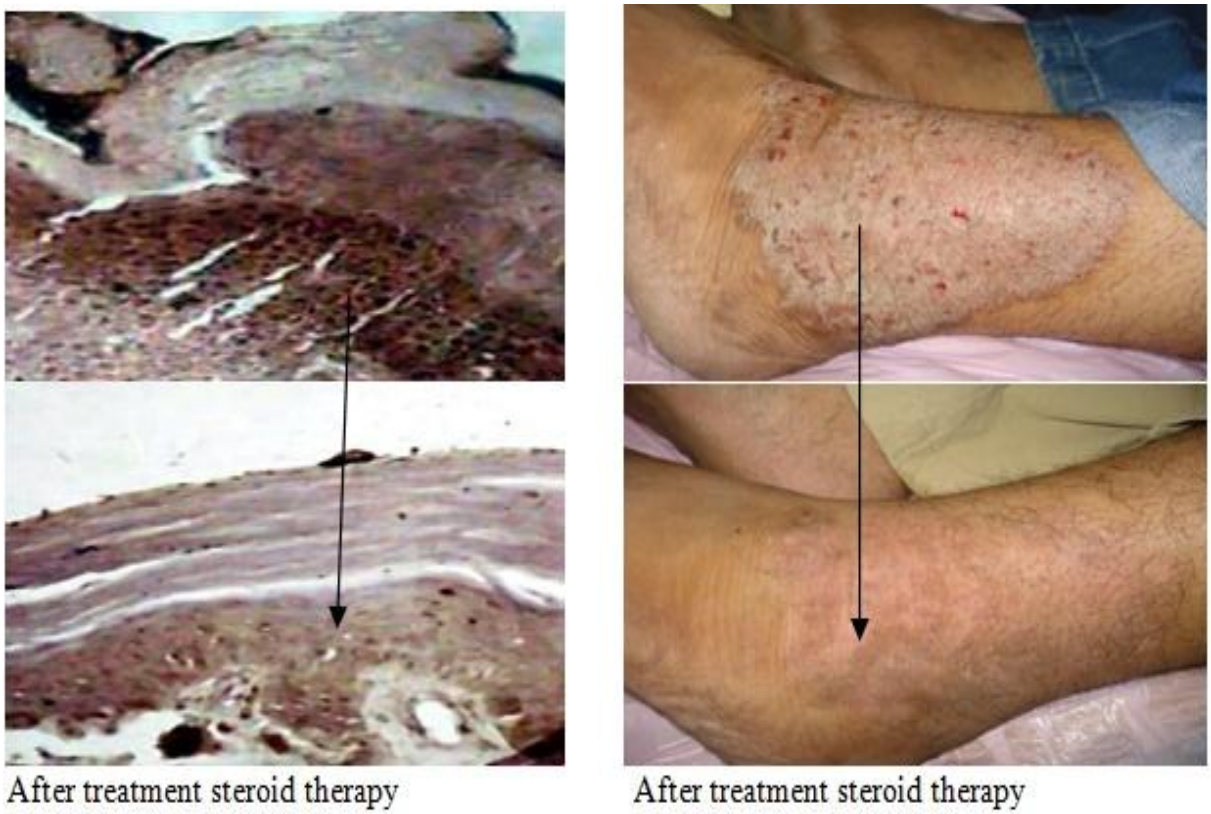

Figure 1. correlation between expression of CCR5 and skin lesion severity

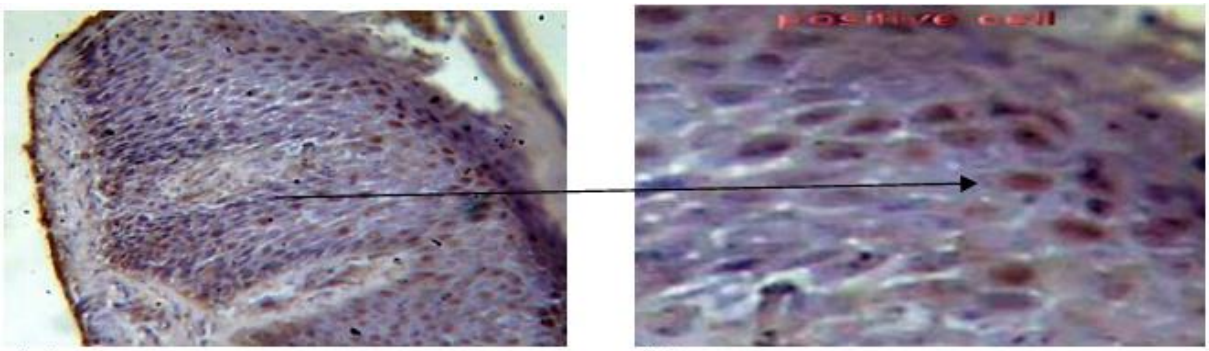

Figure 2A. Immunohistochemical staining low power magnification of 100X, 2B. Immunohistochemical staining: high power magnifications of 400X.

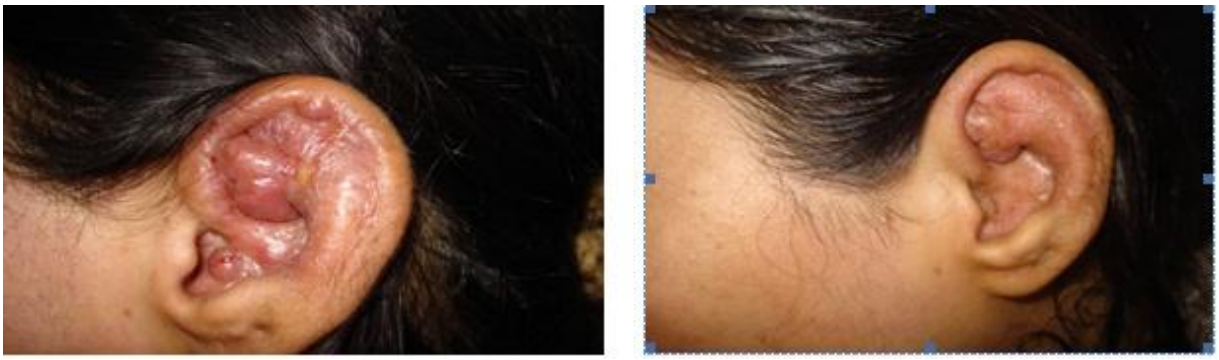

Figure 3. Ear atopic dermatitis after treatment with topical tacrolimus

Discussion

We designed this prospective study to apply this basic immunologic knowledge to confirm previous reported facts but with more practical aims, there has been a paucity of critical prospective studies on AD in which conventional laboratory tests have compared with newer biological markers. The prospective approach is a pre requisite if the prognostic or predictive features of the markers being studied are to be assessed. Also, the clinical indices must be characterized carefully. For this reason we concentrated this study on small but contrasting groups of patients with pronounced differences in the degree of disease activity (the small size of the sample of patients could be criticized). Also, we compared a group of patients at two points of time in order to monitor our putative -markers. However, such an approach will identify only the more robust markers, which in turn may become clinical useful.

In our study, blood eosinophilia is present in most patients with AD correlating roughly with the disease severity, the eosinophil count found to be high at time of diagnosis (mean value= 273.256±120.687) and the count markedly decreased after treatment (mean value= 197.674±72.336) with statistical highly significant difference ($p < 0.001$). Blood eosinophilia was described to be more pronounced in our patients if the AD was associated with respiratory allergic diseases (asthma, allergic sinusitis). As some our patients exhibit normal blood eosinophil counts despite active AD. The determination of eosinophil number in blood is not a reliable tool in establishing the diagnosis of AD but to evaluate the allergic status, this result is in agreement with Breuer, 2001[15].

The total IgE – serum level was found to be positive higher in AD patients at time of diagnosis when compared with post- treatment group. The high frequency of positive results in

pre-treatment group (64%) than in post-treatment group (19%) with statistical high significant difference ($p < 0.001$). The present study confirm a previous one done by Lilic et al 2006 who showed the relevance of total IgE levels are useful for screening for possible allergic disease but failed to establish the place of total IgE as a sensitive test as specific IgE. One of the references quoted (Sinclair and Peters, 2004) [17], although advising that total IgE should be performed as a screening test and is useful in the interpretation of specific IgE tests, because they permit the ascertainment of possible false- negative or false positive results.

The invasion of pathogenic Th2 cells into the skin tissue is critical step in the pathogenesis of acute stage of AD. However, its presently in chronic stage of AD is switching a Th2 cells to Th1 cells, with less significant role of Th2 cells in invasion to skin tissue.

Chemokines such as eotaxin and RANTES are critically involved in the migration of pathogenic T-cells into the skin tissue of AD lesions. However, for human chronic AD, there are very few data on chemokines or chemokine receptors during the course of the disease.

In another T-cell mediated immune disease, psoriasis, Th1-associated chemokine receptors (CCR5 and CXCR3) on peripheral blood lymphocytes or skin tissue have been identified as surrogate markers for the immune activity of the disease. This finding guided us to search for similar phenomena in human chronic AD since surrogate markers for the immune activity are urgently needed in chronic type AD to guide ongoing intervention trial.

These data are readily explained by switching a Th2 cells to Th1 cells with less significant role of Th2 cells. Beside this fact, the increased number of peripheral blood T cells with both T cells type (Th1 and Th2) including increased expression of CCR5+ T cells (Th1) and increased expression (less extent) of CCR3+ T cell (Th2) suggesting a mixed type of immune reaction (type 1 and type 4 allergic reactions) found in chronic AD.

In this study, the relevance of expression of chemokine receptors CCR3 and CCR5 on epidermal cells was investigated in patient with chronic AD and was correlated with disease activity or not.

Our results demonstrated that The percentages of CCR3+ and CCR5+ epidermal cells in patients were significantly higher at time of diagnosis from that post-treatment group patients (CCR3: pre-treatment mean value=26.500±7.835 Vs post-treatment mean value 17.500±7.906, $p \leq 0.05$) whereas high significant difference between two groups of CCR5: pre-treatment mean value=49.500±18.922 Vs post-treatment Mean value 27.5±16.874, $p \leq 0.001$) and was correlated positively with the total serum IgE, eosinophil number and ruption score. These results in agreement with Okazaki, 2002 [18].

In this study, to investigate the effects of tacrolimus on the expression of chemokine receptors (CCR3 and CCR5) in patients with chronic AD.

We found a striking reduction of the Th1-associated chemokine receptors (CCR5) and Th-2 associated chemokine receptors (CCR3) on lesional skin tissue after steroid-tacrolimus treatment from that at the time of diagnosis.

However presence the Th-1 and Th-2 chemokine receptors in the chronic AD which is the poorly defined nature of immune reaction in AD and which does not exactly confirm to one of the well known classic types of immune reaction (type 1, type 4 or mixed). Moreover, certain difficulties are traditionally encountered in the interpretation of any findings related to AD research.

In our study, we used the new immune-modulator topical drug (tacrolimus ointment) at the first time in Iraq in treatment of atopic dermatitis and compared with the old traditional topical steroids therapy for AD.

In our study, we investigated the effects of tacrolimus ointment on chronic AD lesions and compared with effects of topical steroids (Betamethasone valerate) clinically and immunlogically.

Immunologically; our study showed that tacrolimus and topical steroid reduced the expression of chemokine receptors (CCR3, CCR5) on epidermal keratinocytes, nearly in equal percentages and This result is in agreement with Shozo Sakuma, 2001 [19].

Tacrolimus ointment unlike some topical corticosteroids does not cause a decrease in collagen synthesis or skin thickness, nor does it produce skin abnormalities or de-pigmentation. So significant improvements in AD were observed in majority of patients treated with tacrolimus ointment and associated with few side effects unlike topical steroids (Bokersky and Fitzsimmons, 2001) [20].

In this our 2 phase study, clinically, topical steroids treated 10/20 patients showed greater improvement in AD signs and symptoms thereafter, tacrolimus ointment treated 10/20 patients showed improvement in AD signs and symptoms, there were no differences in clinical and immunological events between topical steroids and tacrolimus ointment but significantly longer time to first relapse and significantly fewer disease relapse days.

So our interesting treatment option which goes with Ehrchen, 2008 [21], for patients with stabilized moderate to severe atopic dermatitis, long term intermittent application of tacrolimus ointment to change the skin lesion to normal appearing skin and significantly more effective than steroid at maintaining disease stabilization, with safety profile and very few side effects similar to vehicle.

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Evaluation of Thyroid Function in Patients with Chronic Kidney Disease

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Abstract

Background Thyroid function has been extensively evaluated in patients with chronic kidney disease, however the results are variable.

Objective The study was designed to investigate the thyroid dysfunction in uremic patients clinically and biochemically.

Methods The study was conducted in the department of medicine and dialysis unit in AL-Kadhimiya Teaching Hospital in Baghdad. Three groups were taken sixteen patients with end stage renal disease undergoing regular hemodialysis, 22 patients with chronic renal failure treated conservatively and 21 healthy volunteers with no previous history of thyroid disease and their renal function were normal(control group), serum TT3, TT4 and TSH were estimated in all patients and control group by RIA Kits. The results were tabulated and statistically analyzed using Chisequare and t-test.

Results Fifty nine persons included in this study divided into three groups (regular hemodialysis 16, conservative treatment 22 and the control group were 21). Goiter was demonstrated in 12.5% in hemodialysis group, 4.54% of the conservatively treated group. Uremic patients kept on conservative treatment or on regular hemodialysis showed significant reduction of TT3 and TT4 in comparison to the control group, however the level of TSH didn't show significant alterations, and there were no significant deference in TT3 andTT4 between the patients on conservative management and those maintained on regular hemodialysis.

Conclusions Low TT3 and TT4 are often observed in clinically euthyroid patients with chronic renal failure. These abnormalities do not appear to change significantly after the institution of regular dialysis, on other hand TSH values in clinically euothyroid patients with chronic renal failure were within the normal range, this normal TSH may indicate functional euthyroid status.

Key words Hemodialysis, Chronic renal disease, Triiodothyronin (T3), Thyroxin (T4), Thyroid stimulating hormone (TSH)

Introduction

The kidney normally plays an important role in the metabolism, degradation, and excretion of several thyroid hormones. It is not surprising therefore that impairment in kidney function leads to disturbed thyroid physiology, all levels of the hypothalamic-pituitary-thyroid axis may be involved, including alterations in hormone production, distribution, and excretion, epidemiologic data suggests that predialysis patients with chronic kidney disease have an increased risk of hypothyroidism; many cases are sub clinical [1,2].

Thyroid function has been extensively evaluated in patients with chronic kidney disease, however the result are variable, an increased incidence of goiter in those patients has been reported in studies conducted in China and Turkey, while other centers such as United States, Canada, Great Britain and Australia found the reverse [3-5].

Primary hyperthyroidism is extremely rare, while the prevalence of hypothyroidism is increased in patients with chronic renal failure [6-9].

Some manifestations of hypothyroidism such as pallor, hypothermia and asthenia may also occur in uremia, the exclusion of diagnosis of hypothyroidism on clinical grounds may be extremely difficult, it's basically based on biochemical tests [10].

Most studies of thyroid hormones in clinically euthyroid patients with varying grades of chronic renal failure showed significant decrease in TT3, TT4 and FT3 levels compared with control [11-14]. A low T3 and T4 syndrome is evident when glomerular filtration rate (GFR) is reduced below 30±16 ml/min [15]. Usually there is more distinct suppression of T3 than of T4 [16]. The concentrations of reverse T3 (rT3), the inactive metabolite of T4 in plasma are usually low but normal or even elevated values have been reported by some authors [16,17]. Serum T3 in transplanted patients seems to be higher than control group found in some series [18].

Thyroid binding globulin (TBG) concentrations are usually normal in hemodialysis patients and low or normal in patients underwent continuous ambulatory peritoneal dialysis [19, 20]. TBG levels increased significantly after renal transplantation [21].

Studies of thyroid hormone kinetics revealed normal production rates of thyroid hormone, metabolic clearance rates of the hormone may or may not be increased in patients with end stage renal disease [22-25]. Peripheral deiodination of T4 to T3 is impaired [26], this finding is consistent with the more pronounced decrease of T3 than of T4 in progressive renal failure, and instead there is preferential diversion to inactive metabolites [25].

In contrast to other non – thyroidal illness, rT3 production and metabolic clearance rate are normal, and there is increase extra vascular binding of the hormone, resulting in low normal values [27]. A lot of the studies confirmed that pituitary-thyroid axis is abnormal in uremic patients depending on the observation of the normal thyroid-stimulating hormone concentration despite low TT3 and TT4 [28], and an abnormal response of thyroid stimulating hormone after administration of exogenous thyrotrophin releasing hormone [29-31].

The changes in the temporal organization TSH release in patient with uremia [32,33], in spite of this there is a study which suggested maintenance of pituitary thyroid axis [34].

Methods

The study was conducted in the department of medicine and dialysis unit in Al-Kadhimiya Teaching Hospital in Baghdad. Thirty-eight patients with no previous history of thyroid dysfunction and with varying grades of chronic renal failure were included in this study.

Twenty-two patients were on conservative treatment. Remaining sixteen patients who also had severed renal failure were on regular hemodialysis treatment (RDT). Twenty healthy volunteers with normal renal function and no previous history of thyroid dysfunction were included in this study as a control group.

All patents and control were assessed for possible thyroid dysfunction depending on clinical bases and physical examination

We emphasized on the presence of the following points in the history:

A. A family history of goiter or altered thyroid function.

B. A personal and family history of other organ-specific autoimmune diseases particularly of insulin-dependent diabetes, pernicious anemia, vitiligo, and myasthenia gravis.

C. History of intake of iodine-containing medications, such as amiodarone, lithium carbonate.

D. History of thyroid surgery or radioactive iodine intake.

While physical examination focused on the presence of:

1. Goiter±bruit.
2. Eye signs (exophthalmos, lid retraction….. etc).
3. Pretibial myxedema.
4. Delayed reflexes.

Pallor, weight loss, palpitation, tremor, neurological symptoms and other

manifestations of thyroid dysfunction may also occur in uremia, so they are not regarded as signs of possible thyroid dysfunction in the study group.

All those included in the study underwent estimations of serum total triodothyronine (TT3) and serum total thyroxin(TT4) ,serum thyrotrophin (TSH), and were performed by (T3 [1251] RIA kit REF: RK-6CTI), (T4 [1251] RIA kit REF: RK-5 CTI), (Turbo TSH [1251] IRMA kit REF: RK-ICTI) respectively; these systems provide direct quantitative in vitro administration of L-3,5,3- triodothyronine (T3), thyroxin (T4) and (TSH) human thyroid stimulating hormone in human serum. Patients who were on regular hemodialysis, sample of blood were taken before starting hemodialysis sessions to avoid art factual results caused by heparin.

We made sure that all patients and control group did not receive furosemide before taking blood samples as it is known to influence thyroid function

General information regarding age and the sex of the patients and the control group and duration of renal failure, type of dialysis and associated diseases for the patients, and result of basic laboratory investigations which include hemoglobin level, packed cell volume, blood urea, serum creatinin were integrated in this study

Results of clinical and hormonal assessment of thyroid dysfunction obtained in patients with chronic renal failure were compared with those of the control group by statistical analysis using Chi-square test and t-test, p value < 0.005 considered significant

Results

The total population included (59) patients, (16) 0n regular haemodialysis and (22) on conservative treatment involving peritoneal dialysis and (21) healthy volunteers as control group.

There were (38) men and (21) women, the mean age was (40) years (range 21-65 years).

All those on haemodialysis were hypertensive and one has diabetes mellitus while those on conservative treatment (27.25%) were diabetes and about (77.27%) were hypertensive. Age, sex distribution and other parameters such as: associated diseases (hypertension and diabetes mellitus) and duration of renal failure for the patients and control groups are displayed in table 1.

The result of basic laboratory investigations for the patients and control groups are shown in table 2, both groups of patients showed anemia with more or less similar levels of PCV in contrast with those from the control group who have PCV level within normal range which obviously caused by uremia, on the other hand there was mild improvement in renal function tests among H.D group in comparison with the conservatively treated group.

Regarding clinical assessment of thyroid dysfunction for the patients and the control group (Table 3):

A. No clinical evidence of possible thyroid dysfunction was detected in about (81.25%) of the hemodialysis group, (81.82%) of the conservative treated group and (85.72%) of the control group.

B. The presence of goiter was demonstrated in (12.5%) of the hemodialysis group, (4.54%) of the conservative treated group and it was not demonstrated in the control group.

C. Family history of insulin dependent diabetes mellitus was seen in (6.25%) of the hemodialysis group, (9.09%) of the conservatively treated group and (9.52%) of the control group.

D. A family history of goiter or altered thyroid function was found in (4.54%) of the conservatively treated group and (4.76%) of the control group and no such history was noticed among the hemodialysis group.

All patients on conservative treatment and on regular hemodialysis showed significant reduction in their TT3 and TT4 in comparison with those in control subject (Tables 4 and 5). However TSH level did not show significant alterations (Table 6).

There were no significant differences in TT3 and TT4 between the patients on conservative management and those on hemodialysis, it was found that (68.75%) and (62.25%) of the H.D. patients have TT3 and TT4 levels below normal range respectively, while (54.55%) and (72.72%) of the conservatively treated group have TT3 and TT4 levels below normal range respectively as shown in Table 4 and Table 5. The mean values of TT3, TT4 and TSH were illustrated in Table 6.

Table 1. The demographic features of uremic and control groups

Parameters		Hemodialysis group No. 16	Conservative group No. 22	Control group No. 21
Age range (years) Age mean		21-60 36.2	24-65 42.6	26-50 39.5
Sex	Male	9 (56.25%)	12 (54.5%)	17 (80.95%)
	Female	7 (43.75%)	10 (45.5%)	4 (19.05%)
Hypertension	Yes	16 (100%)	17 (77.2%)	2 (95%)
	No	0	5 (22.72)	19 (90.5)
Diabetes Mellitus	Yes	0	6 (27.27%)	0
	No	16 (100%)	16 (72.72%)	21(100%)
D.O.U range D.O.U mean		(1-3.5) years 2.6 years	(0.34-3) years 1.2 years	0 0

D.O.U = Duration of uremia,

Table 2. The results of the laboratory investigations for the patients and control groups

Investigations		HD patients	Patients on conservative treatment	Control groups	*p* value 1	*p* value 2	Normal values
PCV	Male	31.4±2.1	29.6±2.4	47±3.6	< 0.05	< 0.05	40-54
	Female	30.6±2.9	31.7±2.7	41.4±2.9	< 0.05	< 0.05	37-47
Blood urea mmol/L		31.2±5.7	38.6±6.1	3.5±2.3	< 0.05	< 0.05	2.5-6
Serum creatinine µmmol/L		467±86	531±93	86±30	< 0.05	< 0.05	55-120

PCV = Packed cell volume, HD = Hemodialysis

Table 3. The patients and control groups distributed according to the presence of clinical evidence of possible thyroid dysfunction by history taking and physical examination

Groups	Hemodialysis group	Conservatively treated group	Control group
A	0	1 (4.54)	1 (4.76)
B	1 (6.25)	2 (9.09)	2 (9.52)
C	0	0	0
D	0	0	0
E	2 (12.5)	1 (4.54)	0
F	0	0	0
G	0	0	0
H	0	0	0
Total	3 (18.75)	4 (18.18)	3 (14.28)

A = Family history of goiter or altered thyroid function, B =A personal or family history of other organ specific autoimmune disease, C = History of intake of iodine- containing medications, D = History of thyroid surgery or radioactive iodine intake, E = Goiter± bruit, F = Eye signs, G = peritibial myxedema, H = delayed reflexes

Table 4. Number and percentage of uremic patients and control groups distributed according to the (TT3) level

TT3 level (nmol/L)	Hemodialysis group	Conservatively treated group	Control group
TT3 above normal (> 3.3)	0	0	0
TT3 upper normal (1-2.9)	0	0	2 (9.5%)
TT3 mid normal (1.7-2.49)	0	1 (4.55%)	18 (85.75%)*
TT3 lower normal (1-1.69)	5 (31.25%)*	1 (4.55%)	1 (4.75%)
TT3 below normal (<1)	11 (68.75%)	12 (54.55%)	0

*= statistically significant *p* value < 0.05 using chi- square test

Table 5. Number and percentage of the patients and control group according to the (TT4) level

TT4 level (nmol/L)	Hemodialysis group	Conservatively treated group	Control group
TT4 above normal (0-150)	0	0	0
TT4 upper normal (125-155)	0	0	1 (4.75%)
TT4 mid normal (95-124.9)	0	1 (4.54%)	19 (90.5%)*
TT4 lower normal (65-94.9)	6 (37.75%)	5 (22.72%)	1 (4.75%)
TT4 below normal (<65)	10 (62.25%)	16 (72.72%)*	0

*= statistically significant *p* value < 0.05 using chi- square test

Table 6. TT3, TT4 and TSH mean values for the patients and control groups

Hormones (nmol/L)	Hemodialysis patients	Conservatively treated group	Control group	*p* value 1	*p* value 2	Normal values
TT3	0.69± 0.26	O.78±0.21	2.1±025	< 0.05	< 0.05	1.0-3.3
TT4	48±13	40±11	110±22	< 0.05	< 0.05	65-155
TSH	2.55±0.37	2.23±0.40	2.30±0.45	> 0.05	> 0.05	0.3-3.75

Discussion

There is no significant difference between uremic patients, whether they were kept on hemodialysis or conservative treatment compared with control group in regard to the presence of possible thyroid dysfunction depending on clinical criteria employed in this study apart from the presence of goiter in 12.5% of the hemodialysis group and 4.54% of the conservatively treated group as compared to the absence of such findings among the control group.

These results comply with those found in United States, Canada, Great Britain and Austria which found no significant increase in the incidence of goiter among uremic patients in comparison with general populations, but contradict with the results obtained in republic of China and Turkey which showed significant increase in the incidence of goiter among the uremic patients [3-5], this deference in results might be attributed to the geographical variation in the incidence of thyroid dysfunction, method used in detection of goiter and the size of sample under study.

There is significant reduction in TT3 and TT4 levels in patients with uremia regardless the mode of therapy in comparison with those of control group , this findings was similar to most of the results of investigators who have studied thyroid hormones level in clinically euthyroid patients with varying grades of chronic renal failure [11-14] this reduction in thyroid hormones may be due to the effect of chronic renal failure on the thyroid hormones which include altered peripheral metabolism

like impairment of peripheral deiodInation of T4 which is the main source of T3 resulting in low TT3, possible lowering of thyroxin-binding globulin and possible decrease in the excretion of thyroxin binding to thyroid binding proteins both lead to low TT4 ,on other hand the result were different from those of Lim et al [26], Ramirez et al [34] who found TT4 and TT3 was normal in chronic renal failure. Several factors might be responsible for obtaining controversial results of thyroid hormone levels in chronic renal failure. The most important factors among them are heterogeneity of patient group studied, methodological variations and varying treatment.

In this study it was found that TSH levels didn't show significant alterations between the uremic patients and the control group and they were within the normal range. This result was similar to the most of studies that focused on thyroid function in end stage renal disease [11-14]. The normal TSH level observed in this study may reflect the biochemical euthyroid state of the patients which was also noticed by clinical assessment in this study or maybe due to multiple defect in all levels of the hypothalamic-pituitary-thyroidal peripheral axis in uremic patients which were reported in many previous studies abroad [4,28,29.30, 31,32, 33,35].

It is found that significant deference in TT3 and TT4 between the patients on conservative management and those on hemodialysis these findings are similar to the study done by Pagliacci et al [19] and differ from Verger et al [36] who noticed that slight or no decrease of thyroid hormone in patients with peritoneal dialysis in contrast to hemodialysed patients, this variation with current study might be due to the different definition of conservative therapy (in this study conservative therapy refers to dietary and pharmacological treatment plus peritoneal dialysis as required) while that of the studies done abroad, conservative therapy comprises mainly continuous ambulatory peritoneal dialysis plus the dietary and pharmacological intervention.

In conclusion the abnormalities in thyroid function tests, including low TT3 and TT4 values are often observed in clinically euthyroid patients with chronic renal failure, these abnormalities do not appear to change significantly after the institution of regular dialysis.

On the other hand TSH values in clinically euthyroid patients with chronic renal failure were within the normal range, this normal TSH may indicate functional euthyroid status.

Its recommend that further studies concentrating on improving clinical and biochemical criteria to diagnose thyroid dysfunction in uremic patients are needed.

It is also important to answer the question of what is the effect of thyroxin replacement in uremic patients and diagnosed as to have hypothyroidism.

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Comparative Study of Enzyme Linked Immunosorbant Assay and Agglutination Tests in the Diagnosis of Human Brucellosis in Baghdad

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Abstract

Background Human infection with Brucella spp. had been able to evoke humeral immune response containing both IgG and IgM.

Objective This study designed to compare results obtained from Rose Bengal Test (RBT), Tube Agglutination Test (TAT) and Enzyme Linked Immunosorbent Assay (ELISA) employ serum are described and compared for the detection of human IgG and IgM anti-brucella antibodies.

Methods Serum samples from 105 subjects were collected. 90 were clinically infected with human brucellosis, and 15 were age and gender matched controls. RBT and TAT are the two screening tests routinely recognized, while the 2-mercaptoethanol test (2ME) is the confirmatory assays currently in use. In order to improve the serological diagnosis of human brucellosis, an indirect IgG, IgM and IgG-IgM ELISA kits were evaluated

Results Totally, 90 cases were positive in RBT, from those only 92% shows positive TAT, and by ELISA there are IgG (27.78%), IgM (14.44%) and (57.78%) were Positive for both immunoglobulins.

Conclusion Although RBT and TAT are widely applied tests, they cannot differentiate acute and chronic states of brucellosis. Our data suggest that IgM ELISA may be a suitable test for diagnosis acute brucellosis.

Keywords Brucellosis, ELISA, Tube Agglutination.

Introduction

Brucellosis may present clinically as acute, as chronic after an acute attack, or as chronic and of insidious onset [(1)]. The serological results may differ, depending on the clinical form and stage of the infection [(2)].

Diagnosis is occasionally confounded because of non-specific clinical manifestations, and is confirmed only if brucella species are recovered from blood, bone marrow or other sites [(3-5)]. Brucellosis is usually associated with an intense humoral response [(6,7)]. Immunoglobulin M (IgM) brucella antibody predominates for the first week of the acute infection, after which the IgG antibody level starts to increase, reaches a peak after a few weeks, and predominates over the IgM antibody level until adequate therapy eliminates the infection [(8)]. Isolation of the microorganism is possible only in a minority of the infected patients in the acute phase of the disease [(9)]. Therefore, in the absence of bacteriological confirmation, a presumptive diagnosis can be made on the basis of a single high rising titer of specific antibodies [(8, 10)].

The routine brucella agglutination test is the most frequently used type of serological test for this purpose; however, it does not differentiate between active and inactive disease because it does not differentiate between IgG and IgM agglutinins [(2)]. Among a variety of serological tests, TAT (Tube Agglutination Test) is the most widely used [(17)]. Evaluation of various ELISA assays for IgG and IgM have shown that these techniques are generally more sensitive and specific than conventional tests, while they are able to distinguish specific antibodies of IgM and IgG classes associated with acute and chronic brucellosis [(11)]. The obtained results are always easily interpreted, since they are specific for single immunoglobulin classes [(1)]. On the other hand, they are not routinely available in developing countries, especially in rural areas [(12)]. Thus, this study designed to compare and evaluate the results obtained from different serological procedures for humoral immune response in human brucellosis.

Methods

Subjects

This study included (90) patients complaining of symptoms and signs commonly associated with Brucella infections. All patients were outpatient visitor to the private clinic in Baghdad during March 2008- March 2009; clinical details at presentation were records. All of the patients living in Abu-Ghraib city, an area in which brucellosis is endemic, many of the inhabitants are farmers raising livestock. They were (50) females and (40) male age range from (4-63) years, sera were collected at the first visit and stored in aliquots at -20 °C till analyzed.

Fifteen apparently healthy individuals were age and sex matched with no history of Brucella infection and Rose –Bengal negative test were selected as control group.

Rose Bengal test (RBT)

A drop (30µ) of undiluted serum was placed on circle of the slid, and then adds the same volume of antigen to the drop of the serum, both drops were mixed and the slide was observed for any agglutination within four minutes would indicate the presence of specific of anti-Brucella antibodies.

Tube Agglutination Test (TAT)

Test was performed according to Hausler and Koontz(13) using a standardized suspension of Brucella organisms prepared from *B. abortus*, the 2ME test was performed identically except that 2ME was added to each test tube to a final concentration of 0.05 M, and 0.85% saline was used to dilute the antigen rather than 0.85% saline containing 0.5% phenol. (13-15).

- A 0.9-ml volume of 0.85% saline (containing 0.1 M 2ME for the 2ME test, or 0.5% phenol for the TAT test) was added to a test tube, and 0.5 ml of the same solution was added to four more tubes. Also, 0.5 ml of the same solution was added to an antigen control tube, and 0.75 ml was added to a reading standard tube.
- Two fold serum dilutions beginning with 1:80 were formed by adding 0.1 ml of serum to tube 1, followed by sequential mixing and removal of 0.5 ml of the mixture to the subsequent tube and 0.5 ml of final tube was discarded, The antigen suspension was added in to tubes 1 through 5 and to the antigen standard tube, and 0.25 ml of this suspension was added to the reading standard tube. The reading standard tube was used to simulate 50% clearing of the antigen suspension after the agglutination reaction.
- The final serum dilutions in tubes 1 to 5 were 1:80 to 1:1280, the rack containing the test tubes was shaken 10 times to mix the antigen suspension and serum and incubated at 37 °C for 20 minute, each tube was examined without mixing or centrifugation for agglutination.

Enzyme linked immunosorbent assay (ELISA)

The test kit is provided by (Biocheck®), diluted patients serum,control and calibrater is added to the purified Brucella antigen coated on the surface of microwells, plate incubated for 1hr.at 37°C then the excess non reacted sera removed through three cycle of washing with washing buffer.

While the reacted sera detected by adding 100µ of diluted Horse radish peroxidase (HRP) diluted conjugated secondary antibody, the conjugate used for two ELISA plates were (HRP-anti human IgG for detection of human IgG and HRP-anti human IgM for detection of human IgM), after incubation for 30 min. plate washed with washing buffer and 100µ of substrate Tetramethylbenzidine (TMB) reagent added then incubate in dark place for 30 min at 37°C, reaction will be stopped by addition 100µ stopping solution(H_2SO_4) and read at 450nm. All results above the cut-off value (10 IU/Ml) considers positive.

Statistical analysis

All data were presented as frequency and percentage of categorical data and Chi-square test used for comparison of the results. Cut-off value was measured by calculation the upper limit of the 99% confidence interval, which calculated by the calculation of the mean of the (OD-values) of controls group readings (M) and the standard deviation (SD) and the standard error (SE) [16]. Cut-off value = M + 2.57(SD × SE).

Results

Comparative results of three different methods were used in the diagnosis of human brucellosis. Rose Bengal test considered as a gold standard agglutination test in the diagnosis of human brucellosis, the results showed that 90 sera from the patients with brucellosis showed a positive and 15 negative.

Also all samples were tested by Tube agglutination Test (TAT), A good correlation when Rose Bengal test were compared with the Tube agglutination Test, there were 83 (92%) positive sera and only 7 (8%) considered negative when Ab titer ≤80. All RBT negative sera were also negative for TAT as in table 1.

Table 1. Comparative result between positive and negative Rose Bengal Test with Tube Agglutination Test results

Rose Bengal Positive (90)			Rose Bengal Negative (15)		
TAT Negative 7 (8%)			TAT Negative 15 (100%)		
Titer	No.	Percentage	Titer	No.	Percentage
No agglutination 1:80	3 4	43% 57%	No agglutination	15	100%
Total	7	100%	Total	15	100%
TAT Positive 83 (92%)			TAT Positive 0 (0%)		
1:160 1:320 1:640 1:1280	17 32 23 11	20% 39% 28% 13%			
Total	83	100%	0	0	(0%)

With any positive result for RBT and TAT, 2-mercaptoethanol is performed and the results showed that only (16 patients were positive) and the 74 were negative, this negative results was also seen in 15 negative sera.

For IgG and/or IgM, as tested by ELISA, showed positive results in accordance with the other assays. For RBT positive sera, IgG ELISA (27.78%) showed positive results, IgM (14.44%) and (57.78%) were Positive for both immunoglobulins. While, sera from RBT negative always gave negative results for IgM and only 2 sera were positive for IgG as in table 2.

Table 2. Comparative result of ELISA test results in positive Rose Bengal Test cases.

Titer	Positive RBT cases (90)		
	IgG	IgM	Both (IgG and IgM)
1:16	4 (16%)	3 (23%)	2 (4%)
1:32	5 (20%)	1 (8%)	7 (13%)
1:64	11 (44%)	5 (38%)	24 (46%)
1:128	4 (16%)	4 (31%)	13 (25%)
1:256	1 (4%)	-	6 (11%)
Total	25 (100%)	13 (100%)	52 (100%)

The results of ELISA IgG and IgM showed disagreement with Rose Bengal test 58.26% and 66.96%respectively, while, ELISA results for both IgG and IgM Showed agreement with RBT results (58.26%) see table 4, thus it is becomes clear that both immunoglobulins gives rising titer when they measured by ELISA test and 100% of them were RBT positive (in acute brucellosis) with 100% sensitivity as seen in table 3.

Table 3. The sensitivity, specificity of TAT and ELISA compared with Latex

Test	Latex	
	Sensitivity	Specificity
TAT	100%	100%
ELISA IgG	93%	87%
ELISA IgM	100%	100%
ELISA both	100%	100%

Table 4. Agreement and disagreement between Rose Bengal test and ELISA.

Test	Result	RBT			Agreement	Disagreement
		Positive	Negative	Total		
ELISA IgG	Positive	25	2	27	33.04%	58.26%
	Negative	65	13	78		
	Total	90	15	105		
ELISA IgM	Positive	13	0	13	24.35%	66.96%
	Negative	77	15	92		
	Total	90	15	105		
ELISA both	Positive	52	0	52	58.26%	33.04%
	Negative	38	15	53		
	Total	90	15	105		

Discussion

In the absence of a positive culture, the diagnosis of brucellosis rests on the demonstration of specific antibodies. A variety of serologic tests have been applied to brucellosis, of which TAT is the most widely used [17]. In the TAT test a single serum titer of 1/160 or greater is considered significant [18]. However, early in infection lower titers may be present; therefore, it is important to obtain both acute and convalescent-phase sera. Sometimes agglutination can be masked at low dilutions of serum. If the diagnosis of brucellosis cannot be achieved by TAT because of the low titer of antibodies and the presence of blocking antibodies, Brucella IgG-specific and IgM-specific ELISA test systems have been shown to be an acceptable alternative to TAT for the diagnosis of subacute and chronic brucellosis. The detection of specific immunoglobulins by a single, simple and rapid test is a major advantage of ELISA [19].

Kostoula et al reported that ELISA appears to be more sensitive than the tube agglutination test for the diagnosis of human brucellosis, because this method detects specific IgG, IgM and IgA antibodies [20]. Gazapo et al stated that ELISA IgG and IgM positivity are helpful for epidemiological evaluations, whereas some false positive results can be obtained in classical tube agglutination tests due to the cross reactivity between Brucella spp. and Salmonella spp., Vibrio cholera and Yersinia bacteria [19]. As is well known, incomplete antibodies are commonly seen in subacute and chronic brucellosis, and so ELISA is recommended by some authors as a susceptible test for the diagnosis of such cases and it was asserted that ELISA could detect incomplete antibodies [21,22].

In the present study, ELISA was demonstrated to be more accurate when compared with TAT, indeed, 2 from 15 cases that were negative with TAT were revealed to be positive with ELISA for IgG, however, For RBT positive sera, IgG ELISA (27.78%) showed positive results, IgM (14.44%) and (57.78%) were Positive for both immunoglobulins., therefore, we concluded that it is advisable to perform both IgG and IgM ELISA technique in order to achieve higher accuracy.

The results allow us to conclude that both agglutination based tests we tested are useful as screening tests for the diagnosis of human brucellosis. In addition, ELISA is more reliable than conventional methods because of the sensitivity and the possibility of distinguishing between specific IgG and IgM immunoglobulins.

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Changing Pattern and Incidence of Gallstone Diseases in Al–Kadhymia Teaching Hospital

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Abstract

Background Gallstone diseases remain a common health problem for human, affecting millions of people throughout the world. In Iraq, recent years has shown an increasing number of patients with gallstones with concurrent decreasing age of presentation, risk factors have been assessed taking in consideration the effect of stressful life events that Iraqi peoples had been suffered during these years on the development of gallstone diseases.

Objective To give an idea about, and possible causes for the changing in number, age of presentation and trend of surgical management of gallstone diseases in Iraq.

Methods Clinical assessment and risk factors assay including stressful life events were done for 1226 patients who have been cholecystectomised over seven years from Jun 2002 to Jun 2009 in Al-kadhymia Teaching Hospital.

Results Significant increases in the number of symptomatic gallstone cases were noted, nearly 50% of them presented with abdominal pain, more than half of patients were young in the 3rd and 4th decades, female to male ratio was 5.9:1 and decreasing with age, 74% of patients had BMI > 25% (over weight), impact of stressful life event was obvious in the recent years on nearly two third of patients with gallstone diseases, minimal invasive technique (laparoscopic surgery) was evolving and most of the cases now done by this method.

Conclusions Gallstones diseases are increasing in our country with obvious decrease in the age of presentation, this might be due to stressful life events to which Iraqi peoples had been exposed, and also the revolution of minimal invasive surgery had a great impact in the management of this disease.

Key words Pattern, Incidence, Gallstone diseases.

Introduction

Gall stones are the most common biliary pathology, 10 to 15% of adult population in the USA had gallstones [1], while as high as 60% of pima Indians over 35 years of age [2] and as low as in Japanese women 3.2 % [3], no accurate record of the incidence or prevalence of gall stones in Arab countries [4],for a long time this disease was known to be of fatty fertile fifty female [5], these facts need to be reviewed, there has been a significant increase in the incidence of gallstones in patient under 30s of age in the past 10 years [6] also there is a change toward asthenic female [7].

There was a change in the management approach toward minimal invasive surgery as first option [8]. In this study we tried to evaluate the effect of risk factors on these changes, especially the effect of stressful life events in Iraq in the recent previous war years in which Iraqi peoples had been exposed to different harmful

psychological events that have their obvious effect on many serious illnesses including gallstones, thyroid, and various malignant diseases in Iraq [9].

Methods

A prospective study conducted in Al Kadhymia Teaching Hospital in the period from June 2002 to June 2009, during these years 1226 patients to whom cholecystectomy had been done (open or laparoscopic surgery) for gall stone diseases, other indications have been excluded from the study like trauma and a calculus cholecystitis.

After detailed history and thorough clinical examination, the diagnosis was confirmed by ultrasound examination, special attention was given to risk factors; age, sex, fertility, weight (represented as body mass index, BMI), family history and history of exposure to stressful life events like loss of close relatives, loss of job, loss of house due to sectarian migration and poverty, especially that what happened during and after year 2003 (**guerrilla warfare**). Analysis of these risk factors and their significant effects had been done.

Results

A total number of 1226 patients who had gall stones, over these years a significant increase in the number of admission for patients with gallstone diseases in relation to total admission to the surgical ward e.g. in 2002 it was 7% increased steadily to 16% of the total admission in 2009, the highest incidence noticed in years 2008-2009 about 25% of all cases included in the study, the least number of elective operation is that of year 2003-2004 about 6.5% of all cases as shown in table 1 and figure 1.

Clinically the majority of the patients (43.8%) presented with abdominal pain either epigastric or right hypochodrial, other presentation like non specific dyspeptic symptom, indigestion, heart burn or complication in which acute cholecystits is the commonest (19.4%), incidental finding was in (4.8%) and carcinoma of the gall bladder was the least complication (1.01%) as shown in table 2.

Regarding risk factors

Age range was 17-75 years with a mean age of 45.3 years; the majorities were in the 3rd and 4th decades as shown in table 3.

Sex; the majority of patients were females 1049 compared to 177 males with over all females to males ratio (5.9:1), this ratio decreased with age (2.2:1 for those aged > 50) as number of male patients increases with age, see figure 2.

Fertility: Reproductive data shows that 963 females were fertile ($p < 0.001$) were as only 86 were unmarried or infertile.

Obesity; the relation of obesity, BMI with gallstone formation is compared in table 4, 74% of the overall patients were overweight (BMI > 25), 47% were obese (BMI > 30%), 4.4% were morbidly obese (BMI > 40%) as shown in table (4).

Family history of gall stones diseases (first degree relatives) found in 32% of female patients ($p < 0.01$), while male patients only 5.3%.

Life stress; Definite history of stressful life events was linked to 21% of those patients in the years 2002 to 2004 while 63% of those in years following the war ($p < 0.001$).

Surgical approaches; Minimal invasive surgery played a major role in the management of patient in the last years of the study, while majority of cases in the early years of the study were operated upon by the usual open cholecystectomy as shown in table 5.

Table 1. Distribution of patients by sex and age over the years of study

June to June	No. & % both sex	No. & % females	No. & % males	No. patient aged < 40 yr	No. patient aged > 40 yr
2002-03	101 (8.2%)	83 (6.7%)	18(1.5%)	40	61
2003-04	80 (6.5%)	72 (5.8%)	8(0.6%)	21	59
2004-05	120 (9.7%)	104 (8.4%)	16(1.3%)	63	57
2005-06	175 (14.3%)	153 (12.5%)	22(1.8%)	94	81
2006-07	215 (17.5%)	181 (14.7%)	34(2.8%)	120	95
2007-08	230 (18.8%)	193 (15.7%)	37(3.0%)	145	85
2008-09	305 (25%)	263 (21.4%)	42(3.4%)	242	63
Total	1226 (100%)	1049 (85.6%)	177 (14.4%)	725 (59.2%)	501 (40.8%)

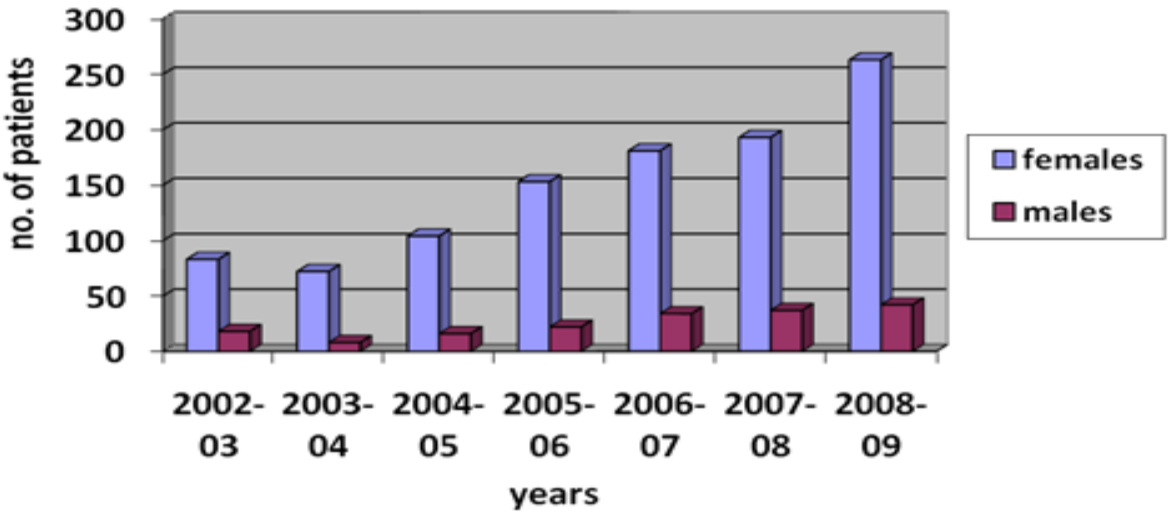

Figure 1. Distribution of male and female patients.

Table 2. Clinical presentations of patients

Symptoms	No.	%
Abdominal pain	537	43.8
Non specific dyspepsia	243	19.8
Complications;		
Cholecystitis	238	19.4
Obstructive jaundice	88	7.2
Empyaemia	28	2.3
Pancreatitis	18	1.45
Carcinoma	13	1.06
Incidental finding	61	4.97
Total	1226	100

Table 3. Distribution by age of presentation

Age / years	Number	%
< 20	43	3.5
20 -29	314	25.1
30-39	368	30
> 40	501	40.8
Total	1226	100

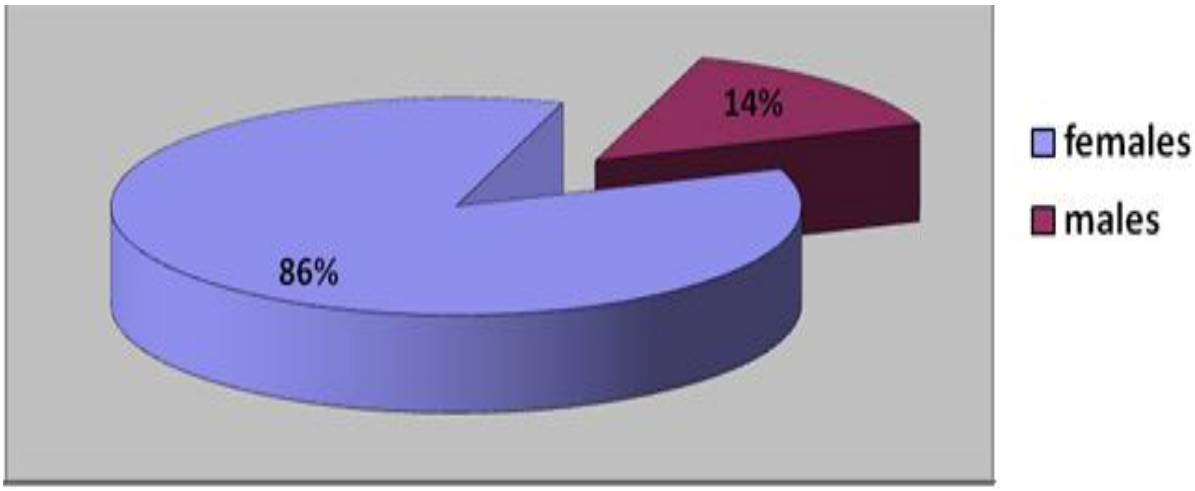

Figure 2. Percentage of male and female patients

Table 4. Distribution of patients by (BMI)

BMI kg/m^2	No. of patients	percentage	*p* value
> 25	907	74%	< 0.001
> 30	576	47%	
> 40	54	4.4%	

Table 5. Distribution of patients by the surgical approaches

Years	Open surgery	%	Laparoscopic	%
1st	78	12.2	23	3.8
2nd	69	10.8	11	1.8
3rd	87	13.6	33	5.5
4th	128	20.1	47	7.9
5th	98	15.4	117	19.8
6th	74	11.6	156	26.4
7th	102	16	203	34.4
Total	636	100	590	100

Discussion

Gallstone disease is one of the most common and costly of all digestive diseases. Survey estimated that 6.3 million men and 14.2 million women aged 20 to 74 in the United States had gallbladder disease [10].

Indeed, cholecystectomy is the commonest surgical procedure in the abdomen in the Western world [1], in relation to the total yearly surgical discharge although the gallbladder operations increases, still cholecystectomies comes next to the frequency of appendicectomies; 1226 versus 1575 subsequently (over 7 years of study), conversely the incidence of gallbladder operations has been increasing and exceeded that for appendicectomies in observation in Jewish General Hospital, Montereal [11].

In the current study, the hospital admission rates for cholecystectomy increased steadily among both sexes which reflect an increase incidence of gallstone in our population (the number of cholecystectomiesed patients has 98% correlation with the true incidence of gallstone [12]), this concurs with the finding in Queen Elizabeth hospital, Hong Kong, which shows an increase incidence of cholecystectomies from year 1981 to 1983 [6], this may be in part due the rise in calorie and fat consumption, decrease in fiber intake, and increased prevalence of the sedentary lifestyle in Iraq especially after year 2003 in Iraq, in Asian population the increase incidence of gallstone disease and hospital admissions for elective cholecystectomy was steady in the past decade due to the similar risk factors [13].

In regard to the spectrum of symptoms of gall bladder diseases, a prospective study conducted at surgical department of Liaquat University of Medical and Health Sciences in Pakistan during 2001 to 2005 showed that 56% of patients presented with abdominal pain, others including acute cholecystits in 36%, acute pancreatitis in 4%, obstructive jaundice and or cholangitis in 0.5% and gall bladder cancer in 0.3% [8]. In this series 43.8% of patients presented with abdominal pain, 19.8% with non specific dyspeptic symptom, 19.4% with acute cholecystits, 7.2% with obstructive jaundice, 1.06 with abdominal mass (proved by histopathology as gall bladder carcinoma) and 4.97% incidentally found.

As most epidemiological studies in western literature, our findings reveil that females have higher frequency of gallbladder disease as males, over all (5.9:1), in western 4:1 [14,15]. In older age group the female to male ratio decreases to 2.2:1 in our study, in western 2:1 [14,1 6, 17], while this females predominance was not found in Korea [18].

Stones are generally reported to be uncommon before the age of 20 years [19], and 40 years is considered as a typical age at clinical diagnosis [20] .This relation to age is supported by the studies that showed that the sensitivity of the gallbladder to cholecystokinin (CCK) decreases with aging [21], in our series 59.2% of patients were younger than 40, 25.1% bellow 30 years old and 3.5% bellow 20 years, these are comparable with that of the study done in Saudi Arabia by Murshid being that gallstones appear to be much more common in Saudi females and appear to occur at a considerably younger age 58% bellow 40 years, 31% bellow 30 years [4], while our previous series in late eighties and nineties of the previous century in Iraq showed that the peak age incidence was between 40-50 years [22,23].

Increased fertility is another important risk factor we found that 92% of female patients were fertile women. This factor appears to exert its influence through the hormonal changes occurring during pregnancy and is translated into a female:male ratio of gallstones which is much higher during the reproductive period than after

the menopause [24]. Ultrasound surveys of pregnant women have found an increase in gallbladder volume and reduced rates of emptying after liquid meals [25]. Others have reported a high prevalence of gallbladder sludge in women who were immediate postpartum although the sludge resolved within a year in most [26]. Jorgensen found a strong trend toward increasing stone prevalence with increasing childbirths, especially among women aged 30 years [27]. These above observations applied strongly to our patient's population with their relatively early menarche, early marriage and high parity [22].

Majority of patients included in the study have BMI higher than 25 kg/m^2. A well-established pathophysiologic link between obesity and gallstone formation is cholesterol-supersaturated bile [28]. Obese people hypersecrete biliary cholesterol, bile salts, and phospholipids, but the rate of cholesterol secretion supersedes that of the other biliary lipids, leading to cholesterol-supersaturated bile [29]. In theory, increased flux of cholesterol from the bile into gallbladder muscle cells stiffens the sarcolemmal membranes, decouples signal transduction, and inhibits gallbladder muscle function [30]. However, ultrasound data on gallbladder volume and emptying in obese humans are conflicting, while most studies suggest increased resting gallbladder volume in obese subjects; some reports demonstrate that these large gallbladders empty normally [31].

The prevalence of symptomatic gallstone disease in the family study was significantly greater ($p <$ 0.01) in females as compared to males, 28% versus 6.6% respectively, which is comparable with our findings; 32% and 5.3% respectively. These data suggest that genetic factors are responsible for at least 30% of symptomatic gallstone disease. However, the true role of heredity in gallstone pathogenesis is probably higher because data based on symptomatic gallbladder disease underestimates the true prevalence in the population [32].

The changes that had been noticed in the incidence and age presentation of gallstones diseases in Iraqi peoples could not be attributed to the usual risk factors in stone formation, we believe that stressful life events (SLEs) might be more significant trigger for stones development. This study was carried over a very stressful period in the history of Iraq, characterized by war and economic sanction. These events put a severe burden on Iraqi people, so we propose, as suggested by previous workers in the field from different countries also subjected to the stress of similar events as in Serbia, Nigeria, or Portugal that stressful life conditions might be the cause of increase incidence of gall stone diseases [9]. In Atlanta – a new study led by Center for Behavioral Neuroscience researchers has determined that social stress leads to gall bladder dysfunction in the form of decreases motility and increases bile retention, a precursor to gallstone development in cichlid fish. The work could hold clues to whether similar stress in humans -- anxiety disorders, or work, status and money worries and the like -- can increase the risk of developing gallstones in Georgia patients [33].

A study done by Geetha et al in China suggested that psychological stress is associated with increased oxidant production and oxidative damage, they noticed that gall stone patients have a high level of oxidative stress in the gall bladder mucosa, a finding that may be related to a decreased activity of functional enzymes in mucosal cells. Such a condition might result in an altered gall bladder absorption and secretion of bile components such as mucins and glycoproteins. The resultant increased risk of bile saturation would further contribute to the progress of gall stone formation [34].

In Pakistan which had passed through war conditions and stress, Naseem, et al reported

that psychological stress is a risk factor for cholesterol gallstones formation [35].

After the introduction of Laparoscopic cholecystectomy (LC) in 1991, its widespread use has completely revolutionized the management of cholelithiasis either simple or complicated and advantages of LC are undoubtable in comparison to open cholecystectomy and it has got economic advantages over open surgery [36], it is obvious from our data results that minimal invasive technique (LC) for removal of gall bladder gradually becomes the standard method over the usual classical open cholecystectomy as shown above in table 5.

Conclusions

The definite rise in the incidence and the decreased age of presentation of gallstones diseases could be due to increasing stressful life conditions affecting Iraqi population, but this should be studied more thoroughly well including all major governmental and private hospitals to be more precise and representative.

In the years following 2003 in Iraq, a more westernized diet had been consumed, this may have an additive effect on the changes of gallstones diseases pattern and it is worthy to study this subject by special dietitian.

The changing behavior toward more minimal invasive technique (laparoscopic surgery) is safe and better especially for the patients.

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The Causative Organisms of Neonatal Sepsis in Al-Kadhimiyia Teaching Hospital

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Abstract

Background Sepsis is common in the neonatal period which may be acquired in utero through the placental or trans-cervical routes and during or after birth and because the immunological system of neonates is not well developed which make this infection serious and fatal if not treated in accurate manner.

Objective To identify the most common causes of sepsis in Al-Kadhthimyia Teaching Hospital and its mortality rate.

Methods Across-sectional study was conducted during the period between 14th of February 2009 to 25th of February 2010 on 127 neonates with sepsis who were diagnosed clinically and they were admitted in Al-Kadhthimyia Teaching Hospital, they were divided into two groups according to the time of appearance of the symptoms which were early onset sepsis and late onset sepsis. Blood was taken from them and sends to Hospital laboratory for culture.

Results The most common clinical presentation in early onset sepsis (EOS) were, poor feeding, lethargy and fever (94.12%, 91.76 %, and 52.94 %, respectively), which is similar to late onset sepsis (LOS) (95.24 %, 92.86 % and 57.14 % respectively). The most common organisms responsible for EOS were Staph. aureus, Enterobacteria, and staph. epidermidis (25.89 %, 21.18 % and 21.18 %, respectively) while in LOS Staph. aureus, Enterobacteria and E. coli (21.43 %, 16.67 % and 11.90 %). The overall mortality was 29.92 % which was slightly more in LOS (30.95 %) than in EOS (29.41%) also it was more common in males than females in both groups.

Conclusion Staph. aureus, Enterobacteria were the leading causes of sepsis in both groups while staph. epidermidis were more common in EOS and E. coli was more common in LOS.

Key words Early onset, late onset, sepsis, neonate

Introduction

Neonatal sepsis is a significant cause of neonatal morbidity and mortality in the newborns particularly in preterm and low birth weight infants [(1)]. The frequency of neonatal bacterial infection ranges from 1-5 per 1000 live birth [(2)].

The epidemiological data from developed countries shows important difference in the incidence, risk factors, causative micro-organisms and antimicrobial sensitivities of pathogens and mortality rate from that of developing countries [(3,4)]. Group B-streptococcal disease is the most important cause of neonatal sepsis in Europe and North America [(5)], but there is preponderance of gram negative organisms in tropical and developing countries [(6)]. The clinical presentations are non specific in both early and late neonatal sepsis including poor feeding, lethargy, temperature instability, respiratory distress, seizures and abdominal distention [(7)].

This study was conducted to determine the clinical presentations, bacteriological profile and mortality rate in neonates admitted in Al-

Kadhimiyia teaching hospital in Baghdad with clinical diagnosis of sepsis.

Methods

This study included 127 suspected cases of neonatal sepsis, admitted to the Pediatric department of Al-Kadhimiyia Teaching hospital during the period between 14th of February 2009 to 25thof February 2010. The diagnosis of neonatal sepsis was clinical depending on the signs and symptoms including poor feeding, lethargy, temperature instability, respiratory distress, abdominal distention and seizures.

Early onset sepsis (EOS) was considered when the onset of symptoms was before one week of life and late neonatal sepsis (LOS) was considered in cases presenting after one week of life. Blood samples were collected from all the cases which were done by careful cleaning of the skin by antiseptic (alcohol) and then put in broth media to be send to the laboratory for culture in chocolate agar, first reading was done after 24 hr, second reading was done in second day, if the two readings were negative third one done after 27 hr and in case of negative result the culture consider to be negative. CRP (C-reactive protein) was not available at laboratory all the time so it was not included in our study.

Pre-term including live born infant delivered before 37 week from the last menstrual period. Term infants are an infant who delivered after 37 week of gestation. Post-term infants are those born after 24 week of gestation.

Statistical analysis

Significance for statistical difference was calculated using P value. P value less than 0.05 was regarded as significant.

Results

A total of 127 newborn with clinical sepsis were evaluated, blood culture reports were positive in 87/127 (89.76%) of the cases, males were affected more than females (59.84% and 40.16% respectively), as shown in table 1.

Table 1. Distribution of patients according to sex and gestational age.

Parameter		EOS		LOS		Total		P value
		No.	%	No.	%	No.	%	
Sex	Male	47	55.39	29	69.04	76	59.84	>0.05
	Female	38	44.61	13	30.96	51	40.16	
	Total	85	100	42	100	127	100	
Gestational age	Pre-term	21	24.70	12	28.57	33	25.98	>0.05
	Post-term	60	70.06	29	69.05	89	70.08	
	Full term	4	5.76	1	2.38	5	3.94	
	Total	85	100	42	100	127	100	

EOS = early-onset sepsis, LOS= late-onset sepsis

We chose our neonates from the pediatric general ward in 6th flower in Al-Kadhhimyia Teaching Hospital, so most of our patients were full term 89 (70.08%), preterm were 33 (25.98%) and post term were only 5 (3.94%). Eighty fife cases (66.9%) had early onset sepsis and 42 cases (33.7%) had late onset sepsis. The majority of newborns with neonatal sepsis presented with lethargy 117 (92.13%), poor feeding 120 (94.49%), fever 69 (54.33%), respiratory problems 54 (42.52%), vomiting 33 (25.98%), seizure 27 (21.26%), jaundice 26 (20.74%) and abdominal distention 11 (8.66%). There was no significant difference in clinical presentation between EOS and LOS (Table 2).

Table 2. Clinical presentation of neonatal sepsis.

Clinical presentation	EOS		LOS		Total		p value
	No.	%	No.	%	No.	%	
	85		42		127		
Poor feeding	80	94.12	40	95.24	120	94.49	>0.05
Lethargy	78	91.76	39	92.86	117	92.13	>0.05
Fever	45	52.94	24	57.14	69	54.33	<0.05
Respiratory problem (respiratory distress)	37	43.53	17	40.48	54	42.52	>0.05
Vomiting	21	24.71	12	28.57	33	25.98	>0.05
Seizure	18	21.18	9	1.43	27	21.26	>0.05
Jaundice	17	20	9	21.43	26	20.47	>0.05
Abdominal distension	8	9	3	7.14	11	8.66	>0.05

EOS = early-onset sepsis, LOS= late-onset sepsis

The most common organisms isolated from blood culture of those with EOS were Staph aureus (25.89%), Enterobacteria (21.18%) and Staph. epidermidis (21.18%). While in LOS Staph. aureus cause (21.43%), Enterobacteria (16.67%) and E. coli (11.9%), (Table 3).

Table 3. Organisms isolated from blood culture from neonate with sepsis.

Microorganism	EOS		LOS		Total		P value
	No	%	No	%	No	%	
Staph. Aureus	22	25.89	9	21.43	31	24.40	>0.05
Enterobacteria	18	21.18	7	16.67	25	19.68	<0.05
Staph. Epidermidis	11	12.94	3	7.14	14	11.26	>0.05
No growth	9	10.59	4	9.52	13	10.34	>0.05
E. Coli	8	9.42	5	11.90	13	10.34	>0.05
Pseudomonas	5	5.89	2	4.77	7	5.51	>0.05
Contaminated	4	4.7	3	7.14	7	5.51	>0.05
Strep. Viridans	3	3.52	4	9.52	7	5.51	>0.05
Strep. Faecalis	3	3.52	3	7.14	6	4.82	>0.05
Proteus	1	6.47	0	0	1	007	>0.05
Klebsiella	1	6.47	2	4.77	3	2.56	>0.05
Total	85	100	42	100	127	100	>0.05

EOS = early-onset sepsis, LOS= late-onset sepsis

In this study the overall mortality rate was (29.92 %)(total number of deaths 38). The total number of death in males was 24 (63.16 %), while the number in females was 4 (36.84). The study also showed that (66 %) of death occur in EOS and (34 %) occur in LOS. Regarding the gestational age, it was found that 25(65.79 %) of death occur in pre term, and the other 13 (34.21 %) occur in full term and there is no death in post term, as shown in table 4.

Table 4. Demographic profile and mortality rate.

Parameter		EOS		LOS		Total	
		No	%	No	%	No	%
Sex	Male	16	64	8	61.54	24	63.16
	Female	9	36	5	38.46	14	36.84
	Total	25	100	13	100	38	100
Gestational Age	Pre-term	17	8	8	62	25	65.79
	Full-term	8	32	5	38	13	34.21
	Post-term	0	0	0	0	0	0
	Total	25	100	13	100	38	100

EOS = early-onset sepsis, LOS= late-onset sepsis

Discussion

For the effective management of neonatal sepsis, knowledge about bacteriological profile play a vital role, in this study we found that EOS was more common than LOS which is similar to other reports [5,9]. Males are affected more than females as documented by other studies [8,9,10].

The international criteria for the clinical diagnosis of neonatal sepsis include lethargy, no sucking, respiratory rate >60, grunting, fever, convulsion and abdominal distention [5]. In this study there is no statistically significant difference in the clinical presentation between, EOS and LOS, as the P values was> 0.05 except in fever where there is statistical difference as the P value was < 0.05, which may be due to difference in the causative agents or due to difference in severity between EOS and LOS.

In this study, blood culture is positive in(89.76%), negative blood culture (10.34%) which could be due to administration of antibiotic before blood collection or may be due to infection by anaerobes. Negative blood culture dose not exclude sepsis and this finding is comparable to other reports, where about (26-30%) of all neonatal sepsis caused by anaerobes [9,10,11].

In this study there is no significant statistical difference regarding the etiological organism between EOS and LOS as the P value is > 0.05, except in Enterobacteria, where the statistical difference is significant as the P value is < 0.05 .This is due to presence of this organism in vagina as normal flora which causes the infection to neonate when he pass through birth canal during delivery.

In this study the predominant organism isolated from blood culture is staph. aureus, which is in agreement with other reports [12,13]. In Europe and North America, group B streptococci are the most common organism [5]. This could be due to increase in nosocomial infections among neonate delivered in developing countries than developed one.

In this study, Enterobacteria is the predominant gram-negative organism in both EOS and LOS, the report of the National Neonatal –Perinatal showed Klebsiella as the predominant gram negative pathogen [14]. A study in Nepal showed that Enterobacteria as well as Klebsiella as the predominant gram negative agents [15].

The mortality rate is (29.92%), and in males the mortality rate is much higher than in females (63.16% *Vs* 36.84% respectively), these results are similar to that found by other studies in Karachi and in Taiwan [15,16], while a study in Italy shows a mortality rate of (6%) only. This increase in mortality rate among male due to the possibility of a sex-linked factor in host susceptibility [7], while the decreased mortality rate in developed country may be due to early detection and availability of specific diagnostic and treatment measures .

Conclusion

It is concluded that Staph. aureus and Enterobacteria are the leading causative agents of sepsis in both early and late onset groups, while staph. epidermidis was more common in EOS and E. Coli was more common in LOS and the mortality rate were more in male and pre term neonate .

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Primary Pulmonary Nocardiosis: A Case Report

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Abstract

A 28-year-old non smoker male who had undergone kidney transplantation and has been taking immunosuppressive drug for the last 15 months was presented to the Hospital of Special Surgery/Baghdad with low grade fever, productive cough, shortness of breath, purulent sputum and bilateral chest crepitation. Laboratory investigations showed low Hb percentage (11.9%) with normal blood urea and creatinine (42 mg/ dL and 0.9 mg/ dL respectively). Chest x-ray revealed homogenous shadow. Initial antibiotic and anti-tuberculosis therapy was not helpful. Laboratory examination of sputum indicated the causative agent was *Nocardia*.

Key words Pulmonary nocardiosis, kidney transplantation

Introduction

Nocardia organism is filamentous gram positive rods. Thirteen species of this organism has been described, of which *Nocardia astroides* being the most usual one found as a pathogen in man [(1)]. Infection takes place by inhalation of airborne bacilli [(2)] or through traumatic inoculation of organism into skin [(3)].

Nocardia astroides is responsible for about 80% of non-cutaneous invasive disease and for most systemic and central nervous system infections [(4)]. Recent reports have shown an increase in the incidence of nocardiosis in humans, probably due to the use of more aggressive diagnostic examinations, the increased use of immunosuppressive treatment such as those with transplanted organs, and those who receiving high doses of glucocosteroids and the appearance of the acquired immunodeficiency syndrome (AIDS) [(5-7)]. Suppression of cellular immunity appears to play a key role in the establishment of *Nocardia* infection [(8)].

Bronchopulmonary or disseminated nocardiosis usually occurs in adults, and male are affected twice as often as female [(9)].

Case presentation:

A 28-year-old kidney transplant patient non smoker white male was admitted to the Hospital of Special surgery/Baghdad on 2/10/1992 with shortness of breath, cough productive of purulent sputum, and bilateral chest crepitation. He was anxious with a temperature of 38˚C, respirations were 22/min heart rate was 100/min, and blood pressure 130/80 mmHg. Hemoglobin concentration was 11.9%, total WBC count 1.7×10^3 /mm^3 with polymorphonuclear leukocytosis, ESR was 64 mm / h.

A chest radiograph showed bilateral homogenous shadow. Relevant past history of renal transplantation for which he was on regular low dose of steroids. Initially, the patient was suspected to be infected with *Mycobacterium*. Therefore penicillin –

streptomycin was tried but with no clinical improvement, as the patient still suffering from cough and breathlessness with high inflammatory markers.

Samples of sputum were taken for three consecutive days and gram stain and modified acid fast stain [10] were done. Furthermore, some samples were used for culturing using solid blood agar.

In gram stain, the organism appeared as beaded branching filaments gram positive (Figure 1). Modified acid fast stain revealed acid fast branching filaments (Figure 2). After incubation at 37˚C for 72 hrs on solid blood agar, there were a small, white compact, dry, gritty colonies adhering to the agar without hemolysis (Figure 3). A 200 ml of brain heart infusion broth was prepared with concentration of 37 gm/liter (Oxoid Company) in 500 ml flask. The broth was inoculated with *Nocardia* from solid blood agar under sterile conditions, and then was incubated at 37˚C for 5 days with daily shaking. *Nocardia* – like organisms were noted after five days of inoculation.

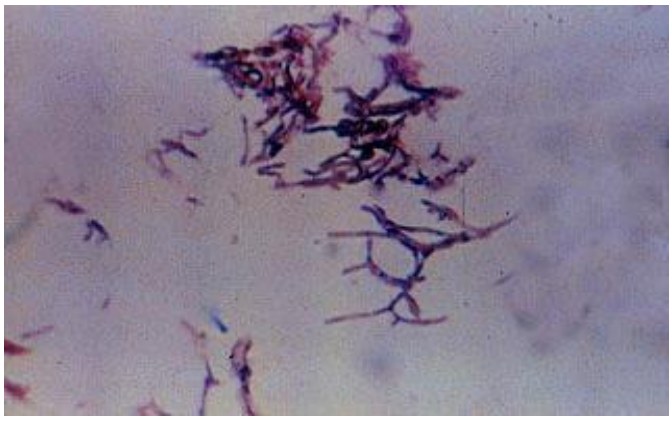

Figure 1. *Nocardia* stained with gram stain

Figure 2. *Nocardia* stained with modified acid fast stain

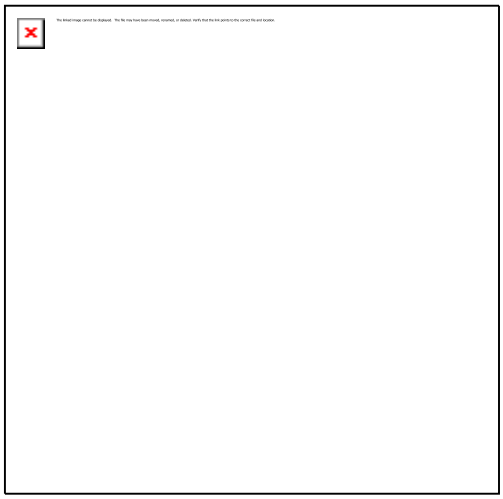

Figure 3. *Nocardia* colonies on solid blood agar

Discussion

Nocardia asteroids are a saprophyte in nature, presents in soil and decaying organic matter. It is a weak aerobic gram positive acid fast filamentous bacterium. The various species of *Nocardia* are pathogens with low virulence, therefore clinically significant disease most frequently occurs as opportunistic in elderly and immunocompromised persons affecting lung, brain, and skin [3] with no race predilection [11].

Pulmonary nocardiosis is difficult to diagnose clinically, as the clinical characteristics are relatively non specific [12]. The radiographic findings are not helpful [13], however, in early nocardiosis there is localized bronchopneumonia, and as the lesion progress, complete lobular consolidation may appear [14, 15]. Sometimes, there is solitary or multiple nodules, abscess or pleural effusion [16].

When pulmonary nocardiosis is suspected, the confirmatory diagnosis depends on sputum examination, although the organism doesn't appear easily in gram stain and modified acid fast stain and we have to do several smears for positive result. On the other hand, sputum culture is more positive more often, but growth may not appear until 3-21 days. So, culture should be kept for at least one month before the possibility of nocardiosis is excluded.

Our patient appears to have had primary nocardiosis, since there was no evidence of any underlying disease process and he responded well to the treatment with trimethoprim - sulfamethaxazol (cotrimoxazole) which are treatment of choice for nocardiosis [12,17,18] , as clinical improvement has been seen with almost normal inflammatory markers after 3 months of treatment.

Early diagnosis of pulmonary nocardiosis is extremely important, as the organism may spread to many parts of the body especially CNS where there will be poor prognosis [3]. Therefore, it is necessary to suspect *Nocardia* as the pathogen in obscure bronchopneumonic illness with apparently sterile sputum.

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