Volume 4 (1) 2005 **ISSN 1681-6579**

IRAQI JOURNAL OF MEDICAL SCIENCES

IRAQI JOURNAL OF MEDICAL SCIENCES is published quarterly by College of Medicine, Al-Nahrain University
All correspondence and subscription information requests should be addressed to:
The Editor of **IRAQI JOURNAL OF MEDICAL SCIENCES**
P. O. Box 14222, Baghdad, Iraq.
College of Medicine
Baghdad, Iraq
Tel and Fax: 964-1-5224368
E-mail: Iraqi_jms@yahoo.com

Advisory Board

Iraqi Journal of Medical Sciences

Aims and Scope

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

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

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 a document officially state that the current work was carried out at the site which provides this certification. The document should be signed by the highest authorized member at that location.**
- Manuscripts submitted to IJMS are subject to editorial evaluation and revision by two 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 two photocopies including figures, tables, and photographs should be submitted. Begin each of following sections on separate page in the following sequence: Title page, abstract and keywords, text, acknowledgments, references, tables, and legends for illustration.
- Manuscript and figures will not be returned to the authors whether the editorial decision is to accept, revise or reject.
- Manuscripts must be accompanied by a covering paper signed by all authors that the paper has not been published in and will not be submitted to any other journal if accepted in IJMS.
- The page should contain (a) title of the manuscript, (b) names of each author (first name, middle initial and family name) including highest academic degree, (c) official academic and/or clinical title and affiliation (d) name and address of the institution where the work was done (e) name and address (E-mail if available) of the author to whom correspondence should be sent.
- **ABSTRACT:** manuscript should include an abstract of not more than 150 words. Structured abstract typed on a separate sheet and consist of background, objective, method, results, and conclusion. Translation in Arabic to be included : (خلفية الدراسة، هدف الدراسة، طريقة العمل، النتائج و الاستنتاج).
- **KEYWORDS:** three to ten keywords should be provided on the same page as the abstract in Arabic and English. As far as possible, be selected from the National Library of Medicine Medical Subject Headings.
- The Arabic abstract should follow the United Medical Dictionary (Council of Arab Ministers of Health/WHO/ Arab Medical Union/ALESCO, 3rd edition.

- 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, J.M.C.: Oxygen toxicity, Oxygen radicals, transition metals and disease. Biochem J, 1984; 219: 1-14.
2. Books: Mann, J.I., Pyorala, K., and Teuscher, A.: Diabetes in epidemiological perspective. London: Churchill Livingstone. 1983.
3. Chapter in book: Phillips, S.J., and Whisnant, J.P.: Hypertension and strock. In: Laragh, J.H., and Brenner, B.M. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

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

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

- After the manuscript has been accepted for publication, authors are required to supply the final version of the manuscript on 3.5" IBM-compatible floppy disk in MS word version 6 or later.
- All corresponding to be addressed to the Chief Editor on the address below:

Chief Editor:
Iraqi Journal of Medical Sciences
College of Medicine,
Al-Nahrain University,
P.O. Box 14222,
Tel. 5231521,
Al-Kadhiymia,
Baghdad,
IRAQ.

IRAQI JOURNAL OF MEDICAL SCIENCES

A MEDICAL JOURNAL ENCOMPASSING ALL MEDICAL SPECIALIZATIONS
ISSUED QUARTERLY

CONTENTS

LEADING ARTICLE

ARTICLES

ESTABLISHMENT OF REPRODUCIBLE LYMPHOBLASTOID CELL LINES

Ismail I. Latif *MSc PhD*, **Layla Al-Omar** *BSc PhD*,
Nidal Abdul-Muhymen *BSc PhD*

Abstract

Background: A whole blood culture started with mononuclear cell fraction would contain several different cell types. Nevertheless, the T-lymphocytes can be cultured specifically and will out grow the others, eventually resulting in highly enriched population.

Objective: The study was conducted as a trial of establishment of lymphocytes cell lines. This series of experiments were done for five selected subjects. The same protocols were applied to all individuals.

Method: One ml of heparinized blood (HB) was cultured in tissue culture flask containing 9 ml stimulation medium. The flask was incubated at 37ºC in CO_2 incubator for 3 days. Then every three days 1ml of whole blood culture from the flasks was subcultured into other tissue culture flask containing 9 ml stimulation medium, until visible suspension of mononuclear cells intervening agglutinated red blood cells were seen within two weeks of culture.

Results: After the whole blood mononuclear cells cultures were maintained up to two weeks, the mononuclear cells were separated. Twenty four-hour incubation of these separated lymphocytes in stimulation medium, pure rich mononuclear cells were obtained and seen under inverted microscope.

Conclusion: A new method for cultivation of lymphocytes from whole blood was developed for the first time and probably no other reported comparable method conducted elsewhere until know. Pure lymphocytes cultures were established, and maintained cell division. The potentiality of human peripheral lymphocytes themselves to act as precursor has been demonstrated *in vitro* by the cloning of established lymphoblastoid cell line. Different procedures for generation of T- cell lines were outlined. No feeder cells were needed in our protocol or the addition of IL2.

Key words: lymphocyte culture, cell line, Phytohemagglutinin

IRAQI J MED SCI, 2005; VOL. 4 (1): 3-9

Introduction

Advances in the understanding of lymphocyte biology have been accompanied by improvement in the techniques of long-term culturing of human T- lymphocytes[1]. Long-term culturing of human T-lymphocytes can lead to establishment of stable line. These cultures respond to produce a wide range of cytokines. More importantly, T-cell lines can be generated which is antigen responsive. Many aspects of the T-cell response to antigen have been described using antigen specific cell lines[2,3,4].

Human lymphocytes can be isolated from whole blood by centrifugation using a commercially available high-density medium. This allows a single step gradient separation of blood, which yields the mononuclear cell fraction[5].

A culture started with mononuclear cell fraction would contain several different cell types. Nevertheless, the T-lymphocytes can be cultured specifically and will out grow the others, eventually resulting in highly enriched population. To achieve this, the antigen reactive cells must be first stimulated with antigen followed by expansion of cell

Dept. Medical Microbiology, College of Medicine, Al-Nahrain University.
Address correspondence to Dr. Ismail I. Latif.
Received 17th December 2003: Accepted 14th March 2005.

numbers using the T- cell specific growth hormone interleukin 2 (IL2). It should be noted that the absolute frequency of antigen response T-cells is low, being between 0.01% and 0.001%, even when blood known to contain relevant T-cells (i.e. blood from an immune donors) is used. Thus, the majority of the cells in the starting culture will die and only the T-cell activated by antigenic stimulation will survive[6].

This stimulation not only selects out the antigen-specific T-cells but result in the cellular expression of the receptor for the IL2[7]. When exogenous source of IL2 is now added to the activated T-cells, they will undergo further round of replication. Since T-cells eventually become refractory to the effect of IL2, they must be restimulated for receptor expression[8]. A T-cell line can thus be maintained in culture by alternate stimulation with antigen and expansion with IL2[5].

Phytohemagglutinin (PHA) activated peripheral CD95+ T-cells are resistant to CD95 mediated apoptosis. After prolonged interleukin-2 treatment, these T- cells become CD95 mediated apoptosis sensitive. In T- cells activated in vitro, up-regulation of bcl-xL, has previously been correlated with general apoptosis resistance[9].

Therefore, this study was conducted as a trial of establishment of lymphocytes cell lines from whole blood.

Materials & Methods

This series of experiments were done for five selected normal adult subjects, their ages ranged from 15 to 45 years, (four males and one female). The same protocol was applied to all individuals. Two ml of venous blood was collected from each subject and placed in sterile heparinzed tube.

One ml of heparinized blood was cultured in tissue culture flask containing 9 ml of **stimulation medium** (SM) (1 x RPMI 1640, penicillin 100 unit/ml, and streptomycin 100µg/ml, 10mM L-glutamine, 20% fetal bovine serum (FBS), 10mM HEPES, 200µl/ml PHA). The flask was incubated at 37ºC in CO_2 incubator. Then every three days 1ml of whole blood culture was subcultured into other tissue culture flask containing 9 ml of SM, until visible suspension of mononuclear cells intervening agglutinated red blood cells were seen within two weeks of culture.

Then, the mononuclear cells were separated by the method described by Boyum (1968)[10], washed twice with serum free medium (1x RPMI 1640, penicillin 100 unit/ml, and streptomycin 100µg/ml, 10mM L-glutamine 10mM HEPES), counted, assessed for viability, then 1ml of 2 x 10^6 cells were cultured in tissue culture flask containing 9 ml of **SM**. Another 1 ml of cells was cultured in tissue culture flask containing 9 ml of **growth medium** (GM) (as stimulation medium without PHA). After 24-hour incubation, cell culture was examined under inverted microscope.

At that time, the cells were counted and assessed for viability. The percent of viable cells were determined at indicated time intervals in both GM and SM, and 2 x 10^6 cells were subcultured in tissue culture flask containing 9 ml of SM or GM, every 72 hour. The lymphocytes culture was maintained continuously by this method.

Results

Preparation of Primary Human Peripheral Blood Mononuclear Cells Culture:

Heparinized blood was cultured in SM, and subcultured frequently as mentioned in materials and methods. The visible suspensions of mononuclear cells intervening agglutinated red blood cells were seen within the second week of culture (figures 1 a & b).

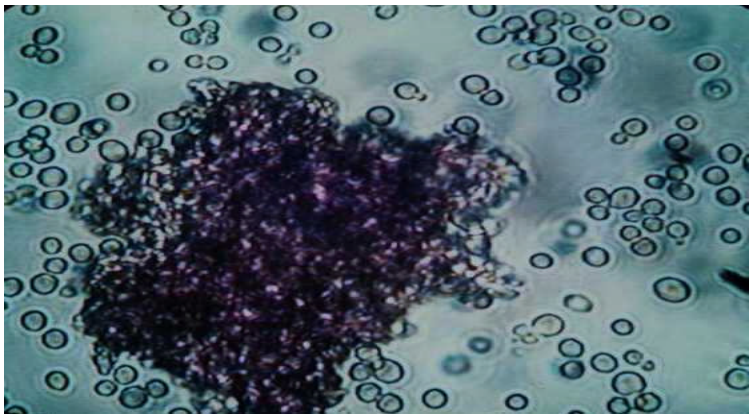


Figure 1a: Photomicrograph Shows Whole Blood Lymphocytes Culture during the first week (X 250)

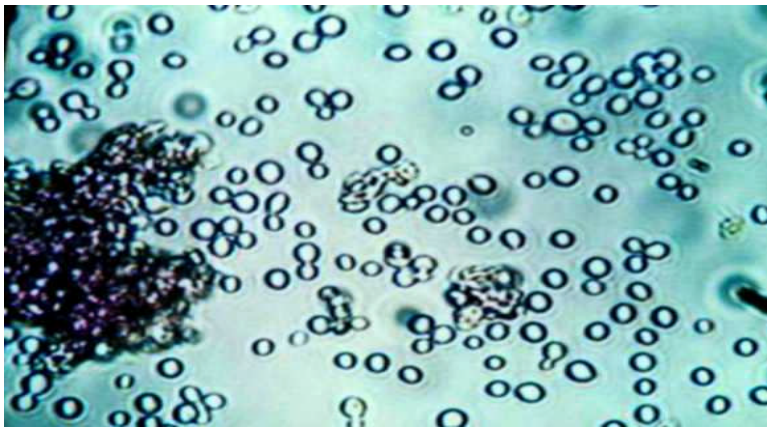


Figure 1b: Photomicrograph Shows Whole Blood Lymphocytes Culture during the second week (X 250)

Purification and propagation of PBMC culture:

After the whole blood mononuclear cell cultures were maintained up to two weeks, the mononuclear cells were separated as described in materials and methods. Twenty-four hour incubation of these separated lymphocytes in SM, pure rich mononuclear cells were obtained and seen under inverted microscope (figure 2).

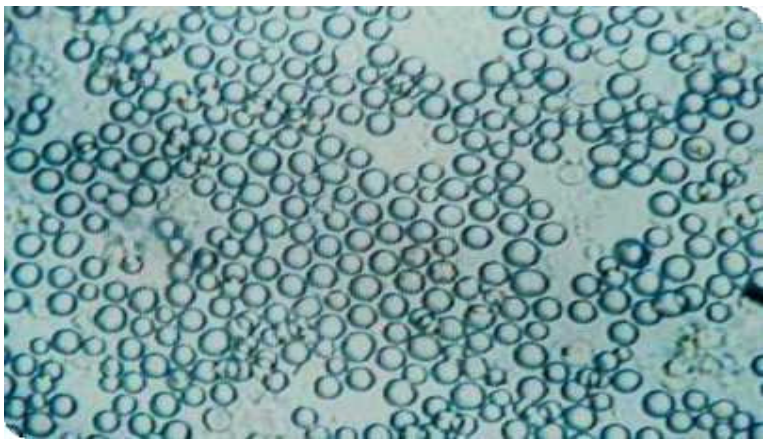


Figure 2: Photomicrograph Shows Purified Stimulated Lymphocytes Culture after the second week of whole blood culture (X 250).

Stimulation was indicated by a rapid increase in the number of cells (count were increased 200 fold within two weeks of subculture of the purified lymphocyte) (table 1 & figure 3).

Table 1: Effect of cell proliferation and cell death on viable count over two week's period, using growth or stimulation medium. The seeding density was 2x 10^5/ml

culture media	Number	Absolute Number of viable lymphocyte count /ml (x10^6)						
		Zero time	Days after culture initiation					
			1	3	5	8	11	14
Stimulation medium	Sample1	0.2	2	25	70	125	250	400
	Sample2	0.2	1.5	20	50	100	200	350
	Sample3	0.2	2.5	30	80	150	300	450
	Sample4	0.2	2	22	75	130	260	410
	Sample5	0.2	2	18	65	110	220	380
	Mean	**0.2**	**2**	**23**	**68**	**123**	**246**	**398**
	SD	**0**	**0.3**	**4.6**	**11.5**	**19.2**	**38.4**	**37**
Growth medium	Sample1	0.2	2	25	60	50	31	3
	Sample2	0.2	1.5	20	39	35	26	2.5
	Sample3	0.2	2.5	30	62	52	35	3
	Sample4	0.2	2	22	60	48	25	3
	Sample5	0.2	2	18	58	47	24	4
	Mean	**0.2**	**2**	**23**	**55.8**	**46.4**	**28.2**	**3.1**
	SD	**0**	**0.1**	**4.6**	**9.4**	**6.6**	**4.6**	**0.5**

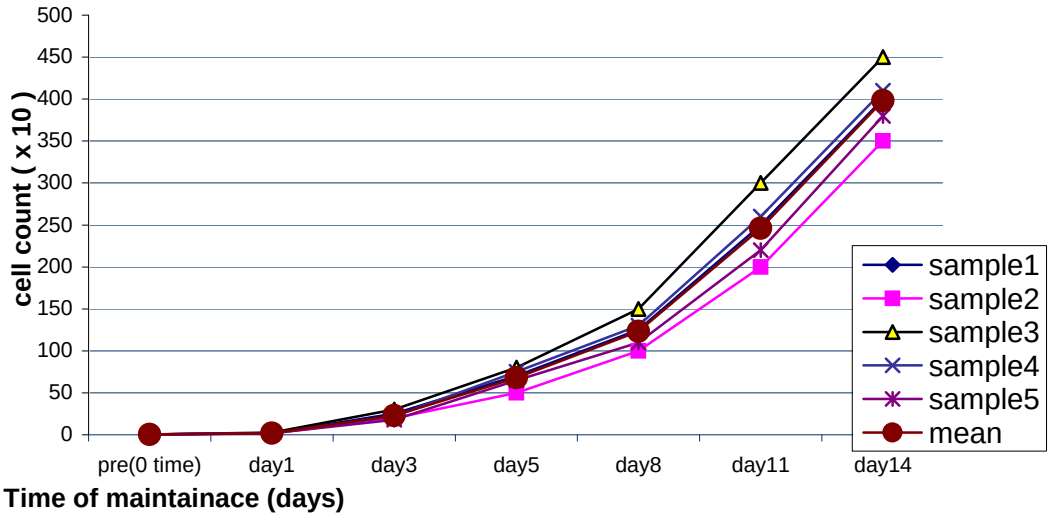


Figure 3: Effect of cell proliferation and cell death on viable count over two weeks period, using stimulation medium, each point represent the mean of 4 values. The seeding density was 2x10 /ml

Many cells became enlarged, and the medium turning yellow 2-3 days after subculture. A clear stimulation event was recognized without the aid of an inverted microscope when the bottom of the tissue culture flask showed a large grayish white central button of cells surrounded by white pinpoint satellilate colonies.

Another (2 x 10 ^{6}/ ml) of cells from day one culture were cultured in growth medium. A reduction in viable cell

counts after day 5 of culture was observed as shown in table 1 & figure 4.

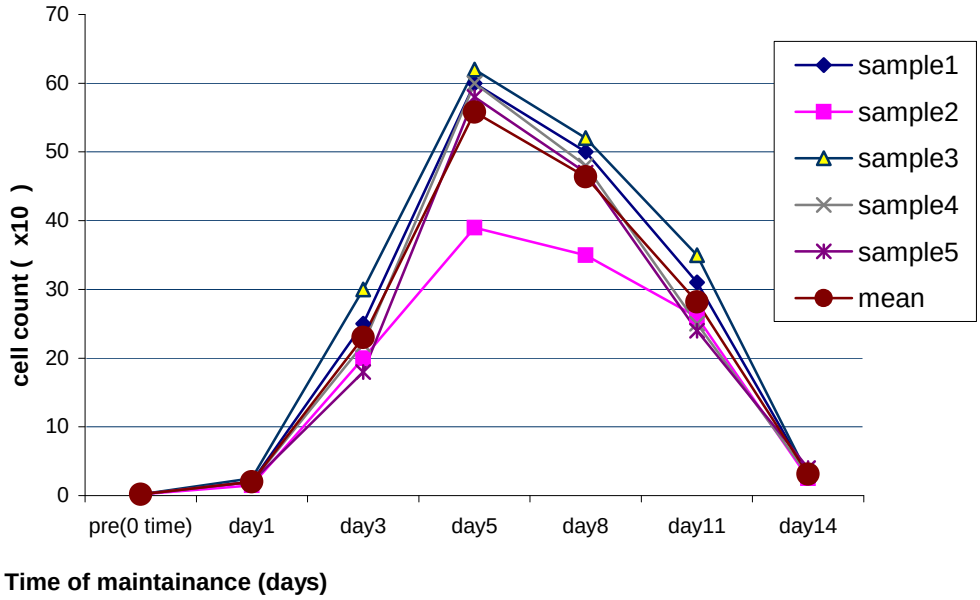


Figure 4: Effect of cell Proliferation and cell death on viable count over two weeks period, using growth medium. Each point represents the mean of 4 values. The seeding density was 2x 10 /ml

All the stimulated cells gave lymphoblast characteristics when stained with Giemsa stain, so actually we can apply the lymphoblastoid cell line as indicated from their morphological appearances.

Discussion

This work probably to be the **first** method to be reported for preparation of continuous lymphocytes culture and probably no other reported comparable method conducted elsewhere till know.

Purification and propagation of PBMC culture:

Human lymphocytes can be isolated from whole blood by centrifugation using a commercially available high-density medium, which relies on lymphocytes being less dense than erythrocytes and granulocytes. This allows a single step gradient separation of blood, which yields the mononuclear cell fraction[5].

Then pure lymphocyte cultures were established, and induced and maintained cell division.

A culture started with mononuclear cell fraction would contain several different cell types. Nevertheless, the T-lymphocytes can be cultured specifically and will out grow the others, eventually resulting in highly enriched population. Beside T-cells, other mononuclear cell types are present in the initial culture. These cells have the capacity to activate T-lymphocyte by processing and presenting antigen to the T-cell receptor (TCR) and provide additional T-cell growth factors[11,12].

T-cells can be activated by some mitogens. Most T-cells are stimulated by PHA, a lectin isolated from kidney beans, or by concavalin A extracted from castor bean. These molecules are able to bind to T- cell surface molecules including the T- cell receptors (TCR) complex and CD2, causing them to cluster on cell surface, there by mimicking the clustering caused by antigen presentation. Such mitogens however will activate the T- cells regardless their antigen specificity[13].

In this assay, PHA was used as mitogen because their stimulation percentage was higher than that of Con A, as indicated early by the results of our

study and easy to be manufactured in our laboratory[14].

Whole Blood Lymphocyte Culture:

We have developed a new method for isolation and cultivation of lymphocytes from whole blood. Heparinized venous blood drowns from normal individuals was added and incubated in SM and the subculture was regularly maintained. Lymphocytes were isolated when lymphocytes suspension was seen by inverted microscope intervening agglutinated red blood cells (figures 1 a & b).

It has been suggested that a cellular components of erythrocytes in whole blood play an important role for the proliferation of the lymphocytes with no effect on their genetic materials. The use of whole blood greatly simplifies lymphocyte culture. In addition, the use of non-separated blood will retain all blood cells, especially RBC, in natural condition thus allowing metabolic activation of environmental pollutants to take place[15,16].

The potentiality of human peripheral lymphocytes themselves to act as precursor has been demonstrated **in vitro** by the cloning of established lymphoblastoid cell line[17].

Choi and Bloom in 1970[18] established CKW–1 cell line involved a mixed lymphocyte culture, they used small inoculums of PGLC-33H cells (cell line from infectious mononucleosis patient) as feeder cells. They also used lysates of previously established lines as a second method of lymphocyte cell line establishment.

Different procedures for generation of T-cell lines were outlined[5]. They used Daudi or any lymphoblastoid cell line that are different from that of donor PBMNCs. Antigen specific T- cell lines were established using foreign antigens such as tetanus toxoid or PPD, restimulation with antigen, with the addition of IL2, and treatment of antigen presenting cells were included[5].

None of them used a whole blood culture for establishment of lymphocytes cell lines. No feeder cells were needed in our protocol or the addition of IL2.

Phytohemagglutinin-activated peripheral T- cells were found to be resistant to CD95 mediated apoptosis, and after prolonged IL-2 treatment, these T- cells became CD95 mediated apoptosis sensitive[9].

Investigations on resting T-cells, i.e., lymphocytes depleted of macrophages and pre-activated cells were made. When PHA was added to these cells resulted in activation with expression of IL-2R (CD25) but not in proliferation. In contrast, addition of PHA plus sheep RBC, which bind to the CD2 receptors, caused IL-2R expression, IL-2 production, and proliferation[19].

A trial of phenotype of the established cell lines was under work. Great benefits can be obtained from this work: this cell lines can be used for virus propagation for example measles virus, different cytokine production, and for different immunological techniques

Monitoring the growth of cultures by direct observation:

Although the growth of the Lymphoblastoid cell lines was, to a large extend, synchronized by stimulation culture, some cultures will grow faster than the others (as indicated in figure 1). It was only by examining the cells on a routine bases with the inverted microscope that the rapidly growing cultures can be identified and cultured accordingly, or by supplementation of GM. In addition, direct observation was the only way of verifying that the cells are actually growing, on day-to-day basis.

References

1. Fathman, C., and Fitch, F.: Long-term culture of immunocompetent cells. In: Paul, W. eds. Fundamental Immunology. Raven press, New York, 1984; p.p. 781-95.
2. Lamb, J., Eckels, D., Woody, J., et al.: Antigen specific human T lymphocyte clone:

induction, antigen specificity, and MHC restriction of influenza virus- immune clone. J Immunol, 1982; 128: 233-8.
3. Modlin, R., Kato, H., Mehra, V., et al.: Genetically restricted suppressor T cell clone derived from lepromatous leprosy lesions. Nature, 1986; 322: 459-61.
4. Fisch, P., Malkovsky, M., and Kovats, S.: T cells. 1990: (Cited in Doyle and Griffiths 2000).
5. Doyle, A., and Griffiths, J.B.: Cell line derivation methods. Cell and Tissue Culture or Medical Research. John, Whiley & Sons, Ltd, 2000; p.p.187-204.
6. Van Oers, M., Pinkester, J., and Zeijlemaker, W.: Quantification of antigen reactive cells among human T lymphocytes. 1978. (Cited in Doyle and Griffiths, 2000).
7. Smith, K.: IL2 inception, impact and implication. Science, 1988; 240: 1169-76.
8. Gullberg, M., and Smith, K.: Regulation of T cell autocrine growth: $T4^+$ cells become refractory to IL2. J Exp Med, 1986; 163: 270-84.
9. Peter, M.E., Kischkel, F.C., Scheuerpflug, C.G., Medema, J.P., Debatin, K.M., and Krammer, P.H.: Resistance of cultured peripheral T cells towards activation-induced cell death involves a lack of recruitment of FLICE (MACH/caspase 8) to the CD95 death-inducing signaling complex. Eur J Immunol, 1997; 27 (5): 1207-12 (Abstract).
10. Boyum, A.: Separation of leucocytes from blood and bone marrow. Scand J Clin Lab Invest, 1968; 21 (Suppl. 1): 97.
11. Oppenheim, I., Leventhal, B., and Hersch, E.: The transformation of column purified lymphocytes with nonspecific and specific antigenic stimuli. J Immunol, 1968; 101: 262-70.
12. Germain, R.: The ins and outs of antigen processing and presentation. Nature, 1986; 322: 687-9.
13. Roitt, I., Brostoff, J., and Male, D.: Immunology. Vol. 6. Gower Medical Puplishing, London, 2001; p.p. 117- 374.
14. Latif, I.I. : Measles vaccination among seropositive individuals: an in vitro and in vivo virological and immunological study. PhD thesis College of Medicine, Al-Nahrain University, 2002.
15. Norppa, H., Vanio, H., and Soria, L.: Metabolic activation of styrene by erythrocytes detected as SCE in cultured human leucocytes. 1983. (Cited in Shubber, E.K., and Al-Allak, A.I. 1986).
16. Jantunin, K., Paakkana, J.M., and Norppa, H.: spontaneous chromosomal aberration and SCE in human lymphocytes. Effect of culture condition. The nucleus, 1982; 29 (3): 92-98. (In Shubber, E.K., and Al-Allak, A.I.).
17. Imamura, T., and Mcore, G.E. : Cloning of human lymphocytes. In Readings in mammalian cell culture. Edited by Pollack, R. Gold, Spring Harbor Laboratory, 1975 ; p.p. 118-22.
18. Choi, K.W., and Bloom, A.D.: Cloning Human Lymphocytes. Nature, 1970; 277 (5254): 171-3.
19. Hofmann, B., Jakobsen, K.D., Odum, N., et al.: HIV-induced immunodeficiency. Relatively preserved phytohemagglutinin as opposed to decreased pokeweed mitogen responses may be due to possibly preserved responses via CD2/phytohemagglutinin pathway. J Immunol, 1989; 142 (6): 1874-80.

ALTERATION IN THE LEVEL OF SOME TRACE ELEMENTS IN THE SERA OF PATIENTS WITH KALA AZAR

Nada M. Al-Basheer[1] *BSc PhD*, **Yahya Y.Z. Farid**[2] *BSc PhD*,
Hussein K.A. Hussein[3] *MSc*, **Huda Th. Hathal**[1] *BSc PhD*

Abstract

Background: Visceral leishmaniasis (Kala-azar) is an important endemic disease in Iraq (WHO 2000) .The disease appears to affect infants and young children mostly under 2 years old .The disease is very dangerous and fatal if it is left without treatment .Trace elements have an important role in the treatment and prognosis of different types of parasitic infection

Objective: Study the relationship between serum trace elements and the disease which may be used as an indicator of the course of the disease

Methods: 14 male and 12 female patients (positive IFAT) their age were between 6 months and 15 years were used in this study .Serum copper (Cu), zinc (Zn), and magnesium were measured using atomic absorption spectrophotometer.

Results: There is a significant increase ($p<0.05$) in serum Cu and significant decrease in serum Zn of kala-azar patients.

Conclusions: Body reaction against parasite infection is associated with different changes in serum level of trace elements.

Keywords: kala-azar, trace elements, copper, zinc, magnesium, visceral leishmaniasis

IRAQI J MED SCI, 2005; VOL. 4 (1): 10-13

Introduction

It has become well established that many trace elements play an essential role in a number of biological processes through their action as activator or inhibitor of enzymatic reaction by competing with other elements and proteins for the binding sites, by influencing the permeability of the cell membrane[1], or through other mechanism[2]. Several biological mechanisms have been proposed to explain how trace elements could reduce the incidence of a number of different cancer and infectious disease[3]. Visceral leishmanasis is an important endemic disease in Iraq[4,5]; the disease appears to affect infants and young children , mainly under 2 years of age , and especially those under 1 year of age[6] . It is very dangerous and it is fatal if left untreated with mortality rat of 1%-29%. The presence of leishmania parasite in reticuloendothelial cells (RECs) of liver spleen and bone marrow is accompanied by physiological and biochemical changes in these organs and trace elements may be one of these changes. Therefore, in this study we aim to find the effect of parasitic infection on the level of trace elements in the sera. This raises the possibility of the use of trace elements as an indicator of the course of disease.

Materials & methods

Twenty-six kala-azar patients were studied depending on their positive IFAT test. The age ranges was between 6 months and 15 years. Twelve patients

[1] **Medical Research Center, Al-Nahrain University** [2] **Dept. Chemistry & Biochemistry, College of Medicine, Al-Nahrain University** [3] **Dept. Chemistry, College of Science, Karabala University**
Address correspondence to Dr.Yahya Y.Z. Farid, E-mail: Yahya1@uruklinik.net
Received 21st December 2002 : Accepted 30th March 2004.

were females and 14 males. Samples were collected from patients attending Al-Kadhimiya teaching hospital.

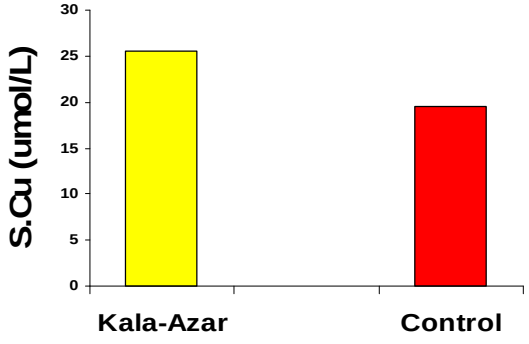
Venous blood samples were collected from patients before administration of any medications. Sera were separated and kept at (-20^{o}C) until used.

Assay: 0.1ml of serum diluted to total volume of 1ml using 6% n-butanol solution and analyzed for their copper and zinc contents using atomic absorption spectrophotometer (Shimadzu AA-646) with an. Copper and zinc hallow cathode lamps were used at wavelengths of 324.75 nm and 213.9 nm respectively. The assay for magnesium estimation was carried out by adding 4.9 ml. of (1% lanthanum chloride) solution to 0.1 ml. of serum. These solutions were aspirated directly into air-acetylene flame and the magnesium hallow cathode lamp were used at a wavelength of 285.2 nm.

Statistical methods: The results were analyzed statistically, and their values were expressed as (mean ± SED).The level of significance was determined by employing (t) test .Only when the p value was less than 0.05; the difference between two groups considered statistically significant.

Results & Discussion

The mean and standard deviation values of serum copper, magnesium, and zinc of healthy controls and patients with kala-azar are presented in Figure 1.



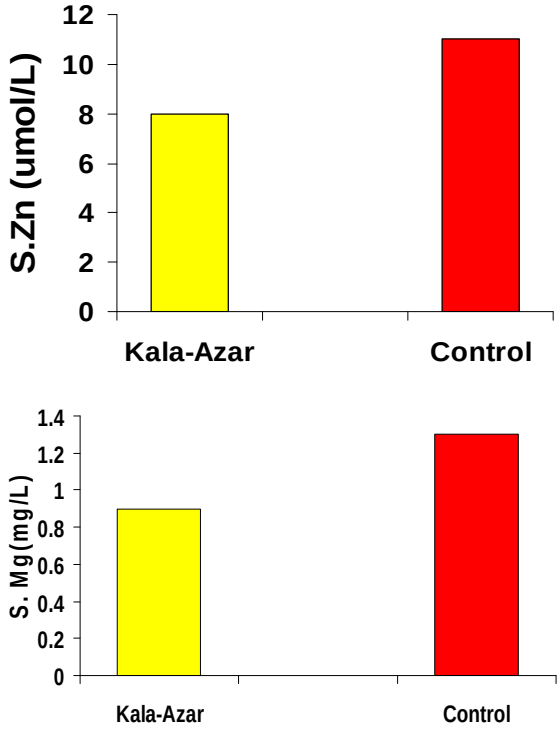


Figure 1: Serum copper, zinc, and magnesium of normal and kala-azar patients

The results showed that there is a significant increase in serum copper in kala-azar patients as compared with healthy controls. This result is due mainly to the body reaction against infection.

Cerruloplasmin which is a copper containing protein is one of the acute phase reactant proteins that increased in inflammation and hence leading to increase serum copper. α-Cerruloplasmin inhibits the oxidant injury of the cells by scavenging the superoxide radicals through dismutation reaction similar to superoxide dismutase enzyme[7] or by reduction the copper ions within the protein[8]. Other workers[9] have proposed that cerruloplasmin acts by converting the reduced iron(Fe^{+2}) to an oxidized form (Fe^{+3}) because it acts as ferroxidase enzyme. Yet, the majority of the antioxidant activity in the serum is dependent on the level of this copper containing protein[10]. There is also other cause explaining the increase of

serum copper in those patients is due to shed copper ions from damaged hepatocells which is the late complication of the disease[11] .This fact is confirmed by reports that recorded an elevation in the level of liver enzymes in the sera of kala-azar patients[12].

There is a significant decrease at ($p<0.05$) in serum zinc in kala-azar patients as compared with healthy controls .This may be due to in part to the hypoalbuminaemia developed in those patients after a period of incubation of the disease[13]. Zinc is transported mainly bound to albumin molecules and the decreased in albumin concentration in serum leads to decrease zinc level in serum. Other reason is associated with the immune changes in kala-azar patients .Those patients have impaired immunity[14] and because there are good evidences about the relationship between the zinc level and immunity[15]; it can be concluded that the zinc is decreased as the immunity attenuated. These results are in agreement with other previous work regarding serum trace elements levels in cutaneous Leishmaniasis patients[16].

Serum magnesium of patients with kala-azar are insignificantly decrease in comparison with healthy controls. Magnesium level is normally kept within narrow limits, which implies close homeostatic control and about 35% of the magnesium in plasma is protein-bound mainly to albumin[17]. Hence this decrease is attributed mainly to the hypoalbuminaemia state in kala-azar patients[13].

Figure 2 shows the relationship between the serum metals as a function to the sex in kala-azar patients. There is a significant increase in serum copper in male patients as compared with female while there is an inverse correlation in serum zinc between males and females. Serum magnesium is not significantly different in both sexes. There is no simple explanation for these results and more investigations are necessary to predict the sexual variance in the level of the metals.

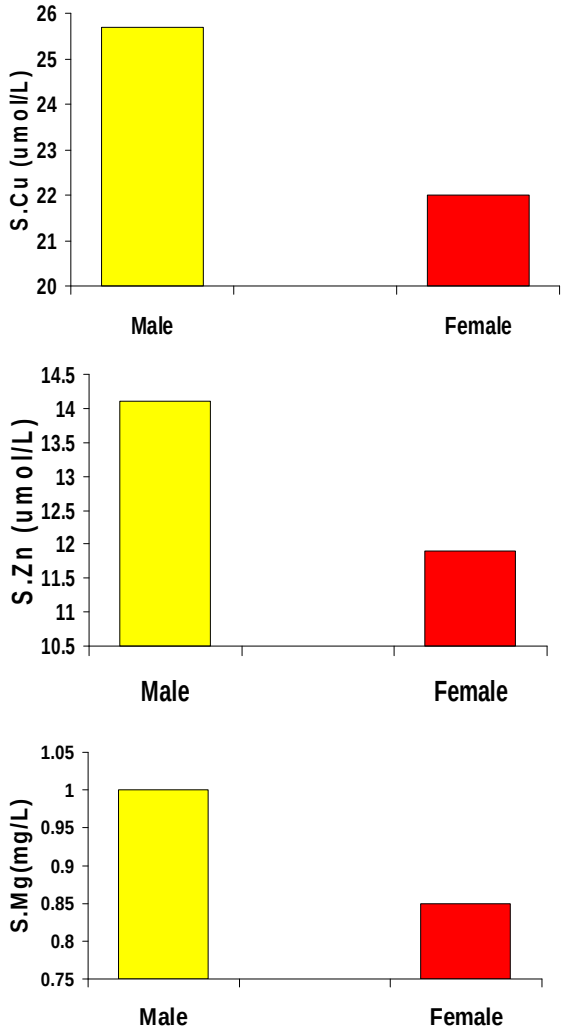


Figure 2: Serum copper, zinc, and magnesium of male and female patients infected with kala-azar

Figure 3 shows that there is age dependence of serum copper but not zinc or magnesium in kala-azar patients. The patients who were of one-year age. Others had a higher serum copper than patients of more than one year of age. This variation may be related to the rate of development and homeopoiesis between these two groups.

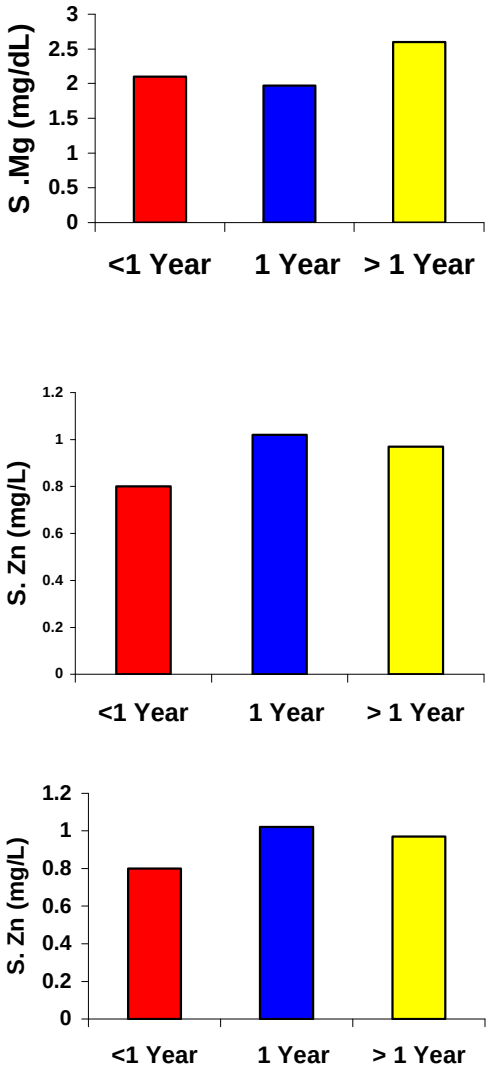


Figure 3: Serum copper, zinc and magnesium of patients of different ages infected with kala-azar

References

1. Neilson, F.H.: Possible functions and medical significance of the abstruse trace metals. In: Inorganic chemistry in biology and medicine. ACS Symposium series No.140. Martell, A.E. (edition), Washington D.C. Am Chem Society, 1980 .
2. McCall, J.T., Golestein, N.P., and Smith, L.H.: Implications of trace metals in human disease. Fed Proc, 1971; 30: 1011-5.
3. Koyama, H.: Trace elements: Mechanistic aspects of anticarcinogenic action. Nippon Rinshow, 1996; 54 (1): 52-8.
4. Taj-Alden, H., Guirges, S.Y., and Falaki, N.: Kala-azar in Iraq: analysis of a new series. J Fac Med Baghdad, 1969; 11 (1): 7-15.
5. Aswad, B., and Jawad, T.: In patient statistics from central children hospital. Iraqi J Ped, 1972; (2): 1-2.
6. Nori, L., and Al-Jeboori, T.I.: Kala-azar in Iraq. An epidemiological and clinical study. J Fac Baghdad, 1979; 15: 72-85.
7. Goldstein, I.M.: Cerruloplasmin: a scavenger of superoxide anion radicals. J Biochem, 1979; 254: 4040-5.
8. Bannester, J.V.: Does cerruloplasmin dismutate superoxide? FEBS Lett, 1980; 118: 127-9.
9. Loustad, R.A.: The protective action of cerruloplasmin of Fe^{+2} stimulated lysis of rat erythrocytes. Int J Biochem, 1981; 13: 221-4.
10. Al-Timimi, D.J., and Drmandy, T.L.: Inhibition of lipid peroxidation by human cerruloplasmin. Biochem J, 1977; 168: 283-8.
11. Edwards, C.R.: Davidson's principles and practice of medicine. 17th ed. Churchil-Livingstone, 1995; p.p. 157.
12. Al-Saffar, N.R., and Al-Mudhaffar, S.A.: Lactate dehydrogenase and other enzymes in kala-azar patients in Iraq. Ind J Med Res, 1979; 70: 598-608.
13. Herwaldt, S.L.: Leishmaniasis. Lancet, 1999; 3 (54): 1191-9.
14. Baqer, H.I., Al-Jeboori, T.I., and Jum.a A.S.: Visceral leishmaniasis: A preliminary study of cell mediated immune response using cytogenic parameters. Iraq J Med Sci, 2001; 1 (2): 119-25.
15. Ripa, S., and Ripa, R.: Zinc and immune function. Minerva Med, 1995; 86 (7-8): 315-8.
16. Kocyigit, A.: Alteration in serum selenium, zinc, copper and iron concentrations and some related antioxidant enzymes activities in patients with cutaneous Leishmaniasis. Biol Trace Elem Res, 1998; 65 (3): 271-81.
17. Whitby, L.G., Smith, A.F., and Beckett, G.J.: Lecture notes on clinical chemistry, 4th edi. Blackwell Scientific, 1988; p.p. 231.

SCHOOL ACHIEVEMENT OF DIABETIC ADOLESCENTS: A PRELIMINARY REPORT

Abdul Hussain M. Al-Hadi[1] *MBChB MSc PhD*, **Jawad K.A. Al-Diwan**[2] *MBChB MSc*, **Eqbal G.A. Ma'ala**[3] *BSc MSc PhD*, **Amjad Niazi**[4] *MBChB PhD*

Abstract

Background: Adolescents struggle to achieve their identity, independence and to cope with all aspect of life. Diabetes may slow the psychological development of adolescents.

Objective: to through a light on the school achievements of adolescents with type I diabetes mellitus.

Methods: 160 diabetic adolescents were included in this study. Full information including age, sex, age of onset, duration, sport activity and admission to the hospital collected. Multiple regression analysis was used to examine the association between the school achievement and the independent variables.

Results: School achievement was significantly associated with visits to diabetic clinics and sport activity.

Conclusion: Visits to diabetic clinics and sport activity improve the school achievement among diabetic adolescents.

Keyword: Adolescents, diabetes mellitus, school achievements

Iraqi J Med Sci, 2005; Vol. 4 (1): 14-17

Introduction

Adolescence period is a transitional period between childhood and adult hood. During this period adolescents struggle to achieve their identity (a major task for this developmental stage), independence and to cope with all aspects of these changes[1,2]. Chronic illness like type I diabetes mellitus {Insulin Dependent Diabetes Mellitus (IDDM)} has an impact on the achievement of the developmental tasks. Diabetes may slow the psychological development of the adolescents, thus affecting their ability to play and enjoy life, share with others, set limits, identify and make commitments. This study was carried out to through light on the school achievement of the adolescents with type I DM.

[1]Dept. Community Medicin, College of Medicine, Al-Nahrain University [2]Dept. Community Medicine, College of Medicine, Al-Anbar University [3]College of Nursing, Baghdad University [4]Iraqi Commission of Medical Specialization.

Address correspondence to Dr. Abdul-Hussain M Al-Hadi

Received 10th July 2004: Accepted 14th March 2005.

Subjects & methods

Adolescents with type 1 DM were enrolled in the study from different diabetic centers (National Diabetic Center at Al-Yarmouk teaching hospital, Diabetic Consultancy Clinic at Al-Kadhmia teaching hospital, Diabetic Consultancy Clinic at Al-Mansour teaching hospital and Diabetic Consultancy Clinic at Ibn Al-Beldy teaching hospital) in Baghdad city. Adolescence period is considered between 12-21 years[3,4]. Each participant was interviewed individually. Questionnaires were checked according to the adolescent's answers. Full information including age, sex, age of onset, duration, sport activity, school activity and admissions to hospital were collected.

A pilot study was done to examine the adolescent's understanding of the questions (instrument items) and to obtain preliminary estimates of the time required for each adolescents and to

determine the validity and reliability of the instrument. The questionnaires were based on the Diabetes Specific Quality of Life Scale[5,6].

Multiple regression analysis was used to examine the association between the dependent variable (school achievement and) with independent variables[7] (demographic variables, visits to diabetic clinic and sport activities). P value less than 0.05 was considered as statistically significant.

Results

A total of 160 diabetic adolescents with type 1 DM were included in the study. Their age range was 12 – 21 years (15.1± 2.3) and 53.8% of them were females. 51.3% of the adolescents had DM for more than five years. The age of onset was less than six years in 78.2% of them. 76.9% of them reported no history of admission to hospital. None of them was in the secondary school, while 33.1% of them were not able read and wrote, 47.5% were in the primary school and 17.5% were in the intermediate school. 53.8% of the adolescents participated in sport activity (Table 1).

Table 1: Characteristics of the diabetic adolescents included in the study

Variable		Number	(%)
Age	≤15 years	98	(61.3)
	>15 years	62	(38.7)
Sex	Male	74	(46.2)
	Female	86	(53.8)
Duration	≤5	78	(48.7)
	>5	82	(51.3)
Age of Onset	≤6	35	(21.8)
	>6	125	(78.2)
Hospital admission	Yes	37	(23.1)
	No	123	(76.9)
School achievement	Not read and write	3	(1.9)
	Primary	53	(33.1)
	Intermediate	76	(47.5)
	Secondary	28	(17.5)
Sport activity	Yes	86	(53.8)
	No	74	(46.2)

School achievement of diabetic adolescents was significantly associated with burden of DM, visits to diabetic clinic and sport activity ($p < 0.05$). School achievement was not associated with age, sex, age of onset and duration of DM. These findings are shown in table 2.

Table 2: Predictors of school achievement among diabetic adolescents

P value	R^2	Predictors
Model I	0.02	NS
Model II	0.015	NS
Model III	0.2	<0.05
Model IV	0.2	<0.05
Model V	0.7	<0.05

Model I: Age, sex, and age of onset, **Model II**: Age, sex, age of onset and duration, **Model III**: Age, sex, age of onset, duration and burden of DM, **Model IV**: Age, sex, age of onset, duration, burden of DM and visits to diabetic clinic, **Model V**: Age, sex, age of onset, duration, burden of DM, visits to diabetic clinic and sport activity

Discussion

The finding that school achievement are not significantly associated with age, sex and duration of diabetes is inconsistent with the findings of Dela Mater[8] and Golden et al[9] . They reported that patients with early onset of diabetes had shown poorer cognitive performance and associated with subsequent learning disabilities. Larsson et al[10] reported that patients in poor metabolic control have a lower educational level. The finding that age and duration of diabetes had no effect on school achievement reflects a good metabolic state achieved in the diabetic clinics included in the study.

Visit to diabetic clinics was positively associated with school achievement. Ryan et al[11] found that children with diabetes missed school twice as much as their peers who do not have diabetes, and the lower school performance was related to the more school absences. Newacheck and Taylor[12] reported an average of annual bed days was 3.6 day and average school absence was 3.1 day among diabetes. In this study, only 23% of the diabetic adolescents were admitted to the hospital (diabetic adolescents with bed days). Visits to diabetic clinics may lead to a good metabolic control reflected by reduced hospital admissions and better school performance.

Faro[1] reported that adolescents are not able to adjust their diabetic regimen to fit their life style. However, better school performance and less bed days found in this study may be attributed to the role of diabetic clinic in monitoring the metabolic control of the diabetic adolescents.

This study shows a positive association between sport activities and school achievement. Campaigne et al[13] found a significant improvements in the glycaemic control in children after training program. Drash[14] reported that maintaining high level of physical fitness provide short and long benefit to individual with diabetes including attendance to good control. In conclusion, visits to diabetic clinic and practicing sport improve the school achievement among diabetic adolescents.

References

1. Faro, B.: The effect of diabetes on adolescent's quality of life. Pediatric Nursing, 1999; 25 (supp.): 147-253.
2. Christain, B.J., D'auria, J.P., and Fox, L.C.: Gaining freedom: self-responsibility in adolescents with diabetes. Pediatric Nursing, 1999; 25: 255-60.
3. Paxaman, J.M., and Zuckerman, R.P.: Laws and polices affecting adolescents health. WHO, editor. Geneve,1987; p.p. 4-10.
4. WHO technical report series. (Health problems of Adolescence: report of WHO Expert Committee), No. 308, 1965.
5. Bott, V., Muhlhauser, I., Overmann, H., and Berger, M.: Validation of a specific quality of life scale for patients with diabetes 1 diabetes. Diabetes Care, 1998; 21: 757-68.
6. Ma'ala, E.G.: Assessment of health related quality of life of diabetic adolescents (Type I diabetes), PhD thesis, College of Nursing, University of Baghdad.
7. Daniel, W.W.: Biostatistics: A foundation for analysis in the health sciences. 7th , New York : John Wiley and Sons. Inc., 1999.
8. Delamater, A.M.: Quality of life in youths with diabetes. Diabetes Spectrum, 2001; 13: 42-46.
9. Golden, M.P., Ingersoll, G.M., Brack, C.I., Russell, B.A., Wright, J.C., and Huberty, T.J.: Longitudinal relationship of a symptomatic hypoglycaemia to cognitive function. Diabetes Care, 1989; 12: 89-93.
10. Larsson, D., Lager, I., and Nilsson, P.M.: Socioeconomic characteristics and quality of life in diabetes mellitus relation to metabolic control. Scand J Public Health, 1999; 27: 101-5.
11. Ryan, C., Langestreet, C., and Marrow, L.: The effects of diabetes mellitus on school attendance and school achievement on adolescents. Child Care Health Devel, 1985; 11: 229-40.
12. Newacheck, P.W., and Taylor, W.R.: Childhood chronic illness, prevalence, severity and impact. Am J Dis Public Health, 1992; 82: 364-71.
13. Campaigne, N.B., Gillian, T.B., Spencer, M.L., Lampoman, R.M., and Schock, M.A.:

Effect of physical activity program on metabolic control and cardiovascular fitness in children with insulin dependent diabetes mellitus. Diabetes Care, 1994; 7: 57-62.

14. Drash, A.L.: Clinical care of diabetes: what is the role of diabetes proffitionals? Diabetic Care, 1984; 17 (suppl): 40-2.

THE ASPARTATE AMINOTRANSFERASE TO ALANINE AMINOTRANSFERASE RATIO AND HCV INFECTION

Al-Kubaisy W. A. *MBchB, PhD*[1] **Habib M.A.** *MBchB, PhD*[2]

Abstract

Background: Hepatitis C virus (HCV) infection is responsible for the majority of cases of post-transfusion hepatitis. Biochemical analysis of liver enzymes used to estimate the severity of liver injury in HCV infected patients.

Objective: To estimate the aspartate aminotransferase to alanine aminotransferase (AST/ALT) ratio as non-invasive parameter for assessment of liver injury in HCV infected patients.

Methods: Two groups of subjects were included in this study. 238 thalassemic children from Al-Zahrawi Hospital (172 were seropositive and 66 were seronegative for HCV specific antibody) and 58 pregnant women (32 were seropositive and 26 were seronegative for HCV specific antibody) as a control group. Serum AST and ALT and AST/ALT ratio levels estimated for each subject.

Results: The mean serum AST/ALT ratio levels for anti-HCV seropositive and seronegative thalassemic children were 3.38 ± 4.34 and 2.56 ± 3.09 respectively, while for anti-HCV seropositive and seronegative pregnant women 1.62 ± 1.34 and 0.59 ± 0.42 respectively.

Conclusion: The mean serum AST/ALT ratio is higher among HCV infected subjects than among the non-HCV infected ones.

Keywords: aspartate aminotransferase to alanine aminotransferase ratio, Hepatitis C virus

IRAQI J MED SCI, 2005; VOL. 4: 18-20

Introduction

Hepatitis C virus (HCV) now considered as the leading cause of post-transfusion hepatitis world-wide[1]. Chronic transfusion recipient, such as patients with thalassemia major have a high frequency of liver disease[2,3]. HCV is responsible for the majority of cases of post-transfusion hepatitis in such patients[1]. Generally, HCV infection is asymptomatic and most often cases are discovered by chance biochemical analysis when a subject found to have raised liver enzymes[4].

Among routine laboratory tests for liver injury is the measurement of liver enzymes. The AST/ALT ratio used to non-invasively assess the severity of the disease in patients with chronic liver disease[5]. Hence, the aim of this study directed to evaluate the liver function impairment among patients infected with HCV by measurement of AST/ALT ratio.

Materials & methods

A total of 238 outpatients and inpatients thalassemic children were included in this study from Al-Zahrawi hospital from November 1996 to August 1997, of those 172 were seropositive and 66 were seronegative for HCV specific antibody. The control group for this study was 58 pregnant women (26 were seropositive and 32 were seronegative for HCV specific antibody). Serum samples collected from each subject and tested for serum ALT and AST levels using Dinitrophenylhydrozol colorimetric method[6]. The AST/ALT levels estimated. Statistical analysis performed using t test.

[1]Dept. Community Medicine, [2] Dept. Medical Microbiology, College of Medicine, Al-Nahrain University.

Address correspondence to Dr. Manal Adnan Habib,

Email: manalviro@yahoo.com

Received 15th May 2004: Accepted 10th July 2004.

Results

As shown in table 1, the mean serum AST/ALT levels for anti HCV seropositive and seronegative thalassemic patient were 3.38 ± 4.34 and 2.56 ± 3.09 respectively with no significant difference (p>0.05). Regarding the control group (pregnant women), the mean serum AST/ALT levels for anti HCV seropositive and seronegative ones were 1.62 ± 1.34 and 0.59 ± 0.42 respectively with statistical significant difference (p<0.005). A highly significant difference was observed when the mean serum AST/ALT levels for anti HCV seropositive thalassemic patient and pregnant women were compared (p<0.005).

Table 1: The mean serum AST/ALT level ± SD* in among different groups

Groups	Number	Mean±SD	P value
Anti HCV seropositive thalassemic patients	172	3.38±4.34	T= 1.407
Anti HCV seronegative thalassemic patients	66	2.56±3.09	Df= 236 (P > 0.05)
Anti HCV seropositive Pregnant women	32	1.62±1.34	T= 3.787
Anti HCV seronegative Pregnant women	26	0.59±0.42	Df = 56 (P < 0.005)
Anti HCV seropositive thalassemic patients	172	3.34±4.34	T = 4.33
Anti HCV seropositive Pregnant women	32	1.62±1.34	Df =202 (P <0.005)
Anti HCV seronegative thalassemic patients	66	2.56±3.09	T= 5.06
Anti HCV seronegative Pregnant women	26	0.59±0.42	Df= 90 (P< 0.005)

*SD: Standard deviation

Discussion

Chronic HCV infection is highly prevalent among thalassemic patient[2]. HCV infection is commonly presents as an asymptomatic liver disease, which over years can cause inflammation, fibrosis and eventual cirrhosis in the liver[7]. The challenge lies in identifying those patients. Who will go on to develop fibrosis and subsequent cirrhosis. Assessment of liver injury, therefore, can be important factor in determining both timing and effectiveness of therapy. Measurement of liver enzymes one of the routine laboratory tests for estimation of liver injury.

The serum AST/ALT ratio of more than one indicates progressive liver injury[8] and correlates with both liver histology and clinical evaluation[5]. Moreover, Reedy and coworkers stated that AST/ALT ratio although specific for liver cirrhosis, should not be the sole determinant to identify cirrhosis[9]. In this study the mean serum AST/ALT ratio among anti-HCV seropositive pregnant women was greater than one and showed a significant difference when compared with that of anti-HCV seronegative pregnant women (p<0.005) reflecting liver injury in the former group. However, the mean serum AST/ALT ratio was higher among anti-HCV seropositive thalassemic patient than among the anti-HCV seronegative ones but with no significant difference (p>0.05). A finding that can be explained on the basis that factors other than HCV infection may be involved in damaging the liver, like iron over load and possibly coinfection with other hepatitis viruses, because those patients are chronic blood transfusion recipients[2,3]. Moreover, the mean serum AST/ALT ratio among anti-HCV seropositive thalassemic patients was significantly higher than that among anti-HCV seropositive pregnant women, a finding that support the statement that serum AST/ALT ratio can be used to assess the severity of liver injury[5].

Although the current gold standard for assessing liver injury and fibrosis is the liver biopsy, however, it is assessed with patient discomfort, potential

complications, and high cost[10]. Even this invasive measure relies on scoring system that may be limited by sampling error, biopsy size and observe reproducibility[9] While measurement of liver enzymes is a non invasive, informative and less costly routine laboratory test. Although ALT level may inflect liver damage, correlation with hepatic fibrosis while elevation in the AST/ALT ratio was observed as patient prognosis for chronic hepatitis to cirrhosis[12-14].

References

1. SpiliopoulouI, Repanti, M., Katinakis, S., Karana-Ginopoulou, A., and Papanastasiou, D.A.: Response to interferon Alfa 2b therapy in multitransfused children with beta thalassaemia major & chronic hepatitis C. EJCMID, 1999; 18: 709-15.
2. Sibal, A., Mishra, D., and Arora, A.: Hepatitis C in childhood. JIMA, 2002; 100(2): 1-6.
3. Prati, D., Zanella, A., Farma, E., DeMattei, C., Bosoni, P., Zappa, M., et al. : A multicenter prospective study on the risk of acquiring liver disease in anti HCV negative patients affected from homozygous B thalassaemia. Blood, 1998; 92: 3460-6.
3. Maio, G., Argini, P.D., Streffolini, T., Bozza, A., Saccol, L., Tosti, M.E., et al.: Hepatitis C virus infection & alanine trasaminase level in the general population: a survey southern Italian town. J Hepatol, 2000; 33: 116-20.
5. Giannini, E., Risso, D., Botta, F., Chiarbonello, B., Fasoli, A., Malfatti, F., et al.: Validity and clinical utility of the aspartate aminotransferase alanine aminotransferase ratio in assessing disease severity and prognosis in patient with hepatitis C virus related chronic liver disease. Arch Intern Med, 2003; 163(2): 218-24.
6. Frankel, S., Retiman, S., and sonnewirth, A.C.: Clinical laboratory methods and diagnosis. Vol. 1, Saint Louis, C.V. Mospy Co., 1970; p.p. 120-2.
7. Douglas, G., and Peter, J.: Non invasive assessment of hepatic fibrosis: How accurate is it? HIV/HCV Coinfection monthly, 2003; 2(1): 1-4.
8. Ustundag, Y., Bilezikci, B., and Booyaciglu, S.: The utility of AST/ALT ratio a non-invasive demonstration of the degree of liver fibrosis in chronic HCV patient on longer-term haemodialysis. Neph Dialy Transpl, 2000; 15(10): 1716-8.
9. Reedy, D.W., Loo, A.T., and Levine, R.A.: Serum HCV-RNA, ALT levels and AST/ALT ratio do not do predict severity of liver disease in chronic HCV infection. American Association for the study of liver disease (annual meeting), 1996.
10. Fontana, R.J., and Lok, A.S.: Noninvasive monitoring of patients with chronic hepatitis C. Hepatology, 2002; 36: s57-s64.
11. Knodell, R.G., Ishak, K.G., Black, W.C., Chen, T.S., Craig, R., Kaplowitz, N., et al.: Formulation and application of a numerical scoring system for assessing histological activity in asyptomatic chronic active hepatitis. Hepatology, 1981; 1: 431-5.
12. Benhamou, Y., Bochet, M., Di Martino, V., Charlotte, F., Azria, F., Coutellier, A., et al.: Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. Hepatology, 1999; 30: 1054-8.
13. Gordon, S.C., Fang, J.W., Silverman, A.L., McHutchison, J.G., Albrecht, J.K.: The significance of baseline serum alanine aminotransferase on pretreatment disease characteristics and response to antiviral therapy in chronic hepatitis C. Hepatology, 2000; 32: 400-4.
14. Persico, M., Persico, E., and Suozzo, R.: Natural history of hepatitis C virus carries with persistently normal aminotransferase levels. Gastroenterology, 2000; 118: 760-4.

STUDY OF DIFFERENT CLINICAL AND DEMOGRAPHIC CHARACTERS OF PATIENTS WITH THALASSEMIA AND THEIR RELATION TO HEMOGLOBIN, SOME MINERALS AND TRACE ELEMENTS AND ALBUMIN LEVELS IN THEIR BLOOD

Enas T. Abdul-Karim[1] *MSc PhD*, **Firyal H. Abdul-Jalil**[2] *BSc PhD*, **Thabit N. Al-Azawi**[3] *BSc PhD*

Abstract

Background: Thalassaemia is considered the most common genetic disorder worldwide. β thalassaemia has emerged as a huge public health problem worldwide. The classic changes of untreated thalassaemia major are now regularly seen only in countries without resources to support long-term transfusion programs.

Objective: To study the different clinical features of patients with anemia attending the center for anemia of Mediterranean origin in Ibn-Albalady Hospital in Baghdad for blood transfusion. To correlate different clinical features with the different demographic characters among the sample patients and with the blood levels of hemoglobin, some trace elements, minerals and albumin.

Methods: Cross-sectional study was conducted in the center for anemia of Mediterranean origin in Ibn-Albalady Hospital, 157 patients were randomly selected using convenient sampling and patients attending the center for blood transfusion. Blood samples were taken from all the studied sample. Tests were done for different serum levels of trace elements, minerals, albumin and hemoglobin.

Results: showed that out of 157 patients studied, there were 112 (71.3%) from Baghdad and 107 (68.2%) from urban areas. The mean age on diagnosis was 1.6 years and that thalassemia major was found in 121 (77.1%), there were 108 (68.8%) who require blood transfusion between 2-4 weeks, and desferol treatment frequency was >4 times\ week in 99 (63.1%) and under nutrition was found in 76 (48.4%) patients.

Conclusion: More centers for thalassemia are to be established in different areas in our country, with increase efficiency as to include gene frequency. Programs based on carrier screening and counseling of couples at marriage, preconception or early pregnancy to be established. Prenatal diagnosis by mutation analysis on PCR amplified DNA from chorionic villi.

Key word: Mineral, Trace elements, Thalassemia

IRAQI J MED SCI, 2005; VOL. 4 (1): 21-37

Introduction

Thalassaemia is considered the most common genetic disorder worldwide. It occurs in a particularly high frequency in a broad belt extending from the Mediterranean basin through the Middle East, Indian subcontinent, Burma, and South East Asia[1]. The estimated genetic frequencies range from 5% to 10% in some areas[2]. Thalassaemia syndrome is described as a series of genetic disorders of hemoglobin synthesis which have in common a reduce output of the globins chain production, it is inherited as an autonomic recessive basis, that to find heterozygous β thalassaemia in both parents of a child with β thalassaemia major[3]. William et al[4] describes the

[1]Dept. Community Medicine [2]Dept. Chemistry & Biochemistry, College of Medicine, Al-Nahrain University [3]Dept. Chemistry & Biochemistry, College of Medicine, Al-Mustansriya University.
Address correspondence to Dr. Enas T. Abdul-Karim.
Received 13th December 2004: Accepted 28th March 2005.

distribution of thalassaemia where the gene frequency for the different thalassaemia and structural hemoglobin variants are high. According to him as the social conditions improve in developing countries and childhood mortality due to infection and malnutrition declines, children with thalassaemia who would previously have died early in life are now surviving long enough to require treatment. The reason for high frequency of thalassaemia throughout the tropics, reflect the advantages that carriers are protected from the consequences of infection with Plasmodium falciparum malaria[5].

Thalassaemia is classified into α, β, δ β, δ, γ δ β subtypes[6]. The severe in effective erythropoiesis results in erythroid marrow expansion to as 30 times the normal level. Both an increase in plasma volume because of shunting through expanded marrow and progressive spleenomegaly exacerbate anemia[7]. Increased erythropoietin synthesis may stimulate the formation of extramedullary erythropoietic tissue, primarily in the thorax and Para spinal region. Marrow expansion also results in characteristic deformities of the skull and face, as well as osteopenia and focal defects in bone mineralization[8].

Marrow hyperplasia leads ultimately to increased iron absorption and progressive deposition of iron in tissues. The marked increase in survival, to the fifth decade of life, patient with well-managed β thalassemia in developed countries represent one of the most dramatic alteration in morbidity and mortality associated with a genetic disease in these countries. Still more than 75 years after the fascinating initial description of peculiar bone changes and other signs and symptoms of the disorder, the β thalassaemia has emerged as a huge public health problem worldwide[7]. The classic changes of untreated thalassaemia major are now regularly seen only in countries without resources to support long-term transfusion programs[9].

The intention of this study is first, to study the different clinical features of patients with anemia attending the center for anemia of Mediterranean origin in Ibn-Albalady hospital in Baghdad for blood transfusion. Second, to correlate different clinical features with the different demographic characters among the sample patients and thirdly, to correlate the different clinical features with the blood levels of hemoglobin, some of the trace elements and minerals, and albumin

Subjects & methods

A coss-sectional study was conducted for the period from 1/9 to 1/12/2002 in the center for anemia of Mediterranean origin in Ibn-Albalady Hospital, 157 patients were randomly selected using convenient sampling and patients attending the center for blood transfusion. Well-studied questionnaire form were used to obtain information regarding different socio-demographic characters.

Blood samples for hemoglobin estimation and estimation of various levels of mineral, trace elements and albumin were taken from the entire studied sample. The blood was left at room temperature for 10 minutes for clotting, centrifuged at 3000 rpm for 10 minutes, and then serum was separated and stored at -20°C until used.

Basal metabolic index (BMI) was classified according to the International accepted range of BMI[10] as follows: under weight <18.5, normal 18.5-24.9, over weight 25-29.9, obese 30.0-39.9 and extremely obese >40.

Chemicals and reagents

All chemical and standard solutions used in this work were the highest analytical grade, and used without purification.

Measurement of trace elements

Serum trace elements were measured by flame atomic absorption spectrophotometry (Schimadzu AA 646). It is simpler and less tedious to perform than the flameless mode where the metal compound in the flame Hollow cathode lamp made of the same metal to be measured. The lamp was used to generate a wavelength of light specific for the same metal to be analyzed. This light is passing through the flame that contains the free atoms of the metal, which absorb a fraction of the light intensity. The light intensity reached to the recorder is directly proportional to the concentration of the free atoms in the flame, which in turn reflects the original concentration of the metal in the solution.

Dilution of the serum was made by deionized water according to the sensitivity of the atomic absorption spectrophotometer in order to avoid the viscosity and to decrease the interference of the protein in serum[11]. Single element hollow cathode lamps were used as line-radiation sources and were operated at currents or energies recommended by the manufacture. Optimum working conditions are given in Table 1.

Table 1: Optimum working conditions for Flame AAS of the elements (Zn, Cu, Fe, Mg, Ca)

Element	Wave length (nm)	Slit width (A˚)	Air Flow (L/min)	Acetylene Flow (L/min)	Lamp Current (mA)	Burner Height (mm)
Zn	213.9	3.8	10	2.4	6	4
Cu	324.7	3.8	10	2.3	7	4
Fe	248.3	1.9	10	2.7	5	6
Mg	285.2	3.8	10	2.4	5	5
Ca	422.7	3.8	10	2.6	8	10

Determination of Zinc and Copper

1000-ppm stock solution was diluted with deionized water to give the following concentrations of the working standard (0.0, 0.4, 0.8, 1.2, 1.6 and 2 ppm) of zinc and copper. Frozen samples were allowed to thaw and come to room temperature then mixed gently.

Samples were diluted 1:10 with 6% butanol as diluents. This method achieved 30% increase in sensitivity compared to use of water only[12]. This effect is due to decrease viscosity and difference in droplet formation and this technique is widely used[13].

Determination of Iron

Serum iron measured using Olson and Hamlin method[14] as follow: Five hundred micoliters of 20% trichloroacetic acid were added to 500 micoliters of serum and heated at 90ºC for 15 minutes, cooled, centrifuged and the iron level in the supernatant determined by flame atomic absorption spectrophotometer at 248.3 nm.

Determination of total iron binding capacity

It is the approximate estimate of serum transferrin, 1 ml of serum was added to saturated iron solution mix them, let stand for 20 minutes. Then add 170 mg of magnesium hydroxycarbonate, wait 20 minutes, shaking intermittently, centrifuge for 10 minutes, and pipette 1 ml of supernatant and measure iron, treating it as serum[15].

Serum Selenium

Selenium in serum was measured by atomic absorption (flameless)[16].

Determination of Magnesium and Calcium

Samples used for determination of magnesium and calcium were diluted 1:50 with 1% lanthanum chloride solution to exclude the effect of serum

phosphate[15]. Standard solution of 1000 ppm was diluted with deionized water. Working standards of magnesium were (0,5,10,15,20,25, and 30 µ mol\L). Working standards of calcium were (0,20,40,60,80,100, and 120 µ mol\L)

Serum Albumin:

Albumin in serum was measured by Bromocresol Green (BCG) method[15] depending on the procedure of Iraqi Sera and Vaccines Institution kit. The measurement of serum albumin is based on its quantitative binding to the indicator bromocresol green (3,3',5,5',tetrabromo-m-cresolsulphonphthalin). The albumin-BCG-complex absorbs maximally at 578 nm.

Hemoglobin determination

The cyanomethaemoglobin using Drabkin test was applied. Five ml of Drabkin solution was added to 0.02 ml whole blood and allow the tube to stand for 10 minutes. The absorbance is measured, against the blank in the photoelectric calorimeter at 540 nm, find the concentration of hemoglobin from the calibration curve with the following working standard of hemoglobin (12 gm \ 100 ml, 10 gm \ 100 ml, 7.5 gm\ 100 ml and 5 gm \ 100 ml) mix volume of standard solution of hemoglobin solution with two volume of Drab kin solution[15].

Statistical analysis:

Frequency tables used statistical tests were done using correlation tests P values < 0.05 were considered significant.

Results

The present sample constitutes 157 patients studied randomly from the Thalassaemic Center (center for anemia of Mediterranean origin) in Ibn- Al-Balady hospital \Baghdad.

Table 2 shows that there were 99 (63.06%) males, and 58 (36.94%) females in the sample, there were 73 (46.5%) patients below the age of 10 years with the mean age 10.8 years, minimal age in years was 0.75 and maximal age 28.0, while there were 88 (56.1%) of patients below the age of one year on diagnosis, and 129 (82.2%) of patients below the age of 3 years on diagnosis, the mean age on diagnosis was 1.6 year mode 0.5, the minimal age in years was 0.08 and the maximal age was 17.

In the sample there were 112 (71.3%) patients from Baghdad, 21 (13.4%) from Diyala, 12 (7.6%) from Wasit, 4 from Karballa, 2 (1.3%) from Najaf and from Anbar, while there was only one patient (0.6%) from Karkuk, Babel and Theekaar governorate. The sample also shows that there were 107 (68.2%) patients from urban and 50 (31.8%) patients from rural areas.

Table 2: Distribution of the sample (157 patients) according to different variables

Variables	Number	percent	Cumulative percent
Age of patient/year			
<10.0	73	46.5	46.5
10-19.9	67	42.7	89.2
20-29.9	17	10.8	100.0
Mean 10.8			
Mode 10.0			
STD 10.0			
Minimum 0.75			
Maximum 28.0			

Table 2: *Continued*

Age on diagnosis/year			
<1.0	88	56.1	56.1
1-2.99	41	26.1	82.2
3-4.99	14	8.9	91.1
5-9.99	11	7.0	98.1
10-19.99	2	1.3	99.4
15-19.99	1	0.6	100.0
Mean 1.6			
Mode 0.5			
STD 1.07			
Minimum 0.08			
Maximum 17.0			
Sex			
Male	99	63.1	63.1
Female	58	36.9	100.0
Address			
Baghdad	112	71.3	71.3
Wassait	12	7.6	79.0
Karkuk	1	0.6	79.6
Diala	21	13.4	93.0
Karballa	4	2.5	95.5
Najaf	1	0.6	96.2
Salah-Aldeen	2	1.3	97.5
Anbar	2	1.3	98.7
Babil	1	1.6	99.4
Thee-Kaar	1	0.6	100.0
Residency			
Urban	107	68.2	68.2
Rural	50	31.8	100.0

Table 3 shows that 121 (77.1%) of patients were suffering from thalassaemia major, 31 (19.7%) from intermedia, and one only from thalassaemia minor, there were also four with Alpha thalassaemia in the sample.

Table 3: The distribution of the sample according to the diagnosis

Variables	Number	percent	Cumulative percent
Thalassemia Major	121	77.1	77.1
Intermediate	31	19.7	96.8
Minor	1	o.6	97.5
Alpha thalassemia	4	2.5	100.0

Table 4 demonstrate that the mean hemoglobin level was 8.4 g\ 100 ml among the patients with the mode being 8.0, and the minimal hemoglobin was 5.0 g\ 100 ml and the maximal hemoglobin reading was 11.0 g\ 100ml.

Table 4: Distribution of the sample according to anemia and age groups

Age groups\years	Frequency	%	Mean Hb	St.d	Minimum	Maximum
< 5	26	16.6	9.1	0.84	7.5	10.5
5-9.99	47	29.9	8.3	1.0	6.0	10.5
10-19.99	67	42.7	8.2	1.12	5.0	11.5
20-29.99	17	10.8	8.2	0.91	7.0	10.0
Total	157	100.0	8.4	1.07	5.0	11.0

Table 5 showed that among the sample group the dominating blood group was group O (51 patients 32.5%), then group B (50 patients 31.8%), while blood group A was found in 42 patients (26.8)%), group AB was found in 14 patients (8.9%). Rh factor was mostly positive in 144 patients (91.7%), while it was negative in 13 (8.3%) patients.

Table 5: Distribution of the sample according to their blood group & Rh factors

Variables	Number	Percent	Cumulative percent
Blood groups			
Group A	42	26.8	26.8
Group B	50	31.8	58.6
Group AB	14	8.9	67.5
Group O	51	32.5	100.0
Total	157	100.0	
Rh factors			
Positive	144	91.7	91.7
Negative	13	8.3	100.0
Total	157	100.0	

Table 6 showed that there were 108 (68.8%) of patients who require blood transfusion between 2-4 weeks, 48 (30.6%) of patients require transfusion in > 4 weeks, while only one patient require transfusion in < 2 weeks time. Desferol treatment were taken > 4 times \week in 99 (63.1%) patients, between 3-4 times \week in 46 (29.3%) of patients, and < 3 times \week in 12 (7.6%) patients.

Table 6: Show type and frequency of treatment given to sample group

Type of treatment &frequency	Number	Percent	Cumulative percent
Blood transfusion			
<2 weeks	1	0.6	0.6
2-4 weeks	108	68.8	69.4
> 4 weeks	48	30.6	100.0
Total	157	100.0	
Desferol			
< 3 times\ week	12	7.6	7.6
3-4 times\ week	46	29.3	36.9
> 4 times\ week	99	63.1	100.0
Total	157	100.0	

Table 7 showed that 76 (48.4 %) of patients were having under nutrition according to the International accepted range of BMI, while 74 patients (47.1 %) were regarded as normal, and only 7 patients (4.5%) were regarded as over weight.

Table 7: Distribution of patients in the sample according to basal metabolic rate (BMI)

BMI	Frequency	Percent	Cumulative Percent
Under weight	76	48.4	48.4
Normal	74	47.1	96.1
Over weight	7	4.5	100
Total	157	100	---

Table 8 showed that there were 126(80.3%) of patients who were pale at time of examination, 93(59.2%) with jaundice, 95(60.5%) with frontal bossing, 76(48.4%) of patients with mongoloid features, 69(43.9%) with liver enlargement, 78(49.7%) with spleen enlargement, while there were only 5(3.2%) with heart failure and 2(1.3%) with diabetes mellitus.

Table 8: Distribution of patients in the sample according to the presence or absence of some clinical manifestation

Variables		Number	Percent	Cumulative percent
Pale	Yes	126	80.3	80.3
	No	31	19.7	100.0
Jaundice	Yes	93	59.2	59.2
	No	64	40.8	100.0
Frontal bossing	Yes	95	60.5	60.5
	No	62	39.5	100.0
Bronze skin	Yes	84	53.5	53.5
	No	73	46.5	100.0
Mongoloid features	Yes	76	48.4	48.4
	No	81	51.6	100.0
Liver enlargement	Yes	69	43.9	43.9
	No	88	56.1	100.0
Spleen enlargement	Yes	8	49.7	49.7
	No	59	50.3	100.0
Heart failure	Yes	5	3.2	3.2
	No	152	96.8	100.0
Diabetes	Yes	2	1.3	1.3
	No	155	98.7	100.0

When the relationship between different clinical manifestation was correlated (Table 9a) it was found that, pale skin was correlated with bronze skin, mongoloid features, diabetes mellitus and heart failure. Jaundice was correlated with frontal bossing, bronze skin, mongoloid features and enlarged spleen. Frontal bossing was correlated with jaundice, bronze skin, mongoloid features; enlarge spleen & frequency of treatment with desferol. Bronze skin was correlated with pale skin, jaundice, frontal bossing, mongoloid features, enlarge spleen. Mongoloid features were correlated with pale skin, jaundice, frontal bossing, and bronze skin and enlarge spleen. Enlarge liver was correlated with enlarge spleen and heart failure. Enlarge spleen was correlated with jaundice frontal bossing mongoloid features bronze skin enlarge liver and heart failure.

Table 9a: The association between different manifestations of patients in the sample

Variables	Pale	Jaundice	F. Bossing	B. Skin	M. Feature	E. Liver	E. Spleen
Pale							
P. Correlation	----------	-.086		-.233**	-.251**	-.306**	-.507**
Significant	-	.285	-.009	.003	.002	.000	.000
Number		157	157	157	157	157	157
Jaundice							
P. Correlation	-.086	----------	.084	.239**	.081	.093	.074
Significant	.285	-	.298	.003	.312	.248	.355
Number	157		157	157	157	157	157

Table 9a: *Continued*

F. Bossing							
P. Correlation	-.073	.311	----------	.112	.045	.029	-.021
Significant	.361	.000		.161	.572	.718	.790
Number	157	157		157	157	157	157
Bronze skin							
P. Correlation	-.302**	.448**	.007	---------	.033	-.028	-.059
Significant	.000	.000	.929		.682	.725	.463
Number	157	157	157		157	157	157
M. Feature							
P. Correlation	-.224**	.414**	.117	-.049	----------	-.049	-.094
Significant	.005	.000	.145	.544		.546	.240
Number	157	157	157	157		157	157
Enlarge liver							
P. Correlation	-.044	.108	-.002	.115	.064	----------	-.087
Significant	.581	.179	.976	.156	.430	-	.282
Number	157	157	154	154	154		154
Enlarge spleen							
P. Correlation	-.083	.254**	.095	.139	.236**	.018	----------
Significant	.300	.001	.237	.082	.003	.824	
Number	157	157	157	157	157	157	
Diabetes							
P. Correlation	-.229**	-.021	.173*	.172*	.030	.217*	.155
Significant	.004	.791	.044	.046	.727	.011	.072
Number	157	157	136	136	136	136	136
Heart failure							
P. Correlation	-.183*	.150	.036	.137	.051	.170	.033
Significant	.021	.060	.662	.093	.539	.038	.687
Number	157	157	150	150	150	150	150

*= Significant at 0.05 level
** = Significant at 0.01 level

Table 9b showed that diabetes mellitus was found in patients with pale skin & heart failure. Heart failure was found in patients with pale skin; enlarge liver & spleen, diabetes mellitus. Treatment with blood transfusion was correlated with mongoloid features, while desferol treatment was correlated with frontal bossing; mongoloid features, and enlarges liver. Blood groups were correlated with enlarge liver; while Rh factors were correlated with enlarge liver & spleen. Basal metabolic rate (BMI) was correlated with jaundice, frontal bossing, mongoloid features, and enlarge spleen.

Table 9b: The association between different manifestations of patients in the sample

Variables	DM	Heart failure	Freq. Blood transfusion	Freq. Desferol	Blood group	Rh. Factor	Hb	BMI
Pale								
P. Correlation	-.229**	-.183**	-.111	-.091	-.055	-.073	-.090	.007
Significant	.004	.021	.165	.292	.504	.375	.265	.930
Number	157	157	157	136	150	150	156	151
Jaundice								
P. Correlation	-.021	.150	.078	.111	.060	.088	.070	-.384**
Significant	.791	.060	.331	.198	.464	.286	.385	.000
Number	157	157	157	136	150	150	156	151

Table 9b: *Continued*

F. Bossing								
P. Correlation	-.027	.072	.95	.173*	.036	.039	.113	-.176*
Significant	.761	.368	.237	.044	.662	.634	.160	.031
Number	157	157	157	136	150	150	156	151
Bronze skin								
P. Correlation	.106	.096	.139	.172*	.137	.155	.159*	-.116
Significant	.187	.230	.082	.046	.093	.059	.048	.157
Number	157	157	157	136	150	150	156	151
M. Feature								
P. Correlation	.117	.115	.236**	.030	.051	.066	.122	-.202*
Significant	.144	.153	.003	.727	.539	.426	.129	.013
Number	157	157	157	136	150	150	156	151
Enlarge liver								
P. Correlation	-.014	.205*	.018	.217*	.170*	.194*	.105	-.141
Significant	.863	.010	.824	.011	.038	.017	.193	.085
Number	157	157	157	136	150	150	156	151
Enlarge spleen								
P. Correlation	.001	.183*	.063	.155	.033	.230**	.121	-.223*
Significant	.993	.022	.430	.072	.687	.005	.133	.006
Number	157	157	157	136	150	150	156	151
Diabetes								
P. Correlation	----------	.303**	.072	-.074	.095	.033	.011	.009
Significant	--	.000	.370	.393	.249	.691	.888	.917
Number		157	157	136	150	150	156	151
Heart failure								
P. Correlation	.303**	----------	.115	.042	.135	.047	-.022	-.145
Significant	.000		.115	.623	.100	.572	.788	.076
Number	157		157	136	150	150	156	151

*= Significant at 0.05 level
** = Significant at 0.01 level

Table 9c showed the correlation between different clinical features and blood level of trace elements, minerals and albumin and it showed that serum copper was correlated with enlarge liver. Serum Selenium level in blood was correlated with frontal bossing, bronze skin, mongoloid features. Hemoglobin concentration in blood was correlated with bronze skin. Serum iron & total iron binding capacity (TIBC) were correlated with pale skin only.

Table 9c: The association between different manifestations of patients in the sample

Variables	Cu	Zn	Selenium	Ca	Mg	Albumin	S. Iron	TIBC
Pale								
P. Correlation	.077	-.046	.093	-027	.073	.053	-.205*	.250**
Significant	.336	.570	.244	.740	.367	.506	.013	.002
Number	157	157	157	157	157	157	146	146
Jaundice								
P. Correlation	-.031	-.077	-.120	.094	-.062	.001	-.125	-.042
Significant	.701	.336	.135	.242	.443	.513	.133	.612
Number	157	157	157	157	157	157	146	146
F. Bossing								
P. Correlation	.039	.080	.197*	.042	-.090	.053	-.091	.011
Significant	.627	.321	.014	.600	.264	.513	.274	.899
Number	157	157	157	157	157	157	146	146

Table 9c: *Continued*

Bronze skin								
P. Correlation	.027	.078	-.168*	.057	.001	.023	-.101	-.067
Significant	.740	.330	.036	.475	.993	.779	.224	.420
Number	157	157	157	157	157	157	146	146
M. Feature								
P. Correlation	-.002	.083	-.169*	.089	.022	.107	-.129	-.026
Significant	.978	.303	.034	.270	.784	.182	.121	.753
Number	157	157	157	157	157	157	146	146
Enlarge liver								
P. Correlation	-.164*	.078	.019	-.135	.021	.028	.108	.104
Significant	.040	.331	.814	.091	.799	.729	.193	.212
Number	157	157	157	157	157	157	146	146
Enlarge spleen								
P. Correlation	-.086	.073	.002	-.085	-.107	-.004	.000	.029
Significant	.284	.360	.980	.290	.183	.960	.999	.724
Number	157	157	157	157	157	157	146	146
Diabetes								
P. Correlation	.005	.006	.020	.086	.086	-.079	.082	.122
Significant	.947	.937	.806	.284	.284	.323	.323	.142
Number	157	157	157	157	157	157	146	146
Heart failure								
P. Correlation	.011	.008	.084	.096	-.012	-.037	.143	.146
Significant	.894	.917	.294	.232	.877	.650	.085	.078
Number	157	157	157	157	157	157	146	146

*= Significant at 0.05 level
** = Significant at 0.01 level

Tables 10a and b showed that the age of the patients was correlated with pale skin, jaundice, frontal bossing, bronze skin, mongoloid features, liver and spleen enlargement, frequency of blood transfusion, diabetes mellitus, heart failure, BMI, and hemoglobin level ion blood. Sex of the patients was correlated with bronze skin, hemoglobin level and blood groups. Address, residency, and diagnosis of the disease were not correlated with any of the studying variables. Age on diagnosis was correlated with BMI only.

Table 10a: The occurrence of different clinical manifestation in the sample of patients in association with different variables

Variables	Pale	Jaundice	F. Bossing	B. Skin	M. Feature	E. Liver	E. Spleen
Age							
P. Correlation	.160*	-.234**	-.208**	-.233**	-.251**	-.306**	-.507**
Significant	.045	.003	-.009	.003	.002	.000	.000
Number	157	157	157	157	157	157	157
Sex							
P. Correlation	-.081	.090	.084	.239**	.081	.093	.074
Significant	.331	.262	.298	.003	.312	.248	.355
Number	157	157	157	157	157	157	157
Address							
P. Correlation	.011	-.085	.010	.112	.045	.029	-.021
Significant	.895	.291	.903	.161	.572	.718	.790
Number	157	157	157	157	157	157	157

Table 10a: *Continued*

Residency							
P. Correlation	-.133	-.036	.007	.048	.033	-.028	-.059
Significant	.097	.633	.929	.550	.682	.725	.463
Number	157	157	157	157	157	157	157
Diagnosis							
P. Correlation	-.005	-.112	.117	-.049	.049	-.049	-.094
Significant	.949	.162	.145	.544	.546	.546	.240
Number	157	157	157	157	157	157	157
Age on diagnosis							
P. Correlation	-.042	-.094	-.002	.115	.064	-.018	-.087
Significant	.603	.244	.976	.156	.430	.820	.282
Number	154	154	154	154	154	154	154

*= Significant at 0.05 level
** = Significant at 0.01 level

Table 10b: The occurrence of different clinical manifestation in the sample of patients in association with different variables

Variables	Freq. Blood	Freq. desferol	DM	Heart failure	BMI	Hb	Blood group	Rh factor
Age								
P. Correlation	-.256*	.024	-.191*	-.203**	.346**	-.216**	-.021	-.119
Significant	.001	.784	-.017	.011	.000	.007	.798	.146
Number	157	136	157	157	151`	156	150	150
Sex								
P. Correlation	-.066	.147	.087	.064	.121	.230**	.197*	.055
Significant	.411	.087	.279	.428	.139	.004	.016	.501
Number	157	136	157	157	151	156	150	150
Address								
P. Correlation	.014	-.064	.058	.031	-.010	.034	.094	.091
Significant	.864	.459	.469	.701	.903	.671	.251	.269
Number	157	136	157	157	151	156	150	150
Residency								
P. Correlation	.030	-.041	-.044	.032	-.002	-.058	-.073	-.021
Significant	.710	.636	.582	.693	.984	.469	.375	.801
Number	157	136	157	157	151	156	150	150
Diagnosis								
P. Correlation	.104	-.020	.045	-.072	-.015	-.029	-.049	-.053
Significant	.193	.815	.574	.369	.850	.720	.550	.523
Number	157	136	157	157	151	156	150	150
Age on diagnosis								
P. Correlation	-.003	-.038	-.100	-.048	.163**	-.067	.146	-,089
Significant	.967	.666	.217	.553	.047	.413	.078	.286
Number	154	133	154	157	148	153	147	147

*= Significant at 0.05 level
** = Significant at 0.01 level

Table 10c showed that only age on diagnosis and frequency of desferol treatment were correlated with serum level of magnesium, while there were no significant correlation between other variables studied with serum level of mineral, trace element and albumin level in blood.

Table 10c: The blood levels of different trace element in patients in association with different variables

Variables	Cu	Zn	Selenium	Ca	Mg	Albumin	S. Iron	TIBC
Age								
P. Correlation	.017	-.076	.052	-.084	.090	.034	.126	.073
Significant	.834	.342	.521	.297	.263	.677	.130	.379
Number	157	157	157	157	157	157	146	146
Sex								
P. Correlation	-.001	.097	-.030	-.156	.020	-.063	-.120	-.126
Significant	.994	.226	.708	.052	.808	.432	.150	.129
Number	157	157	157	157	157	157	146	146
Address								
P. Correlation	-.043	-.043	.007	-.001	-.016	-.117	-.039	-.112
Significant	.592	.592	.926	.989	.840	.146	.643	.180
Number	157	157	157	157	157	157	146	146
Residency								
P. Correlation	.072	-.063	-.037	-.099	-.135	-.140	.078	.060
Significant	.370	.436	.642	.215	.092	.081	.349	.473
Number	157	157	157	157	157	157	146	146
Diagnosis								
P. Correlation	-.037	.065	.063	.030	-.038	-.061	.051	.053
Significant	.645	.416	.435	.711	.634	.444	.541	.522
Number	157	157	157	157	157	157	146	146
Age on diagnosis								
P. Correlation	-.004	-.062	-.095	-.055	.161*	-.072	.076	.072
Significant	.957	.448	.242	.501	.046	.375	.364	.393
Number	154	154	154	154	154	154	144	144

*= Significant at 0.05 level
** = Significant at 0.01 level

Discussion

The present sample was taken from the Thalassaemic Centre in Ibn-Al-Balady hospital in Baghdad; this center is the only center for thalassaemia in Baghdad. The name of the center was changed recently to Center of anemia of Mediterranean Region, for this reasons we can see that patients included in this sample were from ten governorate, but mostly from Baghdad 121(77.1%), and then from Diyala 21(13.4%), Wassiet12 (7.6%).

This could be explained by the fact that patients living in Baghdad and Deilla can more easily come to the center for blood transfusion and treatment and that doctors in these areas are more aware about this condition, so the diagnosis and treatment are more available to patients. On the other hand, patients living in far areas probably will not have the chance for early diagnosis and treatment and die from the disease in early age, or it is very difficult for their families to come to the center in proper time for blood transfusion and therapy. In addition, the prevalence of the disease might be higher in these areas than in other areas in our country, the same observation could be applied to the distribution of the sample according to the residency of patients as the number of patient were 107 (68.2%) from urban areas while they were 50 (31.8%) patients from rural areas.

The finding that more than half of the patients 88 (56.1%) were diagnosed before the age of one year and 129 (82.2%) were diagnosed before the age of three years, this agree with the fact that 77.1% of patients in the sample were suffering from thalassaemia major, most of those patients usually developed severe anemia early in their life[17].

The patients age were mostly <10.0 years (73 patients 46.5%), and there was no patient above the age of 30 years, these finding can be explained by the fact that most of the patients included in the sample were those suffering from thalassaemia major with shorter life period, in a study done by Zurlo etal[18], they found that the over all survival from birth for patients born in 1970-74 was 97.4% at 10 years and 94.4% at 15 years, the most common cause of death was heart disease, followed by infection, liver disease and malignancy. Modell[19] showed that patients who adhere fully to treatment usually complete their education, work, and find a partner, and are expected to live at least until their mid- forties.

In the past decade, treatment of patients with beta-thalassemia has changed considerably, with advances in red cell transfusion and the introduction of iron chelation therapy. This progress has greatly increased the probability for a thalassemic child to reach adult age with a good quality of life[20]. Currently all newborns in the United States are screened for hemoglobinopathies, if a newborn screen returns with large amounts of fetal hemoglobin, alpha hemoglobin, or hemoglobin E, further investigation for thalassemia takes place[21].

In the present sample male patients 99 (63.1%) constituted higher number than females 58 (36.9%), this could be explained by the fact that people especially in developing countries are more concern about their male children than female children.

Treatment of patients with thalassemia major has improved dramatically during the past 40 years; however, the status of these patients remains poorly characterized[22].

The mean Hb. level in the present sample was 8.4 g\dl with the minimal reading of 5.0 g\dl and maximum of 11.0 g\dl, this result is expected since those blood samples were taken from patients who were coming for blood transfusion and accordingly their Hb. level were expected to be low, when the Hb. level was correlated with the clinical manifestation, it was found that it correlated significantly with bronze skin only, while pale looking was correlated with bronze skin, mongoloid feature, diabetes mellitus, heart failure which signified severe complication of thalassemia, and again the patients examined were in the majority suffering from thalassemia major.

Blood group O was the dominating blood group among the sample of patients then comes blood group B, A, AB. The National blood transfusion centre in Baghdad (1988-1993) recorded that blood group O shows the highest percentage (31%) among people attending the blood bank for giving blood then comes group A, B, AB (personal contact), the higher prevalence of blood group B in the thalassemic patients than group A could be due to chance only, or possibly that people with blood group B are more prone to develop thalassemia, a suggestion which need to be studied in a wider and more generalized form, since we could not find a reference which touch this particular point (blood group difference in thalassemic patient and general population).

As for treatment with blood transfusion, it was found that most of the patients (108 68.8%) where having blood transfusion between 2-4 weeks and desferol treatment > 4 times \ week (99 patients 63.1%), which indicate reasonable treatment program, this does not coincide with the severity of clinical manifestation found among the patients, which could be explained by the fact that those patients probably need more aggressive treatment than the one planned for them, on the other hand regular blood transfusion will inevitably lead to multi-organ

hemosiderosis and are attended by risks of blood-born infections[23].

In the present sample the BMI showed that around half of the patients were underweight and only 7 patient were overweight, which means that a good number of patient were suffering from growth retardation, this finding coincide with the finding that large proportion of patients were suffering from thalassemia major with symptomatic manifestation of the disease, and probably those patients are in need of more aggressive treatment than the present one.

The clinical picture of inadequately treated β thalassaemia is characterized by anemia, spleenomegaly, bone changes, and being prone to infection[24], the severity of the disease is extremely variable, and those so called major forms of the illness reflect the severe end of a spectrum that stretches from less severe anemias, which do not require transfusion, through intermediate forms of β thalassaemia to the completely symptom less conditions that are identified only by chance[25].

Clinically patient in the present sample showed high proportion of clinical manifestation of severe thalassaemia, for example, there were 126 (80.3%) of patients who were pale (the mean hemoglobin level was 8.4 g\100ml), 93 (59.2%) of them were with jaundice, 84 (53.5%) with bronze skin.

One of an important issue to be considered during follow up of patient with thalassaemia is bone abnormalities characterized by bone marrow expansion of the medullary cavity, and osteopenia with cortical thicking and trabecular coursing[26], in the present sample, it was found that 95 (60.5%) of patients had frontal bossing, and 76 (48.4%) had mongoloid features (complication of bone marrow expansion).

Hepatospleenomegaly had been mention in many articles[19,24,27-29] especially in those patients suffering from severe form of thalassaemia due to hypertrophy of ineffective bone marrow, in the present sample we find that 69 (43.9%) of patients suffers from liver enlargement, and 78 (49.7%) suffer from enlarge spleen.

There were 5 (3.2%) patients with heart failure in the sample group, it is well Known fact that if excess iron derived from transfusion is not removed patients die in the second or third decade from iron loading of the myocardium[30,31]. In a study done by Ferrara et al[32] 2004, they found that patients with thalassemia major in the study sample showed marked reduction in contractile state and milder LV than in thalassemia intermedia. It is important to notice that classic changes of untreated thalassaemia major are now regularly seen only in countries without resources to support long-term transfusion programs[9].

The striking increases in survival in patients with β thalassemia over the last decade have focused attention on abnormal endocrine function, for example diabetes mellitus was observed in 5% of adults which is due to exhaustion of beta cell and reduce circulating insulin concentration[33,34], in the present sample only 2 (1.3%) of patients were having diabetes mellitus, this could explain by the fact that most of the patients in this sample are of younger age with severe clinical symptom of thalassemia major, which makes the possibility of detecting diabetes mellitus among them less likely than those who are with better treatment programs and older in age.

Growth retardation in early childhood is a consequence of severe anemia. It can be prevented (although not corrected) by an aggressive blood transfusion program[35]. Even in children optimally transfused, however, the preadolescent

growth spurt is delayed and curtailed, so that full potential stature is rarely realized[36]. Growth failure occurs as a result of low somatomedin activity, because the liver synthesizes somatomedin, hemosiderosis has been held responsible for preadolescent growth failure[37].

Filosa[38] concluded that puberty positively influences the bone mineral density only at the start of puberty, while subsequently, the degree of osteoporosis is the expression of widespread and chronic systemic damage due to the hematological phenotype. In the present sample, it was found that 48.4% of patients suffer from under weight, which means that about half of the patients their BMI level were below the normal, this result is expected since 77.1% of patients suffer from thalassemia major and a good percentage of them show different clinical manifestation of thalassemia, also age on diagnosis of the patient was found to be correlated with BMI, which is an expected finding.

Again, the finding of significant correlation between age of patient with most of the clinical feature of thalassemia, type of treatment applied and diabetis mellitus was expected one, while sex was correlated with bronze skin, Hb level, and blood groups only probably because 63.1% of patients were males, and parents of thalassemic children do care about their male children more than the female ones, so different clinical features were correlated less frequently with the sex of the patients.

The finding that the address, residency and diagnosis of the disease were not correlated with any of the studied variables could be due to the fact that the sample size is not big enough to show differences and most of the patients were from urban areas, the same things could be said for the finding that age on diagnosis and treatment with desferol were correlated with magnesium level in the blood, while other variables were not correlated with any of the trace elements, minerals, or albumin level in the patient blood

Recommendation

1. It is very important that more centers for thalassemia are to be established in different areas in our country, with increase efficiency as to include gene frequency
2. To established programs based on carrier screening and counseling of couples at marriage, preconception or early pregnancy, which can be done by simple hematological analysis. These programs are operating in several Mediterranean at risk population, and are very effective, as indicated by increasing knowledge on thalassemia and its prevention by the target population and by the marked decline of the incidence of thalassemia major.
3. Prenatal diagnosis by mutation analysis on PCR amplified DNA from chorionic villi Molecular diagnosis of homozygotes and identification of carrier of beta thalassemia may lead to improved clinical management of patients with the disorder and prevention of the birth of affected homozygotes.

References

1. Lukens, J.N.: The thalassmias and related disorders: Quantitative disorders of hemoglobin synthesis: In Lee, G.R., Forester, J., Lukens, J., Paras-Kevas, F., John, P., Greer, J.P., and Rodgers, G.M.: Wintrobe's clinical hematology 10th edition. Williams & Wilkins, A Waverly Company, 1999; p.p. 1405-48.
2. Weatherall, D.J.: The thalassemias. In: Stamatoyannopoulos, G., Nienhuis, A.W., Majerus, P.H., Varmus, H., 2nd eds. The molecular basis of blood diseases. Philadelphia, W.B. Saunders, 1994; p.p. 157-205.
3. Weatherall, D.J.: Fortnightly review: The thalassemia. BMJ, 1997; 314: 1675.

4. Williams, T.N., Maitland, K., Bennett, S., et al.: High incidence of malaria in a thalassemic children. Nature (in press).
5. Allen, S.J., O'Dennell, A., Alxander, N.D., et al.: Thalassemia protects children against disease caused by other infections as well as malaria. Proc Natl Acad Sci USA, 1997; 94: 14736-41.
6. Babiker, M.M., Bashir, N., and Sarsour, N.: Prevalence of thalassemia in schoolchildren in northeastern Badia, Jordan. East Mediterr Health J, 1999; 5 (6): 1165-70.
7. Olivieri, N.F.: The β thalassemias. N Engl J Med, 1999; 341 (18): 1407.
8. Orvieto, R., Leichter, I., Rachmilewitz, E.A., et al.: Bone density, mineral content, and cortical index in patients with thalassemia major and the correlation to their bone fracture, blood transfusion, and treatment with desferrioxamine. Calcif Tissue Int, 1992; 50: 397-9.
9. Camaschella, C., and Cappellini, M.D.: Thalassemia intermedia. Haematologica, 1995; 80: 58-68.
10. Summenton, C., Shetty, P., Sandle, L.N., and Watt, S.: Nutritional, metabolic and environmental disease In: Haslett, C., Chilvers, E.R., Boon, N.A. et al., Davidson's principle and practice of medicine 19th edition. Churchill Livingstone, 2002; p.p. 298.
11. Alcock, W.N.: Copper: In: Methods in clinical chemistry. Pesce, A.M. and Kaplan, L.A. (editors), Mosby Co. USA, 1987; p.p. 525-38.
12. Meret, S., and Henkin, K.I.: cited by: Gowenlock, H.A., McMurray, R.J., and McLauchan, M.D., Varly's Practical Clinical Biochemistry. 6th Ed. Heinemann Medical Books. London, 1988; p.p. 635-9.
13. Taylor, A., Bryant, T.N.: Cited by Gowenlock, H.A., McMurray, R.J., McLauchlan, M.D.: Varly's Practical Clinical Biochemistry.6th Ed. Heinemann Medical Books. London, 1988; p.p. 636-9.
14. Olson, A., and Hamlin, W.: A new method for serum iron and total iron-binding capacity by atomic absorption spectroscopy. Clin Chem, 1969; 15 (6): 438-45.
15. Gowenlock, H.A., McMurray, R.J., and McLauchlan, M.D.: Varly's Practical Clinical Biochemistry 6th Ed. Heinemann Medical Books. London, 1988; p.p. 627.
16. Koskelo, E.K.: Serum selenium in children during anti cancer chemotherapy. Eur J Clin Nutr, 1990; 44 (11): 799-802.
17. Weatherall, D.J.: ABC of clinical haematology: The hereditary anemias. BMJ, 1997; 314: 492.
18. Zurlo, M.G., De Stefano, P., Borgna-Pignatiti, C., et al.: Survival and causes of death in thalassemia major. Lancet, 1989; 1, 2 (8653): 27-30.
19. Modell, B., Khan, M., and Darlison, M.: Survival in β thalassemia major in the UK: data from UK thalassemia Register. Lancet, 2000; 355 (9220): 2051.
20. Galnello, R.: A thalassemic child becomes adult. Rev Clin Exp Hematol, 2003; 7 (1): 4-6.
21. Catlin, A.J.: Thalassemia: the facts and the controversies. Pediatr Nurs, 2003; 29 (6): 447-9, 451.
22. Chan, L.L., Lin, H.P., Ariffn, W.A., et al.: Providing a cure for beta thalassemia major. Med J Malaysia, 2001; 56 (4) : 435-40.
23. Cunningham, M.J., Macklin, E.A., Cohen, A.R., et al.: Complication of beta-thalassemia major in North America. Blood, 2004; 104 (1): 34-9.
24. Wheatherall, D.J.: Fortnightly review: The thalassemias. BMJ, 1997; 314: 1675.
25. Wheatherall, D.J.: The molecular basis for phenotypic variability of the common thalassemia. Mol Med Today, 1996; 1: 15-20.
26. Gratwich, G.M., Bullough, P.G., Bohne, W.T.T., et al.: Thalassemic osteoarthropathy. Ann Intern Med, 1978; 88 (4): 494-501.
27. Wood, W.G., and Weatherall, D.J.: Developmental genetics of the human haemoglobin. Biochem J, 1983; 215: 1-10.
28. Wood, W.G.: Increased HbF in adult life. Buillieres Clin Haematol, 1993; 6: 177-213.
29. Ho, P.J., Hall, G.W., Luo, L.Y., et al.: Beta thalassemia intermedia is it possible consistently to predict phenotype from genotype? Br J Haematol, 1998; 100: 70-8.
30. Brittenham, G.M., Griffith, P.M., Nienhuis, A.W., et al.: Efficacy of deferoxamine in preventing Complication of iron overload in patients with thalassemia major. N Engl J Med, 1994; 331: 567-73.
31. Olivieri, N.F., Nathan, D.G., Mac Millon, J.H., et al.: Survival in medically treated patients with homozygous β thalassemia N Engl J Med, 1994; 331: 574-8.
32. Ferrara, M., Matarese, S.M., Borrelli, B., et al.: Cardiac involvement in beta-thalassemia major and beta-thalassemia intermedia. Haemoglobin, 2004; 28: 123-9.
33. Italian Working Group on Endocrine Complications in Non-endocrine Diseases. Multicentre study on prevalence of endocrine complications in thalassemia major. Clin Endocrinol (OXF), 1995; 42: 581-6.

34. Cavallo-Perin, P., Pacini, G., Cerutti, F., et al.: Insulin resistance and hyperinsulinemia in homozygous β thalassemia. Metabolism, 1995; 44: 281-6..
35. Logothetis, J.: Body growth in Cooley 's anemia (homozygous beta-thalassemia) with a crrelative study as to other aspects of the illness in 138 cases. Pediatrics, 1972; 50: 92.
36. Borgna-Pignatti, C.: growth and sexual maturation in thalassemia major. J Pediatr, 1985; 106: 150.
37. Saenger, P.: Depressed serum somatomedin activity in β- thalassemia. J Pediatr, 1980; 96: 214.
38. Filosa, A., Di Maio, S., Vocca, S., et al.: Longitudinal monitoring of bone mineral density in thalassemic patients. Genetic structure and osteoporosis. Acta Paediatr, 1997; 86 (4): 342-6.

ANTIOXIDANT STATUS IN THALASSEMIC PATIENTS

Subuh S. AL-Mudalal[1] *MSc FICMS*, **Raad J. Musa**[1] *MSc FICMS*,
Najat AL-Duliami[2] *MSc PhD*

Abstract

Background: increased membrane lipid peroxidation in patients with thalassemia has been reported suggesting that superoxide radicals generated in excess following auto-oxidation of isolated hemoglobin chains is an important contributor to the hemolytic process.

Objective: This study was undertaken to evaluate the extent of lipid peroxidation and antioxidant status of patients with beta-thalassemia in comparison to healthy people.

Methods: Red cell superoxide dismutase (SOD) activity and red cell catalase activity were measured in the biochemistry department for the period from January 2003 to October 2003, 76 patients with beta-thalassemia, 14 patients with beta-thalassemia minor and 19 healthy controls were studied.

Results: Erythrocytes of patients with beta-thalassemia major had significantly higher SOD than control ($p<0.0004$). Red cell catalase activity of thalassemia minor patients was significantly higher than that of the control ($p<0.05$). In thalassemic patients, the more anemic patients have significantly higher SOD activity, but this correlation was not present between anaemic patients & catalase activity.

Conclusion: Red cell superoxide dismutase activity was greatly increased in homozygous beta-thalassemia, and inversely correlated with severity of anaemia.

Keyword: SOD, Catalase, Thalassemia.

IRAQI J MED SCI, 2005; VOL. 4 (1): 38-44

Introduction

Auto-oxidation of biomembranes is considered to be the primary factor involved in cellular senescence and breakdown[1,2]. An increased production of highly activated forms of oxygen released during the oxidation of hemoglobin to methemoglobin in thalassemic red blood cells (RBC)[3-8] has stimulated much interest in superoxide dismutase (SOD) and cellular antioxidant for the control of such deleterious radical reactions. The aim of this study was to evaluate the extent of lipid peroxidation and antioxidant status of patients with beta-thalassemia in comparison to healthy people. Also to find any correlations between the level of these antioxidants with the appropriate time of transfusion.

Patients & Methods

During the period of ten months from January 2003 to October 2003, 109 subjects were included in this study; 76 patients with beta-thalassemia major, 14 patients with beta-thalassemia minor were taken from hematology center of Ibn-Balady Hospital, and 19 healthy controls were taken from laboratory healthy staff in Al-Kadhimiya Teaching Hospital.

Venous blood was collected from patients before blood transfusion and then hematological studies were done including; red cell count , white cell count , mean corpuscular volume and haematocrit were determined in a coulter counter ,MS9. haemoglobin concentrations were measured on a haemoglobinometer. Haemoglobin types and quantitation of different types

[1]Dept. pathology [2]Dept. Chemistry & Biochemistry, College of Medicine, Al-Nahrain University
Address Correspondence to Dr. Subuh S. Al-Mudalal
Received 20th November 2004: Accepted 14th March 2005.

were identified by variant haemoglobin testing system.

Enzyme assay: the method for SOD measurements is based on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium by superoxide radical, which is generated by the reaction of photoreduced riboflavin and oxygen[9]. The method for measuring catalase activity is based on the ability of catalase to decompose hydrogen peroxide[10]. Units of the enzyme assays were reported as mg/g Hb, mg/ml red blood cells.

Statistical Analysis

All values are given as mean + SD (standard deviation). The differences were assessed by unpaired student t-test. P< 0.05 was considered to be statistically significant. The correlation probability and correlation coefficient (R) of any two variables was computed by an AMSTRAD PCW 8256 computer using the AMSTAT computer program.

Results

The mean SOD of erythrocytes of thalassemia major patients was 1478.4 IU/ml, SD (standard deviations) = 346.79, SE (standard error) = 48.09, range: (1012.07-2556.82). The mean SOD of erythrocytes of thalassemia minor patients was 1189.0 IU/ml, SD (standard deviations) = 212.84, SE (standard error) = 56.88, range: (871.51-1568.72). The mean SOD of erythrocytes of healthy controls was 1261.7 IU/ml, SD (standard deviations) = 165.81, SE (standard error) = 40.22, range: (984.58-1547.19).

The mean Catalase of erythrocytes of thalassemia major patients was 5.0 mg/ml, SD (standard deviations) = 1.63, SE (standard error) = 0.23, range: (1.35-8.56). The mean Catalase of erythrocytes of thalassemia minor patients was 6.0 mg/ml, SD (standard deviations) = 1.78, SE (standard error) = 0.48, range: (3.35-10.08). The mean Catalase of erythrocytes of healthy controls was 5.3 mg/ml, SD (standard deviations) = 1.63, SE (standard error) = 0.39, range: (3.58-8.71). Figures 1 and 2 show the mean values of SOD and Catalase activities.

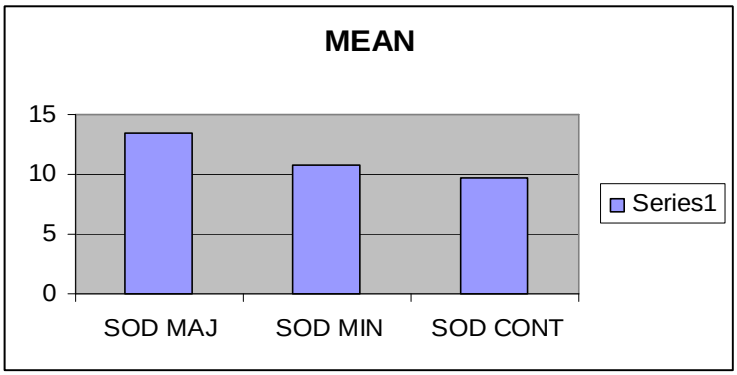


Figure 1: Bar Chart showing mean values of SOD for thalssemia major, thalassemia minor and control subjects

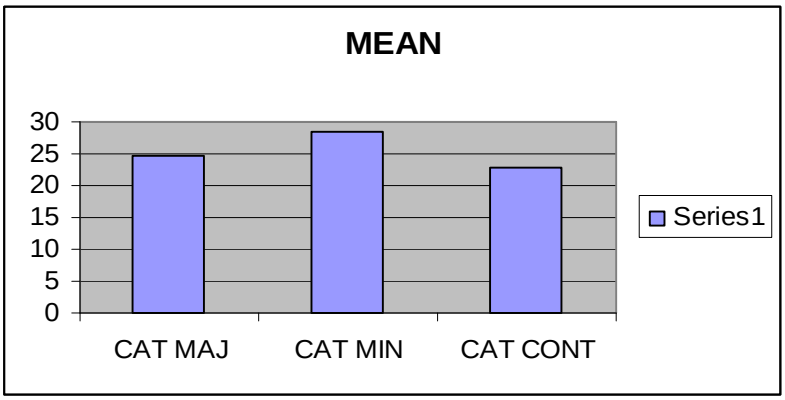


Figure 2: Bar chart showing mean values of catalase for thalassemia major, thalassemia minor and control subjects

Erythrocytes from thalassemia major have significantly higher SOD than control ($p<0.0004$), also erythrocytes of thalassemia minor had a higher SOD than that of the control but statistically was not significant ($p= 0.3138$), while erythrocytes of thalassemia major patients had a higher SOD than that of thalassemia minor patients but statistically was not significant ($p= 0.36817$).

Red cell catalase activity of thalassemia minor patients was significantly higher than that of the controls ($p<0.05$), also was higher than that of thalassemia major patients but statistically was not significant ($p= 0.1234$).

In thalassemic patients, the more anaemic patients had significantly higher SOD activity (Figure-3), but this correlation was not present between anaemic patients and Catalase activity (Figure-4).

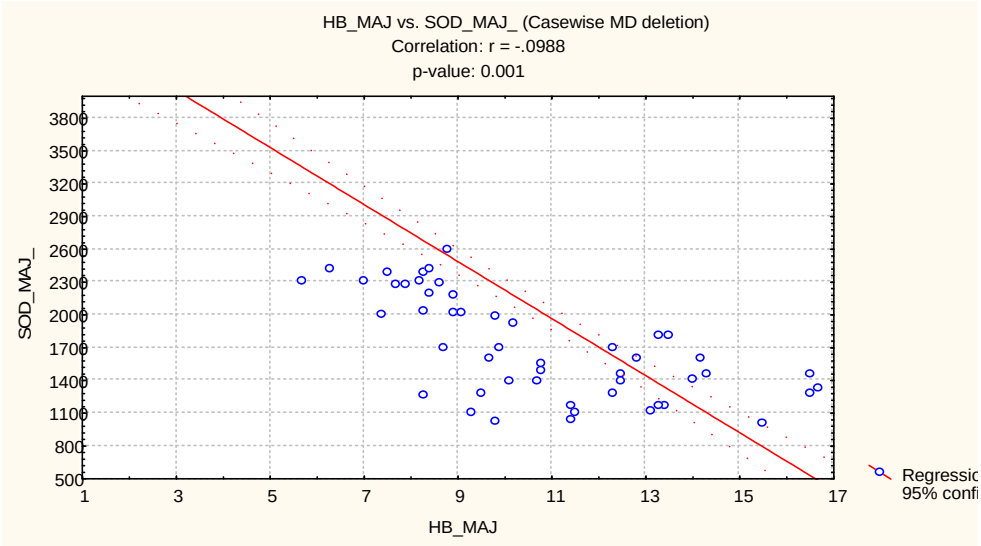


Figure 3: there is negative correlation between hemoglobin (G/dl) and SOD of erythrocytes in thalassemia major patients

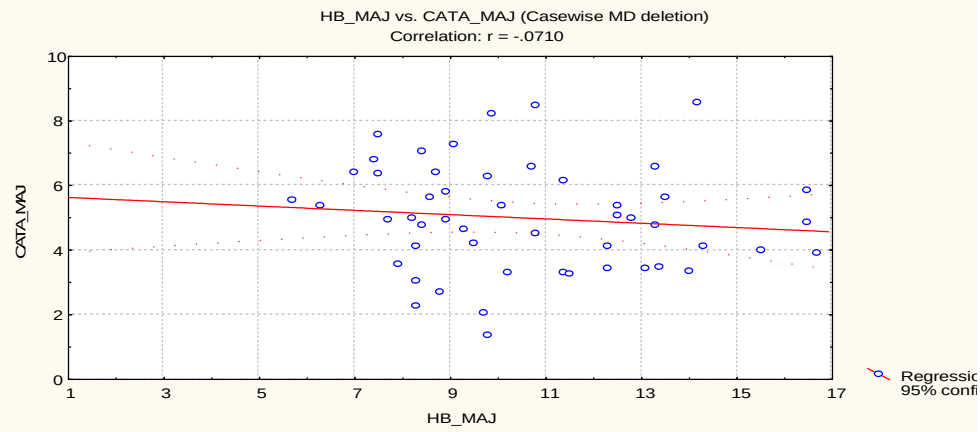


Figure 4: there is no correlation between hemoglobin (G/dl) and catalase of erythrocytes in thalassemia major patients

There was no significant correlation between SOD activity, Catalase activity and the last time of blood transfusion (Figures 5 and 6).

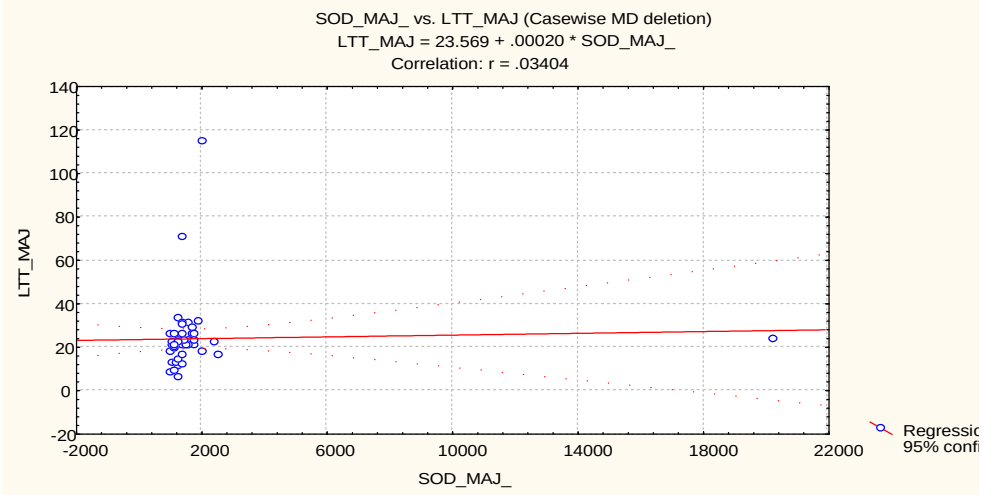


Figure 5: there is no correlation between SOD of thalssemia major patients with the last time of transfusion

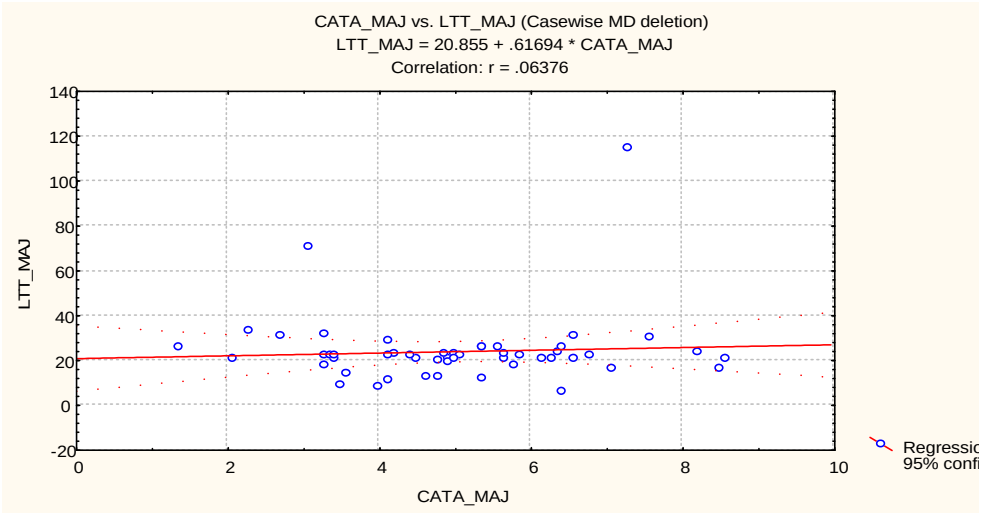


Figure 6: there is no correlation between Catalase of thalssemia major patients with the last time of transfusion

Discussion

In beta-thalassemic red blood cells (RBC), increased precipitation of alpha chain associated with increased oxidation of heme iron has been cited as the principal mechanism for generating activated species of molecular oxygen[3-6], conceivably, these activated forms of oxygen can be formed during transport of oxygen through the membrane as well as within the cell. In this context, the role of small molecules endowed with antioxidant activity would be more effective in protecting membrane lipids from undergoing free radical chain reactions. In contrast, SOD, being effective in the dismutation of superoxide anion in the cell, may be not accessible to the lipid matrix of the membrane due to its size and charged groups. Consequently, the effective radical scavenging action of SOD within the membrane would be markedly diminished[11].

Measurements of the specific activity of SOD in thalassemic RBC show a higher percent inhibition of cytochrome c per milligram of protein as compared with normal individuals. The increase of SOD specific activity found in RBC of thalassemic patients may be associated with increased production of superoxide anion. An induction of SOD was demonstrated in experiments on oxygen toxicity[12-14].

The results of this study concerning the increased specific activity of SOD in thalassemic RBC donot agree with reports from other laboratories(15) which have observed no differences in the level of SOD in normal and thalassemic RBC. Conceivably, the different assay methods employed in such comparison might explain the discrepancy, these methods describes by Nishikimi[22] and by Misra[23], both methods are based on inhibition by the enzyme of color development by chromogenic substance reacting with O_2^-.

In the method of Nishikimi, Nitro Blue Titrazolume acqueous solution giving blue formazan which can be monitored at 560nm. In the method of Misra; epinephrine at pH 10.2 acts both as the source of O_2^- and as the detecting system giving adrenochrome which can be monitored at 480nm. The interesting observation that emerges from this work is that high SOD activities found in thalasemic RBC apparently do not protect them from increased rate of autohemolysis, and polyamines might be more effective in protecting biomembranes against the deleterious effect of free radicals.

The findings of this study indicate that patients with more sever disease, beta thalassemia major, having a greater excess of alpha globin chain, also have higher activities of SOD but lower catalase activity than the milder genotype, beta thalassemia minor. Increased red cell SOD values in thalassemic patients have previously been explained as a reaction to, or compensation for the increased production of superoxide radicals, the amount of which is related to excess globin chain[16,19].

This study showed significantly lower red cell catalase activity (although higher than normal subjects) , in patients with the more sever form of the disease expressed both as per g Hb and per ml red blood cells and that was similar to other study[21] . A possible explanation for lower red cell catalase activity found in the more severe genotype of beta thalassemia is that the greater amount of hydrogen peroxide might produce direct toxic damage to catalase[17,18], the concentration of this is considerably reduced in conditions of high oxidative stress[20].

The increase of erythrocyte superoxide dismutase activities is most likely due to abnormalities specific to thalassemic red cells rather than an increased number of younger red cells for

reticulocytes and nucleated red blood cells did not affect the enzyme activity. Patients with beta-thalassemia major disease with lower haemoglobin concentration had significantly higher superoxide dismutase activities 16.

In all 76 subject with beta-thalassemia major, haemoglobin concentrations and superoxide dismutase activities were inversely correlated (r=-0.60) (p<0.001). This indicates that the amounts of superoxide generated in red cells may, at least partly, determine severity of disease; the increased superoxide dismutase activity in thalassemia is aresponse to superoxide generated in greater amounta because of accumulation of excessive globin chains and iron in the red cells.

Conclusion

1. Red cell superoxide dismutase activity was greatly increased in homozygous beta-thalssemia.
2. Lower red cell catalase activity was found in the more severe genotype of beta-thalassemia.
3. The more anaemic patients had significantly higher SOD activity.

References

1. Michelson, A.: superoxide and superoxide dismutase, Academic Press, New York 1977.
2. Anastassopoulou, J., Anifaantaakis, B., and Anifantakis, Z.: The role of free radical reactions with haemoglobin and thalassemia. J Inorg Bochem, 2000; 79 (1-4): 327-9.
3. Meral, A., Tuncel, P., Surmen-Gur, E., and Ozbek, R.: Lipid peroxidation and antioxidant status in beta-thalassemia. Pediatr Hematol Oncol, 2000; 17 (8): 687-93.
4. Pavri, R.S., Baxi, A., and Bhatia, H.M.: Oxidative injury to the red cell in thalassemia & the protective role of antioxidants. Indian J Med Res, 1988; 88: 356-9.
5. Suthipark, K., Ong-ajyooth, S., and Shumunmsirivath, D.: Oxidative stress and antioxidants in beta-thalassemia/Haemoglobin E. Birth Defects Orig Artic Ser, 1987; 23 (5A): 199-205.
6. Brunori, M., Falcioni, G., Fioretti, E., Giardina, B., and Rotilio, G.: Formation of superoxide in the auto-oxidation of isolated alpha and beta chains of human hemoglobin and its involvement in hemichrome precipitation. Eur J Biochem, 1975; 53: 99-104.
7. Carrel, R., Winterbourn, C., and Rachmile-Witz, E.: Activated oxygen and hemolysis. Br J Hemat, 1975; 30: 259-64.
8. Rotilio, G., Fioretti, E., Falcioni, G., and Brunori, M.: Generation of superoxide radical by heme proteins and its biological relevance; in Michelson, superoxide and superoxide dismutases, Academic Press, New York, 1977.
9. Sun, Y., Oberly, L.W., and Li, Y.: Asimple method for clinical assay of superoxide dismutase. Clin chem, 1983; 34: 497-500.
10. Aebi, H.: Catalase in method of enzymatic analysis. Bergmeyer, H.V., Grable, M., and Moss, D.W., 3rd edition, Volume II, 1983; p.p. 273-85.
11. Avanella, V., Rizza, S., Livolti, R.: Effect of polyamines on autohemolysis: Studies on normal and thalassemic children. Acta Hemat, 1980; 63:226-9.
12. Gregory, E., and Fridovich, I.: Introduction of superoxide dismutase by molecular oxygen. J Bact, 1973; 114: 543-8.
13. Gregory, E., and Fridovich, I.: Oxygen toxicity and superoxide dismutase. J Bact, 1973; 114: 1193-7.
14. Gregory, E., Goscin, S., and Fridovich, I.: Superoxide dismutase and oxygen toxicity in a eukaryote. J Bact, 1974; 117: 456-60.
15. Concetti, A., Massei, P., Rotilio, G., Brunori, M., and Rachmilewitz, E.: Superoxide dismutase in red blood cells: Method of assay and enzyme content in normal subjects and in patients with beta-thalassemia (major and intermedia). J Lab Clin Med, 1976; 87: 1057-64.
16. Yenchitsomanus, P., and Wasi, P.: Increased erythrocyte superoxide dismutase activities in beta-thalassemia/hemoglobin E and in hemoglobin H diseases. J Clin Pathol, 1983; 36: 329-33.
17. Kirkman, H.N., and Gaetani, G.F.: Catalase: tetrameric enzyme with four tightly bound molecules of NADPH. Proc Natl Acad Sci USA, 1984; 81: 4343-7.
18. Eaton, J.W., Martin, B., and Etkin, N.J.: Catalase activity and red cell metabolism. In: Brewer G.J., edition, Advances in experimental medicine and biology, New York: Plenum press, 1972; p.p. 121-31.
19. Gerli, G.C., Mongiat, R., and Sandri, M.T.: Antioxidant system and serum trace elements in alpha-thalassemia and Hb

Lepore trait. Eur J Haematol, 1987; 39 (1): 23-7.
20. Consolini, R., Calleri, A., and Legitimo, A.: Immunological evaluation of patients with beta-thalassemia major .Acta Hematol, 2001; 105 (1): 7-12.
21. Prasartkaew, S., Bunyaratvej, A., Fucharoen, S.: Comparison of erythrocyte antioxidative enzyme activities between two types of hemoglobin H. J Clin Pathol, 1986; 39: 1299-303.
22. Nishikimi, M., and Yagi, K.: The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun, 1972; 46: 849-54.
23. Misra, H.P., and Fridovich, I.: The role of superoxide anion in the autoxidation of epinephtrine and a simple assay for superoxide dismutase J Biol Chem, 1972; 247: 3170-5.

VASCULARIZATION IN PROSTATIC CARCINOMA

Faiza A. Al-Rawi *MBChB MSc FICPath.*

Abstract

Background: Angiogenesis means the creation of new blood vessels, a critical natural process that occurs in the body both in health and in disease.

Objective: Quantitative assessment of tumor vascularization in different grades of prostatic tumors.

Methods: 23 paraffin blocks of prostatic biopsies (8 cases of benign prostatic hyperplasia and 15 cases of protatic carcinoma equally distributed in well, moderately and poorly differentiated (Gleason's grade 2, 3 and 4&5 respectively). Processed routinely and stained with hematoxylin and eosin, elastica Van Gieson, and Masson Trichrome stain. The vascular surface density (VSD), the microvessels number (NVES), and the maximum microvessels number (NVES-MAX) was assessed by means of stereology, and the results were related to grade of tumor differentiation.

Results: NVES and NVES-MAX showed a significant increase with rising tumor grade ranging from 16.1 in BPH to 109.0 microvessels/mm^2 in poorly differentiated (grade 4&5) tumors. Discrimination of different tumor grades was more accurate with NVES-MAX. The VSD was significantly higher in low-grade tumors compared with BPH, whereas there was continuous decrease from low grade (11.6mm^{-1}) to high-grade tumor areas (5.1 mm^{-1}).

Conclusions: The present study shows a correlation between tumor grade and vascularization.

Key words: Prostate cancer, angiogenesis, vascularization.

IRAQI J MED SCI, 2005; VOL. 4 (1): 45-48

Introduction

Rapid cellular proliferation in malignant tumors requires nutritional support guaranteed by a sufficient vascular bed and unrestricted growth of tumors is dependent upon angiogenesis[1,2]. Recent studies have clarified the molecular basis of tumor neovascularization, a process mediated by several growth factors[3]. Angiogenic activity first appears in a subset of hyperplastic tissue before the onset of tumor formation[4,5]. Hyperplasia *per se* does not obligate angiogenesis. Rather, a few hyperplastic tissues become angiogenic correlates closely with subsequent tumor incidence[4,6]. The vessels surface in a tumor is a putative target of tumor cell adhesion and invasion, and consequence local and systemic spread[5-8]. On light microscopic level, the process of tumor angiogenesis results in an increase number of vessels within the tumor tissue, and areas of maximum vascularization, called hot spots[9,10].

Materials & Methods

This study comprises of 23 paraffin blocks of prostatic biopsies (8 cases of benign prostatic hyperplasia and 15 cases of protatic carcinoma equally distributed in well, moderately and poorly differentiated (Gleason's grade 2, 3 and 4 or 5 respectively). Fixed in 10% buffered formalin processed routinely and stained with hematoxylin and eosin, elastica Van Gieson, and Masson Trichrome stain.

Stereological measurements: Areas of unequivocal tumor tissue were marked on the cover slide. Within the tumor 10 areas were randomly chosen for

Dept. Pathology, College of Medicine, Al-Nahrain University

Received 8th May 2004: Accepted 29th November 2004.

assessment of the vascular surface density (VSD), microvessel number (NVES), and Maximum Microvessel number (NVES-MAX)[5].

VSD: The vascular surface density and the stromal volume portion were assessed by means of stereology at a x160 microscopic magnification[5].
An ocular square lattice (periplan x10, leitz Wetzlar, Germany) with 121 points composed of 11 horizontal and 11 vertical test lines with line length (Lg=2.6875Mm) was superimposed on the test fields to be measured, and the number of intersections (Ia) between the test lines and labeled vessels walls was counted (Figure 1). VSD was assessed according to (Barth et al 1996)[5].

VSD=E Ia.2, LR.Vv (STR)

Microvessel number (NVES); in the same procedure step, the number of vessel (N) within the measuring field was counted and the number of vessels per mm2 stroma (NVES) was computed according to
NVES= N/Vv (STR)

Maximum Microvessel number (NVES-MAX)[5]:
To simulate hot-spot measurements, maximum microvessels counts were computed from the arithmetic mean of microvessel counts in the tumor areas that yielded the 30% highest values.

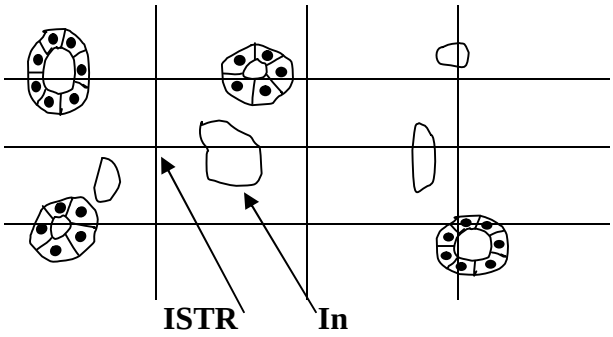


Figure1: Schematic drawing indicating the assessment of the stereological parameters VSD derived from the counts of intersections between test-lines and vessel walls (In) and points superimposed on the stromal compartment (ISTR).

Statistical analysis: Differences between study groups were performed by the analysis of variance procedure; P< .05 was defined to be a statistically significant value.

Results

In low-grade tumor, the vessels formed well-branched network of small capillary vessels, whereas in the intermediate and high-grade tumor the vascular bed was mainly composed of plump less branched vessels (Figures 2 and 3). In BPH, the average number of the vessels (NVES) and (NVES-MAX) was found to be 15.4+1.12 mm^{-2} and 16.1 mm^{-2} respectively.

In prostatic carcinoma, NVES and NVES-MAX increased significantly with rising tumor grade. However, no statistically significant difference could be confirmed between moderately (G3) and poorly differentiated (G4&5) carcinoma (Table 1).
Assessment of the VSD yielded completely different results. In well-differentiated tumors, the VSD reached value significantly higher compared with BPH (11.6 Vs 5.2). While in moderately and poorly differentiated carcinoma, significantly lower values compared with well-differentiated tumors were obtained, and not significantly different from that of BPH (Table 1).

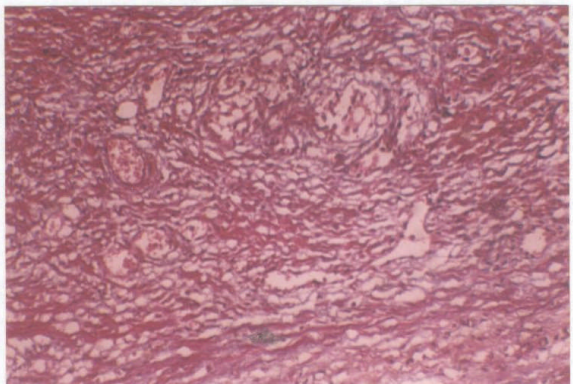


Figure 2: Morphology of low-grade prostatic carcinoma (G2). In this area the NVES is 58, the VSD is 16.3mm^{-1} (Trichrome stain)

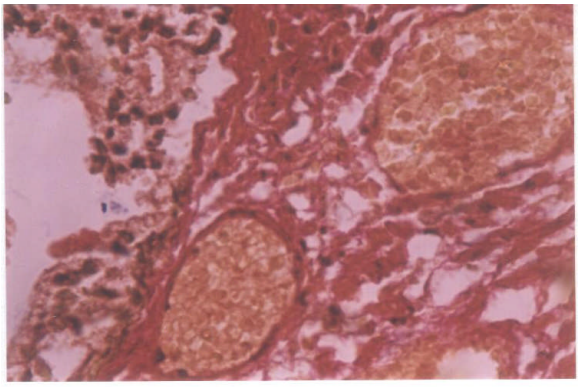


Figure 3: Morphology of high-grade prostatic carcinoma (G4). In this area the NVES is 110, the VSD is 7.3mm^{-1} (Trichrome stain)

Table 1: Vessel Parameters in Different Prostatic carcinoma grades

Method	BPH	Well differentiated carcinoma (G2)	Moderately differentiated carcinoma (G3)	Poorly differentiated carcinoma (G4&5)
VSD(mm^{-1})	5.2+0.2	11.6+1.3	9.2+3.1	5.1+0.6
NVES(mm^{-2})	15.4+1.1	52.7+23.6	75.7+22.6	105.0+0.4
NVES-MAX(mm^{-2})	16.1+2	69.8+21.1	97.5+19.6	109.0+0.4

P values < 0.5.

Discussion

The present study shows a correlation between tumor grade and vascularization, In low grade lesions (G1) the microvessel number increased with increasing tumor grade and data concerning the maximum microvessel number correspond well to those already published[5,7-11]. In contrast to counts of vessel profiles per area, the assessment of VSD showed no significant correlation to tumor grade. This behavior of the vascular surface density is not specific for prostate cancer and also has been reported in renal cell carcinoma[5,9]. In contrast to the microvessel number, the VSD concerns the physiological status of the vasculature as it quantifies the vascular surface available for substrate diffusion[4,5].

In normal tissue and low-grade tumors, the microvessel are well branched and show a high surface/vessel ratio, whereas the microvessel in high grade lesions are plump and non-branched, disclosing a low surface/vessel ratio[5,11]. Although in high-grade lesions the vascular surface is decreased, the vascular bed serves its function to supply the tumor tissue[5,8]. Possible explanations are an increased permeability of the vessel wall and changes of the tumoral vascular bed, which compensates the reduced vascular surface[5].

Counts of microvessel profiles per area unit tissue section provide a significant parameter for the prediction of tumor grade, whereas the VSD primarily reflects the vascular geometry and is only weakly related to the tumor grade. Nevertheless, both methods provide valuable information in studies concerning tumor angiogenesis.

References

1. Bigler, S.A., Deering, R.E., and Brawer, M.K.: Comparison of microscopic vascularity in benign

and malignant prostate tissue. Human Pathol, 1993; 24: 220-6.
2. Craft, P.S., and Harris, A.L.: Clinical prognostic significance of tumor angiogenesis. Ann Oncol, 1994; 5: 305-11.
3. Brawer, M.K., Deering, R.E., Brown, M., ands Jain, R.K.: Prediction of pathologic stage in prostatic carcinoma. Cancer, 1993; 73: 678-87.
4. Folkman, J., Watson, K., Ingber, D., and Hanahan, D.: Introduction of angiogenesis during the transition from hyperplasia to neoplasia. Nature. 1989; 339: 58-61.
5. Barth, P.J., Karl, W., Heinz, H., Kohler, and Adolfo, B.: Assessment of the vascularization in prostatic carcinoma. Human Pathol, 1996; 27: 1306.
6. Weidner, N., Carroll, P.R., Flax, J., Fox, J.B. and Brawer, M.K.: Tumor angiogenesis correlates with metastasis in prostate carcinoma. Am J Pathol, 1993; 143: 401.
7. Dickinson, A.J., Fox, S.B., Persad, R.A., Brawer, M.K., and Mostofi, F.K.: Quantification of angiogenesis as an independent predictor of prognosis in invasive bladder carcinoma. Br J Urol, 1994; 74: 762-6.
8. Weibel, E.R.: Principles and methods for the morphometric study of the lung and other organs. Lab Invest, 1963; 12: 131-55.
9. Kohler, H.H., Barth, P.J., Siebel, A., Bigler, S.A., Deering, R.F., and Braver, M.C.: Quantitative assessment of VSD in renal cell carcinomas. Br J Urol, 1996; 77: 650-54.
10. Asaichi, K., Endrich, B., Gotz, A. and Peter, W.: Quantitative analysis of microvascular structure and function in the melanoma. A-Mel3. Cancer Res, 1981; 41: 1898.
11. Hall, M.C., Troncoso, P., Pollack, A., Folkman, J., Sasse, J., and Graft, P.S.: significance of tumor angiogenesis in clinically localized prostate carcinoma treated with radiotherapy. Urology, 1994; 44: 869-75.

SERUM COPPER AND ZINC LEVELS AND COPPER/ZINC RATIO IN PATIENTS WITH RHEUMATOID ARTHRITIS

Yahya Y.Z. Farid[1] *BSc PhD*, **Alaa K. Mohammed**[2] *BSc PhD*,
Israa S. Mohammed[2] *BSc*

Abstract

Background: Trace elements particularly copper and zinc have a great role in a number of biological processes, therefore estimation of these metals in serum of Rheumatoid Arthritis (RA) patients may ameliorate the understanding of trends in the relationship between the serum copper and zinc levels and activity of disease.

Objective: To determine the serum copper and zinc levels and its relation with the degree of disease activity and compare with a normal group.

Methods: The investigation was carried out on forty-three healthy (25 females, 18 males) and fifty-four(37 females, 17 males) sick adults.The patients were subdivided according to the activity of the disease(Tentative EULAR Criteria) in to two groups. Of them 29 patients (21 female, 8 male) had low disease activity [age range 32-65 years] and twenty-five patients(16 female, 9 male) had high disease activity [age range 22-52 years]. They were compared with 43 healthy control [age range19-64 years].
Serum copper and zinc were determined by using the atomic absorption specrtophotometry.

Results: Serum copper level and copper/zinc ratio in patients with RA in both groups were significantly
increased than the control group. In addition the serum zinc level in patients with low activity RA was significantly decreased and more in high activity disease.

Conclutions: As a result, the alteration of copper and zinc levels in the sera patients with RA can related to degree of disease activity and enable to shed more light on the role of trace elements in both physiological and pathological states.

Key words: Rheumatoid Arthtitis, copper, zinc, and serum.

IRAQI J MED SCI, 2005; VOL. 4 (1): 49-56

Introduction

Metal ions have a great variety of functions in biochemistry. They serve as catalyst in active center of enzymes, as factors stabilizing the structure of the enzymes or as a counter ion to provide electro neutrality. Metal ions in human serum have various functions. They act as the physiologically active part of enzymes (copper in coeruloplasmin) or they are bound to proteins in order to be stored (iron in ferritin) or transported (iron in transferrin)[1].

The trace elements copper and zinc have a significant role in antioxidant protection and immunity. They are constituents in antioxidative enzymes, zinc and copper in cytoplasmic superoxide dismutase[2] and copper in ceruloplasmin[3], which is an important antioxidant in serum[4]. Zinc is important in the maintenance of proper immune response[5,6]. In inflammatory conditions, such as rheumatoid arthritis (RA), there are alterations in blood and serum concentrations of these micronutrients.

Serum zinc was found to be reduced and serum copper increased in adult rheumatoid arthritis and these variations appeared to be associated with the immune inflammatory rheumatoid

[1]Dept. Chemistry & Biochemstry, College of Medicine, Al-Nahrain University [2]Dept. Chemistry, College of Education (Ibn Al-Haitham), University of Baghdad.
Adress Correspondence to: **Dr.Alaa K.Mohammed, E-mail:Alaakareem@maktoob.com
Received 11th December 2004: Accepted 28th March 2005.

process[7]. Neidermeier and Griggs[8] reported low serum zinc values in-patient with RA. Similar observations have been reported by other researchers[9,10] Moreover, both in humans[11] and in animals[12] zinc preparations have been shown to be anti-inflammatory and antiarthritic agents. In contrast, Alegree, et al[13] reported normal serum zinc values in-patient with RA. Low serum zinc and zinc deficiency in patients with RA could be the results of zinc deficient diet, therapeutic regimens, shift of zinc from plasma to other body compartements, and/or increased urinary zinc excretion.

Our purpose was to elucidate the alteration of copper and zinc concentration in sera patient with RA and to evaluate the relation between the levels of copper and zinc with various degrees of disease activity compared with a normal control group.

Materials and Methods

Chemicals: Standard solution for copper and zinc of 1000µg/ml concentration (Atomic absorption grade) were obtained from Aldrich chemical company LTD. All solutions were prepared from analytical grade chemical and distilled deionized water was used throughout.

Apparatus: Serum copper and zinc were measured with a shimadzu- flame atomic absorption model AA-646. Single elements hallow cathode lamps were used as line-radiation sources for individual elements and were operated at current recommended by the manufacturer and the manual of the atomic absorption spectrometer.

Preparation of standard working solutions: On the day of determination, the working solutions were prerared by sequential dilution (i.e.standard 1000µg/ml was diluted to 10µg/ml from which the lower concentration of working solution were prepared sequentially) to obtain the following concentration of the working standard (0.4,0.8,1.2,1.6 and 2.0µg/ml) of copper and zinc.

Frozen sample were allowed to thaw and come to room temperature then mixed gently. Sample were diluted 1:10 with (6%) butanol as diluents, this method achieved 30% increases in sensitivity compared to use of water only[14].

Patient's selection and sample collection:

Subjects: The clinical part of this study was completed between April and August 2004 in the Rheumatology Outpatient Clinic in Baghdad Teaching Hospital-Medicine City. The study population consisted of ninety-seven healthy and sick adults. Fifty-four (37 female, 17 male) from the Rheumatology Out patients' clinic were diagnosed as having RA according to the Rheumatoid Arthritis Disease Activity Index (RADAI) for epidemiological research[15].

Forty-three healthy persons made up the control group (Group1). Age range of the control group was 19 to 64 (mean 44.3±4.2); male/female ratio was 0.72. The patients with RA were divided into 2 groups after evaluation of their disease activity by the Tentative EULAR Criteria[16]. [Number of tender joints: maximum 28; number of swollen joints; maximum 28; pain score:0 (no pain) to 10 (very sever pain), erythrocyte sedimentation and morning stiffness of at least 30 min duration].

Twenty-nine patients (21 female, 8 male) with less than 14 swollen joints and one hour morning stiffness were considered to have low activity of the disease; these patients made up Group 2 and 3. Another twenty-five patients (16 female, 9 male) presenting a higher number of swollen joints and more prolonged morning stiffness, were considered to have a high degree of inflammation and were defined as Group 4 and 5. Characteristics of the

groups 2, 3, 4 and 5 are presented in tables 1 and 2.

Table 1: Characteristics of the Rheumatoid Arthritis Groups (2) and (3) low activity

Data	Group 2	Group 3
Sex	Male	Female
No. of patients	8.0	21.0
Age range (years)	32-65	19-16
Age (years)	55.30±1.90	48.8±2.30
Duration of disease (years)	6.90±1.10	5.90±1.20
Morning stiffness (hours)	0.70±0.20	1.10±0.30
No. of tender joints	15.20±3.0	16.20±2.30
No. of swollen joints	6.10±1.20	8.90±2.0
ESR (mm/h)	36.0±8.0	34.0±2.0
+ve rheumatoid factor	5/8	17/21

Table 2: Characteristics of the Rheumatoid Arthritis Groups (4) and (5) high activity

Data	Group (4)	Group (5)
Sex	Male	Female
No. Of patients	9.0	16.0
Age range (years)	22-52	19-58
Mean age (years)	45.6±7.9	43.8±4.8
Duration of disease (years)	5.2±1.2	6.6±2.3
Morning stiffness (hours)	3.4±1.1	3.3±0.3
No. Of tender joints	18.9±2.3	24.0±0.8
No. Of swollen joints	15.1±3.5	14.2±2.5
ESR (mm/h)	65.0±5.0	78.0±5.0
+ve rheumatoid factor	9/9	16/16

Serum preparation:

Avenues blood samples were drown by utilizing disposable needle and plastic syringes from each patient and control. The blood was allowed at room temperature for 10 minutes for clothing; centrifuged at 3000 rpm for 10 min, then serum was separated and stored at (-20^{o}C) until analysis.

Accuracy and Precision:

The accuracy and precision of the method were checked. Table 3, shows the relative standard deviation and relative error percent for five replicate results obtained for lowest and highest concentration range of each individual constituent.

Table 3:Accuracy and precision of copper and zinc analysis.

Cu added µmol/l	Cu found µmol/l	Relative Standard Deviation %	Relative Error
0.0	9.6	-	-
1.40	11.31,11.21 11.12,11.01 11.11.	1.0	1.36
3.50	13.10,13.12, 13.12,13.15 13.18	0.24	0.24
5.65	15.20,15.25 15.31,15.15 15.12	0.50	0.32
Zn added µmol/l	Zn found µmol/l	Relative Standard Deviation %	Relative Error
0.0	5.44	-	-
2.50	7.85,7.92 7.88,7.90 7.95	0.61	0.47
5.0	10.60,10.51 10.31,10.45 10.51	1.01	0.34
7.20	12.52,12.62, 12.56,12.55 12.60	0.27	0.58

Results and discussion

The current study provides data on copper and zinc levels in Rheumatoid Arthritis sera patients and compared with those of normal subjects. As different degree of inflammation may specifically possess different characteristics of the disease and different levels of these elements, separate calculation were made for each test.

Normal subjects:

Serum total copper and zinc were detected in normal subjects. The normal values for copper and zinc in female

was (18.60±1.24) and (15.07±0.66) µmol/l while they were (15.87±2.1) and (14.07±0.85) µmol/l in male respectively, the results show little differences between females and males for both copper and zinc, so that the total means for both sexes was (17.23±0.91) and (14.57±0.21) µmol/l respectively, as show in table 4.

Table 4: The serum copper, zinc levels of the patients with RA and control

Cases	Serum Cu	
	Mean ± SD µmol/l	Mean ± SD µmo l/l
RA with low activity		
Female (21)	21.39±3.31*	8.36±0.79*
Male (8)	19.69±2.31*	8.07±1.03*
RA with High activity		
Female (16)	32.25±6.12*	5.33±0.92*
Male (9)	29.40±4.46*	4.62±0.98*
Total (54)	25.68±1.22*	6.66±1.23*
Normal		
Female (25)	18.60±1.40	15.07±0.66
Male (18)	15.87±2.0	14.57±0.85
Total (43)	17.23±0.1	14.57±0.21

* = P <0.001

Rheumatoid Arthritis (AR) patients:

The results of sera samples from patients suffered from (RA) compared to these of normal subjects, were generally characterized by increase and decrease levels of copper and zinc respectively.

Serum copper level for patients and normal are shown in table 4. In general the copper concentration in 54 (Fifty-four) patients sera samples was found to be 25.68±1.22 µmol/l, while the corresponding level in 43 (Forty-three) healthy subject was 17.23±1.80 µmol/l. This increase of 49% in copper level was statistically significant (P< 0.001).

As different degree of inflammation may specifically possess different activity of the disease lead to different concentration of copper, separate calculation were made for each characteristics of the disease test. Both in the normal donors and in the various groups of patient, these were significant difference between values obtained for male and female; therefore they were grouped independent. The mean serum copper levels were found to be 21.39±3.11µmol/l and 19.69±2.31 µmol/l for female and male respectively with low activity patient; and 32.25±6.12 µmol/l, 29.40±4.46 µmol/l for serum female and male with active disease.

The magnitude of the increase in the value varied between 24% for female and 14% for male patients with low activity. In contrast, the magnitude of the increase in serum copper level varied between 87% for female and 70% for male patient with active disease.

Figure 1 shows a histogram of the distribution of the serum copper levels in the normal donors and in the (RA) patients grouped according to the activation and severity of disease.

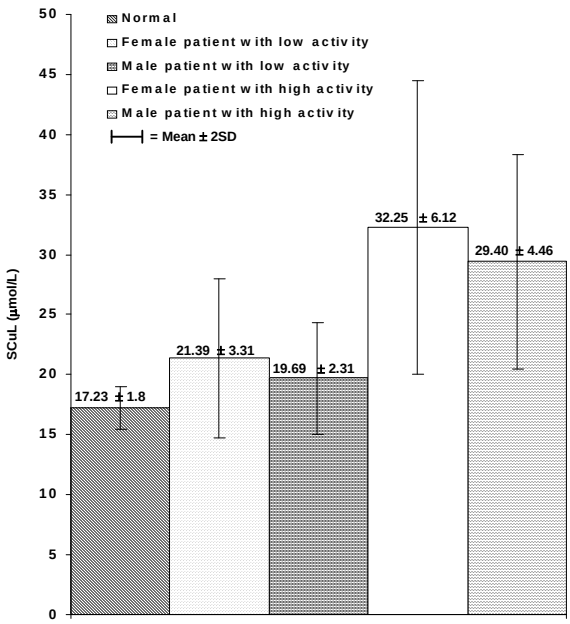


Figure 1: Serum copper level in normal and in patients with RA

The mean level of the serum copper in the total groups of (RA) patients was significantly elevated compared to the levels of the normal donors; nevertheless, only 4 of 21 (20%) and 4

of 8 (50%) for female and male patients with low activity disease have serum copper levels higher than 2SD (standard deviation) above the mean for normal donors. Of these all cases of proven active (RA) had copper serum levels higher 2SD than mean for normal.

The most pronounced changes were found in the levels of serum zinc (Table 4). The mean zinc level in 54 normal was found to be 14.57±0.21 µmol/l, while the mean zinc in all the patients with (RA) was 6.66±1.23 µmol/l, this 56% decrease in the zinc level was statistically significant (P<0.001). The results show no significant differences between values obtained for female and male. The decrease in the zinc serum level was more pronounced when the patients were grouped according to the high activity of the disease, the magnitude of the decease in the value varied between 63% for female and 68% for male with active disease.

Table 5 shows the results obtained for serum copper, serum zinc levels and cu/zn ratios of the patients with RA and the controls.

Table 5: The mean serum copper, serum zinc levels and copper/zinc ratios of the patients and normal.

Cases	Serum Cu µmol/l	Serum Zn µmol/l	Cu/Zn µmol/l
RA with low activity			
female (21)	21.39±3.31	8.63±0.79	2.47±0.52
male (8)	19.69±2.31	8.07±1.01	2.43±0.24
RA with high Activity			
female (16)	32.25±6.12	5.33±0.92	6.05±0.66
male (9)	29.40±4.46	4.62±0.98	6.36±0.41
Total (54)	25.68±1.22	6.66±1.23	3.93±0.43
Normal (43)	17.23±1.80	14.57±0.21	1.17±0.29

It was found a highly significant differences (P <0.001) between the copper/zinc ratio for the control group and that for the patients group, this differences is the result of both higher values for copper and lower values for zinc in serum from the patients group as compared with normal persons. In all cases of proven active disease, the cu/zn ratio was significantly higher as compared to low activity patients group.

Copper and zinc are very effective in DNA and RNA synthesis and cell division. These elements are required for the activation of enzymes such as timidin Kinase, RNA-polymerase and DNA-polymerase.Zinc also play an important role in the catabolism of RNA by regulating RNase activity.These two metals prevent the formation of free radicals capable of mutation with the antioxidant effect[6,17,18].

One approach to the assessment the role of copper and zinc in inflammatory conditions, such as (RA), analogous to that used for other nutrients, is measurement of copper and zinc in the serum or plasma over time because they are co-factors of important enzymes involved in collagen and bone metabolism[19] and immune system function[20]. Results studies have demonstrated that both copper and zinc alterations can be explained by the active inflammatory process and that serum trace elements are measures of disease activity.

Our studies show elevated level of copper in serum of (RA) groups and decrease level of zinc in serum patients with low activity and a more decreased in high (RA) disease activity. Change in serum zinc and copper levels observed in RA patients led some investigators to hypothesis that a marginal deficiency in zinc and copper might contribute to the development of RA and to the progression of the disease itself.

One of hypothesis of decrease zinc and increase of copper in sera of acute or chronic inflammatory processes cause an accumulation of copper and zinc in many body compartments and in the inflamed ares, supporting the hypothesis that the development of inflammation induces an increased body requirement of copper and zinc

containing proteins and enzymes such as metallothioneins, ceruloplasmin and Cu-Zn-superoxide dismutase[7,18,21]. Our results in both RA groups confirm those of studies that observed low plasma or serum zinc in patients with RA[22,23,24], and the results also show that serum copper and zinc levels are correlate to severity of disease.

Low serum and plasma zinc could be regarded as a secondary phenomenon arising from the hypoalbuminemia observed in patients with RA[25]. Levels of both zinc and albumin were found to be decreased by about 12% in plasma and correlated significantly with each other in patients with RA[7].

However, it seems that the decrease in serum zinc is independent of the reduction in circulating albumin[7]. This hypothesis is supported by data showing that only 1.10-2.6% serum albumin in humans' functions in zinc transport and that zinc occupies less than 0.2% of the total zinc binding capacity of the protein[26]. Other investigators attribute zinc deficiency in patients with RA to inadequate nutrition[27].

One of the hypothesis proposed to explain the decreased in plasma or serum zinc is due to redistribution of zinc in the organism, including in particular accumulation of the metal in the liver[28]. In contrast, Alegre et al[13] reported normal serum zinc values in patients with RA, the weakness of that study was the lack of measurement of serum levels in a control group.

Michaelsson and Ljunghall[29] reported decreased zinc concentrations in the serum and epidermis of men with dermatitis herpetiformis, which is considered an autoimmune disease. Svenson et al[10,30] examined zinc concentration in peripheral blood cells; erythrocytes, granulocytes and platelets. Therefore, at this stage of research, it seems that zinc malabsorption is not a specific defect of RA, but rather is common to other inflammatory connective tissue diseases.

Since zinc may act as a stabilizer of various biological membranes by interacting with the extrinsic macromolecule components of the membrane mainly the enzymes and/or directly with the intrinsic structure of the plasma membrane, therefore the low zinc level will affect the membrane stability as well[31].

Zinc deficiency results in blunted cellular immunity, measured by in vitro lymphocyte response to mitogens and delayed cutaneous hypersensitivity testing[32]. In addition, neutrophil chemotoxis is slowed; natural killer cell function is reduced[33,34]. It is possible that zinc deficiency may perpetuate disease in patients with RA through immunocompromised cellular mechanisms. This hypothesis calls for a prospective double blind study of the effect of zinc adminstration on clinical manifestations and cellular immunological functions of patients with RA.

The model involvement of increase in serum copper particulary serum cupro-enzyme and ceruloplasmin which was observed in various specific and non specific pathological condition and was attributed in biological damage caused by superoxide, a radical found in all living tissues, according to this model, superoxide radical or other reducing agents, such as ascorbate, reduced the copper complexes to the cuprous state. In turn, these complexes react with hydrogen peroxidase to form hydroxyl radicals that damage proteins, RNA and most important DNA.Repetitive formation of OH radicals at a specific location-where the copper ions are found is probably the mechanism of this process. These radicals may cause double strand break in the cellular DNA that are not repairable by cellular mechanisms[35].

References

1. Ihnat, M., Thomassen, Y., Wolynetz, M.S., and Veillon, C.: Trace element data reliability in clinical chemistry-interlaboratory trials and reference materials. Acta Pharmacol Toxicol, 1986; 59: 566-72.
2. McCord, J.M., Keele, B.B., and Fridovich, I.: An enzyme based theory of obligate anaerobiosis: the physiplogical function of superoxide dismutase. Proc Natl Acad Sci USA, 1971; 68:1027-30.
3. Delves, T.H.: The microdetermiation of copper in plasma protein fraction. Clin Chim Acta, 1976; 71: 495-500.
4. James, R., Kenneth, G., Linda, A., Ted, R., and Lindsey, A.: Antioxidant Micronutrients and Risk of Rheumatoid Arthritis in a Cohort of older women. Am J Epidemiol, 2003; 157: 345-54.
5. Fraker, P.J., Gershwin, M.E., Good, A.R., and Prasad, A.S.: Interrelationships between zinc and immune function. Fed Proc, 1986; 4: 1474-9.
6. Aaseth, J., Haugen, M., and Forre, O.: Rheumatoid arthritis and metal compound-perspectives on the role of oxygen radical detoxification. Analyst, 1998;123 (1): 3-6.
7. Milanino, R., Frigo, A., Bambara, L.M., Marrella, M., and Pasqualieehio, M.: Copper and zinc status in rheumatoid arthritis: studies of plasma, erythrocytes and urine and their relationship with disease activity markers and pharmacological treatment. Clin Exp Rheumatol, 1993; 11: 271-81.
8. Niedermeier, W., and Griggs, J.H.: Trace metal composition of synovial fluid and blood serum of patients with rheumatoid arthritis. J Chron Dis, 1971; 23: 527-36.
9. Morgenstern, H., and Mavhtey, I.: Serum zinc and copper levels in rheumatoid arthritis (letter). Arthritis Rheum, 1983; 26: 933-4.
10. Svenson, K.L.G., Hallgren, R., Johansson, E., and Lindh, U.: Reduced zinc in peripheral blood cells from patients with inflammatory connective tissue disease. Inflammation, 1985; 9:189-99.
11. Fernandez-Madrid, F.:Copper therapy of inflammatory disorders in men: Special reference to rheumatoid arthritis.In: Milanino, R., Rainsford, K.D., Velo, G.P., eds. Copper and Zinc Inflammation. Dordrecht: Kluwer Academic Publishers, 1989: p.p. 49-67.
12. Whitehouse, M.W., Rainsford, K.D., Taylar, R.M., and Vernon-Roberts, B.: Zinc monoglycerolate: A slow-release source of zinc with antiarthritic activity in rats. Agents Actions, 1990; 31: 47-58.
13. Alegre, C., Baro, J., and Obach, J.: Zinc and rheumatic disease (letter). Arthritis Rheum, 1984; 27: 1073-4.
14. Meret, S., and Henkin, R.I.: Simultaneous direct estimation by atomic absorbtion spectrophotometry of copper and zinc in serum, urine, and cerebrospinal fluid. Clin Chem, 1971; 17: 369-73.
15. Stucki, G., Liang, M.H., Stuki, S., Bruhlmann, P., and Michel, B.A.: A self-adminstered Rheumatoid Arthritis Disease Activity Index (RADAI) for epidemiological research: Psychometric properties and correlation with parameters of disease activity. Arthritis Rheum, 1995; 38: 795-8.
16. Tentative EULAR criteria for disease activity measurement of rheumatoid arthritis (EULAR Core Set). Rheumatol Eur, 1994; 23: 37-9.
17. Heliovaara, M., Knekt, P., Aho, K., Aaran, R.K., Alfthan, G., and Aromaa, A.: Serum antioxidants and risk of Rheumatoid Arthritis. Ann Rheum Dis, 1994; 53 (1): 51-3.
18. Tuncer, S., Kamanli, A., Akcil, E., Kavas, G.O., Seckin, B., and Atay, M.B.: Trace element and magnesium levels and superoxide dismutase activity in Rheumatoid Arthritis. Biol Trace Elem Res, 1999; 68 (2): 137-42.
19. Lappalainen, R., Knuuttila, M., Lmmi, S., and Alhava, E.M.: Zn and Cu content in human cancellous bone. Acta Orthop Scand, 1982; 53: 51-5.
20. Stable, J.R., and Spears, J.W.: Effect of copper on immune function and disease resistance. Adv Exp Med Biol, 1989; 258: 243-52.
21. Mazzetti, I., Grigolo, B., Borzai, R.M., Meliconi, R., and Facchini, A.: Serum copper/zinc superoxide dismutase levels in patients with Rheumatoid Arthritis. J Clin Lab Res, 1996; 26 (4): 245-9.
22. Mussalo-Rauhamaa, H., Konttinen, Y.T., Lehto, J., and Honkanen, V.: Predictive clinical and laboratory parameters for serum zinc and copper in rheumatoid arthritis. Ann Rheum Dis, 1988; 47: 816-9.
23. Dore-Duffy, P., Peterson, M., and Catalanotto, F.: Zinc profiles in rheumatoid arthritis. Clin Exp Rheumatol, 1990; 8: 541-6.
24. Lee, M.S., Kank, H.J., Hong, S.J., Do, J.H., Yoon, C.H., Kim, W.U., et al.: Serum levels of zinc and copper are associated with disease activity in patients with rheumatoid arthritis. J Korean Rheum Assoc, 2003; 10 (14): 413-20.

25. DiSilvestro, R.A., and Cousins, R.J.: Physiological ligands for copper and zinc.Annu Rev Nutr, 1983; 3: 261-88.
26. Foote, J.W., and Delves, H.T.: Albumin bound and Alfa-macroglobulin bond zinc concentrations in the sera of healthy adults. J Clin Pathol, 1984; 37: 1050-4.
27. Kowsari, B., Finnie, S.K., and Carter, R.L.: Assessment of the diet of patients with rheumatoid arthritis and osteoarthritis. J Am Diet Assoc, 1983; 82: 657-9.
28. Cousins, R.J.: Absorption, transport, and hepatic metabolism of copper and zinc: Special reference to metallothionine and ceruloplasmin. Physiol Rev, 1985; 65: 238-309.
29. Michaelsson, G., and Ljunghall, K.: Patients with dermatitis herpetiformis, acneacne, psoriasis, and Dariers disease have low epidermal zinc concentrations. Acta Derm Venereol, 1990; 70: 304-8.
30. Wanchu, A., Sud, A., Bambery, P., Prasad, R., and Kumar, V.: Plasma and peripheral blood mononuclear cells levels of zinc and copper among Indian patients with rheumatoid arthritis. Ann Rheum Dis, 2002; 61: 88.
31. Prasad, A.S., and Oberleas, D.: Trace Elements in human and disease. 1st ed. New York, Academic Press, 1976; 1:Chapter 16, p.p. 269-80.
32. Chandra, R.K.: Golan memorial lecture.Nutritional regulation of immunity and infection: From epidemiology to phenomenology to clinical practice. J Pediatr Gastroenterol Nutr, 1986; 5: 844-52.
33. Chandra, R.K.: Excessive intake of zinc impairs immune responses. JAMA, 1984; 252: 1443-6.
34. Naveh, Y., Lavy, A., and Suissa, A.: Effect of zinc and interferon on impaired natural killer cell activity in Cronhns disease. Trace Elem Med, 1993;10: 39-43.
35. Samuni, A., Chevion, M., and Czapski, G.: Unusual copper induced sensitization of the biological damage due to superoxide radical. J Biol Chem, 1981; 256: 12632-5.

MOTHER AGE AT MARRIAGE AS A DETERMINANT OF REPRODUCTIVE HEALTH

Enas T. Abdul-Karim *PhD*, **Abdul-Hussain Al-Hadi** *PhD*

Abstract:
Background: Early pregnancy and unplanned childbirth may have far-reaching physical, psychological and social consequences for adolescent girls and her off spring. Therefore, public health issues of concern because of the growing cultural and social sensitivity and controversy over reproductive health issues.

Aim of the study: Studying mother age at marriage in association with different socioeconomic characters in the family, which might affect reproductive health of mothers.

Subject & methods: A cross-sectional study conducted taking data obtained from 800 randomly selected healthy mothers, during their visits to five primary health centers, which selected randomly from different areas in Baghdad city; this done during the period from June- November 1994. Information from the mothers obtained using well-studied questionnaire form.

Results: Significant relationship between mother's age at marriage and maternal education, birth interval, father age at marriage, number of pregnancies, under 5 years children in the family, crowding index, degree of consanguinity ($P < 0.001$). Significant relationship found with father occupation, under 5 years death in the family ($P < 0.01$). No significant relationship with number of abortion and type of family ($P > 0.05$) found.

Conclusion: maternal age at marriage is an important determinant of reproductive health of women when it studied with different socioeconomic variables.
Emphasis should be made on young people to have better access to health information schools, and the medical profession needs to work together both to provide information and to help young females to develop confidence to use available information sources.

Key words: reproductive health

IRAQI J MED SCI, 2005; VOL. 4 (1): 57-62

Introduction

National family planning programs have been an important instrument in accelerating global fertility decline and in restricting ultimate world population to a level probably below ten billion. They begin to come into being after 1950 and probably go out of existence in most of the world 'regions by 2050[1]. The 1994 International Conference on Population and Development held in Cairo has generated wide spread commitment to changing family planning programs from categorical and medically focused service organizations to reproductive health initiatives that embrace wide range of social and human services[2]. Teenage pregnancy has reached epidemic proportions in the United States with 1 million pregnancies and more than 500000 live births occurring each year among women under the age of 20[3]. Early pregnancy and unplanned childbirth may have far-reaching physical, psychological, and social consequences for adolescent girls and her off spring and are therefore public health issues of concern[4]. Reproductive health-seeking behavior, source of advice, and access to care issues studied among a sample of clinic- based homeless adolescent women in USA,

Dept. Community Medicine, College of Medicine, Al-Nahrain University
Address correspondence to Dr. Enas T. Abdul-Karim
Received 14th April 2004: Accepted 2nd November 2004.

and it found that adolescent women are among the most vulnerable homeless population in the United States. Homeless youth rarely invited to participate in research aimed at improving their access to appropriate health care, also the culture in which they live and the personal experience of being homeless are often not addressed[5].

A family planning program can be deemed successful if an individual is able to avoid having an unintended pregnancy (or is able to have a wanted child) within the stipulated period and if she experiences no severe reproductive health problems in the process[6].

Because of the growing cultural and social sensitivity and controversy over reproductive health issues, we aimed at studying mother age at marriage in association with different socioeconomic characters in the family, and its effect as a risk factor on reproductive health of the mothers.

Subjects & methods

A cross-sectional study was conducted taking data obtained from 800 randomly selected healthy mothers, during their visits to primary health centers for different reasons, five primary health centers were selected randomly from different areas in Baghdad city, and about 100-150 mothers were included from each center making a total of 800 mothers, which done during the period from June- November 1994.

Information from the mothers obtained and filled by the researcher only, using well studied and preceded questionnaire form, which include information regarding different socioeconomic characters that might be affected by mothers' age at marriage.

Statistical analysis:

Frequency tables used, statistical tests done using Chi-square. P values <0.05 were considered significant.

Table 1: Distribution of the sample by mother's age at marriage and occupation

Variables	< 20 years		> 20 years		Total	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Age at marriage	441	55.1	359	44.9	800	100
Occupation						
House wife	431	53.8	311	38.9	742	92.7
Employed	10	1.3	48	6.0	58	7.3

Table 2: Distribution of different variables in association with mother age at marriage in the studied sample

Variable	Mother's age at marriage						Significant
	< 20 years		> 20 years		Total		
	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Mothers education							
Illiterate	**52**	**6.5**	**41**	**5.1**	93	11.6	
Read &write	**106**	**13.3**	**44**	**5.5**	150	18.8	$\chi^{(2}$=201.1
Primary school	**196**	**245**	**112**	**14.0**	308	38.5	DF =5
Intermediate school	**67**	**8.4**	**45**	**5.6**	112	14.0	P < 0.001
Preparatory School	**18**	**2.3**	**57**	**7.1**	75	9.4	
Diploma/University	**2**	**0.2**	**60**	**7.5**	62	7.7	
Total	**441**	**55.1**	**359**	**44.9**	800	100	
Birth interval							
0 -11.99months	112	14.0	145	18.1	257	32.1	$\chi^{(2}$=22.7
12-17.99 months	73	9.1	59	7.4	132	16.5	DF =4
18-23.99 months	41	5.1	25	3.1	66	8.2	P < 0.001
24-35.99 months	81	10.1	53	6.6	134	16.8	
36+ months	134	16.8	77	9.7	211	26.4	
Total	441	55.1	359	44.9	800	100	
Father age at marriage							
< 20 years	69	8.6	11	1.4	80	10.0	$\chi^{(2}$=29.74
> 20 years	372	46.5	348	43.5	720	90.0	DF=1
Total	441	55.1	359	44.9	800	100	P < 0.001
No. of pregnancies							
1-2	90	11.2	139	17.4	220	27.5	$\chi^{(2)}$ =27.1
3-4	157	19.6	116	14.5	273	34.1	DF=2
5 +	194	24.3	104	13.0	307	38.4	P < 0.001
Total	441	55.1	359	44.9	800	100	
Under 5 years children in the family							
0-2	147	18.4	188	23.5	335	41.9	$\chi^{(2)}$ =31.8
3-4	180	22.5	117	14.6	297	37.1	DF=2
5 +	114	14.2	54	6.8	168	21.0	P < 0.001
Total	441	55.1	359	44.9	800	100	
Crowding index							
< 2	57	7.1	88	11.1	145	18.1	$\chi^{(2)}$ =19.1
2 -5	310	38.8	220	27.4	530	66.3	DF=2
> 5	74	9.2	51	6.4	125	15.6	P < 0.001
Total	441	55.1	359	44.9	800	100	
Degree of consanguinity							
First degree	227	28.4	127	15.9	354	44.3	$\chi^{(2)}$ =27.58
Second degree	23	2.8	24	3.0	47	58	DF=3
Third degree	53	6.6	25	3.1	78	9.7	P < 0.001
No relation	138	17.3	183	22.9	321	40.2	
Total	441	55.1	359	44.9	800	100	
Father occupation							
Student	4	0.5	1	0.1	5	0.6	
Employed\self employed	377	47.2	334	41.8	711	88.9	$\chi^{(2)}$ =11.54
Others	60	7.4	24	3.0	84	10.5	DF=3
Total	441	55.1	359	44.9	800	100	P < 0.01

Table 2: *Continued*

Variable	Mother's age at marriage						Significant
	< 20 years		> 20 years		Total		
	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Under 5 years death of children in the family							$\chi^{(2)}$ =10.16 DF=1 P < 0.01
Yes	60.0	7.5	24	3.0	84	10.5	
No	381	47.6	335	41.9	716	89.5	
Total	441	55.1	359	44.9	800	100	
Number of abortion							$\chi^{(2)}$ =1.28 DF=2 P > 0.05
No abortion	288	36.0	248	31.0	536	67	
1-3	122	15.2	89	11.2	211	26.4	
4 +	31	3.9	22	2.7	53	6.6	
Total	441	55.1	359	44.9	800	100	
Type of family							$\chi^{(2)}$ =4.84 DF=1 P > 0.05
Extended	325	40.6	239	29.9	564	70.5	
Nuclear	116	14.5	120	15.0	236	29.5	
Total	441	55.1	359	44.9	800	100	

Results

Table 1 shows that more than half of mothers (55.1%) were married before the age of 20 years, and that 92.7% of mothers were housewives in the studied sample.

There were significant relationship between mother's age at marriage and maternal education, birth interval, father age at marriage, number of pregnancies, under 5 years children in the family, crowding index, degree of consanguinity ($\chi^{(2)}$=201.1,27.7,29.74,27.1,31.8,19.1,27.58 respectively P <0.001), also there were significant relationship with father occupation, under 5 years death in the family ($\chi^{(2}$=11.54,10.16 respectively P <0.01),while there were no significant relationship between mother age at marriage and number of abortion, and type of family($\chi^{(2}$=1.28, 4.84 respectively P > 0.05)

Discussion

This study was done on mothers from whom data were taken in the year 1994, since that time, many changes have occurred in our country which might have great effect on the Iraqi families especially the reproductive health of mothers, the results presented in this article might be useful for future similar studies to show changes in behavior and reproductive health of mothers during the last 10 years.

The end of the twentieth century is an appropriate half way mark at which to evaluate the twentieth-century National family planning programs, and to assess what changes in them needed for twenty-first century[1].

A reproductive health approach recognizes that the foundation of women's health are laid in childhood and adolescence, and are influenced by factors such as education, nutrition, social roles and social status, cultural practice, and the socioeconomic environment[7].

In the present sample, data taken from 800 women; of them, 441 (55.1%) women were married at the age less than 20 years, which means that more than half of women in the sample were adolescent at time of **their marriage.** According to Sedlecki 2001[8], the reproductive health of adolescent girls is endangered by their sexual behavior, poor acceptance of healthy life styles,

lack of responsibility in sexual relationship, and high prevalence rates of unintended pregnancy and sexually transmitted diseases. Another fact found that 742 (92.7%) of women were housewives.

The finding that there was a significant association between maternal age at marriage and mother education coincide with the finding of other studies[9, 10]. Heck K 2002[11] concluded that public health insurance coverage is critical to ensure adequate health care access and utilization among children of less educated mothers, regardless of family structure.

Significant association was found in this study between mother age at marriage and birth interval (P <0.001), which coincide with other studies[12, 13]. Modin 2002[14] also came to important conclusion that childhood social condition that linked to birth order position seem to have had consequences for their individuals health and survival that extended over the whole life-course.

Under 5s death in the family was significantly associated with maternal age at marriage in this study (P < 0.01), this result coincide with other studies[15, 16].

Number of abortion was not significantly associated with mother age at marriage (P > 0.05), this result does not coincide with the result obtained by Yassin 2000[17], and he found that the incidence of abortion was significantly associated with gravidity, consanguinity, and mother occupation, while recurrent abortion was associated with mothers' age at marriage, consanguinity, and mother occupation.

Also in the present study significant association was found with father age at marriage, degree of consanguinity, number of pregnancies and children, crowding index (P <0.001), father occupation (P <0.01), while there was no significant association with the type of family (P > 0.05). Those results possibly reflect the effect of socioeconomic conditions on maternal age at marriage as a determinant of reproductive health of mothers in the family in the studied sample, in which a good proportion of the women were of low education, housewives, married at a young age, and usually lived with their relative and having a higher number of pregnancies and children; with possibly little knowledge about their reproductive health.

Conclusion

Maternal age at marriage is an important determinant of reproductive health of women when it studied with different socioeconomic variables.

Special attention should be paid to adolescent sexual and reproductive health services, these should include contraceptive counseling in order to prevent pregnancy at a young age, also emphasis should be made on young people to have better access to health information schools, and the medical profession need to work together both to provide information and to help young females to develop confidence to use available information services.

References

1. Caldwell, J.C., Phillips, J.F., and Barkat, K.E.: The future of family planning programs. Stud Fam Plann, 2002; 33 (1): 1-10.
2. Helzner, J.F.: Transforming family planning services in the Latin American and Caribbean Region. Stud Fam Plan, 2002; 33 (1): 49-60.
3. Kettyle, E.P., and Klima, C.: Adolescent emergency contraception: attitudes and practices of certified nurse- midwives. J Midwifery-Woman's Health, 2002; 47 (2): 68-73.
4. Mingadi, P.T., Thembi, I.T., Rans, J.O., Arvidson, A.B., and Ahlberg, B.M.: Quality of maternity care for adolescent mothers in Mbabane, Swaziland. Int Nurse Rev, 2002; 49 (1): 38-46.
5. Ensign, J., and Panke, A.: Barriers and bridges to care: Voice of homeless female Adolescent youth in Seattle, Washington, USA. J Adv Nurs, 2002; 37 (2): 166- 72.

6. Jain, A.: Implication for evaluating the impact of family planning programs with a reproductive health orientation. Stud Fam Plann, 2001; 32 (3): 220-9.
7. Saif, T., and Nassim, J.: The need for a reproductive health approach. Suppl Int J Gynecol Obstet, 1989; 3: 103-13.
8. Sedlecki, K., Markovic, A., and Rajic, G.: Medical aspects of adolescent sexuality. Srp Arh Celok lek, 2001; 129 (5-6): 109-13.
9. Halldorsson, M., Kunst, A.E., Kohler, L., and Mackenback, J.P.: Socioeconomic differences in children use of physician services in the Nordic countries. J Epidemiol Commu Health, 2002; 56 (3) : 200-4.
10. Minkovitz-Cynthia, S., O' Campo-Ptricia, J., Chen-Yi-Hua, and Grason-Holly, A.: Association between maternal and child health status and pattern of medical care use. Ambul Pediatr, 2002; 2 (2): 85-92.
11. Heck, K.E., and Parker, J.D.: Family structure, socioeconomic status, and access to health care for children. Health Serv Res, 2002; 37 (1): 173-86.
12. Polo, V., Luna, F., and Fuster, V.: Determinants of birth interval in a rural Mediterranean population (La. Alpujarra). Spain Hum Biol, 2000; 72 (5): 877-90.
13. Clegg, E.J.: Starting, spacing, and stopping in the reproductive histories of outer Hebridean families. J Biosoc Sci, 2001; 33 (3): 405-26.
14. Modin, B.: Birth order and mortality: a life-long follow-up of 14200 boys and girls born in early 20th century Sweeden. Soc Sci Med, 2002; 54 (7): 1051-64.
15. Desinor, O.Y., Ferrus, A., Deverson, A., et al.: Survey of infant mortality in Mirebalais, Haiti. Sante, 2000; 10 (6): 407-11.
16. Rahman, S., Nessa, F., Ali, R., et al.: Reproductive health of adolescents in Bangladesh. Int J Gynecol Obstet, 1989; 29 (4): 3219-35.
17. Yassin, K.M.: Incidence and socioeconomic determinant of abortion in rural Upper Egypt. Public Health, 2000; 114 (4): 269-72.

THE BIOLOGICAL ROLE OF ZINC, COPPER AND MAGNESIUM DURING THE GROWTH OF EMBRYONIC CHICK LIMB BUDS

Yahya Y.Z. Farid[1] *BSc PhD*, **Hayder J. Mobarak**[2] *MSc PhD*,
May F. Al-Habib[3] *MSc PhD*

Abstract

Background: The limbs bud development is induced by the apical ectodermal ridge. The trace elements were found to have important role in biological growth of tissues such as tumor tissues.

Objectives: This study was done to reveal the possible biological role of trace elements as zinc, copper, and magnesium in the limb development induced by the apical ectodermal ridge.

Methods: Chick embryos of Hamburger and Hamilton stages 20-26 were used. Trace elements were measured in limb buds with and without apical ectodermal thickening.

Results and discussion: The decrease in zinc concentration found in limb buds tissues having no apical ridge suggested a relationship between this trace element and the inductive effect produced by the apical ectoderm for limb buds formation. The detectable concentration of copper only in buds having no apical ridge was correlated with the embryonic organization of the limb buds. The lower magnesium concentration in the limb buds was considered as a criterion for the metabolism accompanies the functioning apical ectoderm during the embryogenesis of the limb buds.

Conclusions: Trace elements may have a significant role in the embryonic process of budding.

Keywords: Limb buds, trace elements, zinc, copper, magnesium, development.

IRAQI J MED SCI, 2005; VOL. 4 (1): 63-66

Introduction

Embryonic development of the limb bud is induced by thickening of the ectoderm at the apex of the bud[1,2]. The limb primordium starts to develop at stage 16 of Hamburger and Hamilton (H&H) corresponding to about 50-60 hours of incubation. It appears as inconspicuous condensation of the mesoderm at the wing level of the chick embryo, followed by formation of the primordia of the leg bud. The toe plate becomes distinct at stage 24 H&H (at about 4.5th day of incubation) prior to the digital plate that develops a stage later. Faint grooves appear in the toe and the digital plates during the 5th day of development (stages 25 &26 H&H). The differentiation of the wing and leg buds exhibit many resemblances, both have apical ectodermal thickened at earlier embryonic periods[3,4]. The centers of codifications appear in the condensed mesenchyme of limb bud at about the end of sixth day of incubation[5].

The biochemical aspects of induction in embryonic development were not investigated meticulously. In this study, a trial has been carried out to reveal the possible biological role of trace elements as zinc, copper, and magnesium in early embryonic development of limb buds induced by the apical ectodermal ridge. Influence of trace elements in body metabolism and their physiological importance have motivated their accurate quantitative

[1]Dept. Chemistry & Biochemistry [2]Dept. Human Anatomy [3]Dept. Human Anatomy-Section of Histology & Embryology, College of Medicine, Al-Nahrain University
Address correspondence to Prof. Yahya Y.Z. Farid.
Received 31st July 2004: Accepted 28th March 2005.

determination in biological tissues[6]. In chick embryos growth of the limb bud and its parts (including the apical ectodermal ridge) were investigated during the period between the onset of its formation (Hamburger and Hamilton's stage 17) and the stage of nerve fiber in growth (H&H stage 22)[7].

Materials & methods

Fertilized chick eggs of Euribrid Lohman and Faobro type were incubated at 38°C in a humid environment. Embryos of Hamburger and Hamilton stages 20-26 were used corresponding to the incubation period of 3-5 days[3]. Sixty embryos were used in this study. Forty five embryos of stages 20-22 having the apical ectodermal thickening were classified as group (A), fifteen embryos of stages 23-26 with no apical ectodermal thickening were classified as group (B). Limb buds of embryos group (A) were excised and collected in a container with 0.5 ml of normal saline in three sets each set containing the buds of fifteen embryos. Similar procedure was done for the limb buds of group (B) embryos, in each of the three sets of containers limb buds of five embryos were collected.

At first the container were weighed empty, then they were weighed with 0.5 normal saline and lastly we weighed the container and normal saline plus the tissue added. The final weight of the tissue was obtained by abstracting the weight of container plus normal saline from the total weight (Table 1).

Table 1: Method of weighing limb buds with AER

Wt. for empty container (gm)	Wt. of the container with normal saline (gm)	Wt. of the container with normal saline + the tissue (gm)
0.8962	1.4182	1.4533
0.8913	1.4171	1.4529
0.8906	1.4167	1.4599

Table 2: Method of weighing limb buds with AER

Wt. for empty container (gm)	Wt. of the container with normal saline (gm)	Wt. of the container with normal saline + the tissue (gm)
0.7404	1.2586	1.2780
0.7511	1.2776	1.2974
0.7415	1.2685	1.2877

Each set of the specimens of limb buds in each groups (group A and B) were mixed with 0.5 ml of normal saline, homogenized by homogenizer for few minutes, and centrifuged. Then after, a known amount of segmentate was treated as following:

1. determination of Zinc and Copper:

One hundred *u*g\ml stock solution was diluted with de-ionized water to give the following concentrations of the working standard (0.0, 0.4, 0.8, 1.2, 1.6, and 2 One hundred *u*g\ml) of Zinc and Copper. Samples were diluted 1:10 with 6% butanol as diluents. This method achieved 30% increase in sensitivity compared with the used of water only[8]. This effect is due to a decrease in viscosity and difference in droplet formation. This technique is more widely used[9].

2. determination of Magnesium:

The samples used for determination of magnesium were diluted 1:50 with 1% lanthanum chloride solution to exclude the effect of segmentate phosphorous[10]. Standard solution of 1000 ppm was diluted with de-ionized water. Working standards of Magnesium were (0, 5, 10, 15, 20, 25, and 30 One hundred *u*g\ml).

Table 3: Mean concentrations of the trace elements

Trace element	Group (A) mean concentration (mg/gm)	Group (B) mean concentration (mg/gm)
Magnesium	0.002	0.037
Zinc	0.007	0.005
Copper	0	0.006

Results and Discussion

Embryonic stages included in-group (A) embryos have well developed ectodermal thickening at the apices of their limb buds[7]. Embryos of group (B) have toe and digital plates in their limb buds[4]. The mean values of the trace elements concentrations measured in this study are shown in table 1.

Embryonic tissue of the limb bud of group (B) shows higher magnesium and lower zinc concentration compared to that of group (A). Buds of group (B) only demonstrate detectable tissue concentration of copper.

It was concluded that many trace elements in tumor tissues exert direct or indirect action that accelerate growth of tumors[11]. Trace elements measured in this study may have a role to accelerate the growth of the limb buds.

Experimental studies showed evidence for special function of the ectodermal apical ridge in limb morphogenesis indicating chemical messengers. Striking and distinctive characteristics of the mesoderm close to the apical ectodermal ridge were found providing possibilities for the understanding of the function of the apical ridge in limb morphogenesis[12].

The higher concentration of the zinc was linked with rapidly growing tumor tissues in many studies; such elevated concentration was suggested to be a sign of increased cellular activities[13,14]. The higher concentration of zinc in limb bud tissues of group (A) having the apical ridge may signify the biological role of this element in motivation of limb development. The decrease of zinc concentration in limb buds of group (B) having no apical ridge may suggest a relationship between this trace element and the inductive effect produced by the apical ectoderm for limb bud formation. Lower zinc concentration of group (B) bud tissues may be a sign of a reduced inductive influence.

The detectable concentration of copper in limb buds of group (B) may indicate the beginning of embryonic organization. It was suggested that concentration of tissue copper is lowered during the metabolic changes of biological stress[15]. Accordingly, the low and undetectable concentration of copper in buds of group (A) may be correlated with the metabolic stressful events accompany the induction of the apical ectoderm for the early rapid growth and budding of limbs. Extirpation studies of the apical ectodermal ridge reveal its essential task during the critical early stages of limb bud formation; no limb was formed if the apical ectoderm is removed[16].

The above interpretation of the copper concentration in limb tissue may be applied to explain the higher magnesium concentration measured in limb buds of group (B) compared to that of group (A). Lower magnesium concentration in buds of group (A) may be a criterion for the metabolism accompanies the functioning apical ectoderm during the embryogenesis of the limb buds.

Budding is an embryological phenomenon observed in many embryonic regions. The concentration of trace elements should be more thoroughly investigated to establish a judgment for the significance of the biological role of trace elements in this embryological process.

References

1. Vogel, A., and Tickle, C.S.O.: FGF-4 maintains polarizing activity of posterior limb bud cells in vivo and in vitro. Development, 1993; 119 (1): 199-206.
2. Altabef, M., Clarke, J.D., and Tickle, C.S.O.: Dorso-ventral ectodermal compartments and origin of apical ectodermal ridge in developing chick limb. Development, 1997; 124 (22): 4547-56.
3. Hamilton, H.L.: Lillies development of the chick, an introduction to embryology. 3rd edition, New York, Henry Holt and company, 1952; p.p. 82-90.

4. Richman, J.M., and Tickle, C.S.O.: Epithelial-mesenchymal interactions in the outgrowth of limb buds and facial primordia in chick embryos. Dev Biol, 1992; 154 (2): 299-308.
5. Wong, M., and Tuan, R.S.S.O.: Interactive cellular modulation of chondrogenic differentiation in vitro by subpopulations of chick embryonic calvarial cells. Dev Biol, 1995; 167 (1): 130-47.
6. Garg, A.N., Singh, V., Wginwar, R.G., and Sagdeo, V.N.: An elemental correlation study in cancerous and normal breast tissue with successive clinical stages by neutron actvation analysis. Biol Trace Elem Res, 1994; 46 (3): 185-202.
7. Fisher, K.R., and Fedoroff, J.: The development of chick spinal cord in tissue culture. II Cultures of whole chick embryo. In Vitro, 1978; 14 (10): 878-86.
8. Meret, S., and Henkin, K.I.: Simultaneous direct estimation by atomic absorption spectrophotometry of copper, zinc in serum, urin and CSF fluid. Clin Chem, 1971; 17: 369-73.
9. Taylor, A., and Bryant, T.N.: Comparision of procedures for determinatiom of cooper and zinc in serum by atomic absorption spectroscopy. Clin Chem Acta, 1981; 110: 83-90.
10. Gowenlock, H.A., McMurray, R.J., and McLauchian, M.D.: Varly's practical clinical biochemistry. 6th ed. Heinemann medical books. London, 1988.
11. Majewska, U., Braziewicz, J., Banas, D., Kubala, K.A., Gozdz, S., Pajek, M., et al.: An elemental correlation study in cancerous breast tissue by total reflexion X-ray fluorescence. Biol Trace Elem Res, 1997; 60 (1-2): 91-100.
12. Carins, J.M.S.O.: The function of the ectodermal apical ridge and distinctive charachterstics of adjecent mesoderm in the avian wing-bud. J Embryo Exp Morphol, 1975; 34 (1): 155-69.
13. Bradley, D.A., and Looi, L.M.: Elevated trace element concentrations in malignant breast tissues. Br J Radiol, 1997 ; 70: 375-82.
14. Yoshida, D., Ikeda, Y., and Nakazawa, S.: Suppression of 9L gliosarcoma growth by copper depletion with copper deficient diet and D-penicillamine. J Neurooncol, 1993 ; 17 (2): 91-7.
15. Tariq, M.A., Qamar, U.N., and Fatima, A. : Concentration of Cu, Cd, Ni, and Pb in the blood and tissues of cancerous persons in Pakisani population. Sci total Environ, 1995 ; 175 (1): 43-8.
16. Calandra, A.J., and MacCabe, J.A. : The in vitro maintenance of the limb-bud apical ridge by cell-free preparations. Dev Biol, 1978; 62 (1): 258-69.

MALE INFERTILITY AND PHYSIOLOGICAL ROLE OF ZINC

Ali T. Al-Baldawi[1] *MBChB FICMS (Chemical Pathology)*, **Nazar A. Naji**[2] *BSc PhD*, **Aqeel A.A. Al-Ani**[2] *BSc MSc*

Abstract

Background: Male infertility is the inability to conceive a baby in the absence of female causes. Most causes reflect an abnormal sperm count or quality. Zinc is a component of semen and plays an important role in the process of reproduction and sexual maturation.

Objectives: To asses the relationship between concentration of zinc in serum, seminal plasma and semen quality among infertile and fertile men to clarify the possible impact of zinc in male reproductive system.

Methods: Fifty-eight male (infertile group) partners who were undergoing investigation for infertility and thirty-seven men (fertile group) whose wife were pregnant and have normal sexual life studied as a control group.
Seminal fluid analysis, reproductive hormones (luteinizing hormone, follicular stimulating hormone and testosterone) were analyzed by using radioimmunoassay method (RIA) while serum and seminal plasma zinc concentration were determined by using colorimetric methods.

Results: All semen properties (count, motility and morphology) for the infertile group were lower than for the fertile group. No significant differences were found in the levels of L.H. and F.S.H. between the two groups while testosterone levels were significantly lower in the infertile men group than in the fertile group ($p<0.005$).
It was also found that the mean level of serum and seminal plasma zinc concentration were significantly lower in the infertile group compared to the fertile group ($p<0.0001$). No correlation was found between the concentrations of zinc in serum and seminal plasma in both groups. A lack of correlation was also found between zinc concentrations in serum and seminal plasma with semen properties.

Conclusion: The study revealed the importance of zinc in fertility through its direct and its indirect effects on spermatogenesis.

Keywords: Male infertility, Zinc, semen, spermatogenesis

IRAQI J MED SCI, 2005; VOL. 4 (1): 67-71

Introduction

Many recent studies have indicated an increasing prevalence of various abnormalities of the reproductive system in human males[1]. Male infertility is as the inability to conceive a baby after 1 year of unprotected sex in the absence of female causes.
Most causes of male infertility reflect an abnormal sperm count or quality. Although it only takes one sperm to fertilize an egg, in an average ejaculate a man will eject nearly 200 million sperm. However, because of the natural barriers in the female reproductive tract only about 40 sperm will ever reach the vicinity of an egg.
There is a strong correlation between the number of sperm in an ejaculate and fertility. In about 90% of the cases of a low sperm count, the reason is deficient sperm production. Unfortunately, in about 90% of cases, the cause for the decreased sperm formation cannot be identified. Other causes of male infertility may include ductal obstruction, ejaculatory dysfunctions, and infections or disorders of the accessory glands[2]. Prostate gland is the largest accessory gland of the male reproductive tract and there is a good deal of research being conducted on the prostate. Yet it

[1]Dept. Pathology-Section of Forensic Medicine, College of Medicine [2]Dept. Chemistry, College of Science, Al-Nahrain University
Address correspondence to Dr. Ali T. Al-Baldawi
Received 14th April 2004: Accepted 29th November 2004

remains one of the least understood structures in the body. One area of study concentrates on the major function of the gland, the production and the secretion of minerals that play an important role in prostate and the reproductive system functions.

Zinc is a component of semen and it is though that at least 1 mg of zinc is excreted by an ejaculum. Zinc plays an important role in the processes of fertility, reproduction and sexual maturation[3]. At a biochemical level, zinc's primary role as a component of zinc metalloenzymes, many enzymes of the cell nucleus involved with genetic information transfer and cellular replication are metallonezymes of zinc. Zinc also participates in RNA metabolism, prostatic fluid and ocular tissues contain the highest levels of zinc in the body[4].

The present study aimed at clarification of the interrelationship of serum and semen zinc levels, in both men with a history of infertility and men with no history of infertility. Also, investigate the possible role of seminal zinc in the regulation of human spermatozoa functions.

Subjects & Methods

Subject selection:

Iraqi men in the age range of 20 to 50 years with no apparent chronic or acute disease were selected for the study, 37 men of known fertility and 58 infertile men were studied.

The infertile men were unable to be fathers of children for at least 1 year; their wives were considered fertile based on extensive gynecologic evaluations. At the first interview for the infertile men, a complete history was obtained, especially with respect to any history of sexual dysfunction. Physical examination was performed to exclude patients with chromosomal abnormalities. None of the subjects were on drug treatment or on especial diet and none of the subjects used vitamin supplements.

Procedures:

1. Semen collection: semen analysis were performed on three separate specimens for each subject, samples were collected after 2-3 days of sexual abstinence by masturbation into polyethylene containers.
2. Serum collection: Blood was drawn into sterile, disposable plastic syringes. After allowing 30 minutes, for blood clotting, the serum was separated from blood cells by centrifugation at 900 g for 5 minutes at room temperature. The serum was decanted and stored in a metal-free polypropylene tube at –20C^{o} until required.
3. Seminal plasma collection: The semen was put into plain tube and after one hour the seminal plasma was separated by centrifugation at 3000 rpm at room temperature for 10 minutes. The seminal plasma was decanted and stored in a metal- free polypropylene tube at -20 ^{o}C until required.
4. Hormonal Assay: Basic endocrine measurements includes serum LH and FSH will testosterone by radioimmunoassay method (RIA)[5] from (CIS Bio International, groups ORIS, B.P. 32-91192. GIF-SUR-Yvette Cedex-France).
5. Zinc analysis: Zinc analysis was done by Jenway 6100 spectrophotometer (model 6100 serial No. 3000; frequency 50/60 Hz, UK. England) according to the methods described by Shibata, S., who said pyridylazo compounds of phenol derivatives are sensitive chromogenic reagents for the analysis of zinc and many other metals[6].

An aliquot of 50μL of serum was mixed with 1ml of the reagent and 10μL of seminal plasma was mixed with 1ml of the same reagent. All tubes were mixed and incubated for 10min at 25^{o}C. Then measured against blank at 560nm using (Cat. No. 0033, Giesse Diagnostics, C.F. 03084750581, P. IVAO

11572610077, Reg. Soc. N. 1103178. Trib- Roma).

Statistical Analysis

The results are presented as (mean ± standard deviation) for better comparison with relevant data in literature

The results within the groups were also presented, as ranges, and the significance of the differences between the groups were calculated using student's t-test two-tailed.

The Pearson's correlations (r, p) and regression equation were calculated between each of the measured parameters to assess the shape of relationship based on the highest (r) value obtained.

Results

The mean±standard deviation and range of semen quality (counts, motility, and morphology), serum reproductive hormones (LH, FSH, Testosterone) and serum zinc and semen zinc are listed in table I.

Table 1: Parameters of semen quality, reproductive hormones, and Zinc concentrations in fertile and infertile men groups

Item	Fertile men group (n=37)		Infertile men group (n=58)		P-value
	Mean±SD	Range	Mean±SD	Range	
Count (60-150x10^6/ml)	96.6±28.3	57-154	31.28±30.0	0-98	S
Motility (>60%)	75.1±11.7	55-95	15.0±12.3	0-60	S
Morphology (>70%)	84.3±5.5	70-90	56.9±31.8	0-90	S
LH (1-5mIu/ml)	3.5±1.0	1.5-5.0	4.7±3.9	0.9-23	Ns
FSH (1-9mIu/ml)	6.0±1.7	2.8-9.8	6.4±6.0	0.9-34.5	NS
Testosterone (8.2-34.6nmol/L)	22.5±4.5	15.8-33.0	18.0±8.2	7.0-44.0	S
Serum Zn (70-120µg/dl)	118.7±16.8	90.6-162	97.5±26.9	36-147.4	S
Seminal plasma Zn (1.2-3.9nmol/L)	3.1±0.4	2.5-3.8	2.1±0.6	0.39-2.7	S

The parameters of semen quality were significantly lower in the infertile men group than in the fertile men group (P<0.0001). No significant differences were found in the mean concentrations of LH and FSH in serum, when fertile men group was compared with infertile men group, but the mean serum testosterone concentration was significantly lower in the infertile men group than in the fertile men group (P<0.005).

The results revealed that serum zinc levels in infertile men group were lower than in the fertile men group (P<0.0001). In addition, seminal plasma zinc levels were lower in the infertile men group than in the fertile men group (P<0.0001).

From table 2, there was no significant correlation between serum zinc and the parameters of semen quality in both groups. Moreover, no significant correlation between semen zinc and the parameters of semen quality in both groups.

Table 2: Values of simple correlation coefficient (r) between Zinc concentration and parameters of semen quality for both groups

Item	Fertile Men Group (n=37)		Infertile Men Group (n=58)	
	Serum Zn	Seminal Plasma Zn	Serum Zn	Seminal Plasma Zn
Count	-0.098	0.058	-0.006	-0.041
Motility	-0.285	0.12	-0.025	-0.187
Morphology	-0.071	-0.368	-0.179	0.099

No correlation was found between semen zinc and serum zinc level in the fertile men group (r=0.075) nor in the infertile men group (r=0.120).

Discussion

Our data are in agreement with those of Bonde et al[7], who reported that not sperm concentration but sperm quality determines fertilizing capacity of spermatozoa.

Although, the most cases of male infertility are nonendocrine in origin. However, routine evaluation of hormonal parameter is not warranted unless sperm density is extremely low or there is clinical suspicion of an endocrinopathy. A scrotal varicocele is the most common causative finding in infertile men[8]. To explain the abnormalities in spermatogenesis with varicocele, the most point have been proposed was the abnormal blood flow can interfere with testosterone production, which in turn can interfere with sperm production and this in agreement with our results that about 25% of infertile men had varicocele.

We did not find any difference in the our data and in those previously published in the literature for that the serum zinc and semen zinc levels were significantly lower in infertile patients than fertile males[9], in contrast to other reports, that unable to find a significant difference in serum and semen zinc levels between fertile and infertile men[10,11].

The lack of correlation between zinc concentration and semen quality found in our study suggests that biochemical complexity of seminal fluid attempts to perform such simple correlations between seminal plasma component and andrological parameters are likely to produce inconsistent results. These effects include no significant correlation between the total amount of zinc per ejaculate and sperm quality[12] with no statistically significant correlation between zinc concentration and the motile sperm concentration[13].

However, our results are in contrast to other studies that showed zinc-related decrease in human semen quality[14,15].

Most previous results of colorimetric methods and the present method found that the results obtained using the proposed method were not statistically different from those obtained by atomic absorption spectrophotometry[16,17].

The high level of zinc found in semen is due primarily to the secretions of the prostate gland and reflects prostatic stores. Serum zinc may be a reasonable indicator of zinc status. The lack of correlation between serum zinc and semen zinc found in our study suggests that mild zinc deficiency may lower serum zinc while the larger prostate zinc stores remain unaffected. However, we obtained 25% had varicocele, which are enlargement of the internal spermatic veins that drain the testes.

The significant decrease of the zinc in seminal plasma of varicocele men were significantly lower than in the normal subjects .This decrease indicated an impairment of the prostatic function or secretion due to decreases the availability of oxygen and nutrient required for sperm live.

Citrate is part of the circular chain of cellular respiration, known as the Krebs's cycle. The Krebs's cycle is the process in which a sequence of enzymatic reactions involving the metabolism of carbon chains of sugars, fatty acids, and amino acids to yield carbon dioxide, water, and high-energy phosphate bonds. The Krebs's cycle provides a major source of adenosine triphosphate energy and produce molecules that are starting point for a number of vital metabolic pathways for the cell, citrate that been chelated with zinc and their evidence that zinc

required for oxygen consumption by sperm.

Varicocele decreases the concentration of zinc and decreases the availability of oxygen for live sperm. Further studies to elucidate the significance of zinc and other factors presents in seminal plasma in different types of causes of male infertility for the functional properties of human spermatozoa appear to be of importance since such studies may give hints to new ways of regulating male fertility.

References

1. Bromuich, P., Cohen, J., Stewart, I., and Walker, A.: decline in sperm counts: An Artifact of Changed Reference Range of "Normal"? BMJ, 1994; 309: 19-22.
2. Wyngaarden, J.B., Smith, L.H., and Bennett, J.C.: Endocrinolgic Diseases Unique to Men, Cecil Textbook of medicine, 20th edition. WB Saunders, Philadelphia, PA, 1996; Vol. 2: 1325-45.
3. Shils, Maurice, E., And Young, Vernon, R.: Essential Trace Elements, Modern Nutrition in Health and Disease, 7th edition. , Philadelphia, lea & febiger, 1988; p.p. 375-92.
4. Chester's, J.K.: Metabolism and biochemistry of zinc, Clinical, Biochemical and Nutrition Aspects of Trace Elements, New York: Alan liss, 1982; p.p. 487-509.
5. Colzieher, J.W., and Dozier, T.S.: Improving the Diagnostic Reliability of Rapidly Fluctuating Plasma Hormones by Optimized Multiple Sampling Techniques. Clin Endocrinol Metab, 1976; 43: 824.
6. Shibata, S.: 2-pyridylazo compounds in Analytical chemistry. In: H.A. Flaschka et al., Chelates in Analytical Chemistry IV, Marcel. Dekker, Inc. New York, 1962; p.p. 1.
7. Bonde, J.P.E., Ernst, E., and Jensen, T.: Relation between Semen Quality and Fertility. Lancet ,1998; 353: 1172-7.
8. Sigman, M., Stuart, S.: Howard's, Male Infertility, Campbell's Urology. 7th edition, 1998; p.p. 1287 -330.
9. Xu, B., Chia, S.E., and Ong, C.H.: Concentrations of Cadmium, Lead, Selenium, and Zinc in Human Blood and Seminal Plasma. Biol trace elem Res, 1994; 40 (1): 49-57.
10. Ward, R.J., and Hendry, W.F.: A Cooperative Study of Zinc, Copper, Cadmium, and Lead Levels In Fertile and Infertile Men. Fertile Sterile, 1983; 40 (5): 670-7.
11. Schoenfeld, C., Ameral, R.D., Dubin, L., and Numberoff, M.: Prolactin, fructose and zinc levels found in human seminal plasma. Fertile Sterile, 1979; 32: 206-8.
12. Lin, Y.C., Change, T.C., Tseng, Y.J., Lin, Y.L., Huoing, F.J., Lung, F.T.: Seminal Plasma Zinc Levels and Sperm Motion Characteristics in Infertile Samples. Change keng I Hsuch Tsa Chia, 2000; 23 (5): 260-6[Medline].
13. Lewis, D.I., Jones, I.A., Arid, M.M., and Biljan, C.R.: Effects of Sperm Activity on Zinc and Fructose concentrations in Seminal Plasma. Hum Reprod, 1996; 11: 2465-7[Medline].
14. Zvit, H., et al: The Cation Composition of The Seminal Plasma and Prostatic Fluid and its Correlation to Semen Quality. Fertile Sterile, 1978; 29 (5): 539-42.
15. Fuse, H., Kazaman, T., Ohta, S., and Fujiuchi, Y.: Relationship between Zinc Concentrations in Seminal Plasma and Various Sperm Parameters, Int Urol Nephrol, 1999; 31 (3): 401-8.
16. Deadre, J., Johnson et al.: Improved Colorimetric Determination of Serum Zinc. Clin Chem, 1977; 23 (7): 1321-3.
17. Fuentes, J., Miro, J., and Riera, J.: Simple Colorimetric Method for Seminal Plasma Zinc Assay. Andrologia, 1982; 14 (4): 322-7.

A RANDOMIZED PROSPECTIVE STUDY COMPARING THE EFFECTIVENESS OF MITOMYCIN C AND MITOMYCIN/S_2 COMPLEX SEQUENTIAL INTRAVESICAL THERAPY FOR SUPERFICIAL BLADDER CANCER

Ahmad A. Al-Azawi[1] *FICMS*, **Abdul-Wahab R. Hamad**[2] *BSc MSc PhD*, **Mahmood H. Hammash**[3] *BSc, PhD*

Abstract

Objective: Comparison of two protocols regarding efficacy and toxicity of intravesical mitomycin C versus mitomycin C and S_2 complex sequential therapy for superficial transitional carcinoma of urinary bladder.

Methods: A prospective, randomized parallel two lines of treatment groups. Seventy-three patients were evaluated after transurethral resection of superficial bladder cancer with median follow-up of 26 months. In group A, 37 patients received intravesical mitomycin C 30mg on day1 weekly for 6 weak and monthly for 12 month, while group B (36 patient) received in addition to mitomycin C, intravesical S_2 complex 3ml on day 2. Statistical analysis performed by Kaplan-Meier methods.

Result: After follow-up of 26 months, 46% of patient given mitomycin C were disease free compared to 70% for that of group B patient who received mitomycin C and S_2 complex, with no significant difference in incidence of the adverse effects.

Conclusion: The results show that intensive intravesical instillation of sequential mitomycin C and S_2 complex; were highly effective in eradication and/or prophylaxis of superficial transitional bladder cancer.

Key ward: Transurethral resection; bacillus Calmitte-Guerin; complete response; mitomycin C, carcinoma in situ

IRAQI J MED SCI, 2005; VOL. 4 (1): 72-76

Introduction

Bladder cancer is the third most prevalent malignant disease among male patients and the tenth among female patients in the United States[1]. Superficial transitional type constitutes 75-80 percent of bladder cancer and 30-60% recur within 6-18 month after trans-urethral resection[2].

Transurethral resection can effectively control the primary tumor, confirm the superficial nature of the disease, provide cytological and histological tumor, characteristics of prognostic significance; and allows for assessment of extent of mucosal involvement by tumor[1,2]. Tumor recurrence and progression related to stage, grade, number, and size, duration of the disease, positive or negative cytology and aneuploidy[1,3].

There is increasing interest in intravesical instillation of cytotoxic agent or immunotherapy bacillus Calmitte-Guerin (BCG) and become common practice after TUR to cure or prevent recurrence of superficial transitional cell bladder cancer (cis; Ta and Tb) in more than 45% of cases[3-5]. Intravesical trials divided into 2 major categories

1. Therapeutic, designed to treat established disease and
2. Prophylactic/adjuvant, designed to prevent recurrence in patients at high risk for tumor recurrence and progression[1,6].

The most effective intravesical therapy is mitomycin C (can achieve complete

[1] Dept. Medicine-Section of Oncology [2] Dept. Chemistry & Biochemistry [3] Dept. Human Anatomy, College of Medicine, Al-Nahrain University.

Address correspondence to Dr. Ahmad A. Al-Azawi.

Received 28th December 2003: Accepted 29th November 2004.

response in 48%) and BCG (60%) after the basic therapy with TUR[5,6]. After TUR, those patients with: transitional cell stage T_1 papillary tumor, multifical stage Ta (grade 1-III), multifocal and/or symptomatic carcinoma in situ (CIS) and persistent positive urine cytology, are require adjuvant intravesical therapy[1,8].

In the present paper, a clinical study of efficacy of therapeutic of cytotoxic and immunotherapy in patient with superficial transitional bladder cancer after TUR and were analyzed for incidence of recurrence, time-free progression according to Japanese Urological Association[7].

We tried in this study to evaluate, investigate the effectiveness and the adverse effects of MMC in comparison with the immunotherpeutic agent S_2 complex in intravesical local therapy of superficial bladder cancer.

MMC selected in our study because of its proved efficacy[8]. S_2 complex (has been developed at Medical Research Center at the Iraqi Medical College), it is a low molecular weight synthetic organs-metalic complex[9]. The high effectiveness of BCG as immunotherapeutic agent encouraged us to use S_2 complex on it base because S_2 complex is a potent immunostimulator and seem to have antitumor activity[9-11].

Patients and methods

Our study included 75 patients with grade 1-III superficial transitional cell bladder (cis, Ta-T1) who had been registered, treated (and then followed-up between 2001-2003 in Al-Kadhymia Teaching Hospital with intravesical installation mitomycin C and S_2 complex, 2 weeks post TUR. We randomized the patients in two groups:

Group A (38 patients) received therapeutic MMC installation (30mg/30ml/1hour) on day 1 weekly for 6 week & then prophylactic regimen, monthly for 12 month. Group B (37 patient) had been receiving additional S_2 complex (3ml, diluted in 30ml normal saline/1hour) on day 2 after each of the eighteen MMC installations.

The response assessed after induction therapy (6 weeks) and after maintenance doses (12 months), complete response defined as no residual carcinoma cystoscopically and negative urine cytological finding, follow-up ranged from 16 to 26 months with median of 18 months.

Statistical analysis was done for recurrence-free rate (disease free-rate), performed by Kaplan-Meier method for both disease group and were tested with the log rank test.

Results

Patient and tumor characteristics:

Seventy-five patients were entered the study between June 2001 to November 2003 with patient and tumor characteristics were equally distributed between 2 arms. Two patients had been lost during the study; one died because of myocardial infarction and the other refused to continue after the second dose. Table 1 Shows 33 patients had grad-II tumor; male to female ratio 7.1:1 and the age range 21-75 years.

Table 1: Clinicopathological profile of 73 patients with superficial bladder cancer

Criteria's of the study group	Number	%
Men	64	87.6
Women	9	12.4
Age ≤ 30	7	10
≤ 50	12	16
≥ 50	54	74
Stage TA	51	70
T1	16	22
cis	6	8
Grade GI	33	45
GII	27	37
GIII	13	18
Multicentericity		
Solitary	49	67
Multiple	15	21
Unknown	9	12

The efficacy of treatment and the time to first recurrence recorded in both groups showed more effective treatment system in the group B who treated with MMC and S_2 complex especially for those with TA and grade-III, (Table 2 and figure 2).

Table 2: Overall results of the study, according to stage and grade of the cancer

Result	MMC Group		MMC+S_2 Complexe group	
	No	**%**	**No**	**%**
Recurrence of tumor	20(37)	54	11(36)	30
Stage				
Cis	1(3)	3	0(3)	0
TI	4(10)	10	3(6)	8
TA	15(24)	41	8(27)	22
Grade				
GI	8(20)	21.5	2(13)	5.5
GII	7(11)	19	6(16)	16.5
GIII	5(6)	13.5	3(7)	8

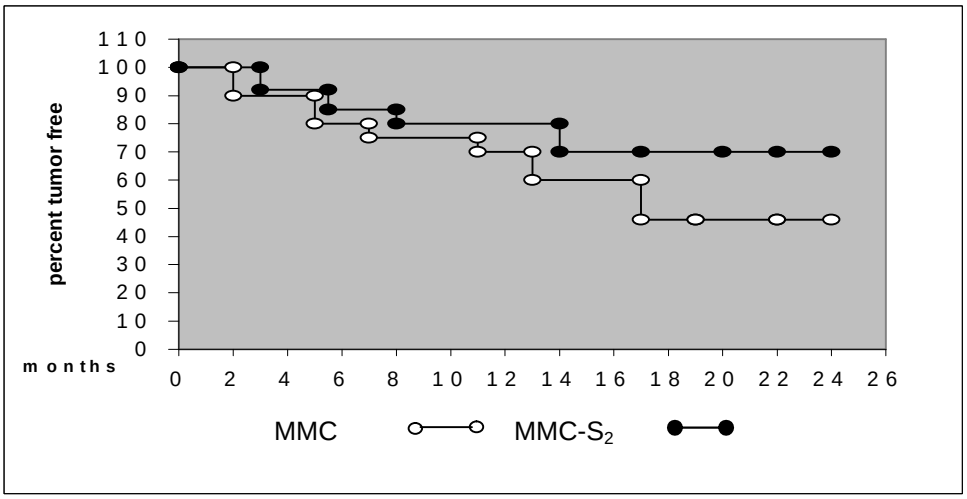


Figure 1: Kaplan Meier curves for time recurrence of patient with papillary tumor cis, pTa, pT1; grade 1 to 3 transitional cell cancer free of tumor after TUR% and adjuvant MMC group and MMC-S2 group.

Figure 2 shows higher percent of patients in group A were remain in remission state after 26 months of follow-up, two patient end with cystectomy and pulmonary metastases in group A while another 3 patients end with total cystectomy and with radiotherapy in group B.

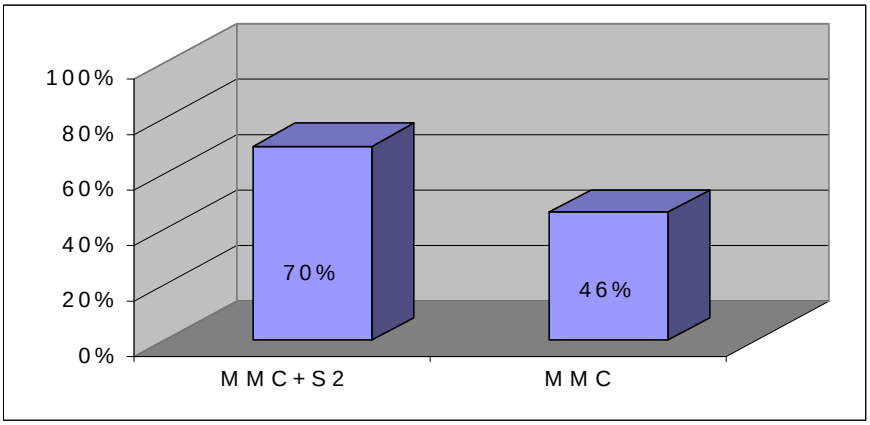


Figure 2: Percentage of patient in remission at the end of the study.

The number and severity of adverse effects were recorded during instillation period are listed in table 3. Mild drug induced cystitis equally reported in both groups.

Table 3: Toxicity

Toxicity	Group A		Group B	
	Number	%	Number	%
Bladder irritation				
Mild	9	24	10	28
Moderate	2	5	3	8
Severe	0	0	0	0
Microscopical hematoria	6	16	7	19
Systemic allergy (mild)	0	0	2	5

Discussion

Administration of adjuvant intravesical chemotherapy after TUR of superficial transitional cell cancer of the urinary bladder (cis: Ta: T1) has become a common practice and confirmed to be effective in reduction of the short and intermediate term incidence of recurrence[12,13].

Intravesical MMC and doxorubicin sequential therapy seemed to be more effective in eradicating carcinoma in situ than single therapy with MMC or doxerubicin and equally affective as BCG[8,15,17].

Topical BCG therapy is now widely accepted as a choice of treatment on base of immunomodulating effect, although systemic adverse and even fatal reaction may occur[7,14,15].

According to those data about the effectiveness of the combination therapy and immunotherapy: The evaluation of sequential MMC and S_2 complex therapy, confirm clearly the efficacy and safety of this regimen in induction (eradication) and maintenance (prophylactic) treatment of transitional cell carcinoma of bladder after TUR, than if those of MMC or BCG is used singly[6,16,17].

Seventy-six clinical studies reviewed, reporting intravesical therapy within last 20 years; their results rate of the net

benefit: thoitepa (8-27%), doxorubicin (12-23%), MMC (13-35%); MMC and doxorubicin sequential therapy (60-65%)[1,7,8], and in comparison to the overall response of MMC and S_2 sequential therapy (70%) and can reduce significantly the number of tumor recurrence within 2 years[1,13].

In addition, S_2 complex is economically available with minimal local and systemic side effect if compared to that of BCG and the majority of patient can tolerate this treatment schedule[7].

Future efforts have to teach us how to select patient for instillation therapy as surprisingly, when patients were separated according to the presence or absence of ABH antigen on the tumor, the more aggressive ABH-negative tumor were found to recur less frequently than ABH positive tumor[14,15].

Many questions remain unanswered in relation to scheme of instillation, when the ideal time to start treatment? What is the optimal duration of treatment? When is the ideal interval between two installations? Those entire questions must be included in longer duration study to evaluate another schedule and real effect of the promising agent S_2 complex[6].

References

1. Harry, W., Vincent, P., and Willet, F.: An overview of intravesical therapy for superficial bladder cancer. J Urol, 1987; 138: 1383-6.
2. Holand, H., and Otto, U.: Mitomycin instillation to prevent recurrence of superficial bladder carcinoma. Eur Urol, 1983; 9: 84-6.
3. Bono, A.V., Hall, R.R., and Denis, L.: Chemoresection in Ta-Tb bladder cancer. Eur Urol, 1996; 29: 385-90.
4. Hartwig, S., Vure, P., and Hans, J.: Long-term follow-up of cytostatic intravesical instillation in patients with superficial bladder cancer. Eur Urol, 1997; 31: 153-9.
5. Sarosdy, M., Soloway, M., and Sheinfeld, J.: Results of a multicenter trial using the BTA test to monitor for and diagnose recurrent bladder cancer. J Urol, 1995; 154: 379-84.
6. Hideaki, S., Iwao, F., and Takumi Y.: Intravesical mitomycin C and doxorubicin sequential therapy for carcinoma in site of the bladder: A longer follow-up result. J Urol, 1994; 151: 27-30.
7. Hideyuki, A., Shiro, H., and Yoshio, A.: Bacillus Calmette-Guerin treatment of existing papillary bladder cancer and carcinoma in sites of the bladder. Cancer, 1995; 75: 552-60.
8. Solsona, E., Iborra, I., and Ricos, J.: Effectiveness of a single immediate mitomycin C installation in patients with low risk superficial bladder cancer: Short and long term follow-up. J Urol, 1994; 161: 1120-3.
9. Ahmad, A., Hamash, M.H., and Hamad A.W.; Carcinoma of unknown primary origin (U.P.C): Treatment with VAC and S_2 complex. Iraqi Medical J, 2002; 3: 75-80.
10. Al-Salihi, A.R., and Hamash, M.H.: Histopathological changes in sequences cell carcinoma after S_2 complex therapy. Proceeding of first scientific symposium on S_2 complex, 1991; 56-74.
11. Mohammad, M.A., and Hamash, M.H.: Evaluation of S_2 complex in animal models: immunological and therapeutic aspects. Proceeding of first scientific symposium on S_2 complex, 1993; 23-32.
12. Carl, L., Bojahan, N., and Peter, E.: A randomized prospective study comparing long-term intravesical instillation of mitomycin C and bacillus Calmette-Guerin in patients with superficial bladder carcinoma. J Urol, 1996; 156: 372-6.
13. Bouffiouk, C.H., Kurth, K., and Bono, A.: Intravesical adjuvant chemotherapy for superficial transitional cell bladder carcinoma: results of 2 European organization for research and treatment of cancer randomized trials with mitomycin C and doxorubicin comparing early versus delayed instillation and short-term versus long-term treatment. J Urol, 1995; 153: 934-41.
14. Donald, L., Daniel, E., and Valerie, D.: Bladder cancer immunotherapy. J Urol, 1982; 128: 931-5.
15. Lamm, D.L., Ther, D.E., and Haris, S.: Bacillus Calmette-Guerin immunotherapy of superficial bladder cancer. J Urol, 1980; 124: 38.
16. Kiemency, L., and Witjes, J.: Predictability of recurrent and progressive disease in individual patients with primary superficial bladder cancer. J Urol, 1993; 150: 60-4.
17. Fukui, I., Sekine, H., and Kihara, K.: Intravesical combination chemotherapy with mitomycin and doxorubicin for carcinoma in sita of the bladder. J Urol, 1980; 141: 531-6.

TUBERCULOSIS OF THE BREAST IN SAMARRA CITY

Harith M. AL- Khateeb *D.G.S, C.A.B.S*

Abstract

Background: Tuberculosis of udder is not a rare disease in animals especially cows but it is rare in human female breast and extremely rare in male breast.

Objectives: Looking for involvement of women breast with tuberculosis.

Methods: From the 1st of July 1992 to the 1st of July 2002, 15 female patients, all in reproductive age were studied for their breast problems under the suspicion of tuberculosis, 14 cases appeared to be tuberculosis diagnosed by FNA cytology and biopsy and treated by anti TB drugs therapeutic trial.

Results: Treatment with anti-tuberculous drugs cured 14 cases.

Conclusion: The tuberculosis of human breast is not very rare disease and should keep in mind when breast infection, ulcers, sinuses and inflammatory masses are not responding to traditional drugs. Tuberculosis of the breast responds very well to anti-tuberculous drugs and cure completely.

Keywords: Breast, tuberculosis, anti-tuberculous drugs

IRAQI J MED SCI, 2005; VOL. 4 (1): 77-80

Introduction

Tuberculosis (TB) is an old and nasty disease, it was common all over the world but it starts to disappear after the discovery of the anti-tuberculous drugs, yet, in the last two decades a flare up in the disease all over the world is noted and reported[1].

Tuberculosis can affect creatures other than human being especially the cows and mainly it affects its udder, the milk production apparatus which cause infection of the milk by TB bacilli which can infect the human if it is consumed without proper sterilization[2,3].

Tuberculosis can affect any system in the body, and many centers reported that human female breast can be involved by the disease usually unilateral and rarely bilateral and there are reported cases in India of male breast TB[4].

Dept. Surgery, College of Medicine, Tikrit University

Address correspondence to Dr. Al-Khateeb H.M., E-mail: khateeb_hm@yahoo.com

Received 21st August 2004: Accepted 27th December 2004.

Hippocrates named tuberculous lesions "pthisis" from the Greek word meaning, "to decay". Sir Astley Cooper was the first one who described a case of breast tuberculosis in 1829 and called it "scrofulous swelling of the bosom"[5]. In 1944, Klossner reported 50 cases of tuberculosis of the breast in women, out of 75,000 with lung involvement. Later on, in 1952, McKeown an Wilkinson described two forms of breast tuberculosis, the primary in which breast infection is the only manifestation of the disease and the secondary one in which the patient had already tuberculosis diagnosed elsewhere[6,7].

Patients & Methods

Fifteen female patients of ages 21 years-46 years, all were married and had breast-fed their children collected along 10 years from July 1, 1992 to July 1, 2002.

The right breast affected in six cases, both breasts affected in one case, and the left breast affected in eight cases including the single case of granulomatus mastitis.

The patients presented as slowly developing breast problem, pain, swelling, and tenderness. In four patients, (26.6%) there was a nipple discharge, pus or serosinguinus in nature. 6 patients (40 %) had local redness and indurations, 3 patients (20%) had a mildly tender mass, freely mobile and not very well marginated. 2 patients (13.3%) had diffuse indurations of a localized sector of the breast.

Ten patients (66.6 %) underwent surgical drainage to their breast lesions but the drainage opening did not heal and changed to chronic ulcer with dark, undermined edges with thin watery discharge. These 10 patients subjected to fine needle aspiration (FNA) cytology, which revealed some acute and mainly chronic inflammatory cells with giant cells of Langhans type.

The rest, five cases underwent open surgical biopsy for their breast lesions, which showed a picture of chronic inflammatory changes with giant Langhans cell also. One case (6.6%) the biopsy showed a picture of granulomatous mastitis.

Nine patients had small palpable mobile lymph nods in the same side axilla, but no one had a chest or lung problem.

Because of no response of these cases to treatment by traditional antibiotics and the lesions, persist for more than 1-2 months, Anti TB drugs therapeutic trial given with a very good response within 2-3 weeks in 14 cases.

The anti TB drugs used are INH tablets 100 mg three times daily, ethambutol tablets 500mg three times daily, refampicin capsules 300mg twice per day for two months then stopping ethambutol and continue on the others for 6-9 months.

Results

The single case, which did not respond to anti TB drugs, was the case of granulomatus mastitis, which responded moderately to steroids.

All tuberculous patients have cured and the affected breasts became normal in shape, size, texture and consistency. All the ten patients who had ulceration of the skin of the breast developed a small scar with a faint white yellowish discoloration at the site of the ulcer after its healing.

One patient developed cholestatic jaundice 2 weeks after starting refampicin capsules due to hepatic toxicity. Thus, the treatment stopped for 3 weeks until the patients condition improved. Again, anti TB regimen started with substituting refampicin by streptomycin injections one gm. i.m daily for 2 months. INH and ethambutol tablets then stopped for other 4 months.

One year after, the patient developed swelling and induration with pain at the same site of the previous lesion. FNA cytology revealed epithelioid cells with Langhans giant cells. So the case regarded as recurrence and new course of anti TB regime started, INH, ethambutol, streptomycin, and pyrazinamid for 2 months. The streptomycin stopped and we continued with the other three drugs for other 4 months.

Including the recurrent case, eight patients became pregnant and breast-fed their babies after delivery with no problems.

The treatment and cure of the 14 patients whose diagnosis depends on the clinical picture, FNA cytology and biopsy suggestion because it is very difficult to reveal the TB bacilli in the examined samples and cultures, and it is the same policy followed in other countries, India and North Korea.

Discussion

Today, TB remains as a major public health problem in the world. According to the WHO, 10 million new cases of active TB occur each year worldwide[8]. HIV infection, chronic diseases, malignancy, transplantation and other

immunosuppressive conditions, aging, and resistant strains lead to increase of TB patients[9,10]. In these groups, tuberculosis may present atypically and sometimes with predominant extra-pulmonary manifestations that result in delays in diagnosis and treatment[11].

Tuberculosis of the breast is a rare disease. It is uncommon even in countries where the incidence of pulmonary and extra-pulmonary tuberculosis is high[12].

Primary form is an infection of the breast through abrasions or through the openings of ducts in the nipple. Secondary form is the result of reverse lymph flow in the axillary lymph nodes or it may be due to direct spread of the infection from intra-thoracic foci[12,13].

Routes of infection include hematogenous spread, lymphatic spread from intra-thoracic foci or from intra-abdominal foci, and by direct extension from adjacent tissues or via abrasions of the skin[9,14].

According to clinical, radiological and pathological appearance of the disease, there are three types of breast tuberculosis:

1. Nodular tuberculosis characterized by a well circumscribed, slowly growing, painless mass. Often the mass, getting larger, infiltrates the skin. At this point, the tumor becomes painful, causing ulceration, and discharge from one or more sinus tracts. This course makes differentiation from carcinoma very difficult.
2. Diffuse type or disseminated tuberculous mastitis characterized by multiple foci, which may lead to sinus formation. The overlying skin thickened and painful ulcers may develop. Axillary lymph nodes frequently infiltrated.
3. Sclerosing type, were excessive fibrosis is the dominant feature. It is slow growing, and suppuration is rare. Clinically there is a hard, painless lump with nipple retraction. Clinical examination usually fails to differentiate carcinoma from tuberculosis[15,16].

FNA cytology has proved to be very useful for diagnosis of breast tuberculosis and reflects the histopathological lesion very accurately[17]. Treatment with standard anti tuberculous drugs has proved to be very successful and avoids unnecessary mutilating surgery[18].

Conclusion

The TB of the breast in human female is not a very rare disease neither in our country nor in other countries so the disease should be kept under suspicion in treating breast lesions.

The response to treatment with standard anti tuberculous drugs is very good and the cure rate is high.

Because it is difficult to reveal the TB bacilli in the specimens from the lesion, the policy of anti TB drugs therapeutic trial should be followed in the treatment.

References

1. Cooper, A.: Illustrations of the diseases of the breast. London: Longman, Rees & Co. 1829.
2. Klossner, A.R.: Uber die Brustdrusentuberculose. Eine pathologisch-anatomische und klinische studie. Acta Chir Scand, 1944; 90 (Suppl. 85): 1-181.
3. Banerjee, A., Green, B., and Burke, M.: Tuberculous and granulomatous mastitis. Practitioner, 1989; 233: 754-7.
4. Tan, K.K., and Tan, T.H.: Tuberculosis of the breast. Singapore Med J, 1988; 29: 271-5.
5. Sheikh, M.Y., Rana, T.A., and Islam, M.U.: Tuberculous mastitis mimicking malignancy: a case report with review of literature. JPMA, 1993; 43: 122-3.
6. Bahadur, P., Aurora, A.L., Sibbal, R.M., and Prabhu, S.S.: Tuberculosis of the mammary gland. JIMA, 1983; 21: 67-80.
7. Rangabhashyam, N., Gnanaprakasm, D., Krishnaraj, B. et al.: Spectrum of benign breast lesions in Madras. J R Coll Surg Edinb, 1983; 28: 369-73.
8. Sharma, A.K., Sree, S., and Misra, S.K.: Tuberculous mastitis: a pragmatic approach to its management. Aust N Z J Surg, 1993; 63: 263-5.
9. The Breast. In: AJ Hardinge Rains, Charles V Mann, eds. Bailey and Love's short practice

of surgery, 20th ed. London, HK Lewis and Co. Ltd., 1989; p.p. 712-39.
10. Wilson, J.P., and Chapman, S.W.: Tubercular mastitis. Chest, 1990; 98: 1505-9.
11. Tabar, I., Kett, K., and Nemeth, A.: Tuberculosis of the breast. Radiology, 1979; 118 (3): 587-9.
12. Baily & Loves Short Practice of Surgery. 23rd edition, 2000.
13. Shinde, S.R., Chandawarkar, R.Y., and Deshmukh, S.P.: Tuberculosis of the breast masquerading as carcinoma: a study of 100 patients. World J Surg, 1995; 19 (3): 379-81.
14. Goksoy, E., Duren, M., Durgun, V., and Uygun, E.: Tuberculosis of the breast. Eur J Surg, 1995;161 (7): 471-3.
15. Makanjuola, D., Murshid, K., Sulaimani, A.l., and Al-Saleh, M.: Mammographic features of breast tuberculosis:the skin bulge and sinus tract sign. Clin Radiol, 1996; 51 (5): 354-8.
16. Oh, K.K., Kim, J.H., and Kook, S.H.: Eur Radiol, 1998; 8 (8): 1475-80.
17. Tabar, L., Kett, K., and Nemeth, A.: Tuberculosis of the breast. Radiology, 1976; 118: 587-9.
18. D'Orsi, C.J., Feldhaus, L., and Sonnefeld, M.: Unusual lesions of the breast. Radiol Clin N Am, 1983; 21: 67-80.

EOSINOPHIL COUNT AND RELATION TO CHILDHOOD ASTHMA

Hussam M. Al-Alwany[1] *DCH CABP*, **Falih Salim**[2] *FIBMS*, **Shatha H. Ali**[1] *CABP*

Abstract

Background: Asthma is a diffuse obstructive lung disease with hyperreactivity of the airways to variety of stimuli. The prevalence and severity of the disease are steadily increasing. In addition to broncho-constriction and viscid secretions, inflammation is an important patho-physiological factor, eosinophil play important role in this inflammation. Eosinophil peripheral blood count in Asthmatic patient is our concern in this study.

Objective: Study the relationship of eosinophil peripheral blood count and Asthma.

Methods: A total number of 55 asthmatic children subjected to a prospective study regarding the severity of asthma, which assessed by clinical examination and spirometry and for each patient a sample of blood, were collected and eosinophil count was calculated.

Results: 35 of a total 55 asthmatic patient found to have moderate to sever asthma of this category 82.8% (29 patients) were having high eosinophil count >400 / mm^3 while 17.2% (6 patient) of less eosinophil peripheral count < 400 / mm^3. The other 20 patients were mild to moderate severity, of these 80% (16 patients) were of low eosinophil count < 400 and 20% (four patients were of high eosinophil count).

Conclusion: There is an increase in eosinophil peripheral blood count in Asthmatics and the more the severe the attack, the more the eosinophil count.

Key words: Asthma, Eosinophil, Severity

IRAQI J MED SCI, 2005; VOL. 4 (1): 81-83

Introduction

Asthma is a leading cause of chronic illness in childhood and responsible for a significant proportion of school days lost because of chronic illness.

Asthma can lead to severe psychosocial disturbance in the family[1]. Asthma is a condition characterized by variation of intrathoracic airway obstruction, which occur spontaneously, or because of treatment. Asthma in fact is an umbrella term rather like hypertension both are clinically important syndromes brought about by several different mechanisms.

The prevalence and severity are steadily increasing in most industrialized nations, presumably as a result of environment change[2]. Asthma defined as a diffuse obstructive lung disease with hyperreactivity of the airways to a variety of stimuli and a high degree of reversibility either of the obstructive process, which may occur spontaneously, or because of treatment. In addition to broncho-constriction and viscid secretions, inflammation is an important pathophysiological factor; it involves eosinophil monocytes and other immune mediators[3].

The eosinophil is a minor species of granulocyte comprising 1-4% of total peripheral blood WBC (normal range 0.04-0.44 x 10^9/L). The eosinophil is the densest leukocyte in the peripheral blood but can also exist as hypodense form common in the blood of patients with asthma or dermatitis. Eosinophils have less phagocytic and bactericidal activity than neutrophils but have an important role in mediating hypersensitivity reactions, bronchial asthma and skin inflammation[4].

[1] Dept. Pediatrics [2] Dept. Pathology, College of Medicine, Al-Nahrain University
Address correspondence to Dr. Hussam M. Al-Alwany.
Received 25th September 2004: Accepted 6th December 2004.

Eosinophil, predominantly provide protection against parasites aided by T-Helper lymphocyte[4].

The intention of the study is to find the relation between eosinophil count in the peripheral blood of patients with asthma and severity of their disease.

Patients and Methods

A total number of 55 asthmatic children subjected to a prospective study regarding the severity of their asthma, which assessed by clinical criteria (mild, moderate and severe) and supported by spirometry measurement of FEV1 taken by portable spirometer in out patient clinic.

From each patient venous blood collected into dik-EDTA tube, and 20-millimeter blood mixed with 400 mm of glacial acetic acid, improved Neubaur chamber used to calculate the total leukocyte count, a blood film was prepared using leishman's stain and the differential WBC count found[5]. The absolute Eosinophil count calculated according to the formula[6].

Absolute eosinophil count = differential eosinophil count x total WBC/100

Results

There was 35 cases with moderate-sever asthma (63.6%), divided into 29 (82.8%) with >400/mm^3 and 6 (17.2%) < 400/mm^3 and 20 cases were mild-moderate asthma (36.4%), divided into 16 (80%) < 400 and 4 (20%) > 400, the difference between the two groups was statistically significant using chi-square.

Severity of Asthma attacks

Parameters	Mild	Moderate	Severe	Respiratory arrest imminent
Talking in alertness	Sentences , may be agitated	Phrases, usually agitated	Words, usually agitated	Drowsy or confuse
Respiratory rate	Increased	Increased	Mark increase	Parodoxical
Accessory muscle	Usually not	Usually	Usually	Paradoxical thoraco-abdominal movement
Wheeze	Moderate , often only end expiratory	Loud	Usually Loud	Absence of wheeze
Pulse / min	<100	100-200	>120	Bradycardia
PEF	over 80%	60-80%	<60%	

Discussion

Many studies suggest the relation of eosinophils to allergy and hyper reactive airway diseases. The increase in the percentage of moderate-severe asthmatic children may be because the mild case did not reach the center and treated as out patients. The following are examples of some international studies about asthma and eosinophil:

Tonnel and colleagues in their study[7] showed the relation of neutrophils and eosinophil increase in asthma in blood and sputum[6]. Lemiere and colleagues[8] discover the increase in eosinophil in peripheral blood with the more exposure to trigger factors (which support this study). Yamada and colleagues said that the high eosinophil count. And Eotaxin (protein secreted in the lung of asthmatic which is a chemo attractant for eosinophil) the more unstable asthma[9]. They found that about 75% of patients were of high eosinophil count.

Also Lilly CM and Colleagues showed that the more unstable acute attack of asthma the more high eotaxin and eosinoph count[8,10]. However good relationship of eosinophil to asthma

eosinophil can increase by many allergic conditions and parasitic infestation, which can bios the study and this, can be overcomed by selection of defined asthmatic by proper history and clinical finding and spirometry.

Conclusion

The increase in eosinophil count in asthmatic patients is correlate with the severity of attack, the more severe the high blood eosinophil

Recommendations

1. Eosinophil count could be used to asses asthmatic severity.
2. Correlation of clinical and lab finding (eosinophil count) give the best assessment by decrease the range of error in the assessment.

References

1. Sly, M.C.: Allergic Disorders. In: Berhman, R.E., Kligman, M.K Aruin, A.N. Nelson textbook of Pediatric. 16th edition, America, W.B Saunders Company 2000; 628-640.
2. Mark, B.Z., Donald, Y.M., and Leung, M.R.: Allergic Disorders. In: William, W.H., Anthony, R.H.: Current pediatric diagnosis and treatment. 14th edition. USA, Philadelphia. Appleton and Lange, 1999; 902-25.
3. Tepas, E.C., and Umetsu, D.T.: Immunology and Allergy. In: Berhman, R.E., Kliegman, M.K. Nelson essentials of pediatrics, WB Saunders Comp., 1999; 254-244.
4. Baltimore, L.S., and Williams, W.K.: Blood Flow and Metabolism. In: Nonn, J.F., Applied Respiratory Physiology, 5th edition, London, Churchill Livingstone Company, 1995; 58-59.
5. Roberts, P.J., Linch, D.C., and Webb, K.D.: Phagocytes. In: Hoffbrand, A.V., Lewis, S.M.: Postgraduate hematology. 3rd edition. Oxford: Heinemann.1999; 309-310.
6. Morstol, A.L., Ernest, B.D., Thomas, J.K., and William, J.W.: Manual of Hematology, 6th Ed. London: McGrow-Hill. 2003; 200-1.
7. Tonnel, D.C., Gosset, P.L., and Tillie, G.I.: Characteristics of inflammatory response in asthma. J Immunol, 2000; 165 (9): 184-5.
8. Lemiere, C.K., Chabarilliez, S., Trudeau, C.P., Taha, R., Maghi, R.D., Nootin, I.G., et al.: Kaka of airway inflammation after repeated exposure to stimuli. J Allergy Clini Immunol, 2000; 206 (6): 163-70.
9. Yamada, H.M., Yamaguchi, M., Yamamato, K.J., NakaJima, T., Hiraai, R., Morita, Y., et al.: Relationship of eosinophil and cationic proteins. J Allergy, 2000; 55 (4): 392-7.
10. Lilly, C.M., Woodrull, P.G., Gamargo, C.A. Nakamora, H., Drazen, J.M., Nodal, E.S., et al.: Increase eotaxins eosinophils in patient with acute asthma J Allergy Clini Immunol, 1990; 104 (4): 786-90.

SERUM CALCIUM LEVEL IN PATIENTS WITH TYPE II DIABETES MELLITUS

Baybeen K. Al-Selevany *PhD*

ABSTRACT

Background: Evidence for a disturbance of mineral metabolism in diabetes has accumulated since the eighties of the last decade. Calcium ion shown to play an important role in the biosynthesis, storage, release and activity of insulin, in addition, to glucose tolerance in human beings.

Objective: To examine if serum calcium level (SCaL) is influenced in patients with type II diabetes mellitus (type 2 DM).

Methods: The study comprised one hundred twenty subjects of both sexes. Sixty patients of newly diagnosed type 2 DM with a mean±SD age of 47.6±11.6 years and 60 healthy controls with a mean age±SD of 35.2±14.3 years. Main outcome measures (SCaL) and serum glucose level (SGL) in fasting blood samples.

Results: Serum calcium level and SGL were significantly higher in newly diagnosed type 2 DM as compared to healthy non-diabetic controls. Furthermore, there was no correlation between SCaL and hyperglycemia in diabetic patients.

Conclusion: Hypercalcemia may be a result of other factors in diabetes mellitus rather than hyperglycemia.

Key words: Type 2 diabetes mellitus, calcium, glucose, hypercalcemia, hyperglycemia.

IRAQI J MED SCI, 2005; VOL. 4 (1): 84-88

Introduction

Approximately 97% of people who have diabetes mellitus have type II diabetes mellitus (DM)[1]. Evidence for a disturbance of mineral metabolism in diabetes has accumulated since the eighties of the last decade[2]. Extracellular calcium provides calcium for maintenance of intracellular calcium ion, bone mineralization, renal function, hormone secretion, and a second messenger affecting enzyme activity[3].

Calcium ion shown to play an important role in the biosynthesis, storage, release and activity of insulin. In addition to, glucose tolerance in human beings[3-7]. Pancreatic ß-cells of the islets of Langerhans are rich in Ca^{+2}[4]. The role of Ca as a mediator of insulin action was originally proposed by Clausen et al[8] and Kissebah et al in the mid seventies[9]. Although some investigators failed to observed a relationship between Ca and insulin action[10-11] diverse aspects of insulin action have been demonstrated to be dependent upon extracellular and cytoplasmic Ca^{+2}[12-15].

Several studies among diabetics of both types I and II have demonstrated abnormalities in Ca^{+2} metabolism[16-18]. Also, type II DM results in altered cellular Ca^{+2} regulation and transport[19]. Homeostasis of Ca^{+2} is disturbed in animal models with diabetes mellitus; nevertheless the effects of DM on Ca^{+2} metabolism in human are controversial. Although some authors have found no significant alterations, apart from excessive urinary loss of Ca^{+2}[20], increased Ca^{+2} absorption has been reported following oral Ca^{+2} loading in type I diabetics[21] and slightly higher levels of serum calcium have been found in type II DM patients than in normal subjects[2, 5, 22].

Dept. Medical Physiology, College of Medicine, University of Mosul, Mosul-Iraq
Address correspondence to Dr. Baybeen K. Al-Selevany, E-mail: gollan_phydept@yahoo.com
Received 23rd October 2004: Accepted 8th December 2004.

The aim of the present study was to examine serum calcium level in newly diagnosed type II diabetics attending Al-Waffa Diabetic Clinic in Mosul .

Patients & Methods

Sixty patients with newly diagnosed type II DM of both sexes with a mean±SD age of 47.6±11.6 years who were attending Al-Wafa Diabetic Clinic in Mosul during the period from September 2002-Febraury 2003, and sixty non-diabetic healthy controls with a mean±SD age of 35.2±14.3 years, were included in this study. No participants were taking any mineral supplements or medication, during the period of the study.

The median and range of the SCaL and SGL in controls and type II diabetics presented in the table 1. The proportion of patients who had hypercalcemia was 81.7%.

Table 1: Median and range for the SCaL and SGL in healthy controls and type 2 diabetics

Parameters (mmol / L)	Controls n = 60		Type II diabetics n = 60	
	median	range	median	range
SCaL	2.22	1.64 - 2.71	3.08	2.21– 4.01
SGL	4.96	3.47 – 6.87	11.84	3.37 – 21.85

Venous blood samples were taken between 8 and 10 a.m. after overnight fast and collected into an acid-rinsed metal-free covered glass test tube and allowed to clot for 1-2 hours at room temperature. After centrifugation, the serum removed. Glucose measurement performed immediately, while serum for estimation of calcium level was stored at -20 ºC in other metal free plastic test tubes for further analysis.

Serum glucose concentration was measured by spectrophotometric enzymatic end point method[23]. Serum calcium level analyzed by dye colorimeteric method, which uses O-cresolphthalein complexone (CPC). In alkaline solution, CPC forms a red chromophore with Ca^{+2} [24]. The kit purchased for estimation of SCaL and SGL was from Biocon Co. (Germany).

Z-test and Pearson's correlation coefficient (r) used for the analysis of the data. The accepted level of significance was at $P < 0.05$.

Results

Serum calcium and serum glucose levels were significantly higher in type II diabetics ($P< 0.001$) compared with those in healthy controls (Table 2 and Figure 1).

Table 2: Mean±SD for SCaL in type II diabetics compared to the healthy non-diabetic controls (data presented as mean±SD)

Variables (mmol/L)	Control (n = 60)	Type II diabetics (n = 60)
SCaL	2.17±0.27	3.11±0.45**
SGL	5.17±0.85	12.62±4.33**

** Significantly different from the respective control value, p<0.001

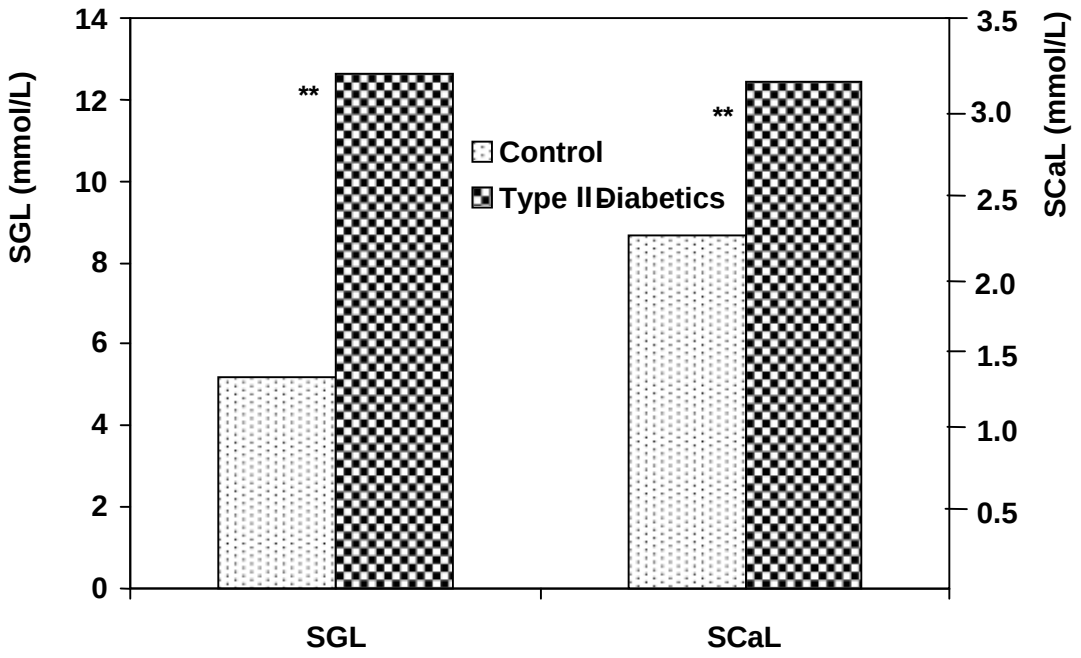


Figure 1: Comparison between mean values of SCaL and SGL in control subjects and type 2 diabetics.

There was no significant correlation between SGL and SCAL (r = - 0.03, p > 0.05) in type 2 diabetics, or in healthy controls (r = - 0.091, p > 0.05).

Discussion

The exact manner by which defective glucose homeostasis interferes with mineral metabolism remains unsettled[2]. A series of reports suggest the existence of altered Ca metabolism in diabetes mellitus[16-18, 25].

Hypercalcemia has been reported by several another to be present in type II diabetics[2,5,22,26] which is in agreement with the observation in the present study, but in contrast with the report of others[27-31].

Hypercalcemia occurs because of an increased flux of Ca^{+2} into the extracellular fluid compartment from the skeleton, intestine, or kidney[23].

The assumed hypercalcemia in this study may be due to dehydration; this is expected in the newly diagnosed diabetics with severe hyperglycemia[32].

Bone mineral loss is another abnormality that recognized as a feature of the diabetic patients. This suggests that hypercalcemia may be as a result of calcium loss from bone[33].

Hypercalcemia in diabetic patients is speculated to be partly attributed to the low level of serum albumin[5,26]. Normally about 47% of Ca^{+2} in the plasma combined with plasma proteins[34].

The tendency of hypercalcemia observed in diabetic patients could be also due to the decreasing renal tubular reabsorption[2,5], and increasing intestinal absorption of calcium[24]. Such inhibition of tubular reabsorption could be caused by hyperglycemia per se[35-36] or by some other direct action of abnormal glucose homeostasis on the renal tubules. The definitive mechanism is still unknown.

Many interactions occur between Ca^{+2} and insulin metabolism. Calcium ion shown to be associated with the conversion of pro-insulin into insulin or

glucose tolerance in human beings and numerous laboratory animals[3-5,7]. This suggests that hypercalcemia may be a result of insulin resistance[3, 6].

The other probable causes of hypercalcemia may include primary hyperparathyroidism which is a common disorder resulting from increased secretion of parathyroid hormone from parathyroid adenomas, carcinomas or hyperplasia.

Parathyroid hyperplasia may be associated with other inherited endocrine disorders (multiple endocrine neoplasia 1 and IIa). Multiple endocrine neoplasia; type I (MENI) which also called wermer's syndrome, this disorder includes tumor or hyperplasia of parathyroid, pituitary, and non- β cells of pancreas.

Hypercalcemia secondary to malignancy is relatively common and occurs in 10 to 20 of patients with cancer. The degree of hypercalcemia is worse with malignant disease (often greater than 3.5mmol/L) than with hyperparathyroidism (serum calcium less than 3.5 mmol /L[37,38].

References

1. Seeley, R.R., Stephens, T.D., and Tate, P.: Anatomy and Physiology. WCB McGraw-Hill companies, 4th edition 1998; p. p. 568.
2. Olukooa, A.O., Adewoye, H.O., and Erasumus, R.Y.: Renal excretion of magnesium and calcium in diabetes mellitus. Cen Afr J Med, 1989; 34 (4): 378-83.
3. Resnick, L.M.: Calcium metabolism in hypertension and allied metabolic disorders. Diabetes Care, 1991; 14 (6): 505-20.
4. Dodson, G., and Steiner, D.: The role of assembly in insulin's biosynthesis. Current Opinion in Structural Biology, 1998; 8: 189-94.
5. Chen, M.D., Lin, P.R., Tsou, C.T., Wang, J.J., and Lin, W.H.: Selected metals status in patients with non-insulin dependent diabetes mellitus. Biol Trace Elem Res, 1995; 50 (2): 119-24.
6. Draznin, B., Sussman, K.E., Eckel, R.H., Kao, M., Rosi, T., and Sherman, N.A.: Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. J Clin Invest, 1988; 82: 1848-52.
7. Howel, S.L., Tyhurst, M., Duvefelt, H., Andersson, A., and Hellerstrom, C.: Role of zinc and calcium in the formation and storage of insulin in the pancreatic β-Cell. Cell Tiss Res, 1978; 188: 107-18.
8. Clausen, T., Elbrink, J., and Martin, B.R.: Insulin controlling calcium distribution in muscle and fat cells. Acta Endocrinol, 1974; 77: 137-43.
9. Kissebah, H.A., Hope-Gill, H., Vydelingum, N., Tullock, B.R., Clark, P.V., and Fraser, T.R.: Mode of insulin action. Lancet, 1975; 1: 144-7.
10. Klip, A.: Is intracellular Ca^{2+} involved in insulin stimulation of sugar transport? Fact and prejudice. Can J Biochem, 1984; 62: 1228-36.
11. Klip, A., Li, G., and Logan, W.J.: Role of calcium ions in insulin action on hexose transport in L6 muscle cells. Am J Physiol, 1984; 247: E297-E304.
12. Plehwe, W.E., Williams, P.F., Caterson, I.D., Harrison, L.C., and Turtle, J.R.: Calcium-dependence of insulin receptor phosphorylation. Biochem J, 1983; 214: 361-6.
13. McDonald, J.M., and Pershadsingh, H.A.: The role of calcium in the transduction of insulin action. In: Molecular Basis of Insulin Action M. Czech, (editor). Plenum Publishing Corp., New York, 1983; p.p. 103-17.
14. McDonald, J.M., Bruns, D.E., and Jarett, L.: Ability of insulin to increase calcium binding by adipocyte plasma membranes. Proc Natl Acad Sci USA, 1976; 73: 1542-6.
15. Pershasingh, H.A., and McDonald, J.M.: Direct addition of insulin inhibits a high affinity Ca^{2+}- ATPase in isolated adipocyte plasma membrane. Nature (Lond.), 1979; 281: 495-97.
16. Ishimura, E., Nishizaw, R., Koyama, H., Shoji, S., Inaba, M., and Morii, H.: Impaired vitamin D metabolism and response in spontaneously diabetic GK rats. Miner Electrolyte Metab, 1995; 21(1-3): 205-10.
17. Frazer, T.E., White, N.H., Hough, S., Santiago, J.V., McGce, B.R., Bryee, G., et al.: Alterations in circulating vitamin D metabolities in the young insulin dependent diabetic. J Clin Endocrinol Metab, 1981; 53: 1154-59.
18. Auwera, J., Deqacker, J., Bouillon, R., Geusens, P., and Nijs, J.: Mineral metabolism and bone mass at peripheral and axial skeleton in diabetes mellitus. Diabetes, 1988; 37: 8-12.
19. Allo, S.N., Lincoln, T.M., Willson, G.L., Green, F.J., Watanabre, A.M., and Schaffer,

S.W.: Non-insulin dependent diabetes induced defects in cardiac cellular calcium regulation. Am J Physiol, 1991; 260 (6 pt 1): C1165-C71.
20. McNair, P., Madshad, S., Christensen, M.S., Faber, O.K., Binder, C., and Transbol, I.: Bone mineral loss in insulin treated diabetes mellitus: studies on pathogenesis. Acta Endocrinol, 1979; 90: 463-9.
21. Hough, S.: Alterations of bone and mineral metabolism in diabetes mellitus. Part II. Clinical studies in 200 patients with type I diabetes mellitus. S Afr Med J, 1987; 72: 120-26.
22. Levy, J., Stern, Z., Gutman, A., Naparsrck, Y., Gavin, J.R., and Avioli, L.V.: Plasma calcium and phosphate levels in an adult non -insulin dependent diabetic population. Calcif Tissue Int, 1986; 39: 316-8.
23. Burtis, C.A., and Ashwood, E.R.: Tietz Fundamentals of Clinical Chemistry .4th edition .Philadelephia: WB Saunders, 1998; p.p. 362-63, 698.
24. Christenson, R.H., Gregory, L.C., and Johnson, L.J.: Appletone and Langens Outline Review Clinical Chemistry. New York: McGraw Hill Companies; 2001: p.p. 128-9.
25. Kawagishi, T., Seklya, K., Okuno, Y., Miki, T., Nishizawa, Y., and Morii, H.: Calcium metabolism in diabetes mellitus. J Nutr Sci Vitaminol, 1991; 37 (suppl): 551-6.
26. Kobbah, A.M., Hellsing, K., and Tuvemo, T.: Early changes of some serum proteins and metals in diabetes children. Acta Paediat Scand, 1988; 77: 734-40.
27. Mather, H.M., Nisbet, J.A., Burton, G.H., Poston, G.J., Bland, J.M., Bailey, P.A., et al.: Hypomagnesaemia in diabetes. Clin Chim Acta, 1979; 95: 235-42.
28. Olukoga, A.O., Erasmus, T.R., and Adewoye, H.O.: Erythrocyte and plasma magnesium concentrations in Nigerians with diabetes mellitus. Ann Clin Biochem, 1987; 24: (Suppl): P2: 248-9.
29. McNair, P., Fogh-Anderson, N., Madsbad, S., and Christensen, M.S.: Decreased serum concentrations of ionized calcium in insulin-dependent human diabetes mellitus. Eur J Clin Invest, 1983; 13(3): 267-70.
30. Fuji, S., Takemura, T., Wada, M., Akai, T., and Okuda, K.: Magnesium levels in plasma, erythrocyte and urine in patients with diabetes mellitus. Horm Metab Res, 1982; 14: 161-2.
31. Isuia, G.C., Ardissone, P., Distefano, M., Ferrat, D., Martina, V., Porta, M., et al.: Bone metabolism in type 2-diabetes mellitus. Acta Diabet, 1999; 36 (1-2): 35-8.
32. Ganong, W.F.: Review of Medical Physiology. Stamford. Lange Medical books/McGraw-Hill. New York, 21th edition, 2003; p.p. 344.
33. Levin, M.E., Biosseau, V.C., and Avioli, L.V.: Effects of diabetes mellitus on bone mass in Juvenile and adult-onset diabetes. N Engl J Med, 1976; 294: 241-5.
34. Guyton, A.C., and Hall, J.E.: Textbook of Medical physiology. Philadelphia. W.B. Saunders Comp., 10th edition 2001; p.p. 899-900.
35. Lindemann, R.D., Adler, S., Viengst, M.J., and Beard, E.S.: Influence of various nutrients on urinary divalent cation excretion. J Lab Clin Med, 1967; 70: 236-45.
36. Prien, E.L., and Ricinati, E.S.: Evidence that glucose injection inhibits net renal tubular reabsorption of calcium and magnesium in man. J Lab Clin Med, 1970; 75: 578-85.
37. Braunwald, E., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L., and Jameson, J.L.: Harrison's Principles of Internal Medicine. New York. McGraw-Hill. 15th edition 2001; p.p. 2184-209.
38. Griffin, J.E.: Pretest Manual of Clinical Endocrinology and Metabolism. New York. McGraw-Hill Book Comp., 1982; p.p. 178-88, 260-61.

CONGENITAL HYPOTHYROIDISM IN AL-KADHIYMIA TEACHING HOSPITAL, BAGHDAD-IRAQ

Nasheit A. Nasheit[1] *CABP*, **Husam Al-alwani**[1] *CBAP*, **Iman S. Mahmoud**[2]

Abstract

Background: Congenital hypothyroidism is one of the most encountered endocrine diseases in childhood and the importance of this diagnosis is because early and adequate treatment prevents developmental retardation and other squeal of the condition.

Objective: Looking for the congenital hyperthyroidism in Iraqi children.

Methods: A retrospective study involved 40 children who were attended Pediatric Endocrine and diabetic Clinic for children in Al-Kadhiymia Teaching Hospital, College of Medicine, Al-Nahrain University Baghdad, Iraq, over period from Jan 1993-Jan 2003 .

Results: Of the 40 patients, 24 were female and 16 male. Female to male ratio 1.5:1.A history of parental consanguinity was positive in 36(80%) of the patients and 25(62.5%) had family history of hypothyroidism, and 24(60%) of the patients were from urban area 16(40%) from rural area west to Baghdad. Ten children (25%) detected in first month of life, 15(37.5%) in first 3 months and 25(62.5%) within the first 6 months. Seven patients had aplasia of thyroid gland, five had ectopia,and 8 had thyroid in normal position . Developmental assessment performed on all patients, also clinical manifestation in 20 patients diagnosed before 6 months of life.

Conclusion: Although we do not know much about the overall prevalence of congenital hypothyroidism in Iraq, the first observation to make is that it is not a rare disease in this country. A genetic explanation suggested by the involvement of multiple siblings and the high rate of consanguinity in this population.

Key Words: Congenital hypothyroidism, Retrospective

IRAQI J MED SCI, 2005; VOL. 4 (1): 89-92

Introduction

Congenital hypothyroidism (CH) results from deficient production of thyroid hormone or defect in thyroid hormone receptor activity. Congenital causes of hypothyroidism may be sporadic or familial, goitrous or non-goitrous. In many cases the deficiency of the thyroid hormone is sever and symptoms develop in early weeks of life, in others lesser degree of deficiency occur or manifestations may be delayed for months[1].

Early treatment of CH particularly within first 3 months of life usually results in normal psychomotor development since the mass neonatal thyroid screening programmes have been introduced in many industrialized countries aiming at prevention of mental retardation by early diagnosis of CH and prompt thyroid replacement therapy[2,3]. However, in most of the developing countries the diagnosis of CH still based on clinical grounds. This may results in a delay in the diagnosis and the initiation of thyroid replacement therapy with consequent mental retardation.

[1] **Dept. Pediatrics, College of Medicine Al-Nahrain University** [2] **Central Arab Child Hospital**

Address correspondence to Dr. Nasheit A. Nasheit e-mail: naazna2003@yahoo.com

Received 5th December 2004: Accepted 14th February 2005.

Patients & Methods

This retrospective study included children who were attended Pediatric Endocrine and Diabetes Clinic in Al-Kadhiymia Teaching Hospital, Baghdad-Iraq, over period of 10 years from Jan. 1993-Jan. 2003. They were clinically diagnosed as having CH. Data recorded includes sex, age ,residency, parental consanguinity, family history of thyroid disease, pregnancy history, place of birth, postnatal fallow up, clinical manifestation, age at diagnosis and treatment, and assessment of mental development by Stanford-Binet scale.

Diagnosis of CH was confirmed by thyroid function tests (T4, T3, TSH and TC99M thyroid scan), the values considered according to the age (some investigation performed out side Iraq Perchlorate discharge test). Skeletal maturity was assessed by X-ray of the knee for infants and other joints for older children. Thyroid function tests made by radioimmunoassay methods using commercially available kits.

Results

Forty Patients with CH, Twenty-four (60%) from urban area and 16(40%) from rural area west to Baghdad. The female to male (24/16) ratio was 1.5:1.

A history of parental consanguinity was positive in 36(80%) of the patients and 25(62.5%) had family history of thyroid disease. In seven families, multiple siblings affected and in none of the patients was maternal history of ingestion of any medication during pregnancy.

Eleven children (27.5%) were detected in first month of life, at age from 1-3 months 6 (15%) and at age 4-6 months 5(12.5%), at age 7-12 months 3(705%), and nine (22.5%) at age from1-3 years and six (15%) were diagnosed after age of 3 years (Table 1).

TC-99M thyroid scan was performed for 20 patients, seven had no thyroid gland (aplasia), five had ectopia and 8 had thyroid gland in normal position (Table 1).

The mean period of fallow up was 2.1 year (range 6 months- 5.5 year). Development assessment performed on all patients: Ten (25%) had moderately severe mental retardation (IQ more than 50), Sixteen children (40%) achieved normal psychomotor development and 14 patients (35%) had mild mental retardation (IQ 50-75; Table-1).

Table 1: Ages at diagnosis, Sex and the development outcome

Age of diagnosis	No.	%	Development outcome			Findings on (TC-99M)scan of the thyroid gland N = 20		
			Normal	Mild mental retardation (IQ 50-75)	Moderatly sever mental retardation (IQ<50)	Normal position	Ectopic gland	Aplosia
< 1 month	11	27.5	8	3	-	1	1	2
1-3 months	6	15	4	2	-	1	1	2
4-6 months	5	12.5	3	1	1	-	1	2
7-12 months	3	7.5	2	1	-	2	-	1
1-3 years	9	22.5	4	3	2	2	-	1
>3 years	6	15	-	1	5	-	1	2
Total	**40**	**100**	**21 (52.5%)**	**11 (27.5%)**	**8 (20%)**	**6 (30%)**	**4 (20%)**	**10 (50%)**
			40 (100%)			**20**		

The clinical manifestation in 20 patients with congenital hypothyroidism diagnosed before 6 months (Table-2).

Table 2: Clinical manifestation in 22 patients with congenital hypothyroidism diagnosed before the age of 6 months

Manifestations	No.	%
Coarse faces	18	81.8
Delayed bone maturation	16	72.7
Constipation	15	68.2
Neonatal Jaundice	12	54.5
Umbilical hernia	11	50
Hoarse cry	8	36.3
Large anterior fontanel	8	36.3
Hypothermia	6	27.2
Macroglossia	6	27.2
Skin mottling	3	13.6

Discussion

Neonatal screening programmes for congenital hypothyroidism have revealed a variable incidence. In Iraq the incidence is not Known, but in USA and other Countries (1/2500)[1,4,5].

Although we do not know much about the overall prevalence of CH in Iraq, the first observation to be made is that this is not a rare disease in this country.

Congenital hypothyroidism can be due to absent or hypo plastic gland 35%, an ectopic gland 43%, or inborn error of metabolism of thyroid hormone 22% 6, while in our series the percentage of ectopic gland is 20%, aplasia is 50% and normal position 30%. Determination of the cause of CH has genetic, epidemiological and prognostic importance[7,8]. The overall sex distributions showed the same pattern of female predominance reported elsewhere[1,9,10], while there was even sex distribution in infants with thyroid aplasia or ectopia presented in the first 6 months of life, similar to the experience of Daoud et al[9]. The most frequent initial clinical manifestation in 22 patients presented in the first months of life was coarse facial features, constipation, prolonged neonatal jaundice, umbilical hernia and delayed skeletal maturation (Table-2) which is similar to those reported by Smith[6,12], while in older age group the main reason for referral was psychomotor retardation, constipation and short stature. Though many of these features are rather non-specific, it is important to notice that most of the hypothyroid infants had several of these features at a time. Forty-two percent of our patients detected in the first 3 months (62.5%) in the first year. These results are similar to those reported by others[11,12].

Children with thyroid gland aplasia or ectopia might present early in life. Nine out of eleven of our patients with either thyroid aplasia or ectopia presented within the first 6 months of life.

Among 21 children who achieved normal mental and motor development, Thirteen received thyroid replacement therapy within the first 6 months of life, and seven of the eight patients with moderately sever mental retardation were diagnosed and received therapy after the age of 1 year(Table-1). This demonstrates the favorable effect of early treatment, which has been confirmed by recent prospective studies in children with CH detected by neonatal thyroid screening[13].

Conclusion

These results demonstrate that CH is not uncommon disease, which is probably due to high rate of consanguinity among our population, and many thyroid investigation if needed are necessary to identify the cause of CH, which has genetic, epidemiology and prognostic importance.

Recommendation

Because of the sequelae of untreated CH are so sever and because the benefits of early treatment have been well-documented eight or national screening programme should keep in consideration the characteristic of neonatal practice in Iraq to achieve the maximum diagnostic benefits.

General practitioners and pediatricians should be aware of this disease and treat such patients early enough to minimize the unwanted sequaelae.

References

1. Lafranchi, S.: Disorders of thyroid gland In: Beherman, R.E., Kligman, R.M., and Jenson, H.B., Nelson textbook of Pediatrics 16th edition W.B Saunders Company, Philadelphia, Pennsylvania 2000; 537: 1696-708.
2. Illig, R., Largo, R.H., Quin, Q., Torresani, T., Rochicciolo, P., and Larsson, A.: Mental development in congenital hupothyroidism after neonatal screening. Arch Dis Child, 1987; 62: 1050-5.
3. Rovert, J., Ehlich, R., and Sorbara, D.: Intellectual outcome in children with fetal hypothyroidism. J Pediatr, 1987; 110: 700-4.
4. Glorieux, J., Dussault, J.H., Letrate, J., Guyda, H., and Morisette, J.: Preliminary results on the mental development of hypothyroid infants detected by the Quebec screening program. J Pediat, 1983; 102-22.
5. Al-Juarayyan, N., Al-Nuaim, A.R., and El-Desouki, M.: Congenital hypothyroidism clinical and laboratory characteristics in infants detected by neonatal screening, Ann Saudi Med, 1992; 12: 86-9.
6. New England Congenital Hypothyroidism Collaborative Characteristics of infantile hypothyroidism discovered on neonatal screening. J Pediatr, 1984; 104: 539-44.
7. Majeed-Saidan, M.A., Joyce, B., Khan, M., Hamam, H.D.: Congenital hypothyroidism in Riyadh Military Hosp. experience Cli Endocrinol (Oxf), 1993; 38 (2): 191-5.
8. Vela-Amieva, M., Gamboa-Cardiel, S., Perez-Androde, M.E., Ortiz-cortes, J., Gonzales-Contreras, C.R., and Ortega-Velazquez, V.: Epidemiology of congenital hypothyroidism in Mexico. Saluo Publica Mex, 2004; 46 (2): 141-8.
9. Daod, A.S., Zaki, M., Al-Saleh, Q.A., Teebi, A.S., and Al-Wadi, S.A.: Congenital hypothyroidism in Kuwait. J Trop Pediatrics, 1989; 35: 312-4.
10. Waller, D.K., Anderson, J.I., Lorey, F., and Cunningham, G.I.: Risk factors for congenital hypothyroidism: an investigation of infants birth weight, ethnicity and gender in California 1990-1998.
11. Jacobsen, B.E., and Beandt, N.J.: Congenital hypothyroidism in Denmark. Arch Dis Child, 1981; 56:134-6.
12. Klett, M.: Epidemiology of congenital hypothyroidism. Exp Clin Endocrinol Diabetes, 1997; 105 (suppl. 14): 19-23.
13. Ordookhani, A., Mirmiian, P., Jajafi, R., Hedayati, M., and Azizi, F.: Congenital hypothyroidism in Iran. Indian J Pediatr, 2003; 70 (8): 625-8.

RESPIRATORY DYSFUNCTION IN PATIENTS WITH MYASTHENIA GRAVIS

Abdul-Kareem A.M.[1] *FRCP*, **Sajid I.K. Al-Hussaini**[2] *FICMS*

Abstract

Background: Myasthenia Gravis, a neuromuscular disorder causing skeletal muscle weakness involving respiratory muscles which some times sever enough to need assisted respiration. Approximately 30% of patients with MG develop some degree of respiratory weakness, mysthenic crisis most often precipitated by systemic infection (40%), thymoma (30%) and aspiration pneumonitis (10%).

Objectives: To evaluate respiratory function status with MG and to determine the triggering factors for respiratory dysfunction and effect of thymectomy.

Method: 50 consecutive cases of MG, 33 females and 17 males with an age range between 16–60 years old, 29 of them underwent thymectomy. The study was done throughout the period October 1999 to June 2001 in Al-Kadhiymia Teaching Hospital.

Results: 46% of patients show respiratory muscles involvement. 47.82% of cases show respiratory involvement in the first 4 years. 68.9% of thymectomized patients show no respiratory muscles involvement while 66.7% of non-thymectomized show respiratory muscles involvement. Infection is the highest triggering factor (39%).

Conclusion: Respiratory dysfunction seems to develop in the first 4 years of the disease course. The incidence of respiratory dysfunction is less frequent in thymectomized patients. Infection is the main triggering factor.

Keywords: Myasthenia Gravis, Neuromuscular, Thymectomy, Respiratory failure

IRAQI J MED SCI, 2005; VOL. 4 (1): 93-98

Introduction

Myasthenia Gravis (MG) is a neuromuscular disorder characterized by fluctuating weakness and easy fatigability of the skeletal muscles that resolves with rest[1]. It is an autoimmune disorder of neuromuscular transmission in which antibodies of IgG class reduce the functional status of acetylcholine receptors at the neuromuscular junction[2].

Myasthenic crisis defined as a marked clinical worsening of mysthenic weakness of the intercostals and diaphragmatic muscles leading to respiratory failure that requires intubations and mechanical ventilation[1,3-6].

Approximately 30% of all patients with MG develop some degree of respiratory weakness. 15-20% will experience at least one crisis; one third of the patients who survive their first crisis will later experience a second one[4].

Myasthenic crisis tends to occur early in the course of the disease. The median interval from onset of the myasthenia gravis to the first crisis is 8 months with almost 75% of cases occurring within

[1]**Section of Neurology-Dept. Medicine, College of Medicine, Al-Nahrain University**
[2]**Al-Numan General Hospital, Adhamiyiah, Baghdad, Iraq**
Address correspondence to Dr. Abdul-Kareem A.M. Al-Sheikhly, E-mail a_mutaleb@yahoo.uk.com, POBox 70041, Kademeyiah Baghdad, Iraq.
Received 16th June 2004: Accepted 14th March 2005

the first 2 years of the disease onset and rarely a respiratory distress is the first manifestation of myasthenia gravis[2-4,6]. Patients are often asymptomatic from the respiratory symptoms point of view in the early stages but as weakness progresses (VC< 30 ml/kg) atelactasis, reduced lung compliance, ventilation perfusion mismatch and hypoxia develop[4].

Respiratory crisis is mediated through two mechanisms of equal importance, respiratory muscles weakness and oropharyngeal weakness which leads to aspiration, infection and inability to clear airway passages[4,5]. Myasthenic crisis is most often precipitated by infection (40%), thymoma (30%), aspiration pneumanitis (10%), change of medications (8%) and spontaneous exacerbation (no risk factor) (30%).

Myasthenic crisis can be avoided in many patients through immunosupression and thymectomy[4].

Pulmonary function tests (PFT) provide objective standardized measurements for assessing the presence and severity of respiratory dysfunction. The most common measurements of lung function through pulmonary function test are the ventilatory capacities (FVC, FEV1, and VC)[7,8].

In MG the respiratory muscles involvement results in restrictive pattern of defect on PFTs[4,6-8]. The best, simplest and most reliable way to evaluate respiratory functions in patients with MG is frequent measurement of VC[9,10].

Normal VC value is 60-70 ml/kg, Patients below these values should be monitored and treated aggressively. Vital capacity of 15ml/kg (~ 1 litter) is generally considered the level of which intubations is needed[4,6-8,10].

Aims of the study

1. To evaluate respiratory function depending on PFTs (VC) to recognize the cases where respiratory muscles are involved.
2. The relationship between respiratory dysfunction in patient with MG with onset of clinical presentation of respiratory dysfunction, thymectomy and the aggravating factors.

Patients & Methods

A prospective cohort clinical study; evaluation of fifty patients with MG, 33 females and 17 males with an age range between 16-60 years, 29 of them underwent thymectomy; throughout the period from October 1999 to June 2001 at Al-Kadhiymia Teaching Hospital

The diagnosis of MG is secured through clinical, tensilon test and neurophysiological assessments. Other investigations that were needed for the study, PFTs, TFT, CT scan or MRI of the chest, Chest X-ray, X-ray of the sinuses, CBP and ESR, vasculitic screening tests and other basic hematological tests

In this study, patients were classified according to their vital capacity into three groups: first, those patients with absent pulmonary function abnormality (VC > 80%), no ventilatory complaint. Second, those patients with mild to moderate impairment in pulmonary function test (V.C. 60-80%) of its predicted value, (V.C. 15-30 ml/kg), in which patients tend to have a low tidal volume and breath faster than healthy persons (RR 25-30 / minute). Third, those patients with severe pulmonary function tests impairment (VC <60%) of its predicted value, (VC <15 ml/kg), with RR >35/minute clinically[4,6,7,11, 12].

Results

Table 1: Percentage of Respiratory muscles involvement in M.G. patients depending on vital capacity

All patients with M.G.	VC> 80% Absent	VC 60-80% Mild -Moderate	VC <60% Sever	Total
Without respiratory muscles involvement	27	0	0	27 (54%)
With respiratory muscles involvement	0	16 (32%)	7 (14%)	23 (46%)

Percentage of patient without respiratory muscles involvement =54%
Percentage of patient with respiratory muscles involvement = 46%

Table 2: Time of onset of respiratory muscles involvement in relation to the time of onset of the disease

MG patients with respiratory muscles involvement	Presenting (respiratory failure)	1-2 years	3-4 years	>5 years
23	2 8.7%	11 47.82%	8 34.78 %	2 8.7 %

The onset of respiratory muscles involvement in relation to the time of onset of the disease is more frequent in the first four years and rare after five years or as a presenting clinical feature (R.F.).

Table 3: Respiratory muscles involvement in relation to thymectomy

All patients with M.G.	Thymectomized		Non thymectomized		Total
	No.	(%)	No.	(%)	
Without respiratory muscles involvement	20	(68.9 %)	7	(33.3 %)	27
With respiratory muscles involvement	9	(31.1%)	14	(66.7%)	23
Total	29		21		50

Most M.G. patients with respiratory muscles involvement were not thymectomized, while most patients without respiratory muscles involvement are thymectomized.

Table 4: Respiratory muscles involvement in relation to aggravating factors

All patients with respiratory muscles involvement	No.	Infection			Pregnancy			Surgery and stress	Drugs	Exercise	Mixed	Total
		UR	LR	Others	1	2	3					
Mild	6	1	0	1	1	0	0	0	1*	2	0	6
Moderate	10	2	1	0	0	1	0	1	0	1	4**	10
Severe	7	2	2	0	1	0	0	0	0	0	2***	7
Total	23	5	3	1	2	1	0	1	1	3	6	23
Total	23	9			3			1	1	3	6	23

*Steroid induced, ** Infection & exercise, *** Infection & surgery.
Infection > Exercise & Pregnancy > Drugs, stress & surgery.

Discussion

In this study, 46% of the cases showed respiratory muscles involvement (respiratory dysfunction) which is more in contrast with other studies: Younger (1997) 16%[13], Thomas et al (1997) 30%[14], Fink (1993) 30%[15], Michael et al (1981) 16%[16].

This higher percentage of involvement in this study is due to, firstly delay in diagnosis and treatment of the disease itself, which leads to a more progression of the disease pathology and thus possibility of involvement of respiratory muscles, and secondly frequent infections in our patients, which is one of the main aggravating factors for myasthenic crisis.

This study shows that the onset of respiratory muscles involvement is more frequent at the first two years in the course of illness (47.82%), and less frequent at the (3-4) years of the course (34.78%) and rare to occur after 5 years of the disease course (8.7%) or as a presenting feature (acute respiratory failure) (8.7%) which agree with other studies[14,17].

This high incidence of respiratory dysfunction in the first four years of the disease course is due to many factors which include: firstly the progression of the disease is more in the first three years, secondly most of the patients are not thymectomized at that time, and if they underwent thymectomy they may not get the full benefit of that operation early.

While low incidence of respiratory dysfunction after five years of the disease course is that, rarely the disease progress after five years and most cases are stabilized and in stationary state, and most patients are diagnosed and treated properly and were thymectomized and had the full benefit of it.

The present study shows that most patients with respiratory muscles involvement (66.7%) are not thymectomized, while most patients without respiratory muscles involvement (68.9%) were thymectomized which means that thymectomy is one of the protective measures against the development of respiratory dysfunction which agree with study done by Stephan (1997)[4] who said that thymectomy is a protective measure to avoid myasthenic crisis. In addition, this is in agreement with other studies about benefit of thymectomy in achieving remission[18,19,20,21,22,23].

In addition, this study shows that infection is the main frequent aggravating factor for respiratory muscles involvement that includes upper respiratory tract infections, urinary tract infections and other infections for milder cases. URTI and LRTI like sinusitis and pneumonia are the main aggravating factors for moderate and severe cases. Some patients have mixed types of aggravating factors like infection, pregnancy and exercise.

Pregnancy (1st and 2nd trimester) was less frequent precipitating factor while drug induced, stress and surgery were the least frequent precipitating factors for respiratory dysfunction which agree with other studies[1,3,14,16,24-27].

The pregnancy plays an important rule in the aggravation of myasthenic weakness and inducing myasthenic crisis especially in the first and second trimesters which is due to, frequent emesis during pregnancy which interferes with absorption of oral medications as drug treatment of myasthenia gravis. It also leads to hypokalemia which can aggravate myasthenic weakness, and the large uterus elevates the maternal diaphragm resulting in relative hypoventilation of the lower portions of the lungs, and lastly, expanded plasma volume and increased renal clearance may require that AchE medications be adjusted during pregnancy[28].

Conclusions

1. The vital capacity as a part of PFTs is a simple test that can give a reliable idea about respiratory muscles involvement in patients with myasthenia gravis.
2. The respiratory dysfunction seems to develop early in the first years of the

course of the illness, while it is less frequent after five years of the course of the illness and rarely as a presenting feature (acute respiratory failure).

3. The incidence of respiratory dysfunction is less frequent in thymectomized patients compared to non thymectomized patients.

4. Infection is the main aggravating factor for the development of respiratory dysfunction followed by pregnancy, exercise, surgery and stress factors in sequence.

Recommendations

1. Patients with moderate or severe respiratory dysfunction should be kept under monitoring in RCU with frequent vital capacity assessment.

2. MG should be considered as differential diagnosis in patient with acute respiratory failure.

3. Thymectomy should be done as early as possible as one of the protective measures to prevent the occurrence of respiratory dysfunction.

4. Search and treatment of infection is essential as immunocompromised patients to prevent myasthenic crisis.

References

1. Drachman, D.B.: MG and other diseases of neuromuscular junction In: Braunwald, E., and Hauser, S.L. Harrison's Principles of Internal Medicine, 15th ed. New York, Mc Graw Hill 2001; p.p. 2515-20.
2. Hagon, J.A., and Cooper, J.D.: Surgical management of M.G. In Sabiston (chief editor). The biological basis of modern surgical practice. 15th ed. Philadelphia W.B. Saunders Company, 1997; p.p. 1933-41.
3. Adam, S.R., Victor, M., and Ropper, A.: MG and related disorders of neuromuscular transmission In: Adam, Victor, and Ropper. Principles of neurology. 6th ed. New York, Mc Graw Hill, 1997; p.p. 1441-2, 1459-75.
4. Stephan, A., and Mayer, M.: Intensive care of the myasthenic patient. Neurology, 1997; 48(Suppl.5): 70-5.
5. Shah, S.M., and Kelly, K.M.: Neuromuscular Disorders Acute Weakness Resulting from Neuromuscular Junction Disorders. In: Shah, S.M., and Kelly, K.M., Emergency Neurology, Principles and Practice. Cambridge University Press, 1999; p.p. 85-90.
6. Weiner, W.J., and Shulman, L.M.: Neuromuscular Disorders MG. In: Weiner, W.J., and Shulman, L.M., Emergent and Urgent Neurology, 2nd ed. Philadelphia. A Walters Kluwer Company, 1999; p.p. 264-67.
7. Thomas, J.: Pulmonary function testing, In: Ronald, D.M. (ed.). Anasthesia 5th ed. San Francisco, 2000; p.p. 885-902.
8. Brakash, U.B.S.: Pulmonary diseases, Pulmonary function tests, In: Barakash, U.B.S., and Habermann, T.M., Mayo Internal Medicine Boared Review, 2000-2001; printed in USA; Lippincott Williams and Wilkins, 2000; p.p. 771-3.
9. Crompton, G.K., Haslett, C., and Chilvers, E.R.: Diseases of the respiratory system, Lung function testing, In: Haslett, C., Chilvers, E.R., Hunter, J.A.A., and Boon, N.A.: Davidson's principles and practice of medicine, 18th ed., UK, Churchill Livingstone, 1999; p.p. 311-2.
10. Donald, J., and David, C.: Interpretation of blood gas measurements and respiratory function test. Medicine, Middle East, 1st series. Feb. 1980; p.p. 667-9.
11. Ferguson, I.T., Murphy, R.P., and Lascelles, R.G.: Ventilatory failure in myasthenia gravis. J Neurol Neurosurg Psychiat, 1982; 45: 217-22.
12. Abdulla, W.Y.: Guideline for ventilatory support in adults with respiratory failure, In: Principles of resuscitation and Intensive care, 1st ed., College of Medicine, University of Busra, Mosol, 1981; p.p 436.
13. Younger, D.S., Bradford, B., and Penn, S.A.: MG: Historical prospective and overview. Neurology, 1997; 48 (Suppl 5): 1-7.
14. Thomas, C.E., Mayer, S.A., Gungor, Y., et al.: Myasthenic crisis: clinical features, mortality, complications, and risk failures for prolonged intubation. Neurology, 1997; 48: 1253-60.
15. Fink, M.E.: Treatment of the critically ill patient with myastenia gravis, In: Ropper A H, Neurological and neurosurgical intensive care, 3rd ed., New York; Raven Press; 1993; p.p. 351-62.
16. Cohen, M.S., and Younger, D.S.: Aspects of the natural history of myasthenia gravis crisis and death. Ann N Y Acad Sci, 1981; 377: 670-7.
17. Oosterhuis, H.J.: The natural course of myasthenia gravis: a long term follow up study. J Neurol Neurosurg Psychiat, 1989; 52: 1121-7.
18. Robinson, C.L.N.: The role of surgery of the thymus for myasthenia gravis. Ann Royal Coll Surgeon Engl, 1983; 65; 3: 145-50.

19. Monden, Y., Nakahara, K., Kagotani, K., et al.: Effects of preoperative duration of symptoms on patients with myasthenia gravis. Ann Thoracic Surg, 1984; 38 (3): 287-91.
20. Jaretzki, A.: Thymectomy of myasthenia gravis: Analysis of the controversies regarding technique and results. Neurology, 1997; 48 (suppl 5): 52-63.
21. Abdel-Kareem, A.M., Al-Kashab, S., Al-Nimer, M.S.M., and Niami, I.A.: Myasthenia gravis thymectomy; early outcome. J Fac Med Baghdad, 1993; 35 (4): 505-12.
22. Al-Shaikhly, K.I., Rustam, H.S., and Mussa, K.I.: Thymectomy in myasthenia gravis. J Fac Med Baghdad, 1994; 36 (4): 555-9.
23. Oosterhuis, H.J.: Observations of the natural history of myasthenia gravis and the effect of thymectomy. Ann NY Acad Sci, 1981; 377: 678-89.
24. Harber and Raw, Myasthenia gravis. Clin Obs Gyne, 1983; 26 (3): 599-604.
25. Bedlack, R.S., and Sonders, D.: How to handle myasthenic crisis, Essential steps in patient care. Postgrad Med, 2000; 107 (4): 211-4, 220-2.
26. Mazia, C.G., Schottlender, J., Menga, G., et al.: Invasive positive pressure ventilation in myasthenic crisis, The summary of the 1999 MGFA Scientific Session, Seattle, Washington, USA, 1999.
27. Masse, J.M.: Treatment of acquired myasthenia gravis, Neurology, 1997; 48 (suppl 5): 46-51.
28. Branch, D.W., and Porter, F.: Autoimmune disease; MG, In: James, D.K., Steer, P.J., Weiner, C., and Gonic, B., High risk pregnancy; Management options, 2nd ed., Philadelphia W.B. Saunders, 1999; p.p. 876-9.

FEMALE HYPERPROLACTINEMIA: ANALYSIS OF PRESENTATION AND DIAGNOSTIC EVALUATION. IS PITUITARY MAGNETIC RESONANCE IMAGING ALWAYS INDICATED?

Sahar L. Halabia[1] *CABOG*, **Ayad Sh. Salih**[2] *DMRD*

Abstract

Background: Hyperprolactinemia (HPRL) is the most common endocrine disorder of the hypothalamic-pituitary-ovarian axis. The most important and common cause is pituitary tumor.

Objectives: 1. Analysis of the presenting features. **2.** Role of MRI in the diagnostic evaluation of HPRL. **3.** MRI measurements and correlation between MRI findings and serum prolactin concentration (PRL) in Iraqi women. **4.** Literature review and work-up for HPRL.

Method: clinical assessment, basal PRL and pituitary and cranium MRI performed in a case-series study for 82 Iraqi HPRL female patients attending gynecologic clinic after excluding secondary HPRL.

Results: sub-fertility, galactorrhoea and menstrual irregularities were the commonest features. MRI abnormalities found in 41.46% of the patients. 88.24% were pituitary abnormalities. Their PRL was significantly higher than those with idiopathic HPRL (p=0.03). Right pituitary adenomas were more common than the left. The pituitary gland occupied 81.73% of the sella turcica in case of pituitary hyperplasia. There was no significant correlation between adenoma size and PRL (p=077), while there was significant positive correlation between pituitary and sella areas in those with normal MRI (p=0.007) as well as those with pituitary hyperplasia (p=0.04).

Conclusion: PRL of 18.5ng/ml considered as the cut-off value to perform high-resolution pituitary and cranium MRI. Primary pituitary hyperplasia may carry a risk of parasellar extension during pregnancy. There is positive correlation between pituitary and sella turcica sizes. MRI considered as the gold-standard imaging method for the pituitary while sella X-ray should be abandoned.

Key words: Hyperprolactinemia, Pituitary adenoma, Pituitary hyperplasia, MR imaging, MR measurement, Iraqi

IRAQI J MED SCI, 2005; VOL. 4 (1): 99-110

Introduction

Hyperprolactinemia (HPRL) is the most common endocrine disorder of the hypothalamic-pituitary-ovarian axis, occurring mostly in women, with a prevalence of 0.4-0.5% in unselected normal adult population to as high as 9-17% in women with reproductive disorders[1,2]. Hyperprolactinemia defined as serum prolactin (PRL) level above the normal range. This is considered as > 19 ng/ml[3], > 20-25 ng/ml[4,5] or > 30ng/ml[6].There is great deal of variability between laboratories in their analytic methods and ranges of normal. Unselected autopsy studies had shown pituitary tumors in 1.5-26.7%. Magnetic resonance imaging (MRI) signs of pituitary tumor found in 10% of normal population. However, clinically significant pituitary tumors affect the health of 8.2-20/100000 people[2, 3, 6-8]. The causes of HPRL fall into five categories:

[1] Dept. Obstetric & Gynecology, College of Medicine, Al-Nahrain University [2] Dept. Radiology, Al-Kadhimiya Teaching Hospital, Baghdad, Iraq.
Correspondence: Dr. Sahar Halabia, c/o Basil Ghazala P.O.Box 3290 Saadoon Baghdad Iraq.
e-mail: ghazalahalabia@yahoo.com
Received 14th April 2004: Accepted 29th November 2004.

First: *Pituitary causes classified to:* Tumors secreting intact hormones that result in clinical syndromes of hormones hypersecretion. These tumors may secrete either *pure* hormones as PRL (termed prolactinoma which constitute 40% of pituitary tumors), growth hormone (GH), adrenocorticotrophic hormone (ACTH) or thyroid stimulating hormone (TSH) or may be *mixed* which express more than one hormone; Non-functioning tumors (non-secreting adenoma NSA) form 25-39% of pituitary tumors. They do not cause clinical syndromes of hormone hypersecretion but indeed produce either silent glycoprotein hormones or their subunits. They may present with functional HPRL. The cause of pituitary tumors is unknown and is not inheritable[8]; Pituitary hyperplasia i.e. global pituitary enlargement which is either primary or secondary to puberty, pregnancy, primary hypothyroidism with secondary HPRL, hypothalamic tumors as GH releasing hormone-secreting tumors or mental depression[3]. Pituitary incidentoloma represented 12% of pituitary tumors. It means incidentally discovered pituitary mass during imaging performed to evaluate conditions not linked to pituitary disease[3, 7, 9].

Second: *Non-pituitary sellar and parasella lesions with functional HPRL*: Structural lesions as empty sella, intrasellar cyst, tumours as craniopharangioma, germinoma, meningioma , chordoma, astrocytoma, lymphoma, metastatic tumors, glioma and vascular abnormalities as aneurysm; Non-structural lesions as sarcoidosis, cranial irradiation, stalk resection, lymphocytic hypophysitis[1,3,4,10].

Third: *Secondary HPRL* to renal impairment, liver cirrhosis, primary hypothyroidism, lesions in dermatomes T 3-5 & mammary line, spinal cord tumors, seizures, ectopic PRL production by malignant lesion as bronchogenic carcinoma and PRL-enhancing drugs[1,10,11].

Fourth: *Physiological HPRL* in response to stress, sleep, food intake, sexual activity and nipple stimulation as well as in pregnancy and lactation. PRL is secreted in pulses every 20 minutes with a diurnal rhythm[1, 4, 10, 12].

Fifth: Lastly *idiopathic HPRL* in 32.8-61%, when no underlying cause is found[4,13,14].

The commonest features of HPRL are menstrual irregularity, galactorrhoea, infertility, pregnancy loss, loss of libido, acne and hirsutism, headaches and osteopenia. It is important to consider HPRL and pituitary adenoma in an expanded range of clinical situations. Baird's in his recent study[15] adds other troublesome complaints as physical and mental fatigue, mood disorder, sleep problem, unexplained pain, emotional behavior dysfunction and decline in sexual function.

HPRL alters the pattern of hypothalamic catecholamines and so suppresses pulsatile secretion of gonadotrophin releasing hormone thus blocking follicular stimulating (FSH) and leutinizing hormones (LH) by blunted or no response to estradiol. In addition, PRL inhibits gonadotrophin effect at ovarian level. There is hypoestrogenism and chronic hyperandrogenism[2,10].The increased mean intracellular pressure (MISP) contributes to the development of headaches even in those with microadenomas[16].

However, our understanding of HPRL has improved greatly since the availability of finer diagnostic imaging. MRI now regarded as the pituitary imaging method of choice that provides the most detailed information and

suggests pathological diagnosis, tumor vascularity and invasiveness to surroundings. It can be used during pregnancy and can be repeated.It has no ionizing radiation like computed tomogram (CT) which also provides less precise information[6], although tissue calcifications are better detected by CT[17]. Nevertheless, even MRI may have pit-falls i.e. false-positive results due to normal anatomic variations or imaging artifacts[7]. For pituitary and cranium, imaging the trend now is to use the high resolution MRI with dynamic contrast enhancement[3, 17].

The primary treatment of prolactinomas whether micro- or macro-adenomas and for idiopathic HPRL is medical therapy when indicated, while surgery is the first treatment for the non-PRL secreting and non-secreting pituitary adenoma as well as for the sellar and parasellar tumors[3,4].

Differentiation between prolactinomas and the other tumors is possible by immunohistochemistry study of the specimen after surgery. Since few prolactinoma patients are now submitted to surgery, dynamic tests of PRL in HPRL patients are useful in suggesting a pathological diagnosis of pituitary adenomas.

Dopamine antagonists domperidone[17], metoclopramide or perphenazine[2] stimulation tests give blunted PRL response in prolactinoma i.e. PRL 30/PRL 0<3 in comparison to PRL response of ≥3 in functional HPRL secondary to the other tumors. The blunted PRL increment of less than 30% after thyrotropin releasing hormone (TRH) stimulation test is typical of macroprolactinoma and excludes other pituitary adenoma, but the cut-off not yet established for microadenoma[3].

In prolactinoma when dopamine agonists are used as bromocriptine suppression test there is decrease of serum PRL by 70%[18].

Macroprolactin, a PRL aggregate with high molecular weight 150-170 Kda is active in vitro but has no or low biological activity in vivo. It is indistinguishable from the low molecular active PRL by routine assay but by special tests as chromatography and others[7,19,20]. Macroprolactinemia is present in 8-46% of HPRL. It found that 78-78.9% of those with macroprolactinemia had normal MRI[6,20] while the others have pituitary lesions.

Because MRI is expensive some studies[17,21] suggest the dynamic tests of PRL and macroprolactin assay in HPRL to be done first and then to select those who need the MRI. While others studies[2,4,6,19,20] concluded that pituitary imaging is necessary in the diagnostic evaluation of HPRL.

The aims of the study of female:

1. Analysis of the presenting features.
2. Role of MRI in diagnosing the cause of HPRL with comparison to X-ray of pituitary fossa.
3. MRI measurements and correlation between MRI findings and PRL in Iraqi women.
4. Literature review and work-up for HPRL

Methods

This case-series study included 172 consecutive Iraqi women with PRL concentration higher than 14ng/ml, who were non-pregnant, non-lactating and not on hormonal contraception attending Al-Kadhmyia Teaching Hospital and private gynecologic and infertility clinics during the period from 1 June 2001 through the fifteenth of March 2003.

For all of them a second basal PRL assay in standardized conditions was performed. A fasting morning specimen drawn after suspending any sexual activity and nipple stimulation for at least four days. In spontaneously menstruating women, the sample drawn on the second or third day of the cycle. While in amenorrheic women,

pregnancy excluded by clinical assessment and pregnancy test before sampling. These measures were to reduce potential confounding physiological factors.

During medical history and examination, we looked for: PRL enhancing drugs, mammary line skin lesion or irritation at dermatomes T3-T5, features of renal, liver and thyroid dysfunction. For all the 120 patients with persistent HPRL, screening with blood urea, serum thyroxine and TSH performed.

Fifty-two women (30.23%) were found to have transient HPRL with the basal PRL ≤14ng/ml while the first PRL concentration mean was 16.67±1.38 (range 14.2-24ng/ml). Twenty-six patients(15.12%) were found to have possible secondary HPRL including: 14 (8.14%) with pharmacological HPRL using amitriptyline HCL, alphamethyldopa, digoxin, cortisteroid, danazol, diphenhydramine or cimetidine; 11(6.39%) had mammary line skin lesion or irritation (one herpes zoster, 3 breast pruritus and 7 used local methods for breast hair removal); one with high blood urea (70 mg/dl). None had chronic liver disease or primary hypothyroidism.

Those 26 patients with secondary HPRL with PRL concentration mean 19.2±2.01 range 14.5-29.3 ng/ml not presented in this study. Another 12 patients (6.98%) excluded from the study because they did not attend for the MRI. The remaining 82 women (47.67%) were the study group with HPRL (basal PRL>14ng/ml) who did not have any of the above factors and for whom pituitary and cranium MRI was performed. For the first thirteen patients lateral view X-ray of the pituitary fossa in addition to the MRI performed during the era when the MRI newly introduced to practice in this hospital. Then sella X-ray abandoned.

For the study group, detailed history and examination performed regarding symptoms possibility related to HPRL as menstrual irregularity, galactorrhoea, breast pain, sub-fertility with ovulatory factor, abortion, acne and hirsutism, dyspareunia and loss of libido. Also features of mass affect including headaches, visual symptoms, cranial neuropathies as third and sixth nerve palsy and cerebrospinal fluid (CSF) rhinorrhoea.

Clinical features of excess or deficiency of GH, ACTH and TSH looked for. Full ophthalmologic examination including visual field campimetry using Goldmann perimetry was requested for 50 patients desiring pregnancy as a base line, also for 5 patients with symptoms of mass effect and 5 with pituitary macroadenoma on MRI for whom neurosurgical opinion was also requested. Enquiry about previous brain trauma, postpartum hemorrhage and birth asphyxia made for those found to have empty sella on MRI.

Prolactin assay*:* PRL measured by immunoradiometric assay (IRMA, reference value for adult females 3.6-14 ng/ml; ImmunoTech, Bechman Coulter Company) with a sensitivity of 0.5 ng/ml, intra-assay and inter-assay variation were ≤2.8 and ≤ 8% respectively.

High Resolution Dynamic Contrast MRI Examination*:* MRI study was performed on 1.5 Tesla superconductive system (philips Gyroscan NTCS) with the use of dedicated phase array head coil. The used protocol included whole brain scan as survey by using T2 Turbo-spin-echo (Tse) axial and then dedicated scanning of: 1-The pituitary gland: Coronal and Sagittal planes T1 weighted Se, repetition time (TR) 625, echo time (TE) 13, slice thickness (SL) 1.5mm, 256x256 matrix, field of view(FOV) 180mm, number of excitation (NEX) 4 and intersection gap 0.3mm. 2-Coronal T2 weighted Tse, TR 6772, TE 150, SL 2mm, matrix

256x256, FOV 200 and NEX 4. 3- Dynamic contrast study on T1 coronal study after administration of 0.1 mmol/kg body weight gadolinium diethylene triamine pentaacetic acid (Gd-DTPA) intravenously.

***MRI Assessment**:* Sella turcica: size, antero-posterior (AP) diameter and height (HT) on sagittal view, the U-shaped configuration, walls and borders. Pituitary: position in the sella, the bean shaped configuration, superior border, size, AP diameter and HT on sagittal views, pituitary homogenisity before and after contrast enhancement, any circumscribed hypo- or hyper-intense area and dynamic sequence for any time differential in the enhancement of different pituitary areas.
Infundibulum (pituitary stalk): position and size. Optic chiasma: position, size and symmetry. Suprasella CSF spaces: symmetry and constriction. Cavernous sinus: symmetry, size and infiltration. Internal carotid arteries: symmetry, size, narrowing or expansion. Neurocranium: temporal lobe, hypothalamus and floor of third ventricle. Sphenoid sinus: margins especially the roof and pneumotization.
Diagnosis of pituitary adenoma and hyperplasia and the SIPAP classification as a grading system for extension was according to the imaging characteristics demonstrated in Ref. 3, 11, 22.23. In this study pituitary hyperplasia was diagnosed when both pituitary sagittal diameters AP and HT were >10mm and >8mm respectively with generalized pituitary heterogeneity with or without enhancement.

Statistical Analysis
Analysis of data was with the personal computer Microsoft Excel program. Results presented as frequencies. Mean and standard error of the mean (M±SEM) was calculated. Student *t-test* and ANOVA used as tests of significance taking $p \leq 0.05$ as significant value. Degree of association assessed by correlation coefficient *r* and linear regression analysis. Sensitivity, specificity and accuracy were calculated using the contingency two-by-two table.

Results

The results of the study group i.e. the 82 patients with HPRL who consulted the gynecologic clinics, for whom pituitary and cranium MRI was performed were presented.

Patients' characteristics: The results presented as M±SEM and (Min-Max).
Age (year) =31.37±0.96 (16-52); weight (kg) =69.93±1.78 (45-105); 10 (17.5%) were obese with BMI>30kg/m^2; 12 (15%) were unmarried; gravidity mean 1.42±0.25 (0-8); parity mean 0.93 (0-7); number of abortions mean 1.74±0.31 (1-6); primary sub-fertility duration (year) 4.83±1.01 (0.9-24); secondary sub-fertility duration (year) 4.82±0.92 (1-16).

Clinical features: The 82 patients had one or more of the features presented in Table 1. Their mean PRL was 39.64±2.47 (16.6-168).

Table 1: Frequencies of clinical features and PRL concentrations of the 82 HPRL patients

Features	Total 82 patients*		PRL ng/ml	
	Number	%	mean±SEM	Min-max
Sub-fertility§	50 (43)	71.43 (61.43)	39±2.52	19.6-118
1ry	32 (27)	45.71 (38.57)	39.82±3.22	19.6-110
2ndary	18 (16)	25.71 (22.86)	37.54±4.13	25.6-95
Galactorrhoea	38 (4)	46.34 (4.88)	41.6±4.48	18.5-168
Menstrual abnormality	31 (19)	37.8 (23.17)	45.47±5.19	22.6-168
Abortion§	19 (2)	27.14 (2.85)	43.29±7.62	22.6-168
Hirsutism/Acne	14 (3)	17.07 (3.66)	37.31±4.26	22.6-84.6
Breast pain	9 (3)	10.98 (3.66)	40.69±5.63	28-78.7
Headache	3 (0)	3.66 (0)	73.63±47.19	24.8-168
Visual symptoms	2 (0)	2.44 (0)	96.4±71.6	24.8-168
Dyspareunia/loss of libido	0	0		

*within parenthesis are the patients presenting symptoms

§ the denominator was 70 instead of 82 because the 12 unmarried patients were not included

Coexisting conditions: Some of the HPRL patients had also polycystic ovary syndrome (PCOS), uterine leiomyoma (LM) or goiter. Their results presented in Table 2. Of the 11 PCOS patients, only one had MRI abnormality. The remaining 10 with normal MRI had PRL concentration of 36.29±3.58, which compared with the PRL of 35.08±2.62 of the 38 patients who had neither PCOS nor MRI abnormality. Two tail student *t-test* revealed no significant difference (*t=0.27 df=20 p=0.79).*Of the 10 LM patients with HPRL 4 had MRI abnormalities. The remaining six LM patients with normal MRI had PRL concentration of 30.12±4.06, which compared with the 36.05±2.37ng/ml of the 42 patients with neither LM nor MRI abnormality. *t-test* revealed no significant difference (*t=1.26 df=9 P=0.24).* The two patients with HPRL and goiter were found to have neither hyper- nor hypo-thyroidism.

Table 2: Frequencies of coexisting conditions and PRL concentration in the 82 HPRL patients

Condition	Total 82 patients		PRL ng/ml	
	Number	%	mean±SEM	Min-max
Polycystic ovary syndrome	11	13.41	35.38±3.37	22.6-50.7
Uterin leiomyoma	10	12.2	30.7±2.75	16.6-45.1
goitre	2	2.44	32±2.9	29.1-34.9

Pituitary MRI Characteristics: MRI findings and their correlation to prolactinemia values presented in Table 3 and Table 4. Of the 48 normal MRI i.e.idiopathic HPRL patients 2 (4.2%) had normal variations: one with left local asymmetry of the pituitary gland and the other with left sided pituitary stalk. Another two had incidental findings in the form of nasopharangeal polyp in one and left sphenoid sinus mucocele in the other. Three (10%) of the 30 patients with MRI abnormality showed convexity or bulge of the upper pituitary margin. In 2 (8.4%) MRI with pituitary adenoma the adenoma areas 99mm^2, 276mm^2 were larger than their sella areas 90 mm^2, 258mm^2 respectively with suprasellar extension grade 1 according to SIPAP classification but no pressure on optic chiasma. One MRI with pituitary microadenoma showed shift of the lower pituitary stalk.

The mean pituitary area, mean sella area and their percentage ratio calculated for the 48 women with normal pituitary MRI and for the 5 women with pituitary hyperplasia. Results revealed

69.79±3.32 mm^2, 107.15±4.83 mm^2 and 65.13% vs. 139.75±38.8 mm^2, 171±38.9 mm^2 and 81.73% respectively.

Only one patient with macroadenoma had restricted visual field and treated medically under the supervision of the neurosurgeon. Opened cranial surgery done with removal of the meningioma and the cerebellar cyst. The latter revealed heamangioblastoma tumor.

Table 3: Pituitary and cranium MRI findings in the 82 HPRL patients

MRI findings	Total 82 patients		PRL ng/ml		P value
	Number	%	mean±SEM	Min-max	
Normal*	48	58.54	35.33±2.19	16.6-110	0.03
Abnormal	34	41.46	45.45±4.87	18.5-168	
Pituitary abnormality§	30	36.59	46.35±5.28	18.5-168	
Microadenoma	20		45.51±7.09	18.5-168	0.57
Right	11				
Left	9				
Macroadenoma	5		57.76±13.68	29.5-95	
Right	4				
left	1				
Hyperplasia	5		38.32±4.68	28-53.9	
Non-pituitary brain abnormality	4	4.88			
Meningioma	1		26.3		
Cerebellar cyst	1		21.7		
Empty sella¤	2		53.4		

***** Those HPRL patients with normal MRI considered to have idiopathic HPRL

§ Microadenoma means: its largest diameter < 10 mm while macroadenoma ≥ 10 mm

¤ One empty sella associates microadenoma

Table 4: Pituitary gland, pituitary adenoma and sella measurements of the 82 HPRL patients

	MRI findings	M±SEM	Min-Max
Normal pituitary	AP(mm)	10.58±0.31	8-16
	HT(mm)	6.61±0.29	4-10
	AREA(mm^2)	69.79±3.32	32-105
Microadenoma	AP(mm)	5.43±0.54	2.2-9.7
	HT(mm)	4.37±0.45	1.2-8.8
	AREA(mm^2)	27.1±5.11	4.2-77.44
Macroadenoma	AP(mm)	16.24±2.19	11-23
	HT(mm)	8.58±1.10	5.1-12
	AREA(mm^2)	146.15±36.2	62.22-276
Hyperplasia	AP(mm)	12±1.08	9-14
	HT(mm)	11.25±2.29	8-18
	AREA(mm^2)	139.75±38.8	81-252
Sella	AP(mm)	1192±0.25	9-16
	HT(mm)	9.37±0.3	6-18
	AREA(mm^2)	113.24±5.32	54-270

AP=antero-posterior diameter HT=height

Correlation Study: These presented in Table 5 and Figure 1.

Pituitary and cranium MRI vs. sella turcica X-ray: Of the 13 patients who had sella X-ray in addition to the MRI, six X-rays showed abnormal findings in the form of calcifications, ballooning or doubling of the floor, erosion of the floor or clinoid process. Calculation from the 2*2 Table 6 revealed 12.5% sensitivity of the sella X-ray, 16.67% specificity and the overall accuracy of only 15.71%.

Table 5: correlation between pituitary and cranium findings and other parameters

MRI findings	Variables		Statistical tests		
	Independent(X)	Dependent(Y)	r	p	n
Pituitary adenoma	Adenoma area(mm^2)	PRL(ng/ml)	0.06	0.77	25
Pituitary hyperplasia	Pituitary area(mm^2)	PRL(ng/ml)	0.4	0.51	5
Normal pituitary	Pituitary area(mm^2)	PRL(ng/ml)	0.28	0.11	33*
Pituitary adenoma	Age(year)	Adenoma area(mm^2)	0.2	0.35	25
Normal pituitary	Age(year)	Pituitary area(mm^2)	0.3	0.09	33*
Normal pituitary	Gravidity	Pituitary area(mm^2)	0.15	0.44	30*
Normal pituitary	Parity	Pituitary area(mm^2)	0.01	0.96	30*
Pituitary hyperplasia	Sella area(mm^2)	Pituitary area(mm^2)	0.96	0.04	5
Normal pituitary	Sella area(mm^2)	Pituitary area(mm^2)	0.46	0.007	33*

r=correlation coefficient p=significant value n=sample size

* In 15 HPRL patients with normal pituitary MRI, the pituitary diameters not measured. The gravidity and parity not mentioned in three of them

Figure 1:correlation between MRI normal pituitary & sella areas

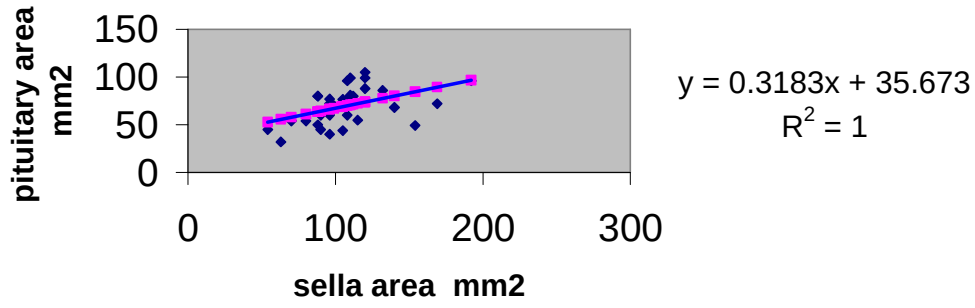


Table 6: Two-by-two table between pituitary MRI and sella X-ray findings

		MRI findings		
		Abnormal	Normal	Total
X-ray findings	Abnormal	*a* 1	*b* 5	6
	Normal	*c* 7	*d* 1	8
	Total	7	6	14

a=true positive b=false positive c=false negative d=true negative

Discussion

with NSAs. Patients lost weight during PRL lowering therapy. In addition, he found that recent weight gain is an indication to measure serum PRL as it may be the presenting symptom of HPRL. In Dublin's study[14] drug-

The entire study group was in reproductive age. 17.54% of them were obese. Green[24] found that the mean weight is significantly higher (P=0.007) in those with prolactinomas than those

LH as well as PRL. Therefore, the central basal hypothalamic deficiency of dopaminergic activity leads to the increased LH/FSH ratio and PRL in PCOS HPRL patient.

PRL produced by human endometrium, myometrium and uterine leiomyoma. Insulin-like growth factor and others mediate this production. Proliferative phase leiomyomas contain elevated levels of PRL in vivo and synthesize PRL in vitro[27]. Could leiomyoma be the source of the high PRL in patients with idiopathic HPRL? The answer may be "NO" as we found no significant difference in PRL level in those with idiopathic HPRL whether they had leiomyoma or not (p=0.24).

PRL is two folds higher in tumoral HPRL than secondary HPRL[29]. This also proved in our study, the mean PRL in MRI abnormality group was 45.45 ± 4.87 ng/ml (Table 3) vs. 19.2±2.01 in those with secondary HPRL. Table 3 showed the significantly higher PRL level in those with pituitary and brain MRI abnormality than those with idiopathic HPRL (P=0.03). This is consistent with Dr. Reba findings[18]. As PRL of 18.5ng/ml was the minimal value for those HPRL with pituitary and brain lesions (Table 3), this figure may be suggested to be considered as the cut-off value at or above it MRI is indicated. In Ref 3 a patient with pituitary adenoma had even a PRL level of 18ng/ml. The mean PRL level in those with pituitary lesions was 46.35±5.28 in our study while in other studies it was 61±16.6[20], 62±13[29] and 79.9±63.6[6].

The results of Table 3 can be compared with Cano et al. study[13] of MRI findings in HPRL which were: pituitary adenoma in 40.6%, questionable nodule in 4.7%, homogenous gland (i.e. pituitary hyperplasia) in 12.5%, 9.4% empty sella and 32.8% normal pituitary gland while Hauche et al study[6] revealed 6.2% pituitary macroadenoma, 32.8% microadenoma and 61% normal induced, HPRL is found in 16% vs. 8.14% in our study which may be explained by the more common use of neuroleptic drugs in developed countries.

The prevalence of HPRL varies according to patient selection and presenting symptom[10]. Our study included patients attending gynecologic clinic: subfertility was the commonest clinical feature followed by galactorrhoea and menstrual abnormality (Table 1) while in other studies[3,10,14] menstrual abnormalities was the commonest followed by galactorrhoea then subfertility but these studies include male and female patients presenting to endocrine centers. We should stress that not all HPRL women have galactorrhoea; it ranges between 40.5-67.4%[3,10,14]. Only 25% of those with galactorrhoea have HPRL[10]. It was found that 20-30% of those with menstrual abnormality had HPRL vs. only 10% of those regularly menstruating[10,21]. Thirty percent of patients with infertility have HPRL[10].There was no significant difference (P=0.14) in PRL concentration between the different clinical features of HPRL (Table 1). PRL level indeed correlates with the severity of the symptoms rather than the type of the symptom[25].

The association between polycystic ovary syndrome (PCOS) and HPRL remains controversial. In one study[10] HPRL was found in 17% of PCOS patients. PCOS found in 13% of the study group (Table 2). There was no significant difference in PRL level in those with idiopathic HPRL whether they had PCOS or not (P=0.79). It seems that these two conditions may coexist and they have independent origin[26]. However, Dr. Luciano 10 explained the relationship between PCOS and HPRL: First, the high oestrone of PCOS stimulates PRL; second, dopamine inhibits the release of

response to treatment on follow-up MRI[22].

It seems that women with HPRL present to the gynecologist early. In this study, no one with pituitary adenoma had suprasellar significant extension, invasion or compression as manifested by MRI, full ophthalmologic and neurological examination. Because of the vague presenting features of HPRL in men, they tend to present late with larger prolactinomas and at older age than women[3]. If we assume that microadenoma increases in size with time to change to macroadenoma then we should find increasing adenoma size with increasing women age but this was not found(r=0.2, p=0.35)Table 5. This is supported by the fact that microadenomas tend to remain the same size[18] and in less than 5% the tumor enlarges slowly; while macroadenomas are already established at time of presentation and in 25% significant tumor growth occurs during follow-up[1,9,12,18]. Adverse pregnancy out-come occurs in <5% in microadenoma as compared to 40% in macroadenoma patients[5]. In those with normal pituitary MRI no correlation (r=0.3, p=0.09) between age and pituitary size was demonstrated in women of reproductive age; while pituitary size is larger in pubertal girls and smaller in postmenopausal women[23].

In women with normal MRI no correlation was found between pituitary area and previous gravidity (r=0.15, p=0.44) or parity (r=0.01, p=0.96) (Table 5). Although there is, 2% and 5% increase in height and width of pituitary gland respectively 2-6 months postpartum as compared to non-pregnant control but the difference is not statistically significant[30]. Post-pregnancy PRL returns to pre-pregnancy level in HPRL women yet it is significantly lower in parous than pituitary .It seams that right pituitary adenomas are more common than left. It was 15 vs. 10 in our study, and twice as frequent in the right as left in Gspones et al study[3].

The relation between PRL level and adenoma size not settled yet. We found no significant difference (P=0.57) in PRL concentration between those with micro- and macroadenoma (Table 3). In addition, there was no correlation between PRL concentration and each of adenoma area(r=0.06 P=0.77) and normal MRI pituitary area(r=0.28 P=0.11) (Table 5). In Arafah et al study[16] PRL levels correlated positively with the MISP but the tumor size did not correlate with MISP or PRL level. While in other studies there is relatively linear relationship between degree of PRL elevation and the size of "True Prolactinoma"[4,13]. Indeed, PRL level correlates positively with the vascular density in prolactinoma. MRI can diagnose pituitary adenoma and its size but not its type. Not all adenomas are secretary and not all secretary adenomas are “True Prolactinomas”. In those with non-PRL, producing mass there is usually large mass and mild functional HPRL[4], which is explained by secondary interruption of the normal PRL inhibiting dopamine traveling via the portal blood vessels down the pituitary stalk due to either compression by the mass or due to increased MISP, which impairs portal blood flow[16].

In women of childbearing age the normal pituitary MRI sagittal diameters are: AP 8-10 (< 10 mm) HT 3.5-8 (2-6 mm) Ref 23 and 29 respectively. The normal sella turcia MRI sagittal diameters are: AP 11-16 mm and HT 8-10 mm[23]. These are comparable to our results in table 4. Variation in different populations is possible. Indeed for each patient more than one diameter basic measures of the pituitary adenoma is necessary to diagnose growth and

ng/ml[3] and >100 ng/ml[2] are 100% diagnostic of "True macroprolactinoma".

Third: If milder HPRL of ≤85 ng/ml is associated with MRI pituitary mass, further dynamic tests of PRL are required to differentiate prolactinomas, which treated primarily medically from other pituitary and brain tumors, which require surgery. Dynamic tests alone do not differentiate tumor induced functional HPRL from idiopathic HPRL. Therefore, they cannot replace MRI.

Fourth: 21.1-22% of those with macroprolactinemia have prolactinomas, usually microprolactinomas[6,20]. Therefore, MRI still indicated to diagnose them with follow-up of their mass effect. However, there is no need to treat those who have macroprolactinemia as they do not have clinical features of HPRL.

References

1. Biller, B., and Luciano, A.: Guidelines for Diagnosis and Treatment of Hyper-prolacrinemia. J Reprod Med, 1999; 44: 1075-84.
2. Crosignani, P.: Management of Hyperprolactinemia in Infertility. J Reprod Med, 1999; 44: 1116-20.
3. Gsponer, J., De Tibolet, N., Deruaz, J.P., Janzer, R., Uske, A., Mirimanoff, RO., et al.: Diagnosis, treatment, and outcome of tumors and other abnormal intrasellar masses. Retrospective analysis of 353 patients. Medicine (Baltimore), 1999; 78: 236-9.
4. Biller, B.: Diagnostic Evaluation of Hyperprolactinemia. J Reprod Med, 1999; 44: 1095-9.
5. Mallmann, E.S., Nacul, A., and Spritzer, P.M.: Pregnancy in Hyperprolactinemic women. Acta Obstet Gynecol Scand, 2002; 81: 265-7.
6. Hauache, O.M., Rocha, A.J., Maia, A.C., Maciel, E.M., and Vieira, J.G.: Screening for macroprolactinemia and pituitary imaging studies. Clin Endocrinol, 2002; 57: 327-31.
7. Glezer, A., d'Alva, C.B., Salgado, L.R., Musolino, M.R., Serafini, P., and Vieira J.G.: Pitfalls in pituitary diagnosis: peculiarities of three cases. Clin Endocrinol, 2002; 57: 135-9.
8. Pituitary Disorders Education and Support-Pituitary disorders-Prolactinoma. Online

nulliparous normoprolactinemic women[5].

There were no previous reports on pituitary size and its correlation with sella turcica size. We found the correlation to be highly significant between pituitary area and sella area in normal pituitary MRI cases (r=0.46, p=0.007) and significant in pituitary hyperplasia (r=0.96, p=0.04) (Table 5, Figure 1). According to our results, the pituitary area occupied 65.13% of the sella turcica in those with normal pituitary vs. 81.73% in those with primary pituitary hyperplasia. As the pituitary doubles its size during pregnancy[1] and increases by 120% in the third day postpartum[31], we may suggest that primary pituitary hyperplasia to be another indication for pre-pregnancy dopamine agonist therapy in order to shrink the pituitary size in a manner similar to the management of macroadenoma. No sufficient information regarding primary pituitary hyperplasia found in all the literatures reviewed in this study.

Simple sella turcica x-ray should be abandoned as a test for the pituitary because of its low sensitivity, specificity and accuracy (Table 6). Interpretation of sella X-ray is vague and incorrect[1,12].

From the results of this study and from reviewing literatures the following work-up suggested in the diagnostic evaluation of female HPRL:

First: whenever clinical features of HPRL are present, basal PRL should be measured and secondary HPRL should be diagnosed before treating the symptoms in order not to miss serious diseases and pituitary or brain tumors.

Second: Then if the basal PRL is > 18.5 ng/ml (as suggested from our study), MRI is indicated in order to differentiate pituitary macro- and microadenoma, pituitary hyperplasia or other brain tumors. MRI diagnosis of pituitary macroadenoma and PRL of > 85

21. Decanter, C., and Wainer, R.: [True and false Hyperprolactinemia: how to discriminate one from the other in infertility management?] Gynecol Obstet Fertil, 2002; 30: 276-81. (Abstract)
22. Edal, A.L., Skjodt, K., and Nepper-Rasmussen, H.J.: SIPAP- A new MR Classification for Pituitary Adenomas. Acta Radiologica, 1997; 38: 30-6.
23. Sohaib, A.A., Bomanji, J., Evanson, J., and Renzrek, R.H.: Imaging of the endocrine system. In: Grainger, R.G. and Allison, D.J. editors. Diagnostic Radiology: A Textbook of Medical imaging. 4th ed. NewYork: Churchill Livingstone; 2001; p.p. 1368-72.
24. Greenman, Y., Tordjman, K., and Stern, N.: Increased body weight associated with prolactin secreting pituitary adenomas: weight loss with normalization of prolactin levels. . Clin Endocrinol, 1998; 48: 547-53.
25. Sanfilippo, J.S.: Implications of Not Treating Hyperprolactinemia. J Reprod Med, 1999; 44: 1111-15.
26. Bracero, N., and Zacur, H.A.: Polycystic ovary syndrome and hyperprolactinemia. Obstet Gynecol Clin North Am, 2001; 28: 77-84.
27. Nowak, R.A., Mora, S., Diehl, T., Rhoades, A.R., and Stewart, E.A.: Prolactin is an autocrine or paracrine growth factor for human myometrial and leiomyoma cells. Gynecol Obstet Invest, 1999; 48: 127-32.
28. Veldman, R.G., Frolich, M., Pincus, S.M., Veldhuis, J.D., and Roelfsema, F.: Basal, pulsatile, entropic, and 24-hour rhythmic features of secondary hyperprolactinemia due to functional pituitary stalk disconnection mimic tumoral (primary) hyper-prolactinemia. J Clin Endocrinol Metab, 2001; 86: 1562-7.
29. Moeller, T.B., and Reif, E.: Normal Findings in CT and MRI. New York: thieme Stuttgard, 2000; p.p. 14-6.
30. Dinc, H., Esen, F., Demrci, A., Sari, A., and Resit Gumele, H.: Pituitary Dimensions and Volume Measurements in Pregnancy and Post Partum. MR assessment. Acta Radiologica, 1998; 39: 64-9.

HTML Document 2003. www. pituitarydisorder.net.

9. Molitch, M.E.: Pituitary Incidentoloma. Endocrinol Metab Clin North Am, 1997; 26: 725- 40.
10. Luciano, A.: Clinical Presentation of Hyperprolactinemia. . J Reprod Med, 1999; 44: 1085-90.
11. Seidl, Z., Obenberger, J., Marek, J., Hana, V., and Vaneckova, M.: MRI-diagnostics and follow-up tool for microprolactinomas. Funct Neurol, 2000; 15: 47-51.
12. Olive, D.: Indication for Hyperprolactinemia Therapy. J Reprod Med, 1999; 44: 1091-4.
13. Cano, S.A., Benito, L. P., Martinez, P.M., Cara, G.M., and Ribes, B.R.: [Pituitary magnetic resonance in women with a clinical suspicion of microprolactinoma.] Rev Coin Esp, 1999; 199: 626-31. (Abstract)
14. Suliman, A.M., Al-Saber, F., Hayes, F., Fiad, T., Culliton, M., and Cunningham, S.: Hyperprolactinemia: Analysis of presentation, diagnosis and treatment in the endocrine service of a general hospital. Ir Med J, 2000; 93: 74-6.
15. Baird, A., Sullivan, T., Zafar, S., and Rock J.: Quality of life in patients with pituitary tumours: A Preliminary Study. - Pituitary Disorders Education and Support, Pituitary News, online serials, Falls 2003. www. pituitarydisorder.net.
16. Arafah, B.M., Prunty, D., Ybarra, J., Hlavin, M.L., and Selman, W.R: The dominant role of increased intrasellar pressure in the pathogenesis of hypopituitarism, Hyperprolactinemia, and headaches in patients with pituitary adenomas. J Clin Endocrinol Metab, 2000; 85: 1789-93.
17. Sawers, H.A., Robb, O.J., Walmsley, D., Strachan, F.M., Shaw, J., and Bevan, J.S.: An audit of the diagnostic usefulness of PRL and TSH responses to domperidone and high resolution magnetic resonance imaging of the pituitary in the evaluation of hyperprolactinemia. Clin Endocrinol, 1997; 46: 321-6.
18. Rebar, R.W.: Following Patients Under Treatment for Hyperprolactinemia. J Reprod Med, 1999; 44: 1100-04.
19. Strachan, M.W., Teoh, W.L., Don-Wauhope, A.C., Seth, J., Soddart, S.J., and Beckett, G.J.: Clinical and radiological features of patients with macroprolactinaemia. Clin Endocrinol, 2003; 59: 339-46.
20. Vallette-Kasic, S., Morange-Ramos, I., Selim, A., Gunz, G., Morange, S., Enjalbert, A. et al.: Macroprolactinemia revisited:a study on106 patients.J Clin Endocrinol Metab, 2002; 87: 581-8.

ASSOCIATION OF MULTIPLE NEUROLOGICAL DISEASES IN YOUNG FEMALE: CASE REPORT

Abdul-Kareem A.M. *FRCP*, **Hasan A. Al-Hamdani** *FICMS*

Abstract
A 20-year-old female was diagnosed as a case of epilepsy since 1995. The patient was diagnosed as a case of myasthenia gravis in April 2002. Since 1999, her complaint became more announcing over two years. Until April 2002, when the patient consulted a neurologist, she was diagnosed as case of Myasthenia gravis.

The patient diagnosed as a case of multiple sclerosis then after. The key to the clinical criteria for the diagnosis was lesions disseminated in space and in time.
This case may represent an association of multiple neurological diseases of dysimmune reaction.

Key worlds: Multiple Sclerosis, Myasthenia and epilepsy
IRAQI J MED SCI, 2005; VOL. 4 (1): 111-114

Introduction

Myasthenia gravis (MG) is not rare, has a prevalence of at least one in 7500. It affects individuals in all age groups, but peaks of incidence occur in women in their twenties and thirties and in men in their fifties and sixties. Overall, women are affected more frequently than men, in a ratio of approximately 3:2[1]. The neuromuscular abnormalities in MG are brought about by an autoimmune response mediated by specific anti-acetylecholine receptor (anti-AChR) antibodies. The anti-AChR antibodies reduce the number of available AChRs at the neuromuscular junctions[2].
Multiple sclerosis affects approximately 350,000 Americans and 1.1 million individuals worldwide. The prevalence of MG is probably not affected by geographic variation on the other hand the prevalence of multiple sclerosis has been shown to vary with geographic latitudes[1].

Using the definition of epilepsy as two or more unprovoked seizures, the incidence of epilepsy is approximately 0.3 to 0.5% in different populations throughout the world, and the prevalence of epilepsy has been estimated at 5 to 10 persons per 1000. Because seizures are common, this clinical problem is encountered frequently during medical practice in a variety of settings[3]. Although epileptic seizures are uncommon in multiple sclerosis, they are more prevalent than in the general population, which may support an etiological relationship. Gurtubay reported two patients where the epileptic seizures formed part of the first episode of their illness[4].

The Case:

A 20-year-old female was diagnosed as a case of epilepsy since 1995. The seizures were uncontrolled. The seizures were generalized tonic clonic in nature without focal symtomatology. Tongue biting and incontinence accompanied the seizures, which were followed by confusion for as long as thirty minutes. She was maintained on 300mg of Carbamazepine twice daily

Dept. Medicine -Section of Neurology, College of Medicine, Al-Nahrain University
Address correspondence to Dr. Hasan A. Al-Hamdani, e-mail: hah_hamdani@yahoo.com
Received 22nd March 2004: Accepted 15th March 2005.

with poor compliance and variable response.

The patient diagnosed as a case of myasthenia gravis in April 2002 Since 1999, increased in severity over two years, the patient started to have diplopia, drooping of upper eyelids, difficulty in chewing and swallowing, nasal regurgitation, inability of combing her hair and difficulty in climbing stairs. Facial weakness produces a "snarling" expression when the patient attempts to smile. Speech has a nasal timbre. The examination reveals limb weakness is often proximal and asymmetric. This weakness, by exertion with diurnal variation. Deep tendon reflexes were preserved. Until April 2002, when the patient consulted a neurologist, and was diagnosed as case of MG. The diagnosis was confirmed by neostigmine test and electroneurophysiological study. She was kept on pyridostigmine, with good improvement. She was advised for thymectony but her family refused.

The patient was admitted to our hospital on June 2002 because of impairment of her balance, clumsiness of the hands, and a feeling of weakness and tiredness in the legs.

She was admitted to the hospital on October 2002 because of an acute onset left sided weakness. Over the course of a few days, her left arm and leg became weak and her left leg become numb. She then noticed that her walking went unsteady. Her symptoms progressively worsened up to the time of her admission to hospital. The examination revealed normal fundi, bilateral facial palsy, of an upper motor neuron type more on the right. All the tendon reflexes were hyperactive and were unequal, being more active on the left side than the right and Babinski sign bilaterally. The superficial abdominal reflexes could not be elicited. Finger nose test revealed intention tremor. The patient was unable to walk steadily along a straight line. Vibration sense was impaired bilaterally over the malleoli and joint sense was impaired in the toes. The remainder of the physical examination disclosed no abnormal findings.

The patient's birth and development were normal. She did not have a history of encephalitis or febrile convulsion .She is unmarried and because of her condition is unemployed. There is family history of MG. Her sister was diagnosed as a case of MG.

Visual evoked potential showed prolonged P_{100} latencies in both eyes, but well preserved waveform. Connective tissue screen normal. Thyroid function test was normal. Brain MRI was normal.

The patient diagnosed as a case of Multiple sclerosis. The key to the clinical criteria for the diagnosis was lesions disseminated in space and in time. She received pulses therapy of methyl prednisolon with Good improvement.

Discussion

This case may represent an association of multiple neurological diseases of dysimmune reaction. The present case may be an example of multiple medical disorders characterized by immune dysregulation and represents the association of MG, multiple sclerosis and epilepsy.

Current evidence indicates that multiple sclerosis is an autoimmune disease that develops in a genetically susceptible individuals who have resided in certain permissive environments[5]. Multiple sclerosis and MG occasionally are found in association with other diseases but rarely in association with each other. Both diseases are immunogenic in origin, and their association is probably not coincidental[6].

There have been numerous case reports of the concurrence of MG and multiple sclerosis[7]. At least 28 cases of MG in combination with multiple

sclerosis have been described in the literature. Margolis and Graves in 1945 described a 43-year-old white female with an 18-year history of transient neurologic signs and symptoms felt to be manifestations of multiple sclerosis fleeting ocular palsies develop that never completely cleared, and she noted worsening of her ocular symptoms with fatigue. She responded to neostigmine with clearing of her ptosis and of much of the ocular palsy[6].

Multiple sclerosis may be present in the MG patient as described by Kean and Hoyt. Patten and associates have described three patients, with an overlap syndrome or both multiple sclerosis and myasthenia gravis. Aita and co-workers described 4 cases of unusual combination of MG and multiple sclerosis[6].

Certainly, these interesting patients with their unusual combination of diseases raise more questions than they answers.

There have been numerous case reports of the concurrence of MG and multiple sclerosis, again suggesting a common autoimmune basis, but the statistical association is not certain[1].

The finding of autoimmune system activation in patients with a seizure disorder has lead to the suggestion that immune mechanisms may play a role in the pathogenesis of some forms of epilepsy[8]. Although epileptic seizures are uncommon in multiple sclerosis they are more prevalent than in the general population, which supports an etiological relationship. Similarly, in a considerable proportion of patients with multiple sclerosis and epileptic seizures, alterations in magnetic resonance and electroencephalogic studies that can be correlated with the clinical features of epilepsy were observed. There is great variability with regard to the type of seizure, point at which this occurs during the course of the disease, degree of recurrence and other aspects[4].

In 1977, Pechadre et al reported that children with epilepsy who were treated with intramuscular injections of immunoglobulin for recurrent upper respiratory tract infections had a decrease in the frequency and severity of their seizures. This has been supported by several reports[9].

The suggestion of Masson[10], may explain how the immune dysregulation affect the nervous system to develop these conditions, by that acquired ionic channel dysfunction resulting from auto-immune aggression. Channelopathies are responsible for muscular diseases certain forms of Mendel's law hereditary epilepsy[10]. It probably plays a part in the clinical, and particularly the sensitive expression (paresthesia and pain) of certain central nervous system affections, such as multiple sclerosis[10].

References

1. Adams, R.D., and Victor, M.: Principle of Neurology, 7th edition, McGraw Hill, 2001.
2. Drachman, D.B.: Myasthenia gravis. In: The Autoimmune Diseases, 3rd edition, Rose, N., and Mackay, I., (eds). Orlando, FL, Academic, 1998; p.p. 637-62.
3. Braunwald, E., Fauci, A.S., and Kasper, D.L.: Harrison's Principle of Internal Medicine, 15th ed. McGraw-Hill, 2001.
4. Gurtubay, I.G., Gila, L., Morales, G., Gallego-Cullere, J.A., Vuso, M.T., and Manubens, J.M.: Multiple sclerosis and epileptic seizures. Rev Neurol, 2000; 30 (9): 827-32.
5. Noseworthy, J.H., Lucchinetti, C., Rodriguez, M., and Weinshenker, B.G.: Medical progress: multiple sclerosis. N Engl J Med, 2000; 343: 938.
6. Scully, R.E., Mark, E.J., and McNeely, W.F.: Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 15-2000. A 69-year-old man with myasthenia gravis and a mediastinal mass. N Engl J Med, 2000; 342 (20): 1508-14.
7. Miller, R.M., Weedon, D., and Robertson, I.M.: Benign hypergammaglobulinaemic purpura of Waldenstrom associated with lymphoid interstitial pneumonitis. Australas J Dermatol, 1998; 39 (4): 238-40.

8. Aarli, J.A.: Immunological aspects of epilepsy. Brain Dev, 1993; 15: 41-50.
9. Aarli, J.A.: Epilepsy and the immune system. Arch Neurol, 2000; 57: 1689-92.
10. Masson, C.: Ion channel abnormalities ("channelopathies") in neurologic diseases. Presse Med, 2002; 31 (6): 244-8.

المجلة العراقية للعلوم الطبية
قائمة المحتويات

المقالات

استحداث طريقة منتجة لخطوط الزرع النسيجي للخلايا اللمفاويه

اسماعيل ابراهيم لطيف، ليلى شعيب العمر، نضال عبد المهيمن

الخلاصة

خلفية الدراسة: ان استنبات الخلايا الوحيدة النواة من محتويات الدم الكامل يؤدي إلى احتواءه على الانواع المختلفة من الخلايا. مع ذلك فان الخلايا اللمفاوية نوع (T) يمكن ان تنمى نوعيا وتتفوق في نموها على الانواع الاخرى من الخلايا لمنتجة في النهاية وسط غني بها.

هدف الدراسة: هدفت الدراسة لاجراء محاولة لابتكار طريقة جديدة لانشاء وعزل خط من الخلايا اللمفاوية من مكونات الدم الكامل. سلسلة التجارب هذه تم اجراءها لخمس اشخاص علما ان الطريقة المستخدمة هي نفسها اجريت على جميع الحالات.

طريقة العمل: تم زرع ١ مل من الدم الكامل المضاف له مادة الهيبارين إلى قارورة الزرع النسيجي والتي تحتوي على ٩ مل من الوسط الزرعي المحفز للخلايا. تم حضانته في حاضنة ثاني اوكسيد الكربون تحت درجة حرارة ٣٧ درجة مئوية ولمدة ثلاثة ايام. اخذ ١ مل من هذا الزرع النسيجي وزرع ثانيتا في قارورة اخرى تحتوي على ٩ مل من الوسط الزرعي المحفز ، كررت هذه العملية كل ثلاثة ايام لحين تكون عالق من الخلايا الوحيدة النواة تتخلل كريات الدم المتجلطة وخلال اسبوعين.

النتائج: تم الحصول على خلايا وحيدة النواة وبصورة نقية والتي تم ملاحضتها بواسطة المكرسكوب. وذلك بعد فترة اسبوعين من المحافظة عليها، وبعد فصلها عن مكونات الدم الاخرى بعد فترة حضانة ٢٤ ساعة والتي تم فصلها بواسطة الوسط الزرعي المحفز للنمو.

الاستنتاج: تم ابتكار طريقة جديدة لعزل وتكثير الخلايا اللمفاوية من محتويات الدم الكامل علما انه لم يتم التطرق إلى هذا الاكتشاف سابقا. وقد تم ملاحظة امكانية عمل الخلايا اللمفاوية نفسها كمادة بادئة في الزجاج وبواسطة استنسال هذه الخلايا، كذلك تم التطرق إلى مختلف الطرق لتكوين خطوط من الخلايا اللمفاوية نوع (T). في هذه الدراسة لم يتم استخدام خلايا مساندة او اضافة (IL_2).

مفتاح الكلمات : زرع الخلايا اللمفاوية، خط الخلايا، فايتوهيماكلوتنين.

قسم الأحياء المجهرية (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٣-٩

التغييرات في مستوى العناصر الضئيلة في مصول مرضى اللشمانية الاحشائية

ندى البشير[١]، يحيى يحيى زكي فريد[٢]، حسين كاظم عبد الحسين[٣]، هدى ظاهر هذال[١]

الخلاصة

خلفية الدراسة: بعتبر مرض اللشمانية الاحشائية من الامراض المستوطنة في العراق و حسب أخر أحصائيات منظمة الصحة العالمية (WHO 2000) و هو يصيب الاطفال دون سن السنتين. و تلعب العناصر الضئيلة دورا في العلاج و التشخيص للانواع المختلفة من الامراض الطفيلية،و ربما تكون كدليل مهم في متابعة المرض و الاستجابة الى العلاج.

هدف الدراسة: دراسة مستوى العناصر الضئيلة في مصول المصابين بمرض اللشمانية الاحشائية، و أيجاد العلاقة بين الاصابة بالمرض ليكون مستوى تلك تم جمع ٢٦ العناصر كدليل يتم من خلالة التعرف على الاصلبة بالمرض و متابعته بعد العلاج.

طريقة العمل: عينة من المرضى من الذكور و الاناث و الذين تراوحت اعمارهم مابين ٦ أشهر الى ١٥ سنة. ثم تم تحديد العناصر الضئيلة التي لها علاقة بالمناعة كالنحاس و الخارصين و المغنيسيوم، حيث تم قياس مستواهم في مصول المرضى مقارنة مع الاصحاء باستخدام مطيافية الامتصاص الذري.

النتائج: اوضحت النتائج بوجود زيادة في مستوى النحاس في مصول المرضى مقارنة بالاصحاء في حين كان هناك نقصان في مستوى كلا من الخارصين و المغنسيوم عند المصابين مقارنة بالاصحاء.

الأستنتاج: تعكس النتائج التغييرات الكيميائية الحياتية المصاحبة للمرض نتيجة للتفاعل الحاصل ما بين الطفيلي و الخلايا المناعية المهاجمة لها.

مفتاح الكلمات: الحمى السوداء، العناصر الضئيلة، النحاس، الزنك، المغنيسيوم، اللشمانية الاحشائية.

[١]مركز البحوث الطبية(جامعة النهرين)
[٢] قسم الكيمياء والكيمياء الحياتية (كلية الطب-جامعة النهرين)
[٣] قسم الكيمياء و الكيمياء الحياتية (كلية الطب-جامعة كربلاء)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ١٠-١٣

اداء المراهقين المصابين بداء السكري في المدارس: تقرير اولي

عبد الحسين مهدي الهادي[١]، جواد كاظم الديوان[٢]، اقبال غانم معلة[٣]، امجد نيازي[٤]

الخلاصة

خلفية الدراسة: يسعى المراهقون الى تكوين الذات و الاستقلالية و تحمل كل تحديات الحياة و داء السكر في فترة المراهقة بؤدي الى تعثر النمو النفسي و تتاثر قابلية المراهق على مواجهة تحديات الحياة و منها اداءه في المدرسة.

هدف الدراسة: توضيح اداء المصاب بداء السكر في المدارس.

طريقة العمل: تم جمع معلومات (العمر، الجنس، فترة المرض، النشاط الرياضي، النشاط المدرسي و مرحلة الدراسة) عن ١٦٠ مراهقا بداء السكر. تم استخدام تحليل الانحدار المتعدد لدراسة الارتباط بين الاداء في المدرسة و العوامل الاخرى.

النتائج: ظهرت علاقة احصائية ذات قيمة معنوية بين اداء المراهق في المدرسة و عدد الزيارات لعيادة السكر و ممارسة الرياضة.

الاستنتاج: عدد الزيارات لعيادة السكر و ممارسة الرياضة تحسن اداء المراهق المصاب بداء السكر في المدرسة.

مفتاح الكلمات: المراهقين، داء السكر، الانجازات المدرسية

[١] قسم طب المجتمع (كلية الطب-جامعة النهرين)
[٢] قسم طب المجتمع (كلية الطب-جامعة الأنبار)
[٣] كلية التمريض (جامعة بغداد)
[٤] الهيئة العراقية للأختصاصات الطبية

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ١٤-١٧

نسبة الاسبارتيت امينوترانسفريز الى الالنين امينوترانسفريز و التهاب الكبد الفيروسي ج

وقار عبد القهارالكبيسي[1]، منال عدنان حبيب[2]

الخلاصة

خلفية البحث: ان التهاب الكبد الفيروسي ج هو المسبب لغالبية حالات التهاب الكبد التي تتبع نقل الدم. ان التحاليل الكيميائية لانزيمات الكبد تستخدم لتقدير مدى تلف الكبد عند المرضى المصابين بالتهاب الكبد الفيروسي ج.

هدف البحث: تقدير نسبة الاسبارتيت امينوترانسفريز الى الالنين امينوترانسفريز (AST/ALT) كطريقة غير مؤذية لتقدير مدى تلف الكبد عند المرضى المصابين بالتهاب الكبد الفيروسي ج.

طريقة العمل: تضمنت الدراسة مجموعتين: المجموعة الاولى تضمنت 238 طفل مصاب بفقر دم البحر المتوسط في مستشفى الزهراوي (منهم 172 لديهم اجسام مضادة و 66 ليس لديهم اجسام مضادة لفيروس الكبد ج). المجموعة الاخرى تضمنت 8 5 امراة حامل (منهم 32 لديهم اجسام مضادة و 26 ليس لديهم اجسام مضادة لفيروس الكبد ج). تم قياس AST, ALT في المصل مع قياس نسبة AST/ALT لكل شخص تضمنته الدراسة.

النتيجة: ان معدل نسبة AST/ALT للاطفال المصابين بفقر دم البحر المتوسط الذين لديهم و الذين ليس لديهم اجسام مضادة لفيروس الكبد ج4.34 ± 3.38 و 3.09 ± 2.56 بالتعاقب بينما كان معدل نسبة AST/ALT للحوامل الذين لديهم و الذين ليس لديهم اجسام مضادة لفيروس الكبد ج 1.34 ± 1.62 و 0.42 ± 0.59 بالتعاقب.

الاستنتاج: ان معدل نسبة AST/ALT عند المصابين بالتهاب الكبد الفيروسي ج اعلى منها عند الغير مصابين بهذا المرض.

مفتاح الكلمات: نسبة الاسبارتيت امينوترانسفريز الى الالنين امينوترانسفريز ،التهاب الكبد الفيروسي ج.

[1] قسم طب المجتمع (كلية الطب-جامعة النهرين)
[2] قسم الأحياء المجهرية (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص١٨-٢٠

دراسة مختلف الأعراض المرضية والديموغرافية لمرضى الثلاسيميا وعلاقتها مع الهيموغلوبين ، بعض المعادن والعناصر الشحيحة في دم المرضى

ايناس طالب عبد الكريم١، فريال حسن عبد الجليل٢، ثابت نعمان العزاوي٣

الخلاصة:

خلفية الدراسة: تعتبر الثلاسيميا من أكثر الأمراض الوراثية انتشاراً في العالم و هي موجودة بنسبة عالية في الحزام الممتد من حوض البحر الأبيض المتوسط و الذي يمر خلال منطقة الشرق الأوسط في أسيا إلى مناطق شبه القارة الهندية و بورما و جنوب شرق أسيا. لاتزال بيتاً ثلاسيميا تظهر كمشكلة صحية عامة في العالم أجمع. كما أن أعراض المرض المتعلقة بعدم أعطاء العلاج الخاص بالمرض لا تزال موجودة في البلدان التي تفتقر إلى المصادر الأساسية التي تدعم برامج أعطاء الدم لفترات طويلة.

هدف الدراسة : دراسة مختلف الأعراض الموجودة عند المرضى المصابين بفقر دم البحر الأبيض المتوسط و الذين يراجعون مركز فقر الدم لحوض بحر الأبيض المتوسط في مستشفى أبن البلدي لأجل إعطائهم الدم. دراسة مدى العلاقة بين مختلف الأعراض الموجودة عند المرضى مع مختلف الصفات الديموغرافية لهؤلاء المرضى. دراسة مستوى العلاقة بين الأعراض الموجودة عند المرضى و مستوى الهيموغلوبين و بعض المعادن و المعادن الشحيحة و الألبومين في دم المرضى.

طريقة العمل: أجريت دراسة عرضية مقطعية للفترة ما بين ٩/١ – ٢٠٠٢/١٢/١ في مركز الثلاسيميا (فقر دم حوض البحر الأبيض المتوسط) في مستشفى أبن البلدي في بغداد. حيث اختير ١٥٧ مريض بصورة عشوائية عند مراجعتهم المركز لغرض نقل الدم استعملت استمارة مدروسة بشكل جيد لملأ المعلومات المختلفة و أخذ نموذج دم من كل مرض و حفظ بعد فصله في درجة حرارة –٢٠مْ لحين الاستعمال. أجريت الفحوصات الخاصة بالهيمو غلوبين و المعادن باستعمال الطرائق المعتمدة لكل مادة.

النتائج : أظهرت النتائج أنه من بين ١٥٧ مريض تمت دراستهم كان هناك ١١٢ (٧١,٣٪) من سكان مدينة بغداد و ١٠٧ (٦٨,٤٪) من مناطق حضرية. كما أن معدل العمر عند التشخيص كان ١,٦ سنة و كانت الثلاسيميا الشديدة تشكل نسبة ٧٧,١٪ من المرضى (١٢١مريض). كان هناك ١٠٨ مريض (٦٨,٨٪) يحتاجون إلى نقل دم في فترة بين ٢–٤ أسابيع و العلاج بالدسفيرول لأكثر من ٤ مرات بالأسبوع عند ٩٩ مريض (٦٣,١٪). كما كان هناك ٧٦ مريض (٤٨,٤٪) يعانون من سوء التغذية. لوحظت وجود مختلف العلامات المرضية بنسبة عالية على المرضى المراجعين للمركز و قد أجري تحليل العلاقة بين مختلف هذه الأعراض و كذلك بينها و بين نسبة الرصاص و السلينيوم، الحديد المجموع الكلي لقابلية الحديد الرابطة و الهيموغلوبين في الدم. و كما وجد أن هناك علاقة بين العمر و الجنس مع مختلف العلامات المرضية عند المرضى.

الأستنتاج: من الضروري جداً تهيئة مراكز عدة في مختلف أرجاء العراق و زيادة كفاءتها المختبرية لتشمل أجراء تحليل تكرار الجينات الخاص بهذا المرض. وضع برامج مرتكزة على أجراء الاختبارات لحاملي المرض، الاستشارة الطبية للراغبين في الزواج و قبل الحمل و في بداية الحمل. التشخيص المختبري للمرض أثناء الحمل باستعمال PCR و فحص DNA المأخوذ من الجنين.

مفتاح الكلمات: المعادن، المعادن الشحيحة، العلامات السريرية، مرضى الثلاسيميا

[1] قسم طب المجتمع (كلية الطب-جامعة النهرين)
[2] قسم الكيمياء و الكيمياء الحياتية (كلية الطب-جامعة النهرين)
[3] قسم الكيمياء و الكيمياء الحياتية (كلية الطب-الجامعة المستنصرية)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٢١-٣٧

العوامل المضادة لاكسدة الكريات في مرضى الثلاسيميا

صبح سالم المدلل[١]، رعد جابر موسى[١]، نجاة عبد الرزاق[٢]

الخلاصة:
خلفية الدراسة: ان زيادة اكسدة شحوم غشاء الكرية الحمراء في مرضى الثلاسيميا يؤدي الى انتاج ايونات السوبر اوكساید بكميات كبيرة و هذا هو سبب تحلل كريات الدم الحمراء في مرضى الثلاسيميا.

هدف الدراسة: اجريت هذة الدراسة لمغرفة حالة اكسدة شحوم غشاء الكرية الحمراء في مرضى الثلاسيميا و كذلك قياس العوامل المضادة لاكسدة الخلية.

طريقة العمل: اجري فحص انزيمات السوبر اوكساید و الكتاليز لستة و سبعون مريضا للثلاسيميا الثقيلة و اربعة عشر مريضا للثلاسيميا الخفيفة و تسعة عشر اصحاء.

النتائج : وجد بان مرضى الثلاسيميا الثقيلة لديهم انزيم السوبر اوكساید نسبة اعلى (و مهم من ناحية احصائية) من الاصحاء، بينما المرضى المصابين بالثلاسيميا الخفيفة لديهم انزيم الكتاليز نسبة اعلى (و مهم من ناحية احصائية) من الاصحاء و كذلك اضهرت النتائج بانه كلما قلت نسبة الهيموغلوبين عند مرضى الثلاسيميا الثقيلة كلما ازدادت نسبة انزيم السوبر اوكساید.

الاستنتاج: انزيم السوبر اوكساید يكون اكثر في مرضى الثلاسيميا الثقيلة و تتناسب كميته بشكل عكسي كلما قلت نسبة الهيموغلوبين.

مفتاح الكلمات: ثلاسيميا، سوبر اوكساید، كتاليز

[١] قسم علم الأمراض (كلية الطب-جامعة النهرين)
[٢] قسم الكيمياء و الكيمياء الحياتية (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٣٨-٤٤

تولد الأوعية الدموية في أورام غدة البروستات

فائزة عفتان الراوي

الخلاصة

خلفية الدراسة: تولد الأوعية الدموية يعني تكوين أوعية دموية جديدة، عملية طبيعية حرجة تحدث في الجسم في الحالة الصحيحة والمرضية.

الهدف من الدراسه: التقييم العددي للأوعية الدموية في أورام غدة البروستات و مقارنتها مع درجة الورم.

طريقة العمل: ٢٣ قالب شمع من نماذج غدة البروستات (٨ تضخم غدة البروستات الحميد و ١٥ قالب أورام خبيثه مقسمة بالتساوي بين ذات تميز جيد، متوسط وضعيف). صبغت منها مقاطع بصبغات هيماتوكسلين و أيوسين، صبغة فان كيزن و ماسون ترايكروم. أحتسبت عدد الأوعية الدموية وكثافتها حسب معادلات رياضية، و قورنت النتائج مع درجة تميز الورم.

النتائج: أزداد عدد الأوعية الدموية والحد الأعلى لها مع زيادة درجة الخبث و تراوحت من ١٦،١ في التضخم الحميد الى ١٠٢،٠ في ألأورام الخبيثه ذات التميز الضعيف.

ألإستنتاج: أظهرت الدراسة علاقة ذات مغزى بين درجة الورم و تولد الأوعية الدمويه.

مفتاح الكلمات: تولد الأوعيه الدمويه،أورام غدة البروستات.

قسم علم الأمراض (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٤٥-٤٨

مستويات النحاس والزنك ونسبة النحاس/الزنك في مصل مرضى التهاب المفاصل الرثوى

يحيى يحيى زكي فريد[١]، علاء كريم محمد[٢]، اسراء سالم محمد[٢]

الخلاصة

خلفية الدراسة: عياثر العناصر وخصوصا النحاس والزنك لها دور كبير في العديد من الفعاليات البايولوجية لذلك تقدير تلك العناصر في مصل المرضى المصابين بالتهاب المفاصل الرثوي ربما تحسن الفهم و الادراك نحو العلاقة بين مستوى النحاس و الزنك و بين فعالية المرض.

هدف الدراسة: تحديد مستوى النحاس و الزنك في مصل المرضى و علاقتة مع درجة فعالية المرض و مقارنة النتائج مع مجموعة الاصحاء.

طريقة العمل: تضمنت ٩٧ شخصا مقسمين الى اربعة و خمسون مريضا (٣٧ اناث،١٧ ذكور) يعانون من مرض التهاب المفاصل الرثوى و مقسمين الى ٢٩ مريضا (٢١ اناث،٨ ذكور) بمدى عمر يتراوح بين (٣٢ —٦٥سنة) و لديهم فعالية قليلة للمرض و ٢٥ مريضا (١٦ اناث،٩ ذكور) بمدى عمر يتراوح بين (٢٢–٥٢ سنة) و لديهم فعالية مرتفعة للمرض و مقارنة النتائج مع ٤٣ شخص سليم بمدى عمر يتراوح بين (١٩–٦٤ سنة). تم تقدير مستوى النحاس و الزنك فى مصل المرضى بتقنية مطيافية الامتصاص الذري.

النتائج: مستوى النحاس في مصل الدم و كذلك نسبة النحاس/الزنك تزداد بصورة ملحوظة فى كلا المجموعتين قيد الدراسة مقارنة بمستواها فى مصل الاصحاء. اضافة الى انخفاض ملحوظ فى مستوى الزنك فى مصل المرضى ذو الفعالية القليلة و بصورة اكبر عند المرضى ذو الفعالية العالية للمرض.

الاستنتاج: ان التغير فى مستوى النحاس و الزنك فى مصل المرضى المصابين بالتهاب المفاصل الرثوى له علاقة بفاعلية و درجة المرض مع تسليط الضوء على اهمية دور العناصر فى الحالات المرضية و الفسيولوجية.

مفتاح الكلمات: التهاب المفاصل الرثوى، النحاس، الزنك، المصل.

[١] قسم الكيمياء و الكيمياء الحياتية (كلية الطب-جامعة النهرين)
[٢] قسم الكيمياء (كلية التربية-ابن الهيثم- جامعة بغداد)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٤٩-٥٦

عمر الأم عند الزواج كمؤشر للصحة الأنجابية

ايناس طالب عبد الكريم، عبد الحسين الهادي

الخلاصة

خلفية الدراسة: إن الحمل المبكر و الولادة الغير مخططا لها قد يكون لها أثار بعيده من الناحية الجسدية و النفسية و الاجتماعية للفتيات المراهقات و أولادهن، و لهذا تعتبر هذه حالة صحية عامة و ذات أهمية خاصة بسبب ازدياد التأثير الحضاري و الاجتماعي و تباين وجهات النظر في الصحة الإنجابية.

هدف الدراسة: هو تحديد عمر المرآة عند الزواج و علاقته بالحالة المعيشية و البيئية و مدى تأثيرها على الصحة الإنجابية عند الأمهات.

طريقة العمل: أجريت دراسة مقطعية عرضية حيث أخذت المعلومات بطريقة عشوائية من ٨٠٠ أم بحالة صحية جيدة و من خلال زيارتهم إلى خمسة مراكز صحية و التي اختيرت بطريقة عشوائية من مدينة بغداد و خلال الفترة من حزيران إلى تشرين الثاني لعام ١٩٩٤. تم اخذ المعلومات من الأمهات من خلال استمارات مدروسة بشكل جيد.

النتائج: أظهرت النتائج إن هنالك علاقة ذات مغزى إحصائي بين عمر المرآة عند الزواج و عدد من المؤثرات مثلا المستوى التعليمي للمرآة، الفترة بين الحمل الأول و التالي، عمر الأب عند الرواج، عدد مرات الحمل و عدد الأطفال دون سن الخامسة في الأسرة، درجة الكثافة الأسرية، درجة القرابة بين الوالدين ($P< 0.001$)، كذاك كان هنالك علاقة ذات مغزى إحصائي مع عمل الزوج، و عدد وفيات الأطفال دون سن الخامسة ($P< 0.01$). بينما لم تكن هناك علاقة ذات مغرى إحصائي مع نوع العائلة و عدد مرات الإسقاط عند الأم ($P >0.05$).

الاستنتاج: إن عمر الأم عند الزواج يعتبر عامل مهم في الصحة الإنجابية للمرآة عندما درست مع مختلف العوامل الاجتماعية و البيئية مما يؤكد على ضرورة أن يكون هناك إقبال من الشباب على المعلومات الصحية، و كذاك العاملين في مجالات الصحة يجب أن يعملوا على توفير المعلومات الضرورية، التي تساعد النساء الصغيرات في العمر لكي تتوفر لديهم الثقة في استعمال مصادر المعلومات المتاحة لهم.

مفتاح الكلمات : عمر الأم، الصحة الإنجابية.

قسم طب المجتمع (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٥٧-٦٢

الدور الأحيائي للنحاس، القصدير و المغنسيوم خلال فترة نماء براعم الأطراف في اجنة الدجاج

يحيى يحيى زكي فريد[١]، حيدر جواد مبارك[٢]، مي فاضل ماجد[٣]

الخلاصة

خلفية الدراسة : ان نماء برعم الاطراف يستحث بالحافة القمية للأديم الظاهر. وجد ان للعناصر الزهيدة دور مهم في النماء الاحيائي للانسجة كما في انسجة الاورام.

هدف الدراسة: اجريت هذه الدراسة للكشف عن الدور الإحيائي المحتمل للعناصر الزهيدة مثل الزنك، النحاس، و المغنيسيوم في نماء الأطراف المستحث بالحافة القمية للأديم الظاهر.

طريقة العمل: استعملت اجنة الدجاج في مراحل هامبركر و هاملتن ٢٠–٢٦. تم قياس العناصر الزهيدة في براعم الاطراف المحتوية و غير المحتوية على التثخن القمي للاديم الظاهر.

النتائج: وجد ان انخفاض تركيز الزنك في أنسجة براعم الأطراف التي لا تمتلك الحافة القمية للاديم الظاهر يشير إلى العلاقة بين هذا العنصر الزهيد و الفعل الحاث لهذه الحافة في تكون الاطراف. ربط تركيز النحاس المكتشف فقط في البراعم التي لا تمتلك الحافة القمية بالتنظيم الجنيني لبراعم الاطراف. ان انخفاظ تركيز المغنيسيوم في براعم الاطراف اعتبر كصفة للأيض المصاحب الحافة القمية الفاعلة للأديم الظاهر خلال التكون الجنيني لبراعم الأطراف.

الاستنتاج: ان العناصر الزهيدة قد تمتلك دور هام في عملية التبرعم الجنينية.

مفتاح الكلمات: براعم الطرف، العناصر الزهيدة، الزنك، النحاس، المغنيسيوم، النمو

[١] قسم الكيمياء و الكيمياء الحياتية (كلية الطب-جامعة النهرين)
[٢] قسم التشريح البشري (كلية الطب-جامعة النهرين)
[٣] فرع الأنسجة و الأجنة – قسم التشريح البشري (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٦٣-٦٦

عدم الخصوبة في الذكور و الدور الوظيفي للخارصين

علي تقي البلداوي[1]، احمد ناجي، عقيل عبد الامير العاني

الخلاصة :

خلفية الدراسة: عدم الخصوبة في الذكور تعرف بأنها عدم القابلية على إنجاب طفل عند غياب السبب عند الاناث. تكمن معظم أسبابه في اختلاف النسب الطبيعية للمني. و يعد الخارصين إحدى مكونات السائل المنوي المهمة لما له من دورا رئيسيا في عملية الخصوبة و الانجاب .

هدف الدراسة: يهدف البحث إلى دراسة العلاقة بين تراكيز الخارصين في مصل الدم و مصل السائل المنوي و جودة السائل المنوي بين الرجال غير المخصبين و المخصبين .

طريقة العمل: اجري البحث على ثماني و خمسون ذكرا متزوجا (مجموعة غير المخصبين) حيث تم إخضاعهم لاستقصاء أسباب عدم الخصوبة و سبعة وثلاثون ذكرا متزوجا (مجموعة المخصبين) لهم زيجات حبلى درسوا كمجموعة سيطرة. تم إجراء تحليل السائل المنوي و الهومونات التوالدية للمجموعتين كما و اجري قياس نسبة تركيز الخارصين في مصل الدم و مصل السائل المنوي باستخدام تحليل مقياس الشدة اللونية .

النتائج: أظهرت الدراسة أن جميع خواص السائل المنوي (العدد، الحركة، الهيئة الخارجية) للرجال غير المخصبين اقل مما هو عليه الحال عند الرجال المخصبين. باستثناء هورمون الشحوم الخصوبي كانت بقية الهورمونات التوالدية (هورمون الاباضة، الهورمون المنبه للجريب) للرجال غير المخصبين ليست مختلفة مما علية الحال عند الرجال المخصبين. كما وجدت معدلات تركيز الخارصين في مصل الدم و السائل المنوي اقل في الرجال غير المخصبين إذا ما قورنت مع الرجال المخصبين. كما أظهرت الدراسة عدم وجود علاقة بين تركيز الخارصين في مصل الدم مع تركيزه في مصل السائل المنوي في كلا المجموعتين .

الاستنتاج: أظهرت الدراسة في ضوء النتائج التي تم التوصل إليها إلى أن الخارصين يسهم في الخصوبة من خلال التأثير المباشر و غير المباشر على عملية الانطاف.

مفتاح الكلمات: عدم الخصوبة، الخارصين، السائل المنوي، عملية الانطاف.

[1] فرع الطب العدلي-قسم علم الأمراض كلية الطب-جامعة النهرين)
[2] قسم الكيمياء (كلية العلوم-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص٦٧-٧١

دراسة عشوائية مستقبلية لتأثير علاج مايتومايسين سي مع مايتومايسن سي بالمقارنة مع مايتوماسين سي/ مركب س٢ متعاقب لغسل المثانة في سرطان المثانة السطحي

أحمد العزاوي[١]، عبدالوهاب رزوقي حمد [٢]، محمود حياوي حماش[٣]

الخلاصة

خلفية الدراسة: يأتي سرطان المثانة بالمرتبة الثالثة في الذكور و بالمرتبة العاشرة في الأناث في الولايات المتحدة الأمريكية. في احايين كثيرة بعد استأصاله تستعمل علاجات كيمياوية في غسل المثانة.

هدف الدراسة: هو مقارنة برنامجين لمدة سنتين لدراسة التأثير و الفعالية و السمية لعلاج غسل المثانة بعقار مايتومايسين سي مقابل من علاج مايتومايسين سي و مركب س٢ تعاقبيا لسرطان المثانة السطحي.

طريقة العمل: دراسة عشوائية مستقبلية لمجموعتين من المرضى المصابين بسرطان المثانة السطحي (٧٣مريضاً) بعد الاستئصال بناظور المثانة عبر الاحليل مع متابعتهم لمدة ٢٦ شهرا. المجموعة الاولى (أ) ضمت ٣٧ مريضاً استلموا علاج مايثومايسين سي (غسل المثانة) ٣٠ ملغ في اليوم الاول/ اسبوعياً لمدة ٦ اسابيع و شهرياً لمدة ١٢ شهراً زائد المرضى في المجموعة (ب) (٣٦مريض) اضيف لعلاجهم مركب س٢ ٣ملم في اليوم الثاني (غسل المثانة). التحليل الاحصائي صمم بواسطة طريقة كابلان–ماير.

النتائج: بعد متابعة لمدة ٢٦ شهرا، ٤٦٪ من المرضى في المجموعة (أ) كانوا خالين من المرض مقارنة مع ٧٠٪ للمرضى في المجموعة (ب) فضلا عن ان التاثيرات الجانبية كانت متقاربة.

الاستنتاج: النتائج بينت ان علاج غسل المثانة بعقار مايثومايسين سي مع مركب س٢ تعاقبياً كانت فعالا جداً في انهاء و/او الوقاية من سرطان المثانة السطحي.

مفتاح الكلمات: مايتومايسين سي ، س٢ ،غسل المثانة ، سرطان المثانة السطحي.

[١] قسم الطب (كلية الطب-جامعة النهرين)
[٢] قسم الكيمياء و الكيمياء الحياتية (كلية الطب-جامعة النهرين)
[٣] فرع الأنسجة و الأجنة-قسم التشريح البشري (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٧٢-٧٦

تدرن الثدي في مدينة سامراء

حا رث مصطفى الخطيب

الخلاصة
خلفية الدراسة: إن تدرن الثدي ليس نادرا في ضرع الحيوانات و خاصة الأبقار و لكنه اقل إصابة لثدي المرأة و عادة لثدي واحد و اندر من ذلك إصابة الثديين معا و حتى ثدي الرجل يمكن إصابته.

هدف الدراسة: البحث عن نسبة اصابة ثدي المرأة بالتدرن

طريقة العمل: تم فحص و تشخيص خمسة عشر مريضة خلال الفترة من ١-٧-١٩٩٢ م الى ١-٧-٢٠٠٢ م كإصابة بتدرن الثدي بواسطة الفحص الخلوي و النسيجي و عولجت بالعقاقير المضادة للتدرن.

النتائج: تم شفاء ١٤ حالة من اصل ١٥ إصابة بتدرن الثدي باستعمال ايزونايزايد، ايثامبيوتول، و ريفادين لمدة شهرين ثم الاقتصار على دواءين منها لمدة ٦-٩ شهور.

الأستنتاج: إن تدرن الثدي عند المرأة يجب إن يوضع في الحسبان عند وجود قرحة أو عقدة التهابية او ناسور او عدة نواسير في الثدي لا تستجيب للعلاج التقليدي و لكنها تستجيب للأدوية المضادة للتدرن و يمكن شفاؤها تماما. و لصعوبة عزل عصيات السل من الانسجة يمكن اعتماد طريقة الاستجابة السريرية للعلاج التجريبي في التشخيص.

مفتاح الكلمات: الثدي، التدرن، أدوية مضادة للتدرن.

قسم الجراحة (كلية الطب-جامعة تكريت)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٧٧-٨٠

تعداد خلايا الايسينوفيل و علاقته بالربو لدى الاطفال

حسام محي العلواني[1]، فالح سالم سرحان[2]، شذى حسين على[1]

الخلاصة

خلفية الدراسة : الربو القصبي هو عبارة عن انسداد في المجاري التنفسية ينتج عن محفزات مختلفة. لقد شهد المرض زيادة في نسبة الاصابة و شدتها في الاونة الاخيرة. في مرض الربو هناك تضيق في المجاري الهوائية مع افرازات كثيفة اضافة الى الالتهاب الذي يلعب دوراً مهماً و هنا لخلايا الايزنوفيل دور مهم.

هدف الدراسة : هو دراسة العلاقة بين مرض الربو و تعداد خلايا الايزونوفيل في عينة الدم.

طريقة العمل : تضمنت الدراسة ٥٥ طفل مصاب بالربو القصبي، و درست شدة الربو لديهم عن طريق العلامات السريرية، و جهاز فحص وظائف الرئة، و اخذت عينة دم وريدي من كل مريض لقياس عدد خلايا الايزونزفيل.

النتائج : بينت النتائج ان ٣٥ مريض كان مصاباً بنوبة متوسطة الى شديدة، و كانت نسبة ٨٢,٨٪ مهم لديهم تعداد خلايا الايزنوفيل عالي (اكثر من ٤٠٠ ملم٣) بينما ١٧,٢٪ كان تعداد الخلايا اقل من ٤٠٠. العشرون مريض الباقين كانوا من ذوات الشدة البسيطة الى المتوسطة و كان ٨٠٪ منهم لديهم نسبة خلايا الايزنوفيل اقل من ٤٠٠ و ٢٠٪ نسبة عالية من الخلايا.

الاستنتاج : هناك زيادة في نسبة خلايا الايزنوفيل في دم مرضى الربو، و تزداد هذه النسبة مع زيادة شدة الربو.

مفتاح الكلمات: الربو القصبي، خلايا ايزنوفيل، شدة المرض.

[1] قسم طب الأطفال (كلية الطب-جامعة النهرين)
[2] قسم علم الأمراض (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٨١-٨٣

مستوى الكالسيوم في مصل دم مرضى السكري من النمط ٢

بيبين خورشيد السليفاني

الخلاصـــة

خلفية الدراسة: يعتبر الكالسيوم من العناصر المهمة في التصنيع الأحيائي، خزن، افراز، و حيوية الأنسولين.

هدف الدراسة: لمعرفة مستوى الكالسيوم في مصل الدم لدى مرضى السكري من النمط ٢ .

طريقة العمل: تمت الدراسة في عيادة الوفاء لداء السكري في الموصل للفترة من تموز ٢٠٠٢ – شباط ٢٠٠٣ و قد شملت ١٢٠ شخصا من كلا الجنسين. ستون مريضا من المصابين بداء السكري من النمط ٢ المشخصون حديثا معدل (± الانحراف المعياري) أعمارهم 11.6±47.6سنة و ٦٠ شخصا أصحاء كمجموعة سيطرة معدل (± الانحراف المعياري) أعمارهم ١٤,٣±٣٥,٢ سنة. تم قياس تركيز الكالسيوم و الكلوكوز في مصل الدم.

النتائج: أظهرت النتائج ارتفاعا ذا معنى في مستوى الكالسيوم و الكلوكوز في مرضى داء السكري من النمط ٢ المشخصين حديثا مقارنة مع الأصحاء، بالإضافة إلى عدم وجود علاقة بين زيادة الكالسيوم و الكلوكوز في مرضى داء السكري.

الاستنتاج: زيادة الكالسيوم في مصل دم مرضى داء السكري من النمط ٢ قد يعزى إلى عوامل أخرى و ليس زيادة الكلوكوز.

مفتاح الكلمات: داء السكري من النمط ٢، الكالسيوم، الكلوكوز، فرط الكالسيوم، فرط الكلوكوز.

قسم الفسلجة (كلية الطب-جامعة الموصل)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٨٤-٨٨

نقص الغدة الدرقية الخلقي لدى الاطفال الذين يراجعون عيادة الغدد الصماء و السكري لدى الاطفال في مستشفى الكاظمية التعليمي

نشأت عزيز نشأت[1]، حسام العلواني[1]، ايمان محمود[2]

الخلاصة

خلفية الدراسة: نقص الغدة الدرقية الخلفية يعتبر من اكثر امراض الغدد الصماء الشائعة لدى الاطفال و التشخيص المبكر له اهمية و ذلك لمنع حدوث تخلف عقلي و بدني في حالة اعطاء العلاج مبكرا.

هدف الدراسة: البحث عن نسبة الأطفال المصابين ب نقص الغدة الدرقية الخلفية في العراق.

طريقة العمل: دراسة راجعة شملت ٤٠ طفلا مصابون بنقص الغدد الدرقية و الذين تم فحصهم و علاجهم في عيادة الغدد الصماء و السكري الخاصة بالاطفال في مستشفى الكاظمية التعليمي في بغداد، العراق للفترة من كانون الثاني ١٩٩٣ –كانون الثاني ٢٠٠٣.

النتائج: ٢٤ من الأطفال كانوا من الأناث و ١٦ ذكر و نسبة الاناث للذكور١:١,٥. زواج الاقارب موجب في ٣٦ (٨٠٪) من المرضى ٢٥ (٦٢,٥٪) لديهم مرض الغدد الدرقية في عوائلهم و ٢٤(٦٠٪) من المدينة و ١٦(٤٠٪) من المناطق الريفية غرب بغداد. عشرة اطفال ٢٥٪ اكتشفوا في الشهر الاول من اعمارهم ١٥(٣٧,٥٪) في الاشهر الثلاثة الاولى و ٢٥(٦٢,٥٪) خلال ستة اشهر الاولى و كان سبعة مرضى لديهم ضمور خلقي و خمسة لديهم انتباذ في الغدة و قد تم تقدير التطور الذهني لدى هؤلاء الاطفال و كذلك الاعراض و العلامات.

الأستنتاج: على الرغم من عدم معرفتنا بنسب الاطفال المصابين بالعراق الا انه من الملاحظات الاولية انه ليس نادر الحدوث و الاسباب الجينية ترجع و ذلك لوجود عدة اشقاء مصابين و النسبة العالية للتزاوج بين الاقارب في العراق. ان نقص الغدة الدرقية الولادي مرض ليس نادر الحدوث و ننصح ببرنامج وطني شامل الفحص حديثي الولادة لتشخيص نقص الغدة الدرقية مبكرا .

مفتاح الكلمات: نقص الغدة الدرقية الخلقي، مراجعة.

[1] قسم طب الأطفال (كلية الطب-جامعة النهرين)
[2] مستشفى الطفل العربي المركزي-بغداد-العراق

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٨٩-٩٢

اختلال وظيفة التنفس لدى مرضى الوهن العظلي الوبيل

عبد المطلب عبد الكريم[١]، ساجد إبراهيم الحسيني[٢]

الخلاصة

خلفية الدراسة: وهن العظلات الوبيل مرض مناعي ذاتي بصيب عضلات الجسم الهيكليه و من ضمنها عضلات التنفس و التي يمكن أن تؤدي الى عجز التنفس و الأحتياج الى التنفس الأصطناعي الميكانيكي في الحالات الشديده.

هدف الدراسه: تقييم وظائف الرئه لمرضى وهن العضلات لمعرفة حالات المشمولين بالأختلال، تبيان العلاقه بين اختلال وظائف الرئه و العوامل المهيجه لذلك الأختلال، تبيان فائدة رفع الغده الزعتريه للتقليل من اختلال وظائف الرئه.

طريقة العمل: تم اجراء دراسه سريريه مقارنه ل(٥٠) حاله من مرضى وهن العضلات الوبيل،٣٣ حاله أناث و١٧ حاله من الذكور و كانت أعمارهم تتراوح بين ١٦–٦٠ سنه. ٢٩ حاله من هؤلاء المرضى خضعوا لعملية رفع الغده الزعتريه. تمت الدراسه في الفتره بين تشرين الأول عام ١٩٩٩ و لغاية شهر حزيران عام ٢٠٠١ في مستشفى الكاظميه التعليمي–بغداد–العراق.

النتائج: ١–٤٦ ٪ من المرضى لديهم اختلال في عظلات التنفس اعتماداً على فحص وظائف الرئه و بالذات السعه الحيويه. ٤٧,٨٪ من هذه الحالات كانت خلال السنين الأربع الأولى من مسيرة المرض السريريه. ٦٨,٩ ٪ من المرضى الذين خضعوا لعملية رفع الغده الزعتريه لم يصابوا بأختلال وظيفة التنفس. الألتهابات الخمجيه هي من أكثر العوامل المهيجه للأختلال (٣٩٪).

الأستنتاج: ان اختلال وظيفة التنفس لدى مرضى وهن العظلات ليست نادره. ان رفع الغده الزعتريه مبكراً ذو فائده ملموسه لمنع حدوث اختلال وظيفة التنفس وخصوصاً في السنوات الأولى من مسيرة المرض السريريه.

مفتاح الكلمات: وهن العظلات الوبيل، الصفيحه العصبيه العضليه، رفع الغده الزعتريه، اختلال التنفس.

[١] فرع الجملة العصبية-قسم الطب (كلية الطب-جامعة النهرين)
[٢] مستشفى النعمان العام-بغداد-العراق

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٩٣-٩٨

الملخصات العربية

فرط برولاكتين الدم في النساء: دراسة تحليلية للملامح السريرية و تشخيصية للاسباب. هل يتوجب اجراء التصوير بالرنين المغناطيسي دائما؟

سحر لويس حلبية

الخلاصة

خلفية الدراسة: فرط برولاكتين الدم هو الاكثر شيوعاً في اضطرابات الغدد الصماء لمحور الوطاء–النخامية–المبيض. حيث تعتبر اورام النخامية من اهم الاسباب و اكثرها شيوعا.

هدف الدراسة: تحليل الملامح السريرية. دور التصوير بالرنين المغناطيسي في التقييم التشخيصي لحالة فرط الهرمون. اجراء قياسات بالرنين المغناطيسي مع بيان العلاقة بين نتائج الرنين و نسبة برولاكتين الدم في النساء العراقيات. مراجعة المصادر و اقتراح طريقة اجراءات التشخيص لحالات فرط الهرمون.

طريقة العمل: اجري التقييم السريري و القياس الاساسي لتركيز البرولاكتين بمصل الدم و كذلك فحص النخامية و القحف بالرنين المغناطيسي في دراسة تعاقبية ل ٨٢ امرأة عراقية مصابة بفرط البرولاكتين عند مراجعتهن لعيادة الامراض النسائية بعد استبعاد حالات فرط الهرمون الثانوية.

النتائج: لقد وجد ان حالات نقص الخصوبة و ثر الحليب و عدم انتظام الدورة الشهرية هي الملامح الشائعة و لقد اظهر الرنين المغناطيسي خللا عند ٤١,٤٦٪ من المريضات و كان ٨٨,٢٤٪ منها بسبب النخامية و التي نتج عنها ارتفاع تركيز البرولاكتين بالدم بصورة معتدة اكثر من الحالات المجهولة السبب $P=0.03$. كما وجد ان غلّوم النخامية في الجهة اليمنى اكثر من اليسرى. تحتل النخامية ٨١,٧٣٪ من مساحة السرج الثركي في حالة فرط التنسج للنخامية. لم تظهر النتائج وجود علاقة معتدة بين حجم الغلوم و مستوى البرولاكتين في الدم و لكن توجد علاقة ايجابية معتدة بين مساحتي النخامية و السرج الثركي في كل من حالة فرط التنسج للنخامية $P=0.04$ و كذلك حالة الرنين المغناطيسي الطبيعي $P=0.07$.

الاستنتاج : يمكن اعتماد تركيز برولاكتين الدم بقياس ١٨,٥٪نغم/مل كحد قاطع لاجراء الرنين المغناطيسي العالي التمييز للنخامية و القحف. ان فرط التنسج للنخامية قد يحمل خطورة الامتداد خارج السرج الثركي اثناء الحمل. هناك علاقة ترابط موجبة بين حجم النخامية و السرج الثركي. ممكن اعتبار الرنين المغناطيسي هو المعيار المذهبي كطريقة لقصور النخامية بينما الصور الشعاعية للسرج الثركي قد بطل استعمالها.

مفتاح الكلمات: فرط برولاكتين الدم، غلّوم النخامية، فرط تنسج النخامية، التصوير بالرنين المغناطيسي، قياسات بالرنين المغناطيسي، عراقيات.

قسم النسائية و التوليد (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ٩٩-١١٠

مزاملة لعدد من الأمراض العصبية لفتاة صغيرة السن: حالة مسجلة

عبد المطلب عبد الكريم، حسن عزيز الحمداني

الخلاصة

الحالة لفتاة تبلغ من العمر عشرون عاما" شُـخصت إصابتها بمرض الصرع سنة ١٩٩٩ و كان نوع الصرع، صرع أكبر بدون علا مات بؤرية.

في نيسان ٢٠٠٢ شُـخص للمريضة أصابتها بمرض وهن العضلات الوبيل. فمنذ سنة ١٩٩٩ حيث بدأت المريضة تعاني من علامات تزداد شـدة بشـكل مسـتمر متمثلة بازدواج النظر، هطول الأجفان، صعوبة في المضغ و البـلـع و صعوبة في صعود السـلم و تمشـيط شـعـر الـرأس. العلامات كانت أكثر وضوحا" عند المساء. و قد أثبت وجود المرض بواسطة الفحـص الكهروفسلجي و بواسطة ملاحظة الاستجابة لعقار النيوستكمين وريديا". و قد وصِـف العلاج بواسطة عقار النيوستكمين عن طريق الفم و أظهرت المريضة تحسـن ملحوظ.

في حزيران ٢٠٠٢ أدخلت المريضة إلى المسـتشـفى بعد ظهور أعراض جديدة مختلفة عن السابق متمثـلة باضطراب التوازن و صعوبة القيام بالأعمال الدقيـقـة بواسـطة اليد و صعوبة في المشي. أدخلت المريضة مرة أخرى إلى المستشفى في تشـرين الأول نتيجـة حصول شـلل في الجهة اليسـرى من الجسم مع خدر في نفس المنطقة. العلا مات كانت تزيد شـدة أثناء تواجدها في المسـتشـفى.

أظهر الفحص السـريري وجود شـلل في العصب السـابع للجهتـين ذا منشأ حركي علوي مع نشـاط لردود الأفعال الوترية في جهتي الجسـم مع ضعف حركي ذا منشأ علوي بالأضافة إلى اعتلال في المخيخ مع اضطراب في الجهاز الحسـي خصوصا" في إحساس التذبذب.

أظهر فحص العصب البصري المثار على وجود زيادة في الاسـتثارة لكلا العينين. فشـخص الإصابة بمرض تصلب الأعصاب المنتشر على أساس كون الحالة تمثل انتشـار الضرر في المكان و الزمان للجهاز العصبي المركزي. أعطي عقار مثيل بردنيزولون للمريضة و أظهرت اسـتجابة واضحة.

الأستنتاج: هذه الحالة قد تمثل مزاملة لعدة أمراض عصبية كأوجه مختلفة للاضطراب المناعي.

فرع طب الجملة العصبية-قسم الطب (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٥ م، المجلد ٤ ، العدد ١، ص ١١١-١١٤

المجلد الرابع، العدد الأول، ١٤٢٤ هـ، ٢٠٠٥م

المجلة العراقية للعلوم الطبية

تعنون المراسلات إلى المجلة العراقية للعلوم الطبية، صندوق بريد ١٤٢٢٢ بغداد، العراق. تلفون و فاكس (٩٦٤-١-٥٢٢٤٣٦٨).

رقم الإيداع في دار الكتب و الوثائق ببغداد ٧٠٩ لسنة ٢٠٠٠

الهيئة الأستشارية

نزار الحسني (الهيئة العراقية للأختصاصات الطبية)

Leading Article

CARDIAC RESYNCHRONIZATION THERAPY (CRT): A NEW PACING THERAPY FOR CHRONIC HEART FAILURE

Ammar Al-Hamdi *FRCP*

It has almost become a cliché to say that chronic heart failure (CHF) is a major health problem all over the world[1] It constitutes 1-3% of the population and 10% in the elderly, and about 5% of all medical admissions[1].

CHF combines a high prevalence with a terrible burden of symptoms and morbidity, all of these contributing to a huge societal and health care costs[1,2].

To compound the problem, patients with CHF lives only a short time, though CHF predominantly afflicts the elderly, it is still a cause of significant premature loss of life and affecting children, young and middle age group[3].

Cardiac fibrosis has been proposed to alter cardiac performance, namely diastolic function, in fact accumulation of collagen fibers within the myocardium is mainly responsible for in an increase in intrinsic myocardial stiffness that may alter left ventricular diastolic properties and the pattern of left ventricular filling.

In addition, fibrosis may affect the conduction system of the heart including left bundle branch block (LBBB) or right bundle branch block (RBBB) and AV conduction delay, which cause biventricular dyssynchrony[4].

Cardiac contraction normally occurs in a spatially and temporally uniform manner, so that all portions of the wall contract synchronously. Biventricular dys-synchrony, which occurs in CHF with intraventricular conduction delay especially in LBBB, is disadvantageous to the heart causing:

1. Inefficient ejection.
2. Abnormal regional loading which alters function and arrhythmogenecity
3. Abnormal mitral valve function causing mitral regurgitation[4].

Resynchronization by means of left or biventricular pacing has been found to improve the timing of regional contraction, enhance chamber systolic function and reduces mitral regurgitation with end result of 40% increase in cardiac output[5].

Biventricular pacing allows significant hemodynamic improvement in most patients with chronic CHF and intraventricular conduction delay, especially LBBB. Growing experiments indicate that the pacing site and the optimization of atrioventricular / intraventricular delay (PR interval optimization) seem to be crucial to obtain short and long term improvement of LV function[5].

Cardiac resynchronization therapy (CRT) has been recently proposed as a supplementary treatment of drug refractory CHF[6].

This new therapy aims to improve quality of life and exercise capacity in patients selected as the basis of a dilated cardiomyopathy and cardiac a synchrony. In this particular heart failure population,

Professor of Cardiology, Dept. Medicine, College of Medicine, Al-Nahrain University
E-mail: amar_alhamdi@hotmail.com

the expected benefits depend on careful placement of the pacing leads, particularly that responsible for left ventricular (LV) pacing.

In practice, the optimal LV lead placement associated with the best hemodynamic improvement is of ten varies from one patient to another due to the variable coronary sinus anatomy.

The LV lead is placed in the lateral vein of the LV. Prior to the implant, echocardiography and more precisely tissue Doppler imaging investigations, may be helpful to identify the ideal LV pacing site, based on the late activated segmental contraction, which in most of the cases turns out to be the midlateral wall[5,7].

Complications observed during LV lead implantation remain low, consisting mainly of phrenic nerve stimulation and coronary sinus perforation in < 1% and should further decrease with the use of new instrumentation dedicated to the procedure[5].

Three randomized trials (PATH-CHF[5], MUSTIC and MIRACLE) have reported similar short, mid and long-term improvement of functional class, exercise capacity and quality of life of patients with CHF chronically paced with CRT.

The significant symptomatic benefits have also been confirmed a large European registry, the COTAK registry, including more than 1000 patients followed up to 6 months after CRT[5].

The indications for CRT are:

1. Advanced heart failure (NYHA class III-IV).
2. Right BBB or left BBB
3. Low ejection fraction with or without mitral regurgitation refractory to medical therapy.

In the Cardiology Unit, Al-Kadhimiya Teaching Hospital CRT is available now moreover, we have implicated 30 CRT over the last 12 months. The results up till now are encouraging; more patients are waiting for implantation of the device.

References

1. Petrie, M., and McMurray, J.: Changes in nations about heart failure. Lancet, 2001; 358: 432-4.
2. Berry, C., Murdoch, D.R., and Murray, J.J.: Economics of chronic heart failure. Eur J Heart Fail, 2001; 3: 283-91.
3. Machntyre, K., Capiwell, S., Stewart, S., Chalmers, J.C.V., et al.: Evidence for improving prognosis in heart failure, trends in case fatality in 66,547 patients hospitalized between 1986-1995. Circulation, 2000; 102: 1126-31.
4. Kass, D.A.: Dys-synchrony, segmental kinetics and vavular incompetence. VII European Scientific Symposium, Lisbon, Portugal, October 26-27, 2001; p.p. 36.
5. Tavazzi, L.: Ventricular pacing: a promising new therapeutic strategy in heart failure, for whom? Eur Heart J, 2000; 21: 1211-4.
6. Farweel, D., Patel, N.R., Hall, A., et al.: How many people with heart failure are appropriate for biventricular resynchronization? Eur Heart J, 2000; 21 (15): 1246-50.
7. Stellbrirle, A.A.: The pacing therapies for congestive heart failure (PATH-CHF) study rational, design and endpoints of a proposed randomized multicenter study. Am J cardiol, 1999; 83 (suppl. 5B): 130D-5D.

Xeloda: Targeted Therapy in Colo-Rectal cancers

Introduction

For the last 50 years, the Fluropyrimidines (the 5-Flurouracil) was the standard chemotherapy in the treatment of colo-rectal cancers. F-Flurouracil was given in different doses and different ways to increase its efficacy and decreases its toxicity.

If 5-flurouracil administered as intravenous bolus it will be effective but its efficacy is limited because the plasma concentration of the drug rapidly fall below the cytotoxic threshold due to rapid degradation of the drug. Continuous intravenous infusion or protracted infusion with folinic acid is superior to intravenous bolus regarding survival benefit.

This method of treatment requires good medical, nursing, pharmacy staff, and it is associated with a lot of complication. Thus, there is real need to find oral drug, which mimic the efficacy of intravenous infusion method without associated side effects.

Capecitabin

Capecitabin "Xeloda" is the first new oral drug of the class Fluropyrimidines. It offers the following advantages:

1. It is capable of mimic continuous intravenous infusion of 5-Flurouracil.
2. Providing convenient method of treatment i.e., oral method.
3. Tumor selective activation:
    a. improve efficacy
    b. reducing side effects
4. Patient preference.

Mechanism of action

Xeloda taken by mouth and absorbed by the stomach unchanged. In the liver, it is cleaved to 5-deoxy-5-Flurocytidin and 5-deoxy-5-Flurouridin. Inside the tumor, these compounds cleaved to active 5-Flurouracil by the effect of enzyme called "Thymidine phosphorylase" which is present in high concentration inside the tumor cells and in low concentration inside the normal tissue. By this way, we expose the tumor cells to high concentration of cytotoxic drug with low concentration inside the normal healthy tissues.

Dosage

It is given in 1250 mg twice daily for 14 days, one-week rest and the cycle repeated every three weeks

Side effects

The side effects usually mild include mild nausea and vomiting, mild diarrhea, hand-foot syndrome and mild myelotoxicity.

References

1. 11th European Cancer Conference (ECCO), Lisbon, Portugal, 2001.
2. 27th European Society of Medical Oncology, Nice, France, 2002.
3. Targeted Therapy in malignancy, sharm Al-Shiekh, Egypt, 2002.
4. Targeted Therapy in malignancy, Jordan, 2005.

Khudhair Al-Rawaqe *DMRD*
Associate Professor of Oncology, College of Medicine, Baghdad University.

Strattera: Non-Stimulant Drug for Attention Deficit Hyperactivity Disorder (ADHD)

European Congress of Neurosciences (Schizophrenia & Bipolar Disorders), Germany-Berlin, 8-10 April 2005

About ADHD

Attention deficit hyperactivity disorder (ADHD) is chronic and impairing disorder affects 3-7% of school-age children and manifest itself in levels of attention, concentration, activity, distractibility, and impulsivity that are inappropriate to the child's age[1]. In addition, 60% of children with the disorder carry their symptoms into adulthood[2]. Experts estimate 4% of adults in the United States, more than 8 million people, have ADHD[3,4].

About Strattera

Strattera, (atmoxetine HCl) regarded as the first therapy option for ADHD. It is a selective norepinphrine reuptake inhibitor, works differently than other FDA-approved treatments for the disorder, all of which are stimulant.

It is not known precisely how Strattera reduces ADHD symptoms, but scientists believe it works by blocking os slowing reabsorption of norepinphrine, a chemical in the brain considered important in regulating tension, impulsivity and activity levels. This keeps more norepinphrine at work in the spaces between neurons in the brain. Improved efficiency in norepinphrine system is associated with improvement in symptoms of ADHD.

It is the only medication indicated specifically for the treatment of ADHD in children, adolescents and adults. Strattera provides full-day symptoms control without insomnia in most children and adolescents.

The American Academy of Child and Adolescent Psychiatry (AACAP) states that decision to place Strattera as a first-line therapy option will have positive impact on the lives of many patients and their families who struggle with this disorder.

Special precautions

Strattera should not be taken at the same time as, or within two weeks of taking, a monoamino oxidase inhibitor, or by patients with narrow angle glaucoma. Patients with a history of high or low blood pressure, increased heart rate, or any heart or blood vessel disease should tell their doctor before taking Strattera. Strattera has not been tested in children less than six years of age or in geriatric patients. Some childrem may lose weight when starting treatment with Strattera. As with all ADHD medications, growth should be monitored during treatment.

Side effects

The most common side effects in children and adolescents in medical studies were upset stomach, decreased appetite, nausea and vomiting, dizziness.

The U.S. Food and Drug Administration approved Strattera on November 26, 2002, for the treatment of ADHD in children, adolescents and adults.

References

1. American Psychiatric Association: Diagnostic and statistical manual disorders, 4th edition, text revision, Washington, DC, American Psychiatry Association, 2000.
2. Schweitzer, J.B., et al.: Attention-deficit/hyperactivity disorder. Med Clin North Am 2001; 85 (3): 757-7.
3. Murphy, K., and Barkley, R.A.: J Atten Disord, 1996; 1: 147-61.
4. United States Census Summary File; 2000.

Mohammed Al-Kureishi *MSc* (Neurosychaitry)