University of Dohuk

College of Medicine

DMJ

Dohuk Medical Journal

The Official Journal of Dohuk College of Medicine

Volume 2 Number 1

2008

Front page picture

Dalal Bridge

is located in Zakho District, Dohuk Governorate, Kurdistan Region, Iraq. There are two opinions about its building time: the first said that it was built in the day of Byzantine Emipre and the other one said that it was built during Abbasside era. The aim of building bridge was to connect Mesopotamia to Anatolia.

Source: Dohuk Directorate of Antiques, Dohuk, Iraq

The journal is also available at the college website
www.med.uod.ac

University of Dohuk

College of Medicine

DMJ

Dohuk Medical Journal

The Official Journal of Dohuk College of Medicine

Volume 2 Number 1 2008

This page is left intentionally

DMJ

Dohuk Medical Journal

EDITORIAL BOARD

Submission of Manuscript:

Manuscripts should be submitted to:

The Editor,
Dohuk Medical Journal,
Dohuk College of Medicine,
Post address: Nakhoshkhana Road 9, 1014, AM, Dohuk, Iraq.
Telephone No.: 00964-62-7224268 EXT 115
E-mail: dmj.med.uod@gmail.com

Electronic submission of articles is also accepted

DMJ

Dohuk Medical Journal

ADVISORY BOARD

This page is left intentionally

DMJ

Dohuk Medical Journal

INSTRUCTIONS FOR AUTHORS

Aims and Scope Dohuk Medical Journal is a peer reviewed journal issued bi – annually by Dohuk College of Medicine. Scientific and clinical researches are the main issues. The journal also publishes short articles, letters to editors, review articles and case reports.

General The Dohuk Medical Journal is a signatory journal to the uniform requirement for manuscripts submitted to biomedical journals, February 2006 (http://www.icmje.org).

To present your original work for consideration three manuscript copies written in English together with Kurdish and Arabic abstracts should be submitted to the editor. All authors are required to provide the manuscript on a CD labeled with the name and title of the paper.

Preparation of the manuscript The manuscript should be typed double spaced as normal text on one side of the paper in single column format, font size 14 pt, paper type A4, 1″ margin at each side and each of the following sections should begin on a new page in the following sequence:

1- **Title page**; should include the following: title, font size 16 pt, each author's full name, academic degree(s), scientific title (if available), institutional affiliation, full contact information including emails. If there are more than one author, article should include author to whom correspondence should be addressed including the scientific title (if available), institution affiliation, address, email, telephone.

2- **Structured abstract**; of no more than 250 words including background, objectives, design (methods), results, and conclusions.
3 – 10 keywords or phrases should be put at the end of each abstract. (Printed in bold font; size12 pt).

3- **Body of the text**; structured in an IMRAD style;
(Introduction, Methods, Results and Discussion).

4- **Acknowledgment** (if any.)

5- **References.**

6- **Tables with legends**.

7- **Illustrations with legends**.

8- **Structured Kurdish abstract including title in Kurdish.**

پێشەکی، ئارمانج، ڕێکێن ڤەکولینێ، ئەنجام، دەرئەنجام

9- **Structured Arabic abstract including title in Arabic.**

الخلفية ، الاهداف ، المواد و الطرق، النتائج، الاستنتاجات

Tables Each table must be typed on separate page and should follow the reference list. All the tables must be numbered consecutively in the order of their first citation in the text. Supply a brief title for each on top and place explanatory matter in foot notes not in the heading (if needed). Tables should be simple and not duplicated in the text. Percentages are included with numbers in the same cells but in brackets.

Illustrations Graphs, line drawing, photographs, printed x rays and other illustrations are accepted only if they add to the evidence of the text. They should be of a high quality and suitable for reproduction. They should be numbered consecutively according to the order in which they have been first cited in the text.

Supply a brief title beneath each illustration. Graphs should have white background, should be non 3-dimensional figure and non-colored; labels for X and Y axis identified.

Numbers and Units Measurements of length, height, weight and volume should be reported in metric units. Temperature in degrees Celsius, blood pressure should be expressed in mmHg and all hematologic and clinical chemistry measurements in SI units.

Abbreviations should be defined on first use and then applied consistently throughout the article. Avoid abbreviations in the title and abstract.

References should be numbered both in text and in the list of references in the order in which they appear in the text. The punctuation of the Vancouver style should be followed; if the original reference is not verified by the author, it should be given in the list of references followed by (cited by) and the paper it was referring to. The titles of journals should be abbreviated according to the style used in Index Medicus. This can be obtained from website (http://www.nlm.nih.gov/). The author is responsible for the accuracy of references. The following are examples of the three most common types of citations:
The article citation: if six authors or fewer list all; if seven or more authors list the first six and then add "et al":
1- Nuwayhid IA, Yamout B, Azar G, Kambris MA. Narghile (hubble bubble) smoking, low birth weight, and other pregnancy outcomes. Am J Epidemiol 1998;148(4):375-83.
Book citation, noting chapter and authors:
2- Arevalo JA, Nesbitt TS. Medical problems during pregnancy. In: Taylor RB, editor. Family medicine: principles and practice. 6th ed. New York: Springer – Verlag; 2003. p. 109-16.
Electronic source:
3- Garfinkel PE, Lin E, Goering P. Should amenorrhoea be necessary for the diagnosis of anorexia nervosa? Br J Psych [serial online] 1996 [cited 1999 Aug 17];168(4):500-6. Available from: URL:http://biomed.niss.ac.uk

Authorship and consent form All authors must give signed consent, which should accompany the manuscript. The letter should say "this manuscript is an unpublished work, which is not under consideration elsewhere in the record. Authors are requested to state an approximate estimate of their contribution in the study.
Authors must declare if they have any competing interests in the study and to specify any funds given to conduct the study (Form No.1).

Ethical considerations When experiments on humans are being reported the whole work in the manuscript should conform to the ethical standards of the responsible committee on human experimentation.

Submission of manuscript

Manuscripts should be submitted to:
The Editor,
Dohuk Medical Journal,
Dohuk College of Medicine,
Post address: Nakhoshkhana Road 9, 1014, AM, Dohuk, Iraq.
Telephone no.: 00964-62-7224268 EXT 115
E-mail: dmj.med.uod@gmail.com
Electronic submission of articles is also accepted

N.B.

* Accepted manuscripts may be altered by the editorial board to conform to details of the journal publication style.

** The Editorial Board of Dohuk Medical Journal accepts no responsibility for statement made by authors in articles published by the journal.

DMJ

Dohuk Medical Journal

CONTENTS

EDITORIAL

THE PREVENTIVE PROGRAM FOR HAEMOGLOBINOPATHIES IN DOHUK: AN OPTION OR A NECESSITY

NASIR A. AL-ALLAWI, MBChB, PhD*

DMJ 2008;2(1):1-4.

Submitted 12 April 2008; accepted 10 June 2008

Key words: β-thalassaemia, Sickle cell disorders, Prevention, Dohuk, Iraq

Haemoglobinopathies are inherited disorders of globin chain synthesis that may either be quantitative (thalasssaemias) or qualitative (Sickle cell, Hb C, Hb D, and others). They are the most frequent single gene disorders worldwide particularly in the Eastern Mediterranean region, including Iraq.[1] Following the earliest reports on thalassaemia major and sickle cell disease from Iraq, in the 1960s,[2,3] these inherited disorders became increasingly recognized as important health problems, imposing a huge burden on the already stretched health services. Studies on the prevalence of thalassaemia carrier state quoted rates ranging between 3.7%-4.5% in various regions of the country.[4-6] While those on sickle cell gene prevalence came only from southern Iraq, where rates reaching 16.0% were reported.[7] Dohuk governorate, which lies at the extreme north of Iraq, has more than 500 subjects with these major haemoglobinopathies registered at its recently established thalassaemia centre. The majority of these patients will need life-long support, with almost a monthly need for blood transfusions and a definite need for cumbersome costly iron chelation therapy.[8] Most of these patients fail to comply to variable degrees with such demanding treatment regime and to keep up with the huge financial and psychological demands on the patients and their families are huge. Despite the best efforts of the health authorities and the dedication of the physicians involved, these patients rarely live beyond the age of 20 years unlike their counterparts in Western countries.[9]

A recent pilot study performed in Dohuk revealed, as anticipated, a high prevalence of β- thalassaemia and sickle cell carrier states among individuals screened in a premarital setting, at 3.7% and 1.2% respectively.[6] Based on the above figures, it was estimated that around 40 newborns would be born with a major hemoglobinopathy per year, adding to more than 500 affected individuals already registered by the health authorities. Such an added number of affected newborns would be a huge cumulative problem, as the quality of health care and the education of the patients and their families improves and thus their survival is prolonged. The problem is further complicated by a consanguinity rate of 27.2% among the

* *Professor of Hematology, Head of Department of Pathology, Dohuk College of Medicine, Dohuk, Iraq*
Email: nallawi@yahoo.com

population of Dohuk, increasing further the risk of having affected children.[6]

Such high prevalence and high consanguinity rates as well as the anticipated numbers of affected newborn, stresses the need to explore all possibilities to tackle this important health problem. While every attempt to provide the best possible care for those affected should be made, this alone does not provide a solution for this growing problem. The best solution is to initiate a preventive program designed to prevent or at least reduce the birth of affected individuals.

The experience of several countries in such a preventive program has shown that achieving such a goal is feasible and cost-effective. Probably the best such example is the program applied in Cyprus and to a lesser extent that applied in Iran. Both programs were based on the use of screening, genetic counseling and prenatal diagnosis to achieve this goal.[10,11]

Applying a similar approach in a pilot study in Dohuk has revealed that a policy of screening of premarital couples using sickling test coupled by determination of red cell indices via an electronic hematology analyzer, followed by hemoglobin electrophoresis in those with positive sickling test or reduced MCV and /or MCH, is an effective feasible and a rapid method for picking up couples at risk of having children with these hemoglobinopathies.[6] This step is to be followed by genetic counseling with the aim of giving the partners at risk the essential knowledge to take an informed decision regarding their marriage. Their first option would be not to go ahead with the marriage (i.e. separate). However such an option has its limitations taking into consideration the social background in Dohuk, particularly because of the fact that arranged and consanguineous marriages are quite common, and the fact that premarital screening is only performed days prior to the actual marriage, and not at engagement. Both these factors make the option of separation between the couples at risk difficult and socially unacceptable, even after appropriate genetic counseling. Previous reports worldwide on the actual impact of genetic counseling of the couples at risk have been conflicting. So that while some have suggested that its impact is minimal, others suggested that it may lead to separation in about half of these couples.[12] What is encouraging is that the latter figure came from Iran, which neighbours Iraq and has comparable cultural and social background.[11]

The second option for the couple is to get married and consider performing prenatal diagnosis in early pregnancy with the prospect of having a therapeutic abortion if they have an affected child. Applying prenatal diagnosis for thalassaemia in any population requires prior knowledge of the mutations that are prevalent in the region, a task which has been addressed by a recently published study.[13] as well as the presence of trained obstetricians and ultrasound specialist to perform chorionic villus biopsy. A religious approval of the principle of therapeutic abortion in early pregnancy is another issue which requires scrutiny, although many scholars from different

sects and religions approve such principle.[9] However, securing approval of the local religious leaders is beyond doubt mandatory. Convincing these leaders may be pivotal in the success of the program.

The above issues emphasize the need for an ambitious educational program which involves all sectors of the community. Among those targeted by such a program are health professionals, religious leaders, legal authorities, students at various levels as well as the public at large. The Educational drive should emphasize on the hardship and the suffering of hemoglobinopathy patients, the value of the premarital testing of haemoglobinopathies (preferably prior to engagement), and the options available to couples at risk. Such information should be integrated in curricula of medical and nursing colleges as well as secondary schools. The local media should be used to promote such ideas, including newspapers, radio and television stations, as well as posters and pamphlets distributed at various settings.

Coupled with such an educational scheme, a preventive program for hemoglobinopathies could be initiated in Dohuk, based on the principle of screening, counseling and prenatal diagnosis. An approach which is considered by many authorities to be the best option for the control and management of inherited haemoglobinopathies.[14] The success of this approach has been demonstrated in Europe where the affected birth rates fell almost 100% between the late 1970s and late 1980s in Cyprus and Sardinia, and about 80% in Greece and Italy.[15] In less developed countries, like Iran, it is believed that births with severe thalassaemia fell to about 30% of the expectations.[11] Moreover, several investigators have shown that this approach is cost-effective, and studies from Cyprus revealed that the cost of eight weeks' preventive program was equivalent to one week's treatment of the thalassaemic population.[10]

Last but not least, any population genetic screening program would not be feasible without the sponsorship and the support of the policy makers. Such sponsorship has been recently portrayed by making premarital screening for hemoglobinopathies mandatory by law by presidential decree in Kurdistan region. Such commitment by the policy makers and all the efforts of those involved in the program, would make us look forwards to the day in which no children with major hemoglobinopathy would born in Dohuk.

REFERENCES

1. Bain BJ. Hemoglobinopathies diagnosis. 1st ed. Oxford: Blackwell Scientific Publication; 2001.
2. Taj-Eldin S, Al-Rabii H, Jawad J, Fakhri O. Thalassaemia in Iraq. Ann Trop Med Parasitol 1968; 62(2):147-53.
3. Baker F, Al-Quasi M. Sickle cell anemia in Iraq: first case report. J Fac Med (Bag), 1964;6(5):26-31.
4. Yahya HI, Khalel KJ, Al-Allawi NAS, Hilmi F. Thalassaemia genes in Baghdad, Iraq. East Mediterr Health J

1996;2(2):315-9.

5. Hassan MK, Taha JY, Al–Noama LM, Widad NM, Jasim SN. Frequency of hemoglobinopathies and glucose- 6-phosphate dehydrogenase deficiency in Basra. East Mediterr Health J 2003;9(1/2):1-8.
6. Al-Allawi N, Al-Dousky A. The frequency of haemoglobinopathies and service indicators for their preventive program in the Dohuk governorate-Iraq. East Meditrr Health J. In Press 2008.
7. Alkasab FM, Al-Alusi FA, Adnani MS, Alkafajei AM, Al-Shakerchi NH, Noori SF. The prevalence of sickle cell disease in Abu Al-Khasib District of Southern Iraq. J. Trop Med Hyg 1981;84(2):77-80.
8. Thalassaemia International Federation. Guidelines to the clinical management of thalassaemia. Nicosia: TIF Publications; 2005.
9. Galanello R, Eleftheriou A, Traaeger-Synedions J, Petrou M, Angastiniotis M. Prevention of thalassemias and other hemoglobin disorders. Vol. I. Nicosia: TIF Publications; 2003.
10. Angastiniotis MA, Kyriakidou S, Hadjiminas M. How thalassaemia was controlled in Cyprus. World Health forum 1986;7:291-7.
11. Samavat A, Modell B. Iranian national thalassaemia screening programme. BMJ 2004;329:1134-7.
12. Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, et al., editors. Disease control priorities in developing countries. 2nd ed. Oxford: Oxford University Press; 2006.
13. Al-Allawi NAS, Jubrael J, Hughson M. Molecular characterization of β-thalassaemia in Dohuk region of Iraq. Hemoglobin 2006;30(4):479-86**.**
14. Alwan A, Modell B. Community control of genetic and congenital disorders. Alexandria: Eastern Mediterranean Region Office Technical Publications; 1997. WHO Series 24.
15. Modell B, Kuliev AM. Service indicators for thalassaemia as a model for cost-benefit analysis of genetics services. J Inherit Meta Dis 1991;14(4):640-51.

ACUTE MYOCARDIAL INFARCTION AND DEPRESSION

SABRI K. SHAIKHOW, MBChB, MRCP, FRCP*,
ALIAS A. HUSSIN, MBChB, CABS**

Submitted 19 March 2007; accepted 12 January 2008

ABSTRACT

Background Depression found to be common after acute myocardial infarction AMI, its recognition and treatment may be important to reduce its consequences.
Objectives To clarify the effect of depression following AMI.
Patients and Methods Prospective study was conducted at the coronary care unit (CCU) at Ibn-Sena ,Mosul teaching hospital . Two hundred cases with AMI ages 25 years up to 75 years were included.
Results Depression was prevalent after AMI, with a higher incidence in females, 57% vs 43% for males. P value < 0.05 and odds ratio: 2.85, this study found that higher percentage of depression was among those with low educational and socioeconomic state and discovered that patients with depression following AMI developed more cardiac complications such as recurrent angina, recurrent AMI, arrhythmia, congestive cardiac failure (CCF) and death more than non depressed patients, 38% of the depressed group needed coronary angiogram vs 23% of non depressed.
Conclusion Patients who developed depression following (AMI) are more prone to complications.

DMJ 2008;2(1): 5-16.

Key words: Acute myocardial infarction, Depression

Symptoms of depression are common after acute myocardial infarction (AMI), might persist over the subsequent months.[1-9]

In some studies the incidence of depression after AMI ranged from 17% to 37% [10,11] which affected the quality of life,[12] increased cardiac morbidity,[13,14] and cardiac events.[15-21] There is also limited evidence that initial distress after AMI predicts outcome for return to work, [22,23] and compliance with medical treatment.[24-26] It is commonly thought that traditional risk factors namely hypertension, high cholesterol, cigarette smoking and physical inactivity can at best explain only 50% of the morbidity and mortality in coronary heart disease.[27] Recently, attention has shifted to mood states such as depression and anxiety as an additional risk factors.[3,9,28] The relationship between cardiac disease and depression is complex, there is some evidence that depression may actually lead to cardiovascular disease and vice versa.[29] Life stress and social isolation were both independently associated with higher mortality risk after AMI.[9,30,31]

Early prediction of psychological problems is an important clinical issue because it is believed that there is considerable "potential for large cost in

* *Assistant professor, Department of Medicine, Dohuk College of Medicine, Dohuk, Iraq*
** *Mosul Directorate of Health, Iraq*

one study, with patients physically ill".[22] This means loss of expenses due to time taken of work.

The higher prevalence of depression in women coupled with these studies suggests that women may have worse post MI prognosis than men[32] and has led to the speculation that gender differences in depression may be responsible for some of the difference in the prognosis.[33,34]

Assessing for depression in a patients with AMI requires an understanding of the risk factors for depression that include female gender, previous history of depression, family history of depression, lack of social support, and loss of functioning, or major life role.[34,35]

Although exactly how mood disturbances adversely affect post MI outcome is unknown, the risk of depression reported in many recent studies had led to speculation about possible mechanisms linking depression and increased cardiac risk as shown in the table 1.[9]

PATIENTS AND METHODS

Two-hundred patients 110 males, 90 females their ages ranged between 25 - 75 years who met established criteria for AMI, were recruited from the coronary care unit (CCU) at Ibn-Sena Mosul teaching hospital, between November 2004 and June 2005.

Patients had to meet at least two of the following criteria for diagnosing AMI.[36]

- Typical ischaemic chest pain lasting at least 30 minutes.
- Evolution of electrocardiogram (ECG) changes, such as ST segment elevation AMI, non-ST segment elevation AMI.[3,13]
- A peak creatine phosphokinase (CK) level greater than 1.5 times the normal limit, or a CK-MB (the myocardial iso enzyme of CK) >25 IU/L. Troponine now considered the standard in diagnosing AMI , but unfortunately not available in our hospital.

Patients were interviewed as soon as they were medically stable, 3-5 days after AMI by applied the questionnaires of DSM IV (Diagnostic and statistical manual of mental disorder 4th ed.[34]

Presence of five or more of the following symptoms most of the day, nearly every day, for one week were considered significant .

- Depressed mood indicated by subject report (feeling sad).
- Marked loss of interest or pleasure in all activities.
- Disturbance of appetite or significant weight loss.
- Sleep disturbance or insomnia.
- Psychomotor retardation or agitation.
- Fatigue or loss of energy.
- Feeling of worthless or inappropriate guilt.
- Decreased ability to think, concentrate or make simple decisions.
- Recurrent thoughts of death with suicidal ideas.
- Symptoms causes clinically significant distress or impairment in social, occupational, or other important functions.

After that we took routine demographic data including age, gender, educational status, socioeconomic status, physical activity, stressful events, marital status.

Table 1. Possible mechanisms linking depression and increased cardiac risk

Possible Mechanism	Specific abnormal finding
Life style and behavior	Decreased adherence to risk-reducing recommendations
Neurocardiogenic	Increased susceptibility to ventricular Arrhythmia
Platelet function	Increased platelets activation
Management	Poor follow up, non compliance with investigations

Also we took information about the disease state of the participant like diabetes mellitus, hypertension, current smoking and high cholesterol. We followed the patients while staying in CCU for one week, comparing statistically all demographic and clinical variables for patients who were depressed with those who were not depressed.

We compared the cumulative incidence of cardiac complication during the initial admission to hospital, as well as the cumulative incidence of readmission for cardiac complications.

Physical activity considered poor for those without work.

A patient was labeled as a smoker if they smoked more than ten cigarettes a day because this considered as risk factor. Hypertension (HT) was considered to be present if systolic blood pressure was > 140mmHg and diastolic blood pressure >90mmHg in sitting position by utilizing the standard mercurial sphygmomanometer.[37] Diabetes mellitus (DM) was considered to be present if the symptoms of diabetes was present plus random blood glucose concentration > 11.2 mmol/L (200mg/dL), or fasting plasma > 7.0 mmol /L (126 mg/dL).[8]

According to the educational status, the patients divided into 4 groups: Illiterate, low educational status (primary school), secondary school and post-graduate (high educational status).

Odds ratio was calculated for depressed and non depressed group and Z-test of one proportion was performed to give P-value of < 0.05 was considered to be significant. Age of patients was expressed as mean ± SD (year).

RESULTS

Depression followed AMI was higher among patient's ages between 50-69 years for both sexes. (Table 2) with higher percentage among females 59(57%), low-educational status, 82(80%), poor socioeconomic status 61(59%), poor physical activity 48(46%), and history of major stressful events 54(52%) (Table 3).

Coronary angiogram was indicated in 40 patients (38%) vs (23%) in patients with AMI without depression.

Recurrent MI was present in 30

patients (29%) vs 15 patients (14%) with AMI without depression.

Congestive heart failure was present in 28 patients (27%) Vs 15 patients (14%) with AMI without depression (Table 4).

Readmission because of angina was present in 27 patients (26%) vs 14 patients (14%) with AMI without depression.

Arrhythmia as a leading cause to death was present in 6 patients (6%) of AMI with depression and only in 3 patients (3%) of non-depressed group (Table 5).

DISCUSSION

The present study shows that depression was profound among patients with AMI, with higher incidence in female group 57% vs 43% in male, and more-likely above the age of 50 years for both sexes. The cause of depression is unknown, this might be explained by the fact that patients with an illness, the death of friend among their age group and the their physical limitations may lead to disturbances of manner may lead to forms of depression which are frequently undiagnosed and untreated.[34,38]

Those patients with low educational status who had AMI, a higher percent of them developed depression, 80% in our study. P-value: 0.000 OR: 7.57. Also patients with low socioeconomic status 59% of them were found to have depression after AMI OR= 4.67.

Patient with poor physical activity who develops AMI higher percent of them had depression 46% vs 10% in non depressed group. OR=7.59 the P-value <0.05. These finding were prevalent among the female group "studies of various cultures have shown that the depression disorder is approximately twice as prevalent in women as in men, regardless of age".[34] The explanation could be due to the fact that these patients were unable to accept such a serious condition and unable to treat them self any more .Same, might apply to depression among those with sever marital or relationship problem and a patients who were unmarried and lived alone.[9,34]

Table 2. Distribution of AMI according to the sex and age

Age	Male N=110	Female N=90
<29	1	0
30-39	7	4
40-49	30	18
50-59	33	28
60-69	30	23
70-75	9	17
Total	110	90

Table 3. Distribution of AMI with depression among the age and sex

Age	Male N = 44	Female N = 59	P-value	OR
<29	0 (0%)	0 (0%)	-	0.00
30-39	0 (0%)	4 (4%)	<0.05	-
40-49	7 (15%)	9 (15%)	N.S	3.30
50-59	18 (40%)	23 (39%)	N.S..	3.83
60-69	14 (31%)	15 (25%)	N.S.	2.14
70-75	5 (11%)	8 (13%)	N.S.	2.86

Table 4. Comparison of characteristics of the depressed and non depressed patients with AMI

Studied group	Depressed N = 103	Non-Depressed N = 97	P-value	OR
Female	59 (57%)	31 (32%)	0.000	2.85
Low education	82 (82%)	33 (34%)	0.000	7.57
Poor socioeconomic	61 (59%)	23 (24%)	0.000	4.67
Physical inactivity	48 (46%)	10 (10%)	0.000	7,59
Major stress event	54 (52%)	09 (9%)	0.000	10.78
Marital status	103(100%)	97(100%)	N.S.	-
Diabetes mellitus	47 (45%)	32(33%)	N.S.	1.71
Hypertension	54 (52%)	53(55%)	N.S.	0.92
Smoking	69 (67%)	67(71%)	N.S.	1.91
High cholesterol	50 (48%)	43(44%)	N.S.	1.18
ST-Segment elevation	90 (87%)	64(68%)	0.005	0.15
CCF	39 (38%)	20(21%)	0.008	2.35

CCF= congestive cardiac

Table 5. Incidence of cardiac complications and death among patients with and without depression after AMI

Cardiac complications	Depressed N = 103	Non depressed N = 97	P-value	OR
Recurrent angina pectoris	30 (29%)	14 (14.4%)	0.021	2.25
CCF	28 (27%)	14 (14.4%)	0.044	2.04
Arrhythmia	36 (34%)	25 (25.8%)	N.S.	1.55
Recurrent MI	5 (5 %)	4 (4.2%)	N.S	1.19
Death	8 (7 %)	6 (6.2%)	N.S.	1.94

Our-study also shows that depression was more in patients with low left ventricular ejection fraction (LVEF) 38% vs 21% without depression P-value: < 0.05 and OR = 2.35 and in patients with ST-segment elevation MI 98% vs 68% for depressed and non depressed group respectively. This might explain the severity of the disease state and limitation of there activities making them to think that they will not cope with the normal life and work.[16]

In this study there were significant numbers of depression after AMI in a patients who had major stressful events before admission to hospital 52%. P-value <0.05 and OR=10.78. These stressful events were of different forms; the coping with such challenges is stated to vary from one individual to another depending on early experience and genetics. [15,39]

Two type of reaction can occur.[40,41] Active defense (fight-flight) reaction and passive defected (depressed) type of reaction. The first one (active defense) reaction which involves a combination of behavioral and neuroendocrine changes in the form of activation of sympathetic adrenal medullar system leading to increased nor-adrenaline, adrenaline, rennin, fatty acid and glucogenolysis. Evidently these changes can lead to rise in blood pressure, cardiac arrhythmias and impaired glucose tolerance.[29] The second reaction (depressed) type of reaction either at the initial or continuous response to depression can follow the defense reaction when the performance to challenge drops off. In this defected reaction, behavioral changes also can occur in combination with neuro-endocrine response in the form of activation of hypothalamic-pituitary adrenal axis. These changes lead to increase cortisol, corticotrophin releasing hormone (CRH).[39-41] Such type of depressed reaction leads to a pattern of disease susceptibility including cardiovascular diseases, type two DM, increased platelet activity.[42-44] There was no significant difference between depressed group and those without depression with the following character: poor marital status, current smoking, diabetes mellitus (DM), high cholesterol level and hypertension (HT). While there was significant positive association between depressed patients with acute myocardial infarction and the following: Recurrent angina 29% vs 14% of non depressed group. P-value <0.05 and OR= 2.25; congestive heart failure 27% vs 14% for depressed and non depressed group respectively. P-value <0.05 and OR=2.04. These can be explained by the mechanism, that psychological factors like depression and symptoms of depression stimulate the adrenal gland to release adrenergic catecholamine and glucocorticoid. [29,39-41] These lead to increase the need of myocardium for oxygen, resulting in myocardial hypoxia which lead to consequent like arrhythmia, sever myocardial Ischemia, congestive heart failure, pulmonary oedema and sudden death. [45,46]

The percentage of death among depressed group was 7% and non depressed group was 6% and the main cause of death was arrhythmia. This suggests that an arrhythmic mechanism

linking depression with acute myocardial infarction, these are based on the idea that the combination of vulnerable myocardium after AMI, and negative emotional arousal could easily trigger fatal ventricular arrhythmia.[47,48]

Some studies shows that patients fulfilling DSM IV symptoms criteria for depression at slight increased in the risk of death and increased risk of complication post myocardial infarction.[9]

CONCLUSIONS AND RECOMMENDATIONS

There was a high percent of depressive after AMI with high incidence of cardiac complication and death. For this reason in hospital identification and treatment of depression post AMI is recommended, and indicate the need for a better understanding of the significance of psychological and behavioral factors after AMI and for the application of current knowledge about the efficacy of psychiatric and psychological treatment.

From this study we recommend treating depression after acute (MI) as this plays an important role in reducing the adverse cardiac complications whuch has been shown in other studies. Selective serotonin re-uptake inhibitors [SSRIs] are the first line agents in the treatment of mild to moderate depression unlike their tricyclic antidepressants; SSRIs have repeatedly been demonstrated to be safe and to have a negligible effect on cardiovascular system, even in cases of over dose.

REFERENCES

1. Frasure- Smith N, Lesperance F, Talajic M. Depression and 18- month prognosis after myocardial infarction. Circulation1995;91:999-1005.
2. Lauzon C, Beck CA, Huynh T, Dion D, Racine N, Carignan S, et al. Depression and prognosis following hospital admission because of acute myocardial infarction. CMAJ 2003;168(5):547-52.
3. Lane D, Carroll D, Lip GYH. Anxiety, depression and prognosis after myocardial infarction. JACC 2003;42(10):1808-10.
4. Lesperance F, Frasure-Smith N, Talafic M. Major depression before and after Myocardial infarction: its nature and consequences. Psychosom Medicine 1996;58:99-110.
5. Lane D, Carroll D, Ring C, Beevers DG, Lip GYH. Do depression and anxiety predict recurrent coronary events 12 months after myocardial infarction? Q J Med 2000;93:739-44.
6. Frasure –Smith N, Lesperance F, Talajic M. The impact of negative emotions on prognosis following myocardial infraction: is it more than depression? Health Psychol 1995;14:388-98.
7. Mayou RA, Gill D, Thompson DR, Day A, Hicks N, Volmink J, et al. Depression and anxiety as predictors of outcomes after MI. Psychosom Med 2000;62:212-9.
8. Marshall FJ. Disorders of mood and behavior. In: Andreoli TE, Charles CJ,

Carpenter CCJ, Robert C, Griggs ME, Joseph Loscalzo, editors. Cecil essential of medicine. 6th ed. Philadelphia: Saunders; 2004. p. 99, 621, 989-90.

9. Ziegelstein RC. Depression in patients recovering from a myocardial infarction. JAMA 2001; 286:1621-7.
10. Lane D, Carroll D, Ring C, Beevers DG, Lip GYH. The prevalence and persistence of depression and anxiety following myocardial infarction. Br J Health Psychol 2002; 7:11-21.
11. Schleifer SJ, Macarn-Hanson MM, Coyle DA. The nature and course of depression following myocardial infarction. Arch Intern Med 1989;149:1785-9.
12. Lane D, Carroll D, Ring C, Beevers DG, Lip GYH. Mortality and quality of life 12 month after myocardial infarction: effects of depression and anxiety. Psychosom Med 2001;63: 221-30.
13. Frasure-Smith N, Lesperance F, Junear M, Talafic M, Bourassa MG. Gender, depression, and one year prognosis after myocardial infarction. Psychosom Med 1999;61:26-37.
14. Strik JIMH, Denollet J, Lousberg, R Honig A. Comparing symptoms of depression and anxiety as predictors of cardiac events and increased health care consumption after myocardial infarction. J Am Coll Cardiol 2003;42:1801-7.
15. Ladwig KH, Roll G, Briehardt G, Budde T, Borggrefe M. Post infarction depression and incomplete recovery 6 month after acute myocardial infarction. Lancet 1994:343:20-3.
16. Denollet J, Brutsaert DL. Personality, disease severity and the risk of long term cardiac events in patients with a decreased ejection fraction after MI. Circulation 1998;97:167-73.
17. Moser DK, Dracup K. Is anxiety early after MI associated with subsequent ischemic and arrhythmic events? Psychosom Med 1996;58:395-401.
18. Lesperance F, Frasure-Smith N. Depression and CAD, Time to move from observation to trails. JAMA. 2003;168(5):570-1.
19. Lesperance F, Frasure Smith N, Talajic M, Cameron O. Major depression before and after myocardial infarction: it is nature and consequences. Psychosom Med 1996;58: 99-112.
20. Irvine J, Basinski A, Baker B, Jandciu S, Paquette M, Cairns J. Depression and risk of sudden cardiac death after AMI: testing for the confounding effects of fatigue. Psychosom Med 1999;61:729-37.
21. Frasure-Smith N, Lesperance F. Depression and other psychological risks following myocardial infarction. Arch Gen Psychiatry 2003;60:627-36.
22. Schleifer SJ, Macari-Hinson MM, Coyle DA, Slater WR, Kahn M, Gorlin R, et al. The nature and course of depression following myocardial infarction. Arch Intern Med 1989;149:1785-9.
23. Frasure-Smith N, Lesperance F. Depression and anxiety increase physician costs during the first post MI year. Psychosom Med 1998;60: 99.
24. Ziegelstein RC, Fauerbach JA, Stevens

SS. Patients with depression are less likely to follow recommendations to reduce cardiac risk during recovery from a myocardial infarction. Arch Intem Med 2000;160:1818-23.

25. Jenkins CD. Epidemiology of cardiac disease. J Consult Clin Psychol 1988;56:324-32.
26. Ruberman W, Weinblatt E. Psychosocial influences on mortality after myocardial infarction. N Engle J Med 1984;311:552-9.
27. Tibblin G, Lidstm B, Ander S. Emotion and Heart disease. In: Elsevier, editor. Physiology, emotion and psychosomatic Illness. Ciba foundation symposium 8. Netherlands: Koninklijke Van Gorcumand Comp. Assen.; 1972. p. 325.
28. Ruberman W, Weinblatt E, Goldberg JD, Frank CW, Chandhery GS, Shapiros. Ventricular premature complexes and sudden death after MI. Circulation 1981;64:297-305.
29. Lloyd GG, Sharpe MC. Medical Psychiatry. In: Haslett C, Chilvers ER, Hunter, editors. Davidson's principles and practice of Medicine. 19th ed. Edinburgh: Churchill Livingstone; 2002. p. 262.
30. Greenland P, Reicher-Reiss H. In-hospital and 1 year mortality in 1,524 women after MI: comparison with 4,315 men. Circulation 1991;83:484-91.
31. Carney RM, Freedland KE, Smith L. Relation of depression and mortality after myocardial infarction in women. Circulation 1991;84:1876-7.
32. Reus VI. Mental disorders. In: Braun WE, Fauci AS, Kasper DL, editors. Harrison's principles of internal medicine. 15th ed. New York: McGraw-Hill; 2001. p. 2542-57.
33. Guck TP, Michaelg-Kavn. Assessment and treatment of depression following myocardial infarction. Am Fam physician 2001;64:641-8, 651-2.
34. Garas S, Zafari AM, Dric-Vanderbush FHCC. Myocardial infarction. Medicine 2002; 4: 1-26.
35. Saeed AK, Al-Dabbagh TQ. Type 2 diabetes and it is association with hypertension and depression in an IRAQI population. Ann Saudi Med 2003;5:254-9.
36. Millon T. Pathological behavior reaction. In: Millon T, editor. Modern psychopathology- a biosocial approach to maladaptive learning and functioning. 8th ed. Philadelphia: WB Saunders;1969. p 458.
37. Gold PW, Goodwin FK, Chrousos GP. Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress. N Engl J Med 1988;319:413-20.
38. Bjrntorp P. Stress and cardiovascular disease. Acta Physiol Scand Supply 1997;640:144-8.
39. Henry JP. Biological basis of the stress response. News Physiol Sci 1993;8:69-73.
40. Musselman DL, Marzec UM, Manatunga A. Platelet reactivity in depressed patients treated with paroxetine: prelimiary findings. Arch Gen Psychiarty 2000;57:875-82.

41. Musselman DL, Tomer A, Manatunga AC. Exaggerated platelets reactivity in major depression. Am J Psych 1996;153:1313-7; cited in serebruany VL, O'conner CM, Gurbel PA. Effects of selective serotonin re-uptake inhibitor on platelets in patients with coronary artery disease. Am J Cardiol 2001;87:1398-400.
42. Avorn J, Everitt DE, Weiss S. Increased antidepressant use in patients prescribed Blockers. JAMA 1986;255:357-60.
43. Stein PK, Carney RM, Freedland KE. Sever depression is associated with markedly reduced heart variability in patients with stable cornary heart disease. J Psychosom Res 2000;48:493-500.
44. Fielding R. Depression and acute MI: a review and reinterpretation. Soc Sci Med 1991; 32:1017-27.
45. Lane D, Carrol D, Ring C, Beevers DG, Lip GYH. Do depression and anxiety predict recurrent coronary events 12-month after MI? Q J Med 2000;63:221-30.
46. Verrier RL. Behavioral stress, myocardial ischemia and arrhythmias. In: Zipes DP, Jalife J, editors. Cardiac electorphysiology from cell to bed side. Toronto: WB Saunders; 1990. p. 343 – 52.
47. Vandern Brink RH, Van Melle JP, Honig A, Schene AH, Crijns HI, Lamber FP. et al. Treatment of depression after myocardial infarction and the effects on cardiac prognosis and quality of life. Am Heart J 2002; 144(2):219-25.
48. Black SA, Goodwin JS, Markides KS. The association between chronic disease and depression symptomatology in older Mexican Americans. J Gerontal A Biol Sci Med Sci 1998;53(3):M188-94.

پوختە

وەرمبوونا ماسولکێت دلی یا دژوار و پوسیدەیی یا دەرونی

پێشەکی: توشبوون ب پوسیدەیی یا دەرونی پشتی وەرمبوونا ماسولکێت دلی یا دژوار یا بەربەلاڤە. دەستنیشانکرنا حالەتا و چارەسەریا وان یا فەرە چونکو پوسیدەیی یا دەرونی دبیتە ئەگەرێ چەندین نەخوشییێن دلی.

ئارمانج: دیارکرنا رولی پوسیدەیی یا دەرونی پشتی وەرمبوونا ماسولکێت دلی یا دژوار.

ڕێکێن ڤەکولینێ: ڤەکولینەکا ئایندەیی هاتە ئەنجامدان ل نەخوشخانا گشتی بەشێ چاڤدیریا دلی ل مویسل بو 200 نەخوشا ییێن تووشی وەرمبوونا ماسولکێت دلی یا دژوار بووین ژ رەگەزێ مێ و نێر د ژیێ 25-75 سالا.

ئەنجام: ئەڤێ ڤەکولینێ دیارکر کو دناڤ بەرا ئەڤان نەخوشادا پوسیدەیی یا دەرونی و پتریا وان ژنن (57٪) و مێر (43٪) (P value 0.05, OR 2.85) و ئەڤێ ڤەکولینێ دیارکر کو ئەڤ پوسیدەییێ یا دەروونی پشتی وەرمبوونا ماسولکێت دلی یا دژوار پتریان وان ئەون ئەڤێن ئاریشێن خێزانی هەین و ئەو چینێن کێم روشەنبیر. ئەنجامێت ڤێ ڤەکولینێ دیارکر کو دناڤبەرا ڤان نەخوشاندا ئەوێن تووشی پوسیدەییێ یا دەروونی بووین پشتی وەرمبوونا ماسولکێت دلی هندەك تێکداچوونێن دلی چێدبن وەکو بەردەوامبوونا (ژبحە یْدریە) و وەرمبوونا (احتشاء) ماسولکێت دلی و تێکدانا لێدانا دلی (چربات القلب). و مرن پتر یا هەیی دناڤ وان کەسێن کو نوکە توشبووین ب دامابوونا دەرونی.

دەرئەنجام: پێتڤی یە نیشانکرنا زوی بهێتە کرن بو پوسیدەییێ یا دەروونی پشتی وەرمبوونا ماسولکێت دلی یا دژوار و چارەسەری یا پێتڤی یی ژبەر نەچێبوونا تێکداچوونێن دیارکری.

الخلاصة

احتشاء عضلة القلب الحاد والكأبة النفسية

خلفية البحث: تحدث الكآبة النفسية بشكل واسع بعد أحتشاء عضلة القلب الحاد. تشخيص الحالة و معالجتها ضروري جداً لأن الكآبة النفسية تؤدي الى اختلاطات قلبية كبيرة.
الهدف: بيان تأثير الكآبة النفسية بعد احتشاء عضلة القلب الحاد.
المرضى و طرق البحث: اجرت دراسة مستقبلية فى المستشفى العام شعبة العناية القلبية فى الموصل لمئتان مريض مصابون باحتشاء عضلة القلب الحاد رجالا ونسساء تتراوح اعمارهم بين 25-75 سنة.
النتائج: بيينت الدراسة حدوث حالات كأبة حادة بين هؤلاء المرضى وبنسبة اكثر بين النساء عن الرجال 57 % الى 43% (P value 0.05 O.R 2.85). وثبتت الدراسة ان الكأبة النفسية بعد احتشاء عضلة القلب الحاد اكثر بين بين اللذين لديهم مشاكل عائلية وزوجية والطبقات الاقل ثقافة. نتائج الدراسة بيينت ان ان المرضى الذين يعانون من الكأبة النفسية بعد احتشاء عضلة القلب تحدث لديهم مخالطات قلبية مثل تكرار ذبحة صدرية واحتشاء عضلة القلب واضطرابات لضربات القلب والوفيات بنسبة اكثر من الذين لايعانون من الكأبة النفسية.
الاستنتاج: التشخيص المبكر للكأبة النفسية بعد احتشاء عضلة القلب الحاد ومعالجتها ضرورى لمنع حدوث المخالطتات المذكورة .

PATHOGENESIS OF *Campylobacter jejuni* INFECTION WITH EMPHASIS ON ULTRA STRUCTURAL CHANGES

MUNA S. AL-DELAIMI, BVM, MSc, PhD*,
RAJI H. AL-HADITHI, MD,MSc, American board **

Submitted 14 May 2007; accepted 12 January 2008

ABSTRACT

Aim Studying comprehensively the pathogenesis of local isolate of *C.jejuni* in our country with their details by using ultrastructural studies depending on suitable protocol and animal model.

Methods Newly local isolate of *C.jejuni* (CJM6) which isolated from children was used to study the pathogenesis of such bacteria after oral administration of 1.7x10 viable cell /ml for gnotobiotic mice which were as animal model .

Results Under scanning electron microscopy (SEM), the earliest colonization with huge numbers of *C.jejuni* appear at 24 hours post inoculation (P.I), and early adherence at 48 hours P.I with normal mucosal appearance. The mucosal and edema and loss of microvilli in some areas of epithelial surface were observed 3 and 4 days after inoculation , due to early penetration of *C.jejuni*. In 5 days after inoculation, the mucosa reveled irregular opening of cecal crypts with reduction of goblet cells numbers as well as destroyed cecal mucosa. While 6 and 7 days P.I revealed patchy erosion and necrosis with persistence adherence and colonization. Under transmission electron microscopy (TEM) the colonization was seen within the mucous environment of cecal epithelium, with normal appearance of cellular details. 3 and 4 days P.I some cases showed mixed healthy cells and other showed abnormalities of microvilli, as well as presence of free invasive *C.jejuni*, within epithelial cytoplasm, while deeper crypts were seen to be heavily colonized. Degenerative changes included partial loss of surface mucosal microvilli with numerous invasive *C.jejuni* mucosal goblet cells, while others seen within membrane vacuole in cytoplasmic epithelial cells 5 days P.I. At later stage of infection degenerative changes of microvilli ranged from elongation, fusion swelling, budding to abnormal shortening and microvillous-cytoplasmic extrusion toward *C.jejuni* within cecal lumen as well as exfoliated microvilli and apical cytoplasm into lumen.

Conclusion Gnotobiotic mice improved to be suitable model for studying pathogenesis by producing transient bacteremia, diarrhea and intestinal lesions resembling that which may occur in human. EM (SEM & TEM) is an important in increasing our understanding of disease pathogenesis which include colonization, adherence, penetration, multiplication and invasion as well as producing several pathological changes.

DMJ 2008;2(1): 17-30.

Key words: Pathogenesis, Campylobacter jejuni, Ultra structural changes

Campylobacteriosis is recognized world wide as the most common form of acute bacterial gastrointestinal infection.[1] The majority of infection is

* *Lecturer, Dohuk College of Veterinary Medicine, Dohuk, Iraq*
** *College of Medicine, Jordanian University of Science and Technology*
Correspondence author: *Muna S. Al-Delaimi, , Dohuk College of Veterinary Medicine, Dohuk, Iraq. Email: micro_path @yahoo.com*

attributed to the "thermophilic *Campylobacter*" which include *C. jejuni, C. coli, and C. lari.*

C. jejuni is now recognized world wide as a major cause of enteritis, accounting for (95%-99%) of cases, while *C. coli* and *C. lari* responsible for the remaining (1%-5%) of cases. In developing countries, *C. jejuni* is the third most common cause of diarrhea in children after enterotoxigenic *E. coli* and rotavirus. While in developed countries, *C. jejuni* gastroenteritis is usually more common than *Salmonella* or *Shigella* infection.[2] Although C.jejuni is an important cause of diarrhea through out the world , the pathogenic mechanism associated with *Campylobacter* enteritis remain ill-defined .[3-5] The mechanism by which *C. jejuni* causes diarrhea have been postulated from studies of clinical syndromes. Toxin production is proposed mechanism in patients with acute watery diarrhea. Another mechanism, involved penetration and proliferation within the intestinal epithelium and clinically the stool contain blood and inflammatory cells. A third mechanism, termed translocation, the organism penetrates the mucosa, resulting in minimal damage. [6,7] Much effort has been invested to elucidate the pathogenic mechanism of *C. jejuni*, four major virulence properties were recognized: motility, adherence, invasion, and toxin production .[8]

In addition there are few, attempts to find appropriat animal model,the following animals have been tested as model for studies on *C. jejuni* pathogenesis: cattle, poultry, monkey, swine, and none is completely satisfactory as a model of Campylobacteriosis due to their size, cost and they are immpractical for use in most laboratories. RITARD method is useful for studying pathogenesis and immune response but not suitable for screening large numbers of strains for difference in virulence factors,[8] while Humphy *et al.*[9] suggested that hamster might extremly valuable small animal model for *Campylobacter* infection, contrary to the reports by Aguero-Reseafeld *et al.*[10] were unable to induce diarrhea or colitis in hamster.Blaser *et al.*[11] showed that oral infection of adult mice does not induce disease,while Fauchere *et al.*[12] demonstrated that gnotobiotic mice are better model than holoxenic animals. According to as mentioned above, the aims of present study are to isolation of *C. jejuni* from children depending on most appropriate media as well as studying comprehensively the pathogenesis of local virulent isolate in our country with their details by using several methods depending on suitable protocol and animal model.

MATERIALS AND METHODS

A- Bacterial isolation by using:

*Selective media for primary isolation:

a- Skirrow medium .[13]
b- Preston s medium .[14]
c- Butzler medium .[15]
d- Sheep blood agar .[16]
e- Blood – free charcoal – based selective media .[17]
f- Newly modified charcoal –

cefoperazon deoxycholate selective media .[18]

B- Identification of *C.jejuni* by:

1- Modified gram s stain.
2- Biotype test .[19]

C- Experimental protocol :

a – Laboratory animals:

Eighty Swiss white mice[20] weeks, with offered food and water ad libidum, were divided into seven groups, each group contained 10 mice and the 8th group was left as a control. All animals were checked to be free of pathogens before beginning of experiment.

b – Determination of LD50 of CJM 6:

Pure *C. jejuni* isolates were grown over night at 370c under microaerophilic condition in trypticas Soya broth followed by concentration by centrifugation at 5000xg for 20 minutes and suspended in sterile phosphate buffered saline to give the suspension ranging from 106 to 1010 cfu / ml. Five groups of mice (6 mice for each group) were inoculated via gastric feeding tube with serial 10 fold bacterial dilution and as follow 106, 107, 108, 109and 1010 bacterial cells /ml.

c – Antibiotic treatment and experimental infection of mice:

All mice were given antibiotics ad libidum in drinking water. The antibiotics throughout the whole experiment included: Kanamycin (Sigma) 0.1 gm / ml, Vancomycin (Sigma) 0.05 mg /100ml, and ampicillin (Sigma) 0.1gm /100ml.[15] After 24hr from initial infection and until the end of experimental period, fresh fecal specimens in sterile distal water, then cultured directly on (SK, Ps, BZ, CCDA, CSM, and filtration method), all plates were incubated at 42^0c/48 hr.except for filtration method were the plates incubated for 5 days, under microaerophilic condition.

d – Preparation of tissues for SEM and TEM:

Tissue specimens were immediately cut into approximately 0.5 cm for SEM and 2mm for TEM then fixed in phosphate buffer 3% gluteraldehyde (PH 7.4). Following fixation, tissues were transferred to PBS, then dehydrated through graded acetone series followed by fine dehydration in a BIORAD critical point drying apparatus by using liquid carbon dioxide. The dried specimens were subsequently mounted on stubs and gold coated for viewing by EOMSEM, then examined and photographed under PHILIPS SIS SEM.

RESULTS

Ultra structural finding:

A-Appearance of cecum under SEM:

Cecum specimens of control mice showed normal appearance of mucosa containing goblet cells. No *Campylobacter* –like organism or intestinal flora could be seen on the surface of mucosa .While crypt opening are round and have a uniform size

and shape, so the crypt of cecum were closely packed (Figure 1), also the crypt unit were uniform.

All infected animals showed abnormal appearance of mucosa with various degree of colonization and adherence of *C. jejuni* on the epithelial surface, with pathological changes through the experimental period .Earliest colonization was observed 24 and 48 hours (P.I) with large numbers of *C. jejuni*, with early adherence of *C. jejuni* to the cecal epithelial surface, while the gross appearance of mucosa, the epithelial surface were similar to these seen in control animals. Under low magnification-on of SEM, the mucosa showed pronounced swollen, the microvilli present in some areas and lost from the cell surface in other areas of cecal mucosa of animals which scarified 3 and 4 days P.I. Some areas were normal under low magnification of SEM, while under high magnification showed abnormal appearance, with numerous *C. jejuni* invading the mucosal surface. 5 days P.I. cecal crypts had irregular opening correlated with loosely attached epithelial cells to the luminal surface of mucosal folds with reduction numbers of goblet cells (Figure 2). As well as mucosal micro erosion with various size and shape which characterized by destroyed cecal mucosa and large numbers of *C. jejuni* were observed within this area, which looked like" worm eaten" areas (Figure 3).The general appearance of cecal mucosa of infected animals 6 and 7 days P.I reveled a patchy erosion and necrosis which were another pathological changes which appear as"window"on the cells, with obvious adherence of *C. jejuni* on the normal mucosal areas (Figure 4). The colonization and adherence of *C. jejuni* remain at later times in the infection, with marked decrease number of surface goblet cells. Large number of *C. jejuni* was found associated with mucous secretion and directly attached to the epithelial surface and crypt region. Otherwise, the orientation of *C. jejuni* associated with mucosa in most horizontal, while others attached end-to end appearance (Figure 5). However cross section under SEM showed invaded *C. jejuni* in submucosal region.

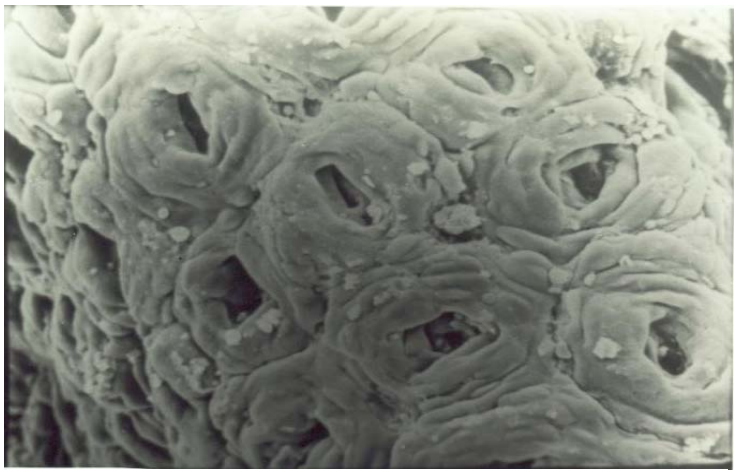


Figure 1. Normal appearance of cecal mucosa containing goblet cells, no *Campylobacter* – like organism or intestinal flora seen (SEM; x 600)

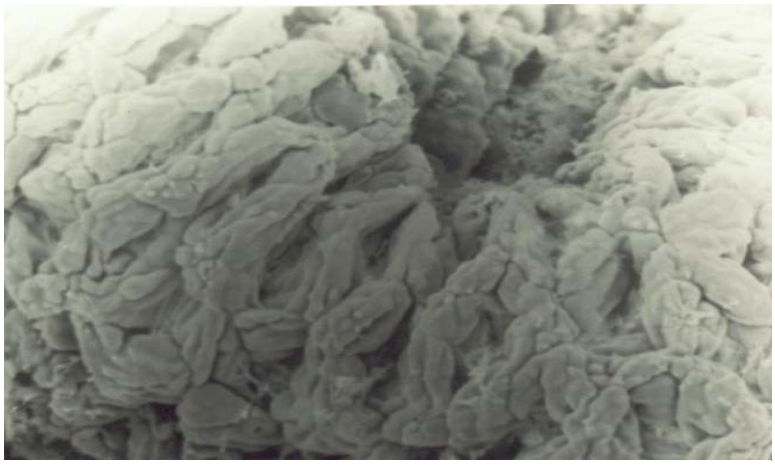


Figure 2. Cecum of infected mouse at 5 days, showed irregular opening crypts with loosely attached epithelial cells to the luminal surface of mucosal fold with reduction numbers of goblet cells (SEM ;x3120)

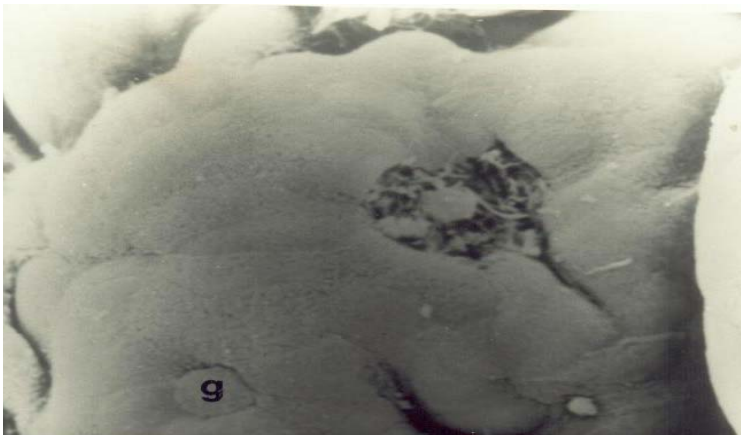


Figure 3. Mucosal microerosion characterized by destroyed cecal mucosa with numerous *C.jejuni,* with normal appearance of goblet cell(g) at 5 days P.I (SEM; x 1930)

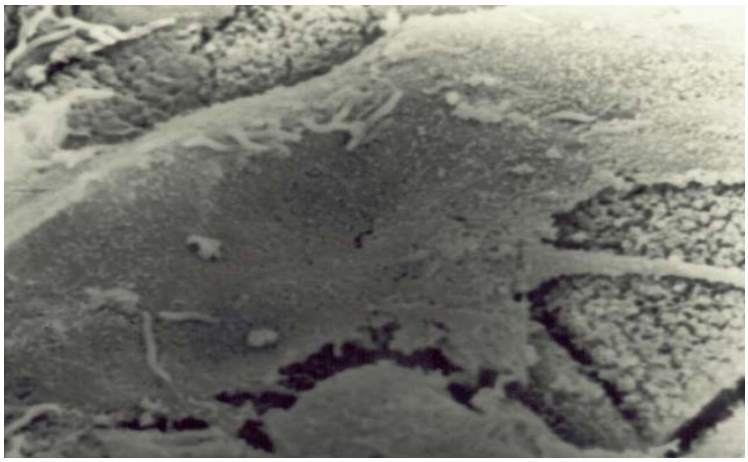


Figure 4. Patchy erosion and necrosis of cecal mucosa which appear as "a window" on the cell, with adherence of *C.jejuni* on the normal area (SEM; x 5000)

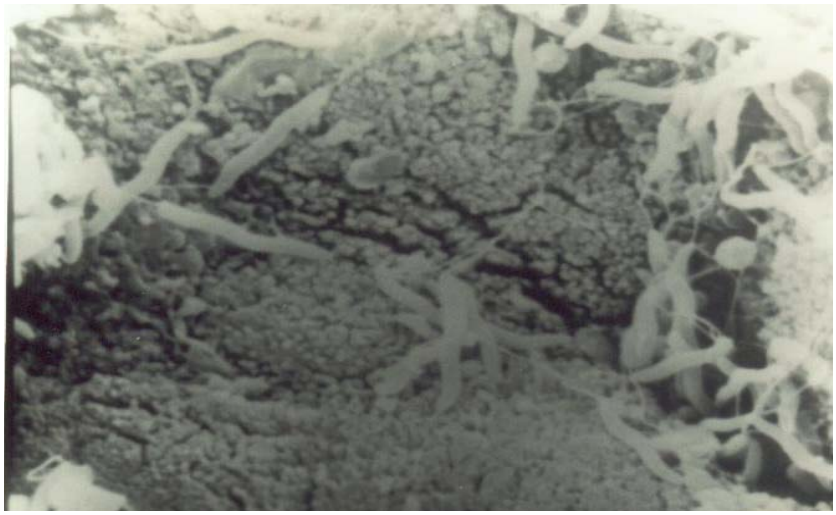


Figure 5. Different orientation of C.jejuni, including horizontal and attached end – on appearance (SEM ; x 9600)

B-Appearance of cecum under TEM:

Pathological studies by TEM help to understand cellular reaction to injury which couldn't seen by light microscope (LM) and SEM. In our study the results revealed to considerable spectrum of cellular alteration ranged from colonization, adherence to various degrees of invaded organisms with earliest sub lethal cell damage. In addition, exfoliated epithelial cells are seen, within the intestinal lumen with degenerative change of microvilli. Ultrastructural studies of cecal epithelium of control mice showed normal columnar cells of surface epithelium which had orderly microvillus border with uniform length. These cells contained basal nuclei, perinuclear Golgi, reticuloendoplasmic reticulum and a abundant mitochondria, and normal cristae. Initially in an infected animals 24 and 48 hours PI, numerous *C. jejuni* in different location at cecal region were observed *i.e.* several bacteria arranged free in mucous environment of cecal epithelium, some of them near epithelial microvillus, others very close proximity to the tissue surface or in close association with microvillus, with characteristic size and shape of *C. jejuni i.e*. S-shaped and spiral form. While endoplasmic reticulum, mitochondria were normal and cytoplasm had normal density slightly damaged epithelial cells. Similar pathological changes were demonstrated in animals which were sacrificed at 3 and 4 days included: damaged cells and healthy appearing cells were seen. Some cells had normal microvillus with pronounced cellular edema appeared as large intracellular vacuole; other cells had microvilli which were distorted, irregular in length tufted or otally lost with multivacuolation. In addition to presence of more than one invaded bacteria free within the epithelial cells cytoplasm. The deeper parts of crypts were seen to be

heavily colonized. On the other hand, nuclear chromatin showing dense staining and fragmentation due to nuclear necrosis was seen. Where as the animals sacrificed at 5 days PI revealed partial loss of surface mucosal microvillus with numerous degenerative change including intracytoplasmic vacuolation and prominent swelling of mitochondria. Numerous *C. jejuni* which colonization to the mucosal epithelial cell, others invading goblet cell within mucosal epithelial cells (Figure 6). Sometimes, intracellular *C. jejuni* were seen within membrane vacuole in the cytoplasm of epithelial cell (Figure 7), also, found free in the sub mucosa, with increased cellular of lamina propria and sub mucosa due to infiltration of neutrophil, plasma cell and eosinophil which were similar to the observation under L.M.At 6 and 7 days PI various degree of degenerative changes of microvillus were seen ranged from elongation, fusion swelling, budding, to abnormal shortening and denudation. Occasionally, *C. jejuni* were observed lying close to the microvillus, cytoplasmic degeneration characterized by project the cecal epithelial cytoplasm into the gut lumen and necrosis with multivaculation. Otherwise, microvillous-cytoplasmic extrude toward *C. jejuni* which located in the cecal lumen (Figure 8). Degeneration of microvillus and apical cytoplasm occurred, damaged cells exhibiting swollen endoplasmic reticulum and loss of microvilli are exfoliated into lumen of the cecum (Figure 9), similar organism were frequently found deep within the crypt (Figure 10).

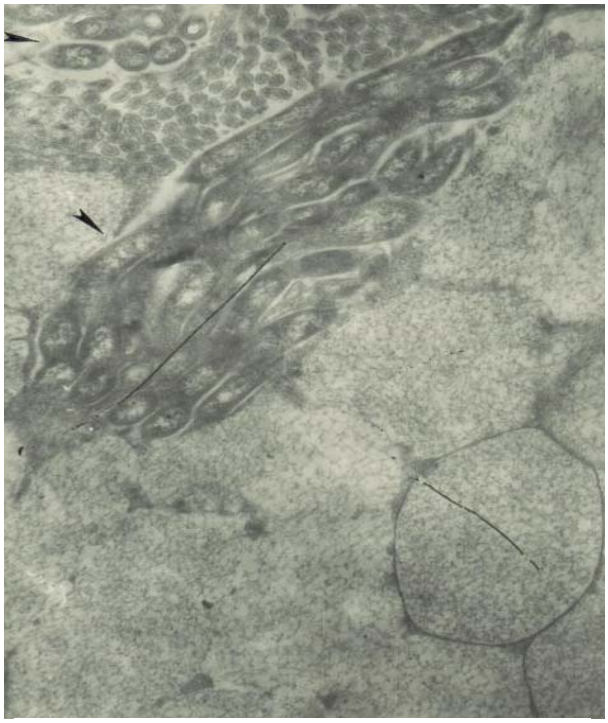


Figure 6. Numerous *C.jejuni* colonized (arrow) epithelial cells, others invaded epithelial goblet cell at 5 days P.I (TEM; 16000)

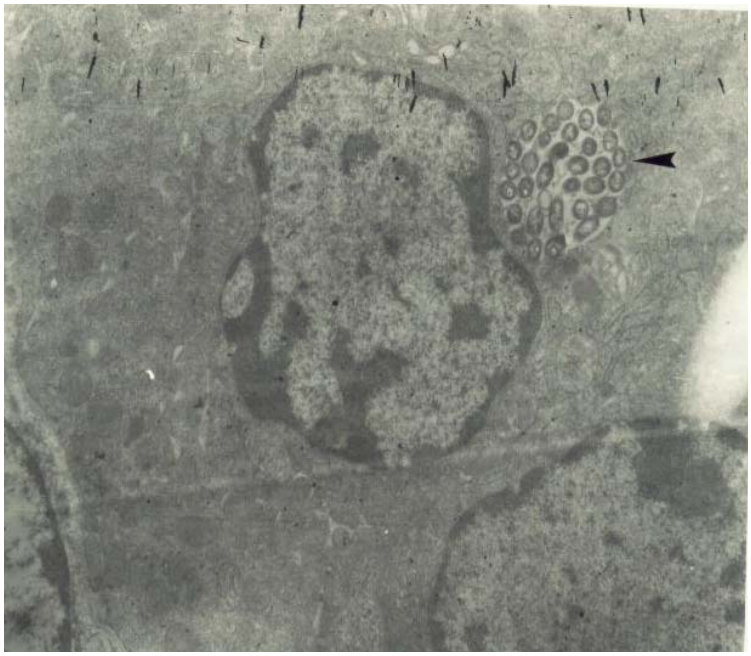


Figure 7. Intracellular *C.jejuni* seen within membrane vacuole (arrow) in cytoplasm of epithelial cell at 5days P.I (TEM; x 8700)

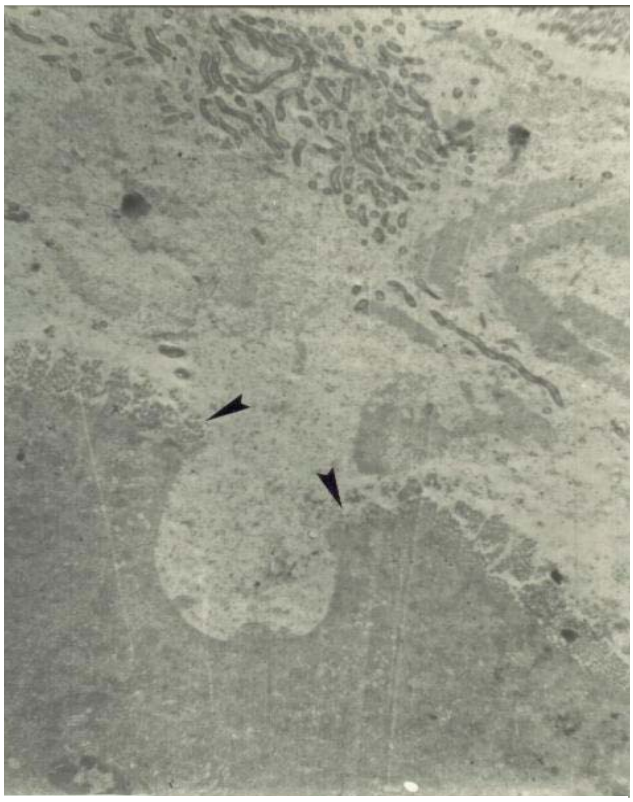


Figure 8. Microvilli-cytoplasmic extrusion (arrow) toward *C.jejuni* which located in the cecal lumen at 7 days P.I (TEM; x3400)

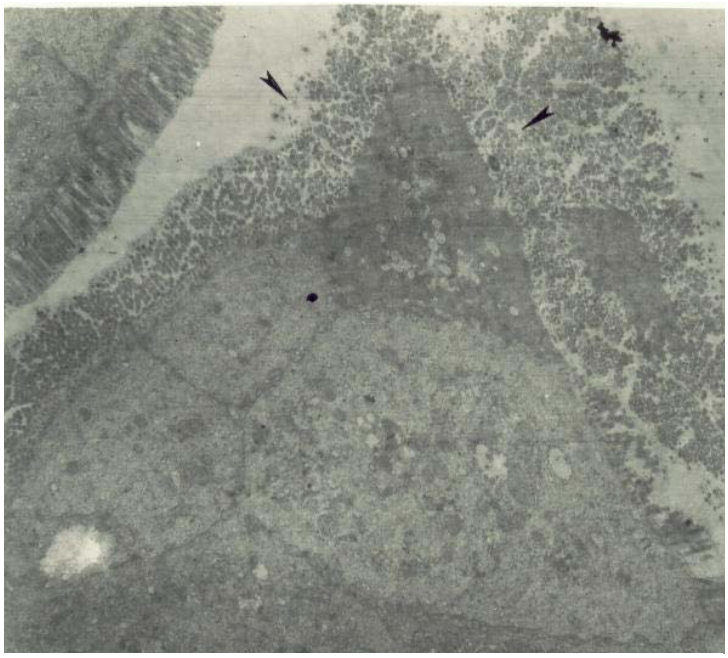


Figure 9. Exfoliated damage epithelial cell into lumen (arrow) of cecum at 7 days P.I. (TEM; x 4400)

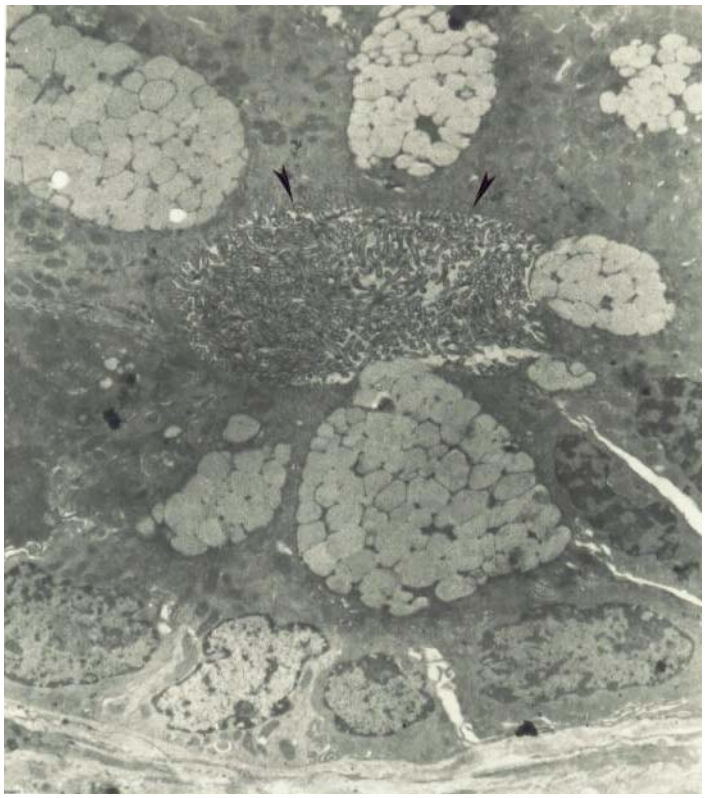


Figure 10. At late stage of infection invaded *C.jejuni* (arrow) in deep cecal crypt (TEM ; x 4400)

DISCUSSION

In this study mucus colonization was seen as early as 24 hours PI with huge numbers of *C. jejuni* which was attributed to participation spiral morphology of *Campylobacte*r and especial mode of motility as well as chemo attraction of such organism to cecal mucosa which provided a good environment. Following colonization of the mucus blanket, *Campylobacter* can adherence to the cecal mucosa and its goblet cells, which suggest that adherence would be the early stage of pathogenesis of Campylobacter infection which agree with Fauchere *et al.*[12] observation, but differs with Lee *et al.*[21] who demonstrated that adherence and invasion may not be needed for colonization. While, Gao *et al.*[22] reported that adherence of *C. jejuni* to the intestinal epithelial cells was only found in chronic infectious model. Our observation showed abnormalities of microvillus and extrusion of cytoplasmic mucosal epithelium, as well as presence of *C. jejuni* within membrane-bound vacuole and free in the cytoplasm. Similar results were observed in experimentally infected infant macaque monkeys, which have been observed *C. jejuni* invade colonic epithelial cells and have been found within membrane- bound vesicles and free in cytoplasm [23] and in hamster model .[9] Campylobacter invasion has been studied in vitro by using several cell lines.[3,24,25] This am plies that following colonization and adherence to the intestinal surface *C.jejuni* disturbed normal absorptive capacity of the intestine by damage epithelial cell surface function may be directly affected by toxic substance produce by *C.jejuni* in the intestinal lumen and\ or by cell invasion, which indicate that invasion considered an important step involved in the pathogenesis of *Campylobacter* infection.Konkel *et al.*[3,25] suggested that several new bacterial proteins synthesized during interaction with epithelial cells in culture *i.e. Campylobacter* can express more than one antigen and these antigens may act individually or may in concert to promote pathogenesis. On the other hand, present study provides evidence that *C. jejuni* has ability to penetrate the mucosal surface between microvillus and through goblet cells without any evidence to internalize via intracellular junction complex. This finding accordance with those reported by Russell *et al.*,[23] but differed with that observation by Humphrey *et al.*, [9] who demonstrated that *C.jejuni* can penetrate the cecal epithelium of hamster via a tight junction. Following invasion, the multiplication of organisms within epithelial cells, then may translocated across intestinal barriers to lamina propria leading to obvious pathological lesions which check as described earlier.Cytoplasmic organelles associated with organisms located within cytoplasm similar that described by Trumps and Arstila .[20] In otherwise, the marked swelling of E.R may refer to ability of inter cellular *C.jejuni* to disturbed cellular processes such as ion and water transport mechanism by secreting cytotoxin, enterotoxin or hemolysin.[23] Intestinal infection with *C.jejuni* correlated with an intense

inflammatory response including polymononuclear leukocyte (PNM's), which infiltrated the lamina propria of cecum of infected animals. These observations indicated that a positive correlation between number of intracellular bacteria and number of intraepithelial PNM's, the survival of *C.jejuni* in cell for an extended period which may attribute to the pathogenicity of this organism. While cryptitis is an indication of the number of inflammatory cells which migrate through an anatomically intact epithelial lining of crypt from the surrounding lamina propria.During the period of our experiment May organism were present in the deep crypt lumen, as well as, at lat stage of infection invaded the cryptal goblet cells. These findings may explain persistence of *C.jejuni* at later period of infection, due to that these organisms provided with the energy and carbohydrate source *i.e*. mucin.

REFERENCES

1. Pearson AD, Healing TD. The surveillance and control of *Campylobacter* infection. Comm Dis Rep CDR Rev 1992;2(12):R133-9.
2. Ashkenazi S, Cleary TG. *Campylobacter* In: Nelson WE, Behram NE, Kliegman RM, Avrin AM, editors. Nelson textbook of pediatrics. 15th ed. Philadelphia: WB Saunder Company; 1996. p.784-800.
3. Konkel ME , Mead DJ, Cieplak Wjr. Kinetic and antigenic characterization of altered protein synthesis by *C. jejuni* during cultivation with human epithelial cells. J Infect Dis 1993;168:948-54.
4. Nachamkin I. *Campylobacter* and *Arcobacter*. In: Murvay PR, Baron EJP, Faller MA, Tenover FC, Yolken RH, editors. Manual of clinical microbiology. Washington, DC: ASM press; 1995. p.483-91.
5. Ketley JM. Pathogenesis of enteric infection by *Campylobacter*. Microbiology 1997;143:5-21.
6. Levine MM, Kaper JB, Black RE, Clements ML. New knowledge of pathogenesis of bacterial enteric infection as applied to vaccine development. Microbiol Rev 1983;47:510-50.
7. Wassenaar TM. Toxin production by *Campylobacter* spp. Clin Microbiol Rev 1997;10:466-76.
8. Walker RI, Cald well MB, Lee EC, Guerry P, Trust TJ, Ruiz-Palacious GM. Pathophysiology of *Campylobacter* enteritis. Microbiol Rev 1986;50(1):81-94.
9. Humphrey TJ. Techniques for the optimum recovery of cold injury *C. jejuni* from milk or water. J Appl Bacteriol 1986;61:125-32.
10. Aguero-rosenfeld ME ,Yang XH, Nachamkin I. Infection of adult Syrian hamsters with flagellar variants of *C. jejuni*. Infect Immun 1990;58: 2214-9.
11. Blaser MJ, Duncan DJ, Warren GH, Wen-Lan L. Experimental infection of adult mice. Infect Immun 1983; 39(2):908-16.
12. Fauchere JL, Veron M, Lellouch-Tubiana A, Fister A. Experimental

infection of gnotobiotic mice with *C. jejuni*: Colonization of intestine and spread to lymphoid and reticuloendothelial organs. J Med Microbiol 1985;20:215-24.

13. Skirrow MB. *Campylobacter* enteritis: a"new"disease. Br Med J 1977;2:9-11.
14. Butzler JP, Skirrow MA. *Campylobacter* enteritis. Clin Microbiol Rev 1979;10(3):737-65.
15. Goossen H, De Boeck M, Butzler JP. A new selective medium for the isolation of *C. jejuni* from human feces. En J Clin Microbiol 1983;2:389-94.
16. Steel TW, Mc Dermott SN. The use of membrane filters applied directly to the surface of agar plates for the isolation of *C. jejuni* from feces. Pathogology 1984;16:263-5.
17. Karmali MA, Simor AE, Roscoe-Fleming PC, Smith SS, Lane. Evaluation of blood free, charcoal based, selective medium for the isolation of *Campylobacter* organisms from feces. J Clin Microbiol 1986;23:456-9.
18. Hutchinson DN, Bolton FJ. Improved blood free selective medium for the isolation of *C. jejuni* from fecal specimens. J Clin Pathol 1984;37:956-7.
19. Lior H. New extended biotyping scheme for *C. jejuni*, *C. coli* and *C. laridis*. J Clin Microbiol 1984;20:636-40.
20. Trump BF, Arstila AV. Cellular reaction to injury. In Lavia MF, editor. Principles of pathobiology. 2nd ed. New York: Oxford University press; 1975. p.9-96.
21. Lee A, ORourke JL, Barrington PJ, Trust TJ. Mucus colonization as a determinant of pathogenicity in intestinal infection by *C. jejuni* a mouse cecal model. Infect Immun 1986;51:536-46.
22. Gao JX, Ma BI, Xie YI, Hunag DS. Electron microscopic appearance of the chronic enteritis of mice. Clin Med J 1991;104(12);1005-10.
23. Russell RG, Odonnoghue M, Blake DC, Zulty JJ, Detolla LJ. Early colonic damage and invasion of *C. jejuni* in experimentally challenged infant Macaca mulatta. J Infect Dis 1993;168:210-5.
24. Konkel ME, Babakhani F, Jones LA. Invasion-related antigens of Campylobacter jejuni. J Infect Dis 1990;162(4):888-95.
25. Konkel ME, Hayes SF, Joens LA, Cieplak W Jr. Characteristics of the internalization and intracellular survival of *C. jejuni* in human epithelial cell cultures. Microb Pathog 1992;13:357-70.

پوختە

چاوانیا چێبونا نەساخیێ ژ *C. jejuni* دگەل گوهورینێن هویر

ئارمانج: هەرچەند *C.jejuni* هویەکێ گرنگێ زکچونێ یە ل سەرانسەری جیهانێ، چ ڤەکولینێن گشتی نینن کو بزانین چاوا ئەو نەساخێ چێد کەت.

ریکێن ڤەکولینێ: ئەڤ ڤەکولینە هاتیە ئەنجام دان بو زانینا چاوانیا چێبونا نەساخیێ یا Local isolate(CJM6) پشتی پێدانا برێکا دەڤی یا viable cell / ml 1.7x10 بو مشکی gnotobiotic کو هاتبو بکار ئینان وەك گیانەوەرێ نمونە ل ژێرScaning electron Microscopy.

ئەنجام: زویترین کوم بون (colonization) دگەل ژمارەکا مەزن ژ *C.jejuni* دیار بون د 24 دەم ژمێرا پشتی کوتانێ (post inoculation P.I) وهەڤگرتنا زوی د 48 دەم ژمێرا دگەل دیار بوونا پەرەیی لیچکی یێ ئاسایی (normal). mucosal appearance) ئاڤبەندا پەردەیێ لیچکی(mucosal edema) و ژ دەست دانا microvilli ل هندەك جهێن epithelial surface هاتنە دیتن 3,4 روژا پشتی کوتانێ ،ژ بەر زوی کون کرنا *C.jejuni* پێنجەمین روژا کوتانێ ،ل سەر mucosa چەند کونێن نەرێک بێن ،دگەل کێم بوونا ژمارا goblet cells ،هەروەسا هەلوەشینا cecal mucosa دیار بون.لێ6,7 روژا , patchy erosion , دگەل necrosis (P.I) هەڤگرتن و کوم بونەکا بەدەوام دیلر بو.ژێرTransmission electron Microscopy(TEM) کوم هاتنە دیتن دناڤ ناوەندێ mucous یێ cecal epithelium دگەل دیاربونا ئاسایی یا cellular details . 3,4 روژا P.I ل هندەك حالەتا خانەیێن ساخلەم بێن تێکەل دیار بون و هندەکێن دیتر نە ئاسایی بوونا microvilli و هەروەسا هەبوونا *C.jejuni* یا هێرشی (invasive) دیار بو دناڤ epithelial cytoplasm،لێ crypts بێن کویرتر هاتنە دیتن کو بشێوەکی زور کوم ببون.گهورینێن گەنی (Degenerative changes) ئەڤەبون:ژ دەست دانا کیم یا (surface mucosal microvilli) دگەل ژمارەکا زور یا *C.jejuni* بێن هێرشی، mucosal goblet cells ،لێ ئەوین دیتر دناڤ ڤالاتیا چەرهکی (membrane vacuole) بین خانەیێن cytoplasmic epithelium 5 روژا (P.I) هاتنە دیتن.د قوناغا دیتر یا نەساخیێ،گهورینێن گەنی بونا microvilli هاتبونە زنجیرەکرن ژ: دریژبوون (elongation) ، پەژبون(پوڤ بونا) ئیگرتی (fusion swelling) وەرار بو کورت بونا نەئاسایی (budding to microvillous cytoplasmic extrusion, abnormal shortening) بەرەڤ *C.jejuni* دناڤ ڤالاتیا ریڤیکا کورەدا (cecal lumen) و هوروەسا microvilli بێن قاشکری:(exfoliated) و سایتوپلازمی لوتکەیی (apical cytoplasm) بو ناڤ ڤالاتیێ.

الخلاصة

استخدام المجهر الالكتروني لدراسة امراضية جراثيم *C. jejuni*

الهدف: دراسة موسعة لامراضية العزلات المحلية لجراثيم C.jejuni في القطر وبصورة مفصلة باستخدام المجهر الاليكتروني (الماسح والنافذ) من خلال اعتماد بروتوكول مناسب لذلك باعتماد الحيوانات المختبرية .

طريقة البحث: اجريت هذه الدراسة باستخدام عزلة محلية (CJM6) حيث عزلت من اطفال يعانون من الاسهال في مستشفى الطفل المركزي لدراسة امراضية تلك الجرثومة بعد اعطائها تجريبيا عن طريق الفم لفئران خالية من الجراثيم (1.7 ×10 خلية / ملم) لاعتمادها كنموذج لهذه الدراسة .

النتائج: بينت الدراسات الدقيقة للاعور باستخدام المجهر الالكتروني الماسح بدء استعمار اعداد ها ئلة من جراثيم *C. jejuni* بعد 24 ساعة من الاصابة , اما بدء الالتصاق فقد ظهر بعد 48 ساعة من الاصابة مع سلامة الطبقة المخاطية فيما لوحظ بعد اليوم الثالث والرابع من الاصابة ودمة الطبقة المخاطية وفقدان الزغيبات في بعض اجزاء الطبقة الظهارية نتيجة لبدء اختراق الجرثومة . اما في اليوم الخامس من الاصابة فقد اظهرت الطبقة المخاطية للاعور عدم انتظام فتحات الخبيئات مع اختزال اعداد الخلايا الكاسية وتبدو بعض اجزائها مقضومة . فيما كانت الافات بعد اليوم السادس والسابع تتمثل بانتشار بقع التاكل والنخر واختزال واضح لاعداد الخلايا الكاسية , مع استمرار استعمار والتصاق جراثيم *C. jejuni*. ويظهر المجهر الالكتروني النافذ استعمارا جرثوميا لاعداد ها ئلة من *C. jejuni* ضمن المخاط المغطي لسطح الاعور مع ظهور خلاياه بالشكل الطبيعي في بداية الاصابة . وبعد اليوم الثالث والرابع من الصابة نلاحظ مزيجا من الخلايا السليمة والخلايا المصابة التي اظهرت تغيرات في زغيباتها مع غزو عدد من جراثيم *C. jejuni* لهيولى الخلايا الظهارية , بينما استعمرت الخبيئات العميقة بشكل كثيف. وفي اليوم الخامس من الاصابة تغزو جراثيم *C.jejuni* الخلايا الكاسية للطبقة المخاطية وبعضها تتواجد في داخل فجوة ضمن هيولى الخلايا المعوية. وفي المراحل المتقدمة للاصابة تراوحت التغيراتالتنكسية للزغيبات من الاستطالة والتورم والتبرعم الى قصرها غير الطبيعي وانبثاق الزغيبات والهيولى معا باتجاه الجرثومة فضلا عن توسف الزغيبات وتقمم الهيولى باتجاه *C. jejuni* المتواجدة في تجويف الاعور .

الاستنتاجات: اظهرت الفئران المختبرية الخالية من الجراثيم انها مناسبة لدراسة امراضية جراثيم *C.jejuni* وذلك من خلال ظهور تجرثم الدم المؤقت والاسهال والافات المعوية المما ثلة في الانسان نتيجة الاصابة بهذه الجرثومة. وقد اظهرت الدراسة الحالية اهمية المجهر الالكتروني (الماسح والنافذ) لدراسة امراضية هذه الجراثيم والتي تضمنت الاستعمار والالتصاق والاختراق والتكاثر والغزو الجرثومي فضلا عن احداث العديد من التغيرات المرضية.

EVALUATION OF SERUM COPPER STATUS IN PATIENTS WITH CHRONIC HEART FAILURE

BAYBEEN K. ALSELEVANY, BSc, MSc, Ph.D*,
SHATHA A. HASSAN, BSc, MSc,**
ABDUL-AZIZ A. AZIZ, MBChB, MSc, Ph.D***

Submitted 1 April 2007; accepted 21 April 2008

ABSTRACT

Background Chronic heart failure is one of the main cardiovascular diseases that has increased prevalence in the recent years and it has been projected that chronic heart failure will be a major cause of morbidity and mortality in the future. Recent researches demonstrate the importance of certain trace elements in the pathogenesis of cardiovascular disorders. Among these elements is copper metal. It is considered as a strong antioxidant**.**

Objectives This study was undertaken in order to investigate the serum copper level in patients with chronic heart failure compared to healthy individuals, and to find whether there is any relationship between serum copper level and patients with chronic heart failure.

Patients and methods A case series study was conducted on 53 patients (37 males, 16 females) with chronic heart failure, with a mean age of 52.23± 13.1 years who randomly selected from patients admitted to medical wards and Cardiac Care Unit of Ibin-Seena Teaching Hospital in Mosul city during the period from July 2006 to December 2006. The study also included 32 healthy volunteers (18 males, 14 females) with a mean age of 41.31± 14.72 years, as a control group. Serum copper concentration was measured in patients with chronic heart failure and healthy controls.

Results The results indicate that patients exhibited significant decrease in the serum copper level ($p<0.001$) as compared to the healthy controls. Also the results showed that there is no statistically significant difference in the concentration of serum copper between males and females in patients with chronic heart failure ($p>0.05$).

Conclusions and Recommendations Chronic heart failure is a multifactorial syndrome. Several factors had been found to contribute to the development of this syndrome. Low serum copper level may be one of these contributing factors, probably by elevating blood pressure, impairing different tissue formation and inducing high serum cholesterol level. Measurement of serum copper level might provide additional and useful laboratory test for the assessment of the patients with chronic heart failure and oral copper may have a role in therapy.

DMJ 2008;2(1): 31-40.

Key words: Chronic heart failure, Serum copper status

Chronic heart failure (CHF) is one of the main cardiovascular diseases that has increased prevalence in the recent years and it has been projected that CHF

**Assistant Professor, Department of Medical Physiology, Mosul College of Medicine, Mosul ,Iraq*
***Assistant Professor, Department of Basic Dental Science, College Of Dentistry, Mosul, Iraq*
****Assistant Professor, Department of Medical Physiology, Kufa College of Medicine, Najaf, Iraq*
Correspondence author:*Baybeen K. Alselevany, Department of Medical Physiology, Mosul College of Medicine, Mosul, Iraq. Email: bselevany@yahoo.com*

will be a major cause of morbidity and mortality in the future.[1] The concept of CHF involves primarily impairment in functional capacity of cardiac muscle with a wide variety of neurohumoral disorders.[2] Recent researches demonstrate the importance of certain elements in the pathogenesis of cardiovascular disorders. Among these elements is copper metal.[3] Copper is the third most abundant mineral in the human body, it acts a cofactor for many enzymes.[4,5] it is also considered as a strong antioxidant.[6,7]The liver is the main regulator of copper in the body ,accordingly liver diseases or altered plasma ceruloplasmin level may contribute to low plasma copper level (PCuL).[8,9] Copper is widely distributed in foods[10] thus nutritional copper deficiency is rarely observed except in patients receiving total parentral nutrition,[11] or in people who are consuming high acid content diet and are stored in cans for a long time,[12] or high dose of supplements of zinc, vitamin C, and iron containing diets.[13] It also occurs in those who are receiving copper – lowering agents like tetrathiomolybdate and D-penicillamine.[14, 15]

Copper deficiency has been associated with nephrotic syndrome,[16] a variety of vascular abnormalities, hypochromic anemia,[17] and impairment of blood supply to cardiac muscles with subsequent heart disease.[18]

On other hand hypercuperaemia is a condition that is associated with hyperceruloplaminaemia.[19]

Ceruploplasmin is an acute phase protein that is increased in a variety of neoplastic and inflammatory states; leukemia, lymphoma; primary biliary cirrhosis and rheumatic arthritis, marked hypercuperaemia are formed in acute and chronic cases of liver diseases' infections.[9,20,21] High level of ceruploplasmins occur in pregnancy due to high estrogens, and with oral contraceptives when the agent contains estrogen as well as progesterone[8,22] and increased with copper intoxication.[11]

The present study is an attempt to evaluate the serum copper status in patients with CHF and to find if there is any relationship between SCuL and CHF.

PATIENTS AND METHODS

The study was conducted on 53 patients [(37 males(69.8%),and 16 females(30.2)] with CHF, with a mean age of 52.23± 13.1 years who were randomly selected from patients admitted to medical wards and Cardiac Care Unit (CCU) of Ibin-Seena Teaching Hospital in Mosul city during the period from July 2006 to December 2006**.**

Chronic Heart failure was documented by electrocardiography as well as electrocardiogram and clinical findings. The severity of the CHF was determined by the criteria of the New York Heart Association.[23] Accordingly 18 patients were of class I CHF, 20 patients were of class II and 15 patients were of class III CHF. Four patients were maintained on digoxin and moduretic, 10 patients were maintained on digoxin, moduretic and captopril and the last 3 patients were maintained on digoxin frusemide and captopril. The study also included 32 healthy volunteers [(19 males (59.4%), and 13 females (40.6%)] with a mean age of

41.31± 14.72 years as control group. All controls were scrutinized for the absence of any cardiac or renal disease by thorough history and physical examination.

Five ml. of venous blood was obtained from a suitable forearm vein into plain tubes, the tubes centrifuged for 30 minutes , the serum then separated and kept in capped plastic tubes in deep freeze (-20°C) until analysis. Serum copper concentration was estimated in patients with HF and healthy controls using a Pye Unicam Model SP9 Atomic Absorption Spectrophotometer.[24]

Standard statistical methods were used to determine the mean, standard deviation (SD) and range. The unpaired Z- test , unpaired student t- test and Chi-square tests were used. All values quoted as the mean ± SD.The accepted level of statistical significance was considered at $p<0.05$.[25]

RESULTS

Figure 1 shows the frequency distributions of SCuL in patients and controls.

In recent study, the mean ± SD of SCuL in patients with CHF was 5.58 ±2.44 µmol/L, while in controls was 16.02 ±5.57 µmol/L. SCuL in patients with CHF was significantly lower in comparison with the controls ($p<0.001$) as shown in table 1.

The reference range (mean ± 2SD) of SCuL was calculated to be 4.88-27.16 µmol/L.In control group 25 subjects (78.1%) had SCuL within the reference range, 5 subjects (15.6%) had SCuL less than the lower limit of the reference range, and 2 subjects (6.3%) had SCuL higher than the reference range. On the other hand in patients with CHF all 53 patients (100%) had SCuL lower than reference range ($p<0001$) (Table 2 and Figure 2).

The results of current study also revealed that SCuL was lower in female patients with CHF as compared to the male patients. The mean±SD for PCuL in females was 5.58± 2.44 µmol/L, while in male patients was 5.65±2.56 µmol/L, but the differences was statistically non-significant ($p>0.05$) as shown in Table 3.

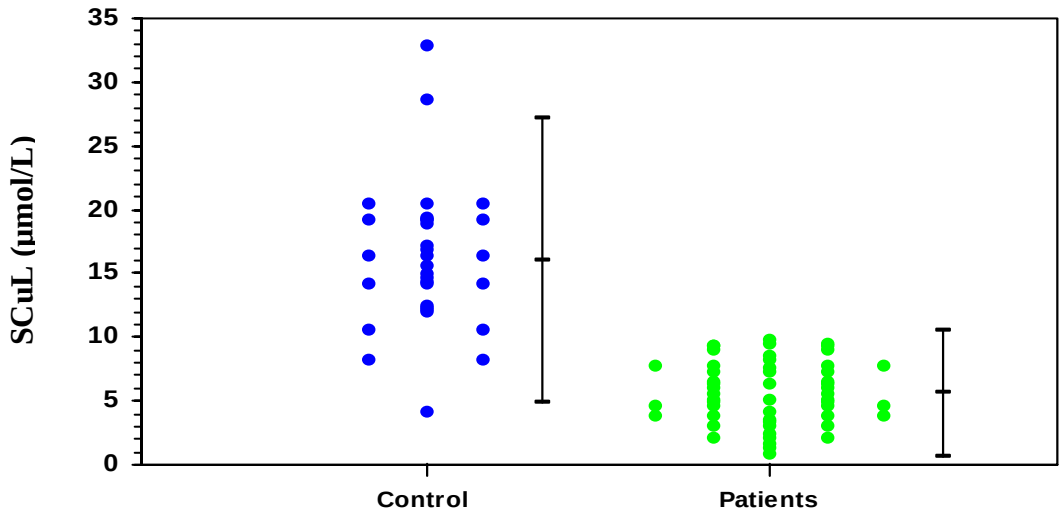


Figure 1. Distribution of SCuL in patients with CHF and controls
Bars represent mean ± 2SD

Table 1. Mean ±SD for SCuL in patients with CHF compared with healthy control group

Variable	Controls n=32	Patient n=53	P-value
	mean ± SD	mean ±SD	
SCuL (µmol/L)	16.02 ±5.57	5.58 ±2.44*	<0.001

**significant difference from control value, p<0.001*

Table 2. Number and percentage of controls, and patients subdivided into three subgroups according to the level of PCuL

Groups	Controls n=32	Patients n=53	P- value
	n (%)	n (%)	
Level I Normal level	25 (78.1)	0 (0.0)	< 0.0001
Level II subnormal	5 (15.6)	53 (100.0)	
Level III Above normal	2 (6.3)	0 (0.0)	

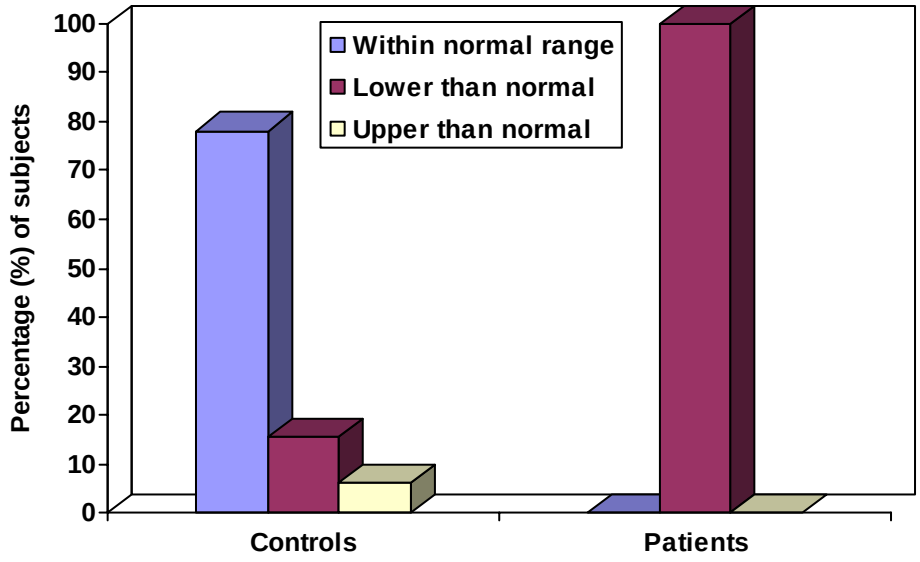


Figure 2. Distribution of serum copper level in patients andcontrols according to normal range

Table 3. Comparison between SCuL in males and females in patients with CHF

Sex	Males n=32	Females n=53	P-value
	mean ± SD	mean ±SD	
SCuL (µmol/L)	5.65±2.56	5.40 ±2.23^{NS}	> 0.05

NS: non-significant difference, (p>0.05)

DISCUSSION

Copper is an essential trace element required by aerobic organisms, it plays an important role in energy production, connective tissue formation, neurotransmitter synthesis iron metabolism and as antioxidant.[5] The results of the current study show a significant decrease in SCuL in patients with CHF in comparison with the healthy controls which is in agreement with the findings observed by other investigators.[26,27] There is more than one explanation for the mechanism of copper deficiency in enhancing cardiovascular diseases. Copper deficiency is usually associated with decreased myocyte fragility and increased myocyte size leading to decrease passive stiffness of cardiac myocytes and cardiac tissues with subsequent cardiac hypertrophy and cardiomyopathy.[28] Many studies in animals maintained on low copper diet revealed decreased connective tissue content of the heart in these animals.[29] In addition to these observations altered Na^+-K^+ ATPase activity[30] and decreased cross linking of elastin and collagen[31] may also contribute to decreased cardiac myocyte functional capacity with subsequent impaired pumping capacity of the heart and finally heart failure.

The abnormal levels of copper in patient with CHF are probably due to changes in the concentration of caeruloplasmin in the plasma.[8,9] Ceruloplasmin is a major carrier protein for copper. About 90-95% of total copper is incorporated into caeruloplasmin while the rest is bound to albumin and amino acids.[32] There is a linear relationship between SCuL and plasma caeruloplasmin.[16] This indicates that hypocupraemia and hypercupraemia are conditions that are related to changes in the level of plasma ceruloplasmin.[19]

Hypocupraemia in patients with CHF is mainly due to hypocerulopalsminaemia.[19] Caeruloplasmin is low in two major inherited abnormalities of copper metabolism such as Wilson's disease and Menkes"kinky-hair syndrome" , with protein loss such as nephritic syndromes and malabsorption,[16,33] and with some cases of advanced liver diseases in which decrease in serum proteins have

occurred.[8,9] Drug effect may also contribute to low SCuL,[14,15] in addition most of elderly patients with chronic debilitating diseases used to consume different combination of ionic that may contains large doses of zinc, vitamin C,and iron which predispose to decrease SCuL[13]; and lastly hypocupraemia in patients with HF may be nutritional.[11]

The findings of this study may indicate that patients with CHF are liable to develop low SCuL; therefore it is recommended that copper supplementation can be added to the treatment of patients with heart failure.

In the current study a non-significant difference is found in SCuL between females and males with CHF.This agrees with the finding by other researcher.[34]

CONCLUSIONS AND RECOMMENDATIONS

Chronic heart failure is a multifactorial syndrome. Several factors had been found to contribute to the development of this syndrome. Low serum copper level may be one of these contributing factors, probably by elevating blood pressure, impairing different tissue formation and inducing high serum cholesterol level.

Measurement of SCuL might provide additional and useful laboratory test for the assessment of the patients with CHF and oral copper may have a role in therapy.

REFERENCES

1. Ahmed A. American College of Cardiology / American Heart Association. Chronic heart failure evaluation and management guidelines relevance to the geriatric practice. *J Am Geriatric* Soc 2003;51:123-6.
2. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. *N Engl J Med* 1999;341:577-85.
3. Li y, Wang L, Schuschke DA, Zhou Z, Saari J, Kang Y. Marginal dietary copper restriction induces cardiomyopathy in animals. *J Nut* 2005;135:2130-6.
4. Schilsky ML. Diagnosis and treatment of Wilson's disease. *Pediatr Transplant* 2002;6:15-9.
5. Turski ML, Thiele DJ, Drosophila Ctr1A. Functions as a copper transporter essential for development. *Biol Chem* 2007;282(33):2417-26.
6. Klevay LM. Heart failure improvement from a supplement containing copper. *Eur Heart J* 2006;27(1):117-8.
7. Allen KG, Klevay LM. Copper: an antioxidant nutrient for cardiovascular health.*Curr Opin Lipid* 1994;5:22-8.
8. Milne DB. Trace elements. In: Bruits CA, Ashood ER, Teitz, editors. Fundamentals of clinical chemistry. 15th ed. Philadelphia: W.B. Saunders Company; 2001. p. 568-84.
9. Reinhold JG. Trace elements: a selective survey. *Clin Chem* 1975; 21(4):476-500.
10. Dunlap WM, James GW, Hume DM. Anemia and neutropenia caused by copper deficiency. *Am Inter Med* 1974;80(4):470-6.
11. Turnund JR. Copper. In: Shills ME, Olson JA, Shike M, Ross AC, editors.

Modern nutrition in health and disease. 9th ed. Philadelphia: Walters Kluwer Company;1999. p. 241-52.

12. Abdel –Mageed AB, Ocheme FW. The effect of various dietary zinc concentrations on biological interactions of zinc, copper and iron in rats. *Biol Trace Elem Res* 1991;29:239-55.
13. Ramadurai J, Shapiro C, Kozloff M, Telfer M. Zinc abuse and sideroblastic anemia. *Am J Hematol* 1993;42:227-28.
14. Henriksen K, Karsdal M, Delaisse JM, Telfer M. Rankle and vascular endothelial growth factor (VEGF) induce orthoclase chemotaxis through an ERK1/2-dependent mechanism. *J Biol Chem* 2003;278:48745- 53.
15. Matsumoto Y, Tanaka K, Hirata G, Hanad M, Masuda S, Shuto T. Possible involvement of pathway vascular endothelial growth factor- Flt -1-focal adhesion kinase in chemotaxis and cell proliferation of orthoclase precursor cells in arthritis joints. *J Immunol* 2002;168:5824-311.
16. William DM. Copper deficiency in humans. *Semin Heamatol* 1983;0(2):118-28.
17. Medeiros DM, Liao Z, Hamlin RL. Copper deficiency in genetically hypertensive cardiomyopathic rat: electrocardiogram, functional and ultra structural aspects. *Am J Nut* 1990;121(7):1026-34
18. Klevay LM. Cardiovascular disease from copper deficiency. *J Nut* 2000;130:489-99.
19. Taylor A. Trace elements in human disease. Clinic in Endocrinology Metabol. London: W.B. Sunders Company, 1985; 4(3):518-728.
20. Milne DB. Laboratory assessment of trace element and minerals status. In: Bog den JD, Klevay LM. Clinical nutrition of the essential trace elements and minerals. Totowa: Human Press; 2000. p. 69-91.
21. Sinha SN, Gabrieli ER. Serum copper and zinc levels in various pathological conditions. *Am J Clin Pathol* 1970;54:270-577.
22. AL-Sulevany BK. Plasma zinc and copper in iron deficiency anemia in pregnancy [Ph.D. thesis]. Mosul, Iraq: Mosul Univ.; 1996.
23. ACC/AHA task force on practice guidelines. Guidelines for evaluation and management of heart failure. *JAM Coll Cardio* 1995;25:1376-98.
24. Milner BA, Whiteside PJ. Introduction to atomic absorption spectrophotometer. England: Pye – Unicam LTD; 1984.
25. Harris M, Taylor G. Medical statistics made easy. USA: Martin Duntiz; 2004.
26. Klevay LM. Diets deficient in copper and zinc? *Med Hypothesis* 1979; 3:1323-526.
27. Elkoubi P. Copper. *J Chir* (Paris) 1989;125(4):248-57.
28. Heller LJ, Mhrman DE, Prohaska JR. Decreased passive stiffness of cardiac myocytes and cardiac tissue from copper deficient rat's hearts. *Am J Physiol Heart Circ Physiol* 2000;278:H1890.
29. Medeiros DM, Bagby D, Oveck G,

McCormick R. Myofibrillar, mitochondrial and valvular morphological alterations in cardiac hypertrophy among copper deficient rats. *J Nutr* 1991;121:815-24.

30. Huang W, Chih –Chia L, Wang Y, Askari A, Klevany LM, Chiu TH. Altered expression of cardiac Na/K ATPase isoforms in copper deficient rates. Cardivasc Res 1995;29:563-68.
31. Borg TK, Klevag LM, Gay RT, Siegal R, Bergin ME. Alteration of the connective tissue network of striated muscles in copper deficient rats. *J Mol Cell Cardiol* 1985;17(12):1173-83.
32. WHO. Trace elements in human nutrition and health. Geneva: WHO; 1996.
33. Mirvis DM, Goldberger AL. Electrocardiography. In: Zorab R, Grey L, Reilly E, Ostroff A. Heart disease: a textbook of cardiovascular medicine. 6th ed. Philadelphia:W.B.Sunders Company; 2001. p. 82-126 .
34. Hamed AA. Assement of certain trace elements (zinc and copper) and minerals (magnesium and calcium) in patients with angina pectoris compared to those of myocardial infarction in Mosul [Ph.D thesis]. Mosul, Iraq: Mosul Univ.; 2006.

پوختە

سەنگاندنا ئاستێ کەرەستێ سفری دناڤ خوینێ دا ل نەخوشێن تووشی لاوازیا دلی یا دوم دریژ بووین

پێشەکی: لاوازیا دلی یا دوم دریژ دهێتە بەرنیاس کو ئێک ژ نەخوشییێن دلی ییێن سەرەکی نە کو بەربەلاڤبوونا زێدە یا هەی، و دهێتە پیشبینکرن کو دێ بێتە ئێک ژ ئەگەرێن سەرەکی ییێن نەخوشیێ و مرنێ ل ئایندەی دا. چەند ڤەکولینا دیارکریە گرنکیا هندەك کەرەستا ل نەخوشییێن دلی و دناڤ ئەڤان کەرەستان کەرەستێ سفری کو دهێتە هژمارتن فاکتەرەکێ (antioxidant) ب هێز.

ئارمانج: لێ گەریانا ریژا سفری دناڤ خوێنا نەخوشێن کو لاوازیا دلی یا دوم دریژ هەی بەرەواركرن دگەل چەند کەسێن ئەڤ نەخوشییە نەی و هەروەسا دیارکرنا کا چێ پەیوەندی دناڤبەرا سفری و لاوازیا دلی یا دوم دریژ هەیە.

ریکێن ڤەکولینێ: ڤەکولینا زنجیرەکا حالەتا هاتە کرن لسەر 53 نەخوشا (37 ژ رەگەزێ نێر و 16 ژ رەگەزێ مێ) کو تووشی لاوازیا دلی یا دوم دریژ بووین. تێکرای ژیێ نەخوشا 52.23 ± 13.1 سال بوو کو ب شێوێ کورانە هاتینە رەوانەکرن بو قاتێ هناڤا و یەکا ڤەژاندنا دلی ل نەخوشخانا ابن سینا ل موسل ژ تەممووزا 2006 تا کانونا ئێکێ 2006. هەروەسا 32 کەس وەك کونترول هاتنە وەرگرتن (18 ژ رەگەزێ نێر و 14 ژ رەگەزێ مێ) کو تێکرای ژیێ وان 41.31 ± 14.72 سال بوو. ریژا سفری دناڤ خوینێ دا هاتە پیڤان ل نەخوشییێن تووشی لاوازیا دلی یا دوم دریژ بووین و هەروەسا ل گروپێ کونترول.

ئەنجام: هاتە دیارکرن کو نەخوشا کێم بوونا پتر یا هەی ل ئاستێ سفری دناڤ خوینێ دا ($p < 0.001$) بەرەواركرن دگەل کونترولا. هەروەسا ئەنجاما دیارکر کو چێ جیاوازییێن گرنک نەبوون ل ریژا سفری دناڤ خوینا دناڤبەرا رەگەزێ نێر و مێ دا ل نەخوشێن کو تووشی لاوازیا دلی یا دوم دریژ بووین ($p < 0.05$).

دەرئەنجام و پشنیار: لاوازیا دلی یا دوم دریژ نەخوشییەکا کو ژ گەلەك فاکتەرا چێت بیت. گەلەك فاکتەر هاتینە دیارکرن کو ئەگەرێ ڤێ نەخوشیێ نە. کێم بوونا ریژا سفری دناڤ خوینێ دا دبیت ئێک ژ ئەگەرا بیت و دبیت ژ بەر بلندبوونا فیشارا خوینێ دا و کاتیکرنێ لسەر چێبوونا شانێن جورارو جور و بلند بوونا ریژا کولترولێ دناڤ خوینێ دا.

پیڤانا ریژا سفری دناڤ خوینێ دا دبیت بهێتە هژمارتن ئێک ژ پشکنینێن مڤا بن بو سەنگاندنا نەخوشێن تووشی لاوازیا دلی یا دوم دریژ بن و وەرگرتنا سفری د ریبا دەڤی دبیت رولەك هەبیت و چارەسەرییێ دا.

الخلاصة

تقييم مستوى النحاس في مصل مرضى عجز القلب المزمن

الخلفية: عجز القلب المزمن يعتبر واحداً من أهم الأمراض القلبية الوعائية والأكثر انتشاراً في السنوات الأخيرة ومن المتوقع أن يكون عجز القلب المزمن السبب الرئيسي لزيادة نسبة الوفيات في المستقبل. البحوث الحديثة وضحت أهمية قسم من المعادن النادرة ودورها في النشوء المرضى للاضطرابات القلبية الوعائية. بين هذه المعادن هو عنصر النحاس الذي يعتبر أقوى مانع للتأكسد .

الأهداف: تهدف الدراسة إلى اختبار مستوى النحاس في أمصال المرضى المصابين بعجز القلب المزمن مقارنة مع الأصحاء و لمعرفة فيما إذا كان هناك علاقة بين مستوى النحاس وعجز القلب المزمن.
دراسة سلسلة من الحالات.

المرضى وطرق البحث: أجريت دراسة سلسلة حالات حيث شملت الدراسة 53 مريضا (37 ذكراو16 أنثى) مصابين بعجز القلب المزمن ، معدل أعمارهم 52.23± 13.1 سنة واللذين تم اختيارهم عشوائيا من المرضى الراقدين في الردهات الباطنية ووحدة العناية القلبية لمستشفى ابن سينا التعليمي في الموصل للفترة من تموز2006 إلى كانون الأول 2006. كما شملت الدراسة 32 شخصا من الأصحاء المتطوعين كمجموعة سيطرة معدل اعمارهم41.31± 14.72 سنة. تم قياس مستوى النحاس في المصل لدى المرضى والمجموعة الضابطة.

النتائج: أظهرت نتائج هذا البحث بصورة واضحة نقصا معنويا (احتمالية <0.001) في مستوى النحاس في مرضى عجز القلب المزمن مقارنة مع الأصحاء. كما أظهرت النتائج عدم وجود تأثير معنوي للجنس على تركيز النحاس في مصل المرضى المصابين بعجز القلب المزمن (احتمالية>0.05).

الاستنتاج و التوصيات: هناك العديد من العوامل التي تؤدي إلى عجز القلب المزمن وربما يكون انخفاض مستوى النحاس في المصل واحدا من تلك العوامل المساهمة وذلك بارتفاع ضغط الدم ، خلل في تكوين أنسجة الجسم المختلفة وتحفيز ارتفاع مستوى الكولسترول في المصل.
يجب قياس مستوى النحاس في المصل كفحص اضافى لتقييم المرضى المصابين بعجز القلب المزمن وإعطاء عنصر النحاس عن طريق الفم كعلاج للمرضى المصابين.

BIOLOGICAL AND ANALYTICAL VARIATION OF SERUM LIPID PROFILE

WAAD-ALLAH S. MULA-ABED, MBChB, MSc, FRCPath *,
SABA K. SALEH, BVM, MSc**

Submitted 10 December 2007; accepted 21 April 2008

ABSTRACT

Background Dyslipidaemia is a major risk factor for coronary heart disease which can be assessed by measuring serum lipid profile. Biological variation has an important effect on the interpretation of all laboratory investigations, including lipid profile.
Objectives To define the biological and analytical components of variation for the different parameters of serum lipid profile.
Methods The present study was conducted in Mosul City in northern Iraq, from 1st February to 30th April 2004. Fasting venous blood was collected from each of 10 apparently healthy volunteers (6 men and 4 women, aged 22-40 years), at 8-10 am following an overnight fast, at intervals of one week for 10 weeks. Sera were separated and stored frozen, in duplicate. Measurement and calculation of the different components of serum lipid profile were made including: triglycerides (TG), total cholesterol, HDL-C, LDL-C and ratios of total cholesterol: HDL-C, LDL-C: HDL-C and TG: HDL-C.
Results The intra-individual (CV_I) and inter-individual (CV_G) variation were 21% and 37% for TG, 7.5% and 16.7% for total cholesterol, 11.2% and 24.5% for HDL-C, 13.7% and 28.3% for LDL-C, 13.1% and 25.4% for total cholesterol: HDL-C, 25.9% and 34.7% for LDL-C: HDL-C, and 27.2% and 40.7% for TG: HDL-C respectively. The indices of individuality, as reflected by CV_I/CV_G, for these parameters were all <1.0 (0.57 for TG, 0.45 for total cholesterol, 0.46 for HDL-C, 0.48 for LDL-C, 0.52 for total cholesterol: HDL-C, 0.95 for LDL-C: HDL-C and 0.85 for TG: HDL-C). The analytical goals for imprecision, as reflected by analytical variation (CV_A), was 6.3% for TG, 4.0% for total cholesterol, 5.2% for HDL-C, 7.8% for LDL-C, 5.8% for total cholesterol: HDL-C, 5.7% for LDL-C: HDL-C and 5.9% for TG: HDL-C. The critical difference calculated as $2.77(CV_A^2 + CV_I^2)^{1/2}$ was 60.7% for TG, 23.5% for total cholesterol, 34.2% for HDL-C, 43.6% for LDL-C, 39.7% for total cholesterol: HDL-C, 73.3% for LDL-C: HDL-C and 97.5% for TG: HDL-C.
Conclusion The biological and analytical components of variation showed marked individuality. This together with the index of individuality supports the limited usefulness of using the conventional population-based reference range for interpretive criteria. The critical differences also confirm that single determination of lipid profile may have limited value in screening purpose.

DMJ 2008;2(1): 41-53.

Key words: Lipid profile, Biological variation, Analytical variation

Lipoproteins are spherical particles that are made up of hundreds of lipid and protein molecules where triglycerides and cholesterol ester (non-polar) comprise the

***** *Senior Consultant and Head, Department of Chemical Pathology, Royal Hospital, PO Box 1331, Seeb 111, Muscat, Sultanate of Oman.*
***Assistant Lecturer, Department of Biochemistry, Mosul College of Medicine, Mosul, Iraq*
Correspondence author: *Waad-Allah S. Mula-Abed, Department of Chemical Pathology, Royal Hospital, PO Box 1331, Seeb 111, Muscat, Sultanate of Oman. E.mail: drsharef@hotmail.com*

core of the lipoprotein, while phospholipids, small quantity of free unesterified cholesterol and apoproteins (polar) occupy the surface of the lipoprotein. There are many types of apoproteins that are present in the lipoprotein, of which apo A1, B, C and E are the most important. The lipoproteins are classified by their density into five main classes: Chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).[1]

Coronary heart disease (CHD) constitutes one of the main health problems, representing the leading most common disease and hospital-based mortality.[2] Extensive medical research has identified hyperlipidaemia as a major risk factor for heart disease with an established clinical correlation between hyperlipidaemia and the incidence of CHD.[3] The risk factors for CHD include, other than hyperlipidaemia or dyslipidaemia,[4] family history,[5] age and sex,[6] hypertension,[7] diabetes mellitus and insulin resistance,[8] obesity,[9] lack of physical exercise,[10] and cigarette smoking.[11]

There are certain factors that may affect lipid measurement. These include pre-analytical factors such as fasting,[12] posture,[13] sample processing and sample type.[14] Intra-individual factors include diet,[15] obesity,[9] exercise,[16] smoking,[11] and alcohol consumption.[17] All these factors, pre-analytical and intra-individual, which vary within the same individual as well as between individuals, will result in a proportion of intra-individual and inter-individual variation.[18] In addition, an analytical variation is associated with the different components of lipid profile which is added to the individual variation.

The aim of the current study was to define the biological (intra-individual and inter-individual) and analytical components of variation for serum lipid profile in a sample of 10 apparently healthy individuals.

PARTICIPANTS, MATERIALS AND METHODS

The present study was conducted in Mosul City in northern Iraq, during from 1st February to 30th April 2004. Fasting venous blood was collected at 8-10 am following an overnight fast, from each of 10 apparently healthy volunteers (6 men and 4 women, aged 22-40 years). The specimens were collected at intervals of 1 week during 10 weeks period. To minimize pre-analytical variation, the same phlebotomist collected the blood specimens. Also, to minimize the analytical variation, all specimens from each individual were assayed in a single batch, using the same lots of reagents. Sera were separated and stored frozen until analysis, in duplicate. Measurements of serum lipid components were made and in addition, a number of indices for certain lipid parameters were calculated or derived from the measured values.[19, 20] The analytical work was performed in the Department of Clinical Biochemistry, College of Medicine, University of Mosul, Iraq, including:

1. Measured parameters:

a. Triglycerides (TG).
b. Total cholesterol.
c. HDL-cholesterol.

2. Derived parameters:
a. LDL-cholesterol.
b. Non-HDL-cholesterol.
c. Total cholesterol**:** HDL-cholesterol.
d. LDL-cholesterol**:** HDL-cholesterol.
e. TG**:** HDL-cholesterol.

Serum TG and total cholesterol were measured by enzymatic methods[21] using kits from bioMerieux (France). Serum HDL.C was measured following the precipitation of the apoprotein B containing chylomicrons and lipoproteins of VLDL and LDL by phosphotungstic acid in the presence of magnesium ions.[22] The supernatant obtained after centrifugation that contains HDL was determined using the cholesterol enzymatic reagents from bioMerieux (France). Serum LDL-C is calculated by the Friedwald formula,[23] using total cholesterol, HDL-C and TG values; whereby:

LDL-C (mg/dl) = total cholesterol – HDL-C – (TG × 0.2)

Or, LDL-C (mmol/L) = total cholesterol – HDL-C – (TG × 0.455)

Serum non HDL-C is calculated by subtracting HDL-C value from total cholesterol value as recommended by the NCEP III[19]. Certain indictors or ratios of lipid profile parameters are calculated by dividing the corresponding value of lipid components. This includes total cholesterol: HDL-C (so-called atherogenic index), LDL-C: HDL-C, and TG: HDL-C. All these biochemical analyses were performed in the Clinical Chemistry Laboratory, Department of Biochemistry, College of Medicine, University of Mosul, Iraq.

Standard statistical methods were used for the analysis of data.[24] The mean and SD were calculated for each parameter of serum lipid profile from each participant. The data were inspected for any outlier (defined as values outside ±3 SD from the mean). There was no outlier and all results were within the mean ± 2 SD. The duplicate data were then analysed for variance. The total variance was dissected into analytical (CV_A), biological intra-individual (CV_I) and biological inter-individual (CV_G) components. The index of individuality (CV_I/CV_G) and critical difference ($2.77\ (CV_A^2 + CV_I^2)^{1/2}$ were also calculated.[25]

RESULTS

The individual duplicate results (with their means) of serum lipid profile each week for 10 weeks and for the 10 healthy volunteers are presented in table 1 and figures 1-5. The mean and SD for each parameter in each individual and the CV were calculated for the 10 weeks and this represents the intra-individual variation (CV_I). The overall variation of each parameter of lipid profile involving all individuals represents the inter-individual variation (CV_G). The analytical variation (CV_A) for each parameter was calculated from the differences in the duplicate results. The individuality index (CV_I/CV_G) and critical difference [$2.77\ (CV_A^2 + CV_I^2)^{1/2}$] were also calculated and presented in table 2.

The CV_I and CV_G are 21% and 37% for TG, 7.5% and 16.7% for total cholesterol, 11.2% and 24.5% for HDL-C, 13.7% and 28.3% for LDL-C, 13.1% and 25.4% for the total cholesterol: HDL-C, 25.9% and 34.7% for LDL-C:HDL-C, and 27.2% and 40.7% for TG: HDL-C respectively. The indices of individuality are 0.57 for TG, 0.45 for total cholesterol, 0.46 for HDL-C, 0.48 for LDL-C, 0.52 for total cholesterol: HDL-C, 0.95 for LDL-C: HDL-C and 0.85 for TG: HDL-C. The analytical goals for imprecision, as reflected by analytical variation (CV_A), are 6.3% for TG, 4% for total cholesterol, 5.2% for HDL-C, 7.8% for LDL-C, 5.8% for total cholesterol: HDL-C, 5.7% for LDL-C: HDL-C and 5.9% for TG: HDL-C. The, changes required for the critical differences to be significant ($p<0.05$), are **:** 60.7% for TG, 23.5% for total cholesterol, 34.2% for HDL-C, 43.6% for LDL-C, 39.7% for total cholesterol: HDL-C, 73.4% for LDL-C: HDL-C and 97.5% for TG: HDL-C.

Table 1. Values of serum lipid profile presented as mean ± SD for serial 10 weeks intervals for 10 healthy individuals

Subject	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TC: HDL-C	LDL-C: HDL-C	TG: HDL-C
1	180.8 ± 30.7	142.5 ± 6.4	40.5 ± 2.67	65.9 ± 9.5	3.52 ± 0.21	1.63±0.26	4.48±0.83
2	73.4 ± 14.4	144.7 ± 9.1	53.5 ± 4.53	76.3 ± 9.1	2.72 ± 0.28	1.44±0.24	1.39±0.34
3	136.3 ± 30.1	207.9 ±12.1	38.4 ± 3.48	142.3 ± 12.1	5.4 ± 0.35	3.72±0.33	3.58±0.33
4	118.9 ± 25.4	216.2 ± 8.8	52.5 ± 4.55	139.9 ± 8.8	4.14 ± 0.30	2.68±0.29	2.28±0.49
5	157.7 ± 35.8	149.7 ± 8.1	33.6 ± 3.41	84.4 ± 10.9	4.47 ± 0.37	2.53±0.39	4.74±1.17
6	73.5 ± 32.8	169.8 ±22.6	39.4 ± 7.14	115.4 ± 22.3	4.4 ± 0.79	3.0±0.79	1.87±0.80
7	152.1 ± 21.2	155.6 ± 5.2	29.25 ±5.43	95.6 ± 10.2	5.47 ± 0.92	3.39±0.76	5.37±1.30
8	195.1 ± 31.1	155.3 ±11.0	27.85 ±3.54	88.4 ± 10.7	5.65 ± 0.79	3.22±0.58	7.15±1.64
9	86.4 ± 19.0	156.6 ± 9.7	41.7 ± 5.00	98.6 ± 9.8	3.81 ± 0.51	2.40±0.39	2.14±0.69
10	126.6 ± 26.4	182.5 ±14.3	51.1 ± 4.35	101.9 ± 15.6	3.54 ± 0.39	2.0±0.38	2.47±0.44

Table 2. Mean ± SD of serum lipid profile with calculated components of variations and derived indices (analytical, intraindividual, interindividual, index of individuality and critical differences)

Analyte	Mean ± SD	Analytical Variation CV_A (%)	Intra Individual Variation CV_I (%)	Inter Individual Variation CV_G (%)	Index of Individuality (CV_I/CV_G)	Critical Difference (%) $2.77(CV_A^2+CV_I^2)^{½}$
Triglyceride (mg/dl)	130.1 ± 48.6	6.3	21.0	37.0	0.57	60.7
Cholesterol (mg/dl)	168.1 ± 27.2	4.0	7.5	16.7	0.45	23.5
HDL-C (mg/dl)	40.8 ± 9.8	5.2	11.2	24.5	0.46	34.2
LDL-C (mg/dl)	100.9 ± 26.9	7.8	13.7	28.3	0.48	43.6
TC:HDL-C	4.3 ± 1.06	5.8	13.1	25.4	0.52	39.7
LDLC:HDLC	2.61±0.85	5.7	25.9	27.2	0.95	73.4
TG:HDLC	3.55±1.98	5.9	34.7	40.7	0.85	97.5

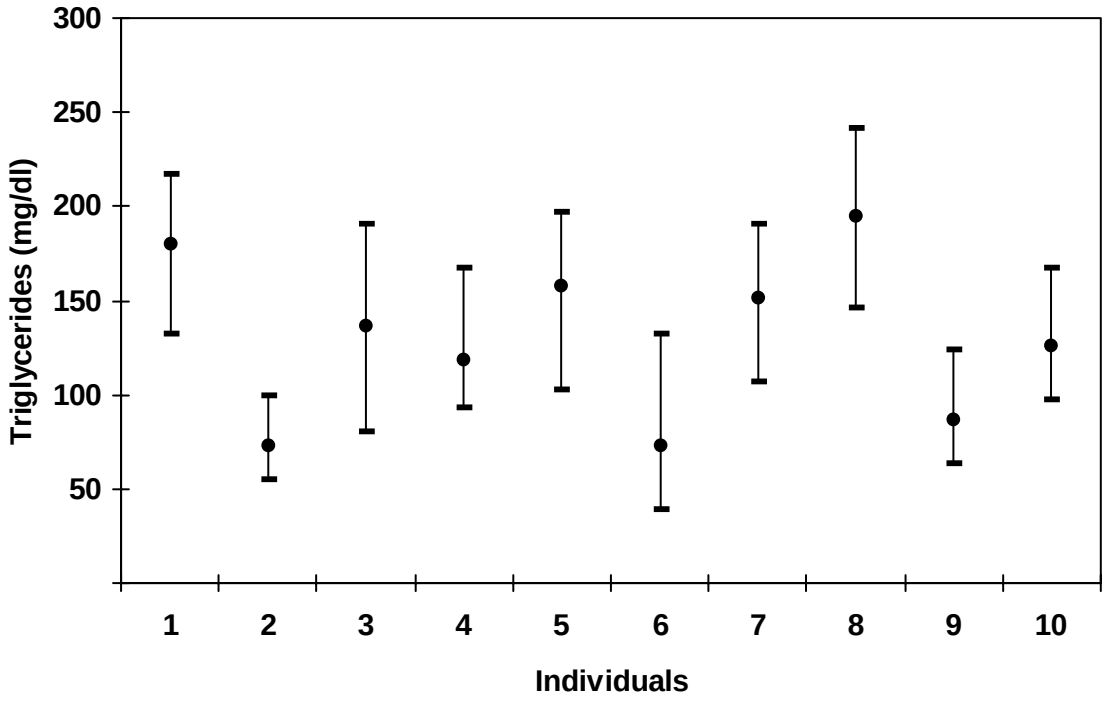


Figure 1. Mean and range for serum triglycerides in 10 healthy individuals

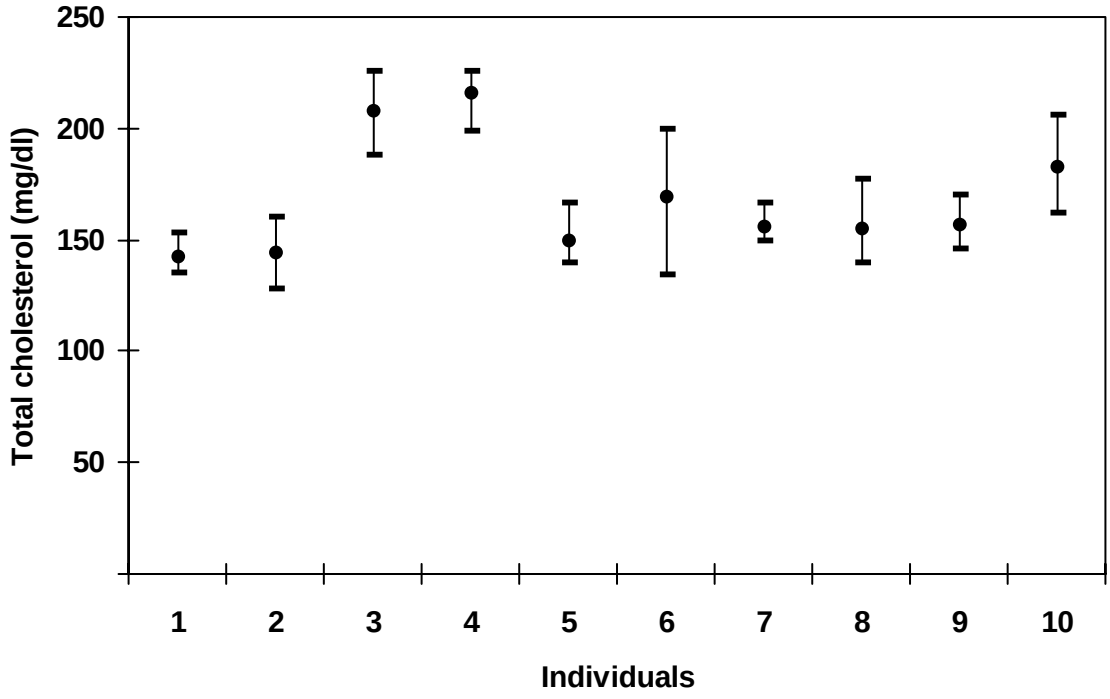


Figure 2. Mean and range for serum total cholesterol in 10 healthy individuals

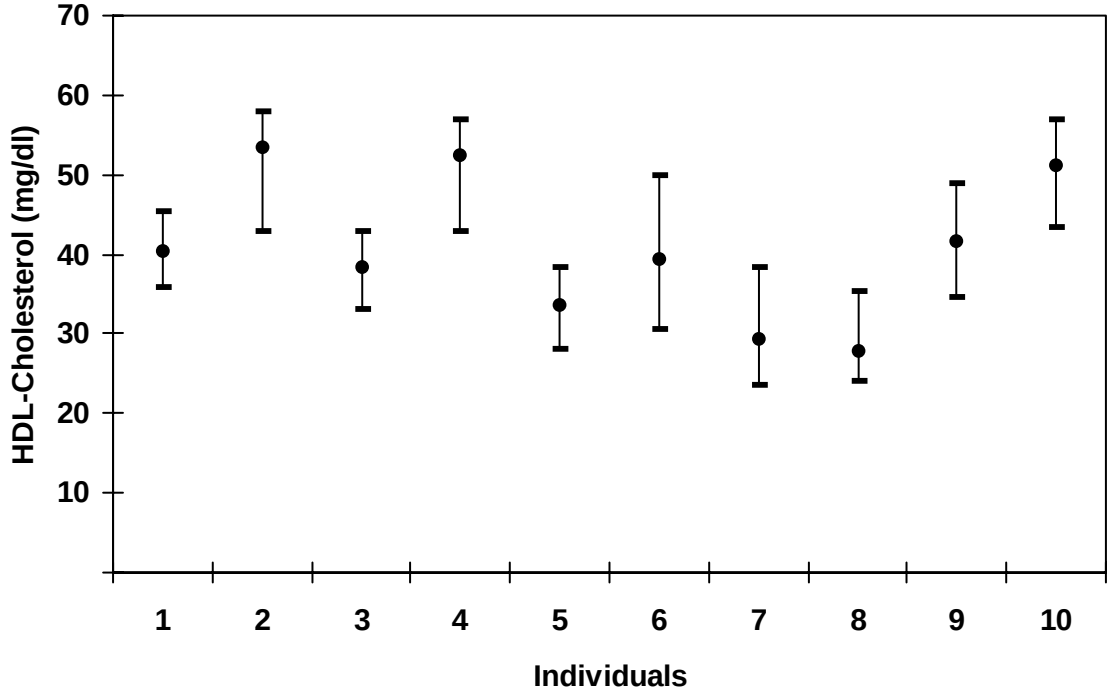


Figure 3. Mean and range for serum HDL-cholesterol in 10 healthy individuals

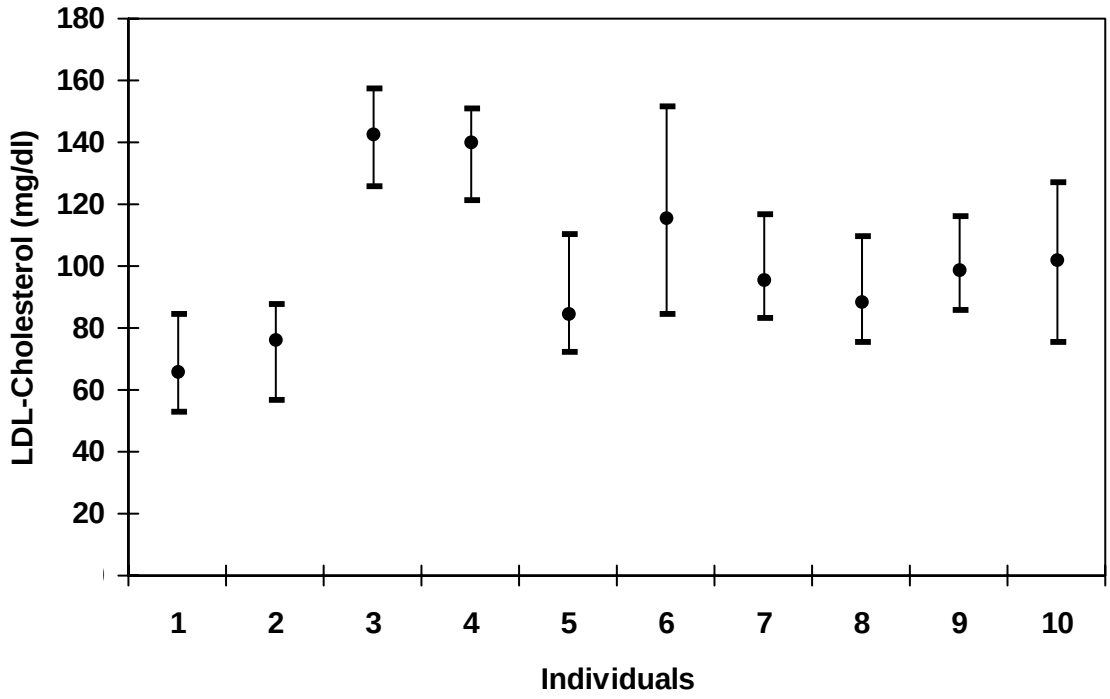


Figure 4. Mean and range for serum LDL-cholesterol in 10 healthy individuals

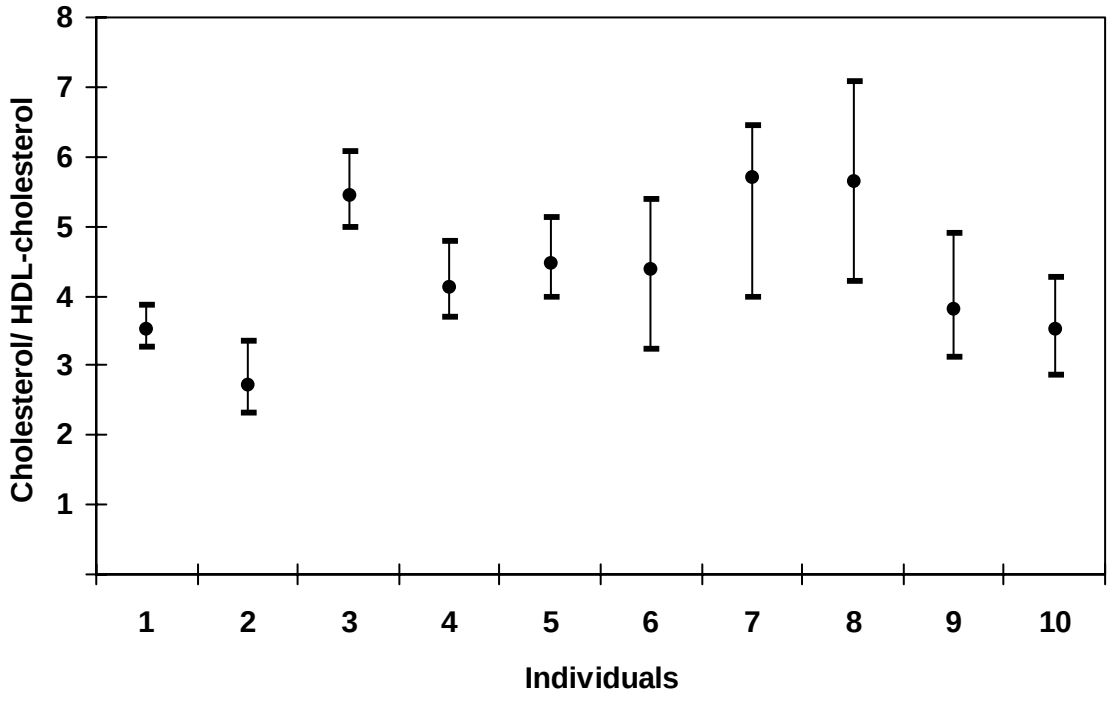


Figure 5. Mean and range for serum cholesterol: HDL-cholesterol in 10 healthy individuals

DISCUSSION

There are several risk factors for CHD including, among other risk factors, dyslipidaemia in form of high LDL-C, low HDL-C and high triglycerides levels. These lipid abnormalities can be modified, hence identifying and correcting them can reduce the risk.[3,4] Biological variation has an important effect on the interpretation of different laboratory investigations, including lipid profile.[18] There is also a growing interest in evaluating the cut-off limits for the desirable thresholds of serum cholesterol (total, LDL and HDL) and triglycerides according to different recommendations and clinical trials, with the trend is always towards lowering these limits.[19, 26]

The current study is an attempt to study the biological variation in serum lipid profile in a set of ten healthy volunteer individuals. The intra-individual and inter-individual variation are 21% and 37% for TG, 7.5% and 16.7% for total cholesterol, 11.2% and 24.5% for HDL-C, 13.7% and 28.3% for LDL-C, 13.1% and 25.4% for total cholesterol: HDL-C, 25.9% and 34.7% for LDL-C: HDL-C, and 27.2% and 40.7% for TG: HDL-C respectively. In comparison with other studies, the range for intra-individual variation was 17.8-22.3% for TG, 5.0-8.2% for cholesterol, 7.1-10% for HDL-C and 7.8-13.6% for LDL-C as reported by others[27,28] which are in agreement with the values observed in our study. Also, Ford[25] had reported an intra-individual and inter-individual variation of 4.9% and 17.3% for cholesterol and 5.5% and 27.2% for HDL-C respectively. An individual day-to-day variability of total cholesterol of 5%, TG of 20%, LDL-C of 8% and HDL -C of 10% was reported by Bookstein *et al*.[29] This range of variability in serum lipid profile within and between individuals may raise the attention to use single measurement if total cholesterol is < 185 mg/dl (4.8 mmol/L), between 215-225 mg/dl (5.5-5.8 mmol/L) or above 255 mg/dl (6.6 mmol/L) and if LDL-C at around 116 mg/dl (3 mmol/L). However, values near the NCEP cut-off points may require repeated measurement as also recommended by Bookstein *et al*.[29] The reported intra-individual variation within 1 year showed a range of change of 12.9-40.8% for TG, 3.9-10.9% for cholesterol and 3.6-12.4% for HDL-C.[30]

In the present study, the indices of individuality, calculated as the ratio CV_I/CV_G[31] are 0.57 for TG, 0.45 for total cholesterol, 0.46 for HDL-C, 0.48 for LDL-C, 0.52 for total cholesterol: HDL-C, 0.95 for LDL-C: HDL-C and 0.85 for TG: HDL-C. Harris[32] stated that, if the index of individuality is <0.6, then the use of the traditional population-based reference range is of little value and may be misleading. For these components of lipid profile, the reference ranges, therefore, may have little value in the interpretation of the data. Hence, it has long been recommended not to derive reference intervals for total cholesterol and lipoprotein cholesterol fractions. Subjects with cholesterol concentrations within the reference intervals may still be associated with an increased risk of CHD. Accordingly, the appropriate desirable

levels of serum lipid profile have been recommended by a variety of international or national health authorities or societies including the British Hyperlipidaemia Association and the American NCEP, for risk assessment. On the other hand, if the index of individuality is > 1.4, then the population-based reference ranges are recommended to be derived for the interpretation of the results. [32] This index value was not achieved by any component of lipid profile in our study. The analytical goals for imprecision, as reflected by analytical variation, were acceptable for the examined analytes. The CV_A for TG was 6.3%, total cholesterol 4%, HDL-C 5.2%, LDL-C 7.8% and total cholesterol: HDL-C 5.8%. The acceptability of CV_A is indicated when the valve is less than or equal to one-half the intra-individual variation.[33] Lower CV_A can be obtained when automated methods for the determination of serum lipid profile are used.

The changes required for the difference to be significant ($p<0.05$) can be calculated as the critical difference as $2.77(CV_A^2 + CV_I^2)^{1/2}$. The critical differences for TG, total cholesterol, HDL-C, LDL-C and total cholesterol: HDL-C was 60.7%, 23.5%, 34.2%, 43.6% and 39.7% respectively. This critical difference is important in decision making when assessing changes in results for monitoring treatment. This difference is attributed to analytical performance of the assay as well as the intra-individual variation of the parameter within the subject. While the latter is being an unavoidable and unadjustable variable which is a reflection of the biological variation in human, the former can be controlled if efforts are continuously concentrated to minimize it. The analytical goal is always to minimize the analytical bias which is easily achieved in automated procedures. The wide ranges can be decreased by improving analytical goals particularly through the use of automated methods. Repeated specimen collection and analysis particularly in individuals with borderline values can reduce the confidence limits of the results and improve their validity.

In conclusion: The biological and analytical components of variation for serum lipid profile showed marked individuality. This together with the index of individuality supports the limited usefulness of using the conventional traditional population-based reference range. The critical differences also confirm that single determination of lipid profile may have limited value for screening purposes. A wider study including bigger sample of participants is recommended for further implementation and extrapolation of results.

REFERENCES

1. Rifai N, Bachorik PS, Albers JJ. Lipid, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B.Saunders Company; 1999. p. 809-860.
2. Carroll K, Majeed A, Firth C, Gray J. Prevalence and management of coronary heart disease in primary care: population-based cross-sectional study

using a disease register. J Public Health Med 2003; 25(1): 29-35.
3. American Heart Association. Heart and stroke facts. New York: American Heart Association; 1991.
4. Maniosy ET. Health and nutrition education in primary schools of Crete: change in chronic disease risk factors following a 6-years intervention programme. Br J Nutr 2002;88(3): 315-24.
5. Hhatnagar D. Diagnosis and screening for familial hypercholesterolaemia: finding the patients, finding the genes. Ann Clin Biochem 2006;43(6):441-56.
6. Jousilabti P, Vartiainen E, Tuomilehto J, Puska P. Sex, Age, cardiovascular risk factors, and coronary heart disease. Circulation 1999;99:1165-72.
7. Wilson PWF, Castelli WP, Kannel WB. Coronary risk prediction in adults (Framingham Heart Study). Am J Cardiol 1987;59:91-4.
8. Grundy SM. Small low density lipoprotein, atherogenic dyslipidemia, and metabolic syndrome. Circulation 1997;95: 1-4.
9. Eckel RR. Obesity and heart disease. Circulation 1997;96:3248-50.
10. Mayer-Davis EJ, D'Agostino RD Jr, Karter AJ. Intensity and amount of physical activity in relation to insulin sensitivity, the insulin resistance: atherosclerosis study. JAMA 1998; 279(9):669-74.
11. Zieske AW, Takei H, Fallon KB, Strong JP. Smoking and atherosclerosis in youth. Atherosclerosis 1999;144(2):403-8.
12. Cohn JS, McNamara JR, Cohn SD, Ordoras JM, Schaefer EJ. Post prandial plasma lipoprotein changes in human subjects of different ages. J Lipid Res 1988;29:469-79.
13. Miller M, Bachorik PS, Cloey TA. Normal variation of plasma lipoprotein: postural effects on plasma concentration of lipid, lipoprotein and apolipoprotein. Clin Chem 1992;38:569-74.
14. Cloey T, Bachorik PS, Becker D, Finney C, Lowry D, Sigmund W. Re-evaluation of serum plasma differences in total individual concentration. JAMA 1990;263:2788-9.
15. Grundy SM, Denke MA. Dietary influences on serum lipid and lipoprotein. J Lipid Res 1990;31:1149-72.
16. Marti B, Suter E, Riesen WF, Tschoppa Wanner HU, Gutzweller. Effects of long term self-monitored exercise on the serum lipoprotein and apolipoprotein profile in middle age men. Atherosclerosis 1990;81:19-31.
17. Superko HR. Effects of acute and chronic alcohol consumption on post prandial lipemia in healthy normotriglyceridemic men. Am J Cardiol 1992;69:71-4.
18. Fraser CG. Biological variation: From principles to practice. 1st ed. Washington: AACC Press; 2001.
19. Warnick GR, Myers GL, Cooper GR, Rifai N. Impact of the third cholesterol report from the adult treatment panel of the National Cholesterol Education Program on the clinical laboratory. Clin Chem 2002;48:11-7.
20. Wood D, Durrington P, Poulter N.

Joint British recommendations on the prevention of coronary heart disease in clinical practice. Heart 1998;80(Suppl 2):S1-S29.

21. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase coupled method for the colormetric determination of serum triglycerides. Clin Chem 1983;29:538-42.
22. Lopes VMF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high density lipoprotein separated by three different methods. Clin Chem 1977;23:882-4.
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without Use of the ultracentrifuge. Clin Chem 1972;18:449-552.
24. Armitage P, Berry G, Matthews JNS. Statistical methods in medical research. 4th ed. Oxford: Blackwell Scientific Science; 2002.
25. Ford RP. Essential data derived from biological variation for establishment and use of lipid analyses. Ann Clin Biochem 1989;26:281-5.
26. Laker MF. Cardiovascular disease prevention: the new joint British Societies guidelines'. Ann Clin Biochem 2006; 43(5):335-9
27. Kafonek SD, Derby CA, Bachorik PS. Biological variability of lipoproteins and apolipoproteins in patients referred to a lipid clinic. Clin Chem 1992; 38:864-7.
28. Brown SA, Boerwinkle E, Kashanian FK, Swanson N, Patsch W. Variation in concentrations of lipids, lipoprotein lipids and apolipoproteins A1 and B in plasma from healthy women. Clin Chem 1990;36:207-10.
29. Bookstein L, Gidding SS, Donovan M, Smith FA. Day to day variability of serum cholesterol, triglycerides and high density lipoprotein cholesterol levels. Impact on the assessment of risk according to the National Cholesterol Education Program guidelines. Arch Intern Med 1990;150(8):1583-5.
30. Demacker PNM, Schade RWB, Jansen RTP, Von't Laar A. Intra-individual variation of serum cholesterol, triglycerides and high density lipoprotein cholesterol in normal humans. Atherosclerosis 1982;45;255-66.
31. Costongs GMPJ, Janson PCW, Bas MB. Short term and long term intra-individual variations and critical differences of chemical laboratory parameters. J Clin Chem Clin Biochem 1985;23:7-16.
32. Harris EK. Statistical aspects of reference values in clinical pathology. Prog Clin Pathol 1981;8:45-66.
33. Fraser CG. Desirable performance standards for clinical chemistry tests. Adv Clin Chem 1983;23;299-339.

پوخته

جیاوازییێن بایولوجی و شلوفەیی ییێن چەوراتی دناڤ خوینێ دا

پێشەکی: تێکدانێن چەوراتی دناڤ خوینێ دا دهێتە هژمارتن ژ فاکتەرێن مەترسیێ بو توشبوونێ ب نەخوشییێن دلی ییێن ئەکلیلی. جیاوازییێن بایولوجی کاتیکرنێ دکەتە لسەر ریژا چەوراتی دناڤ خوینێ دا و دڤێت لڤێ چەندێ یێ هشیار بیت.

ئارمانج: دیارکرنا پێکهاتییێن جیاوازییێن بایولوجی و شلوڤەیی ییێن ریژا چەوراتی یێ دناڤ خوینێ دا.

ڕێکێن ڤەکولینێ: ئەڤ ڤەکولینە هاتە کرن ژ 2004/2/1 تا 2004/4/30. نموونە هاتە وەرگرتن ژ خوینا وەریدی ژ هەر دەھ پێشبەرا ئەوێن چێ نەخوشی نەبوون (6 نێر 4 مێ د ژیێ 22-40 سالی دا) و ب شێوێ حەفتیانە و بو ماوێ 10 حەفتیا. نموونە هاتنە وەرگرتن ژ سەعەت 8-10 بەری نیڤرو پشتی روژی گرتنێ. ریژا چەوراتی دناڤ خوینێ دا هاتە پێڤان کو پێکهاتی بوو ژ ترایگلسیراید، کولسترول، کولسترولێ یێ گەلەك تیراتی و یێ کێم تیراتی. هەروەسا ریژێن کولسترول:کولسترولێ یێ گەلەك تیراتی، کولسترولێ یێ کێم تیراتی:کولسترولێ یێ گەلەك تیراتی، ترایگلسیراید: کولسترولێ یێ گەلەك تیراتی هاتە وەرگرتن.

ئەنجام: ریژێن جیاوازییێن بایولوجی دناڤ و دناڤبەرا کەسان دا 21٪ و 37٪ بو ترایگلسیراید، 7.5٪ و 16.7٪ بو کولسترول، 11.2٪ و 24.5٪ بو کولسترولێ یێ گەلەك تیراتی، 13.7٪ و 28.3٪ بو کولسترولێ یێ کێم تیراتی.13.1٪ و 25.4٪ بو ریژا کولسترول:کولسترولێ یێ گەلەك تیراتی لدویف ئێک. دەلائیلێن جیاوازییێن شلوڤەیی بو پێکهاتییێن چەوراتی دناڤ خوینێ دا تا رادەکی مەقبوول بوون بو نموونە ریژا وێ 6.3٪ بو ترایگلسیراید، 4٪ بو کولسترول، 5.2٪ بو کولسترولێ یێ گەلەك تیراتی، 7.8٪ بو کولسترولێ یێ کێم تیراتی و 5.8٪ بو ریژا کولسترول:کولسترولێ یێ گەلەك تیراتی. جیاوازی یا کریتیکال یا باوەرپیکەر لسەر جیاوازییێن بایولوجی دناڤ کەسان دا و جیاوازییێن شلوڤەیی 60.7٪ بو ترایگلسیراید، 23.5٪ بو کولسترول، 34.2٪ بو کولسترولێ یێ گەلەك تیراتی، 43.6٪ بو کولسترولێ یێ کێم تیراتی و 39.7٪ بو ریژا کولسترول:کولسترولێ یێ گەلەك تیراتی.

دەرئەنجام: ڤەکولینێ ئاشکراکر کو پێکهاتییێن جیاوازییێن بایولوجی و شلوڤەیی ییێن ریژا چەوراتی یێ دناڤ خوینێ دا جەندین جیاوازی ییێن هەین دناڤبەرا کەسان دا و ئەڤە ژی پەسەندکەت کێم مفایا ب کارئینانا پیڤەرێن ییێن تەقلیدی و جیاوازیا ییێن کریتکال دوباتکر کو پشکنینێن تاك ییێن چەوراتی یێ دناڤ خوینێ دا مفایەکێ کێم یێ هەی د ڤەکولینێن سەرژمیری دا.

الخلاصة

التباين البايولوجي والتحليلى لشحوم مصل الدم

خلفية البحث: يعتبر اضطراب شحوم الدم من عوامل الاختطار للاصابة بمرض القلب الاكليلي، وقد يتأثر قياس شحوم مصل الدم، شأنها شأن الفحوصات المختبرية الاخرى، بالتباين البايولوجي الذي يجب الانتباه اليه عند تفسير النتائج.
الأهداف: تعريف مكونات التباين البايولوجي والتحليلى للقيم المختلفه لشحوم الدم
المنهجية: أجريت الدراسة خلال الفترة من 2/1 لغاية 2004/4/30، تم اخذعينة دم وريدى من كل من المتطوعين الاصحاء العشرة (6 ذكور و4 إناث بأعمار 22-40 سنة) أسبوعيا ولمدة عشرة أسابيع. جمعت نماذج الدم فى الساعه 8-10 ق. ظ في حالة الصوم من كافة المتطوعين في الدراسة. تم فصل المصول وحفظت مجمده بشكل مزدوج لحين اجراء التحليل. اجرى قياس وحساب المكونات المختلفه لشحوم مصل الدم التي شملت ثلاثي الغليسريد، الكولسترول الكلي، الكولسترول رفيع الكثافة، الكولسترول خفيض الكثافة، وكذلك نسب الكولسترول/ الكولسترول رفيع الكثافة، الكولسترول خفيض الكثافة / الكولسترول رفيع الكثافة و ثلاثي الغليسريد/الكولسترول رفيع الكثافة.
النتائج: كانت قيم الاختلاف البايولوجي داخل وبين الأفراد 21% و 37% لثلاثي الغليسريد، 7.5% و 16.7% للكولسترول الكلي، 11.2% و 24.5% للكولسترول رفيع الكثافة، 13.7% و 28.3% للكولسترول خفيض الكثافة، و13.1% و 25.4% لنسبة الكولسترول\الكولسترول رفيع الكثافة على التوالى. أما نسبة الاختلاف داخل الفرد / الاختلاف بين الأفراد "عامل الفردية" لهذه القيم فكانت اقل من 0.6 مما يقلل من أهمية اعتماد المجالات المرجعية التقليدية وربما يعطي استخدامها دلائل خاطئة عند الرجوع إليها في تفسير النتائج. كانت دلائل الاختلاف التحليلي لمكونات شحوم الدم مقبولة حيث كانت قيمها 6.3% لثلاثي الغليسريد، 4% للكولسترول الكلي، 5.2% للكولسترول رفيع الكثافة، 7.8% للكولسترول خفيض الكثافة، و5.8% لنسبة الكولسترول:الكولسترول رفيع الكثافة. أما الفروقات الحرجة المعتمدة على الاختلاف البايولوجي داخل الفرد والاختلاف التحليلي فكانت 60.7% لثلاثي الغليسريد، 23.5% للكولسترول الكلي، 34.2% للكولسترول رفيع الكثافة، 43.6% للكولسترول خفيض الكثافة، و39.7% لنسبة الكولسترول\الكولسترول رفيع الكثافة.
الاستنتاج: أظهرت الدراسة أن لمكونات التباين البايولوجي والتحليلي لشحوم مصل الدم فوارق واضحة بين الأفراد مما يؤيد محدودية فائدة استخدام المجالات المرجعية التقليدية وان الفروقات الحرجة أيدت بأن الفحوصات المفرده لشحوم الدم ذات دلالة محدودة في الدراسات المسحية.

IDENTIFICATION OF CANCER STEM CELLS IN PAEDIATRIC BRAIN TUMOUR GLIOMAS

RAMADHAN T. OTHMAN, MBChB, MSc*,
DEEMA HUSSEIN, BSc, PhD **
BETH COYLE, BSc, PhD ***

Submitted 2 January 2008; accepted 21 April 2008

ABSTRACT

Background Brain tumours are the leading cause of cancer mortality in children and remain difficult to cure despite advances in surgery and adjuvant therapy.
Objective To identify cancer stem-like cells in established cell lines from three paediatric brain tumour (PBT) gliomas.
Setting Queen's Medical Centre/ Nottingham city/ UK.
Methodology Three glioma cell lines were studied including one high grade glioblastoma multiforme (BT4), one well differentiated oligodendroglioma (Olig1), and one recurrent ependymoma (EPN1). A control cell line of mouse neural stem cells (C17.2) was also included for comparison.
Results Established tumour cell lines maintain stem cell marker (nestin and Sox2) expression when grown as monolayers in 15% foetal bovine serum/Dulbecco's modified Eagle's medium. Cells derived from these cell lines are able to form neurospheres when cultured in serum-free stem cell media containing basic fibroblast growth factor and human epidermal growth factor. These neurospheres are self-renewable and re-form new neurospheres when dissociated and cultured in fresh medium supplemented with growth factors. The percentages of neural stem cell marker CD133 positive cells were determined by flow cytometry analysis of neurospheres from the three cultured cell lines, BT4 47.2% ± 10.5, EPN1 40.4% ± 8.9 and Olig1 48.7% ± 2.9 (mean ± standard error). Under conditions promoting differentiation, cells derived from neurospheres were multipotent giving rise to neurons, astrocytes, and oligodendrocytes, at different levels for each tumour cell line.
Conclusion Paediatric gliomas contain cancer stem-like cells that are able to self-renew and differentiate into three neural lineages

DMJ 2008;2(1): 54-70.

Key words: Paediatric brain tumour, Cancer stem cells, CD133

Tumours of central nervous system are the most common solid tumours in children. Brain tumours are considered the most common cause of cancer related death in children.[1, 2] They are a diverse group of tumours represented by about thirteen histological types.[3] The majority of brain tumours develop from glial cells.

**Oncology Department, Azadi Teaching Hospital, Dohuk city, Dohuk, Kurdistan region, Iraq*
***Lecturer, Human Development, Division of Child Health, Nottingham University Medical School*
**** Human Development, Division of Child Health, Nottingham University Medical School*
Correspondence author: *Ramadhan T. Othman, Oncology Department, Azadi Teaching Hospital, Dohuk city, Dohuk, Kurdistan region, Iraq. Email: ramadhan.othman@yahoo.com*

The most common glial brain tumours are astrocytomas (52%) they are usually arising in the cerebral hemispheres. They cover a wide spectrum of degrees of malignancy, ranging from the often slow growing pilocytic astrocytoma to the highly malignant glioblastoma multiforme (GBM), but low grade tumour predominate.[4] Ependymoma represents approximately 9% of childhood brain tumours, and originate from the wall of the ventricular system along the entire craniospinal axis.[5] Oligodendrogliomas account for 5-10% of paediatric brain tumours (PBTs), the frontal lobes are involved most frequently followed by parietal and temporal.

Most PBTs are treated by surgery and radiotherapy. The role of chemotherapy in the management of these tumours has become important in the last few decades.[6] Despite new advances in brain tumour therapy, treatment related morbidity and mortality remain high.[7] Therefore further studies are required to better understand the biology of brain tumours and to determine the key cells in the tumour population that maintain tumour growth. This will give us insight into the mechanism of tumourigenesis and will allow us to trace back the cell of origin in normal brain, hence providing a possible target for a future treatment.

It has been demonstrated that cells with stem-like characteristics can be isolated from different types of brain tumours that could explain the origin of these tumours. CD133, a marker of normal neural precursors, has been used for the enrichment of cancer stem-like cells from brain tumours.[8-11] Moreover, Singh *et al* demonstrated that intracranial injection of 100 CD133 positive cells from PBTs was sufficient to initiate tumour in the non-obese diabetic, severe combined immunodeficient mouse brain. The resultant tumour could be serially transplanted and had properties similar to the patient's original tumour, whereas injection of 10^5 CD133 negative cells failed to instigate tumour.[12] These observations showed that these cells have the key characteristics of stem cells, and most importantly they have cancer-initiating capacity as would be expected of brain tumour stem cells (BTSCs). These findings raise the possibility that these cancer stem cells could be the cause of brain tumour initiation and maintenance.

In the current study, we addressed the issue of whether newly established glial tumour cell lines contain cells with features similar to NSCs. Cells were isolated and characterized from three well-characterized subtypes of PBT gliomas.

METHODS AND MATERIALS

CULTURE OF PRIMARY BRAIN TUMOURS. Brain tumour samples were obtained from the Queen's Medical Centre as approved by the Local Research Ethics Committee. Tumour cells that had been successfully used to establish a cell line were grown in Dulbecco's modified Eagle's medium DMEM/L-glutamine medium (Sigma), supplemented with 15% foetal calf serum (FCS) (Invitrogen). The cell lines were maintained in standard humidified 5% CO_2-air incubator at 37 ºC.

Cells were grown as a monolayer attached to the base of 75cm² flasks and were harvested using trypsin/ Ethylene diamine tetracetic acid (EDTA) and split (1:20) every 3-4 days into fresh medium. The control mouse neural stem cell line (C17.2) was cultured in 15%FCS/DMEM media supplemented with 5% horse serum (Invitrogen); 2mM glutamine (Gibco); 15000 unit/15gm penicillin/ streptomycin (Invitrogen); 750 µg of Fungizone (Invitrogen); and 150 mg Gentamycin (Invitrogen).

NEUROSPHERE CULTURE. Tumour cells grown as a monolayer were washed with Hank's Balanced Salt Solution (HBSS) (Sigma), dissociated and resuspended into serum-free stem cell media (SCM): DMEM high glucose (Sigma); 23% of Ham's F-12 solution (Invitrogen); 2% B27; and 5ng/ml heparin (Sigma). SCM was supplemented with human recombinant epidermal growth factor (hEGF) (20ng/ml; Invitrogen), and human basic fibroblast growth factor (bFGF) (20ng/ml; BD Bioscience) to promote neurosphere growth. Primary neurospheres were washed with HBSS and dissociated either by TrypLE Select (Gibco) or mechanically by glass pipette into single cells and reseeded (1:2) in 75cm² flasks in 10 ml volume of SCM supplemented with growth factors.

FLOWCYTOMETRY ANALYSIS OF CD133 EXPRESSION. Neurospheres were dissociated into a single cell suspension, and resuspended in 100 ml calcium free phosphate buffered saline (PBS) containing 0.5% bovine serum albumin and 4 mM of (EDTA). Non specific binding was blocked using 10 µl of human FCR blocking reagent (IgG; Miltenyi Biotec). 10 µl of either PBS or mouse anti-CD133-PE (phycoerythrin conjugated antibody; Miltenyi Biotec) was added, and cells incubated in the dark for 10 minutes at 2-5 ºC. CD133 staining was analyzed using a Coulter Epics Altra flow cytometer. Data analyses were carried out using FlowJo program version 7.1.1. These experiments were performed three times, and the mean and standard error (SE) were calculated.

DIFFERENTIATION ASSAY OF TUMOUR NEUROSPHERES. Neurospheres were washed with HBSS, and dissociated into a single-cell suspension. These cells were then plated in chamber slides at a concentration of 1×10^2cell/ml. Differentiation of neurospheres was induced by plating cells in 8 well chamber slides in various differentiation conditions and incubating for 7 days. In each case dissociated neurospheres were plated in SCM supplemented with 3% of FCS in the absence of hEGF and bFGF. Cells were either plated in SCM+3% FCS in chambers pre-coated with laminin (5ng/ml; Sigma) or fibronectin (4ng/ml; Sigma), or in SCM+3% FCS supplemented with LIF (20ng/ml; Chemicon), PDGF (10ng/ml; Sigma), or RA (100ng/ml; Sigma). Tumour cell lines grown as monolayers were also plated on chamber slides at a density of 1×10^3cell/ml in DMEM/FCS media and incubated for three days.

IMMUNOCYTOCHEMICAL STAINING OF DIFFERENTIATED

NEUROSPHERES AND MONOLAYERS. After seven days (three days for monolayers), cells were fixed with 4% paraformaldehyde for 10-15 minutes at room temperature. Non-specific binding was blocked and cells were permeabilized by incubating them for 1 hour at room temperature in 5% normal goat serum (NGS) (Invitrogen), and 0.25% Triton X-100 in PBS. Cells were then incubated with primary antibody prepared in 2% NGS and 0.1% Triton X-100 in PBS overnight at 4 ºC. The following antibodies were used: rabbit anti-Ki-67 (1:200; Lab Vision); mouse anti-nestin (1:50; BD Bioscience); mouse anti-Sox2 (1:50; R&D); mouse anti-CNPase (1:500; Sigma); rabbit anti-MAP2 (1:750; Sigma); and rabbit anti-GFAP (1:200; DAKO). Cells were then washed with PBS, followed by incubation with Alexa Fluor 555 goat anti-rabbit antibody (1:500; Molecular Probe) or Alexa Fluor 488 goat anti-mouse antibody (1:500; Molecular Probe) for 1 hour in dark at room temperature. Finally, chamber slides were washed with PBS and slides were counterstained with 4', 6-diamidino-2-phenylindole (DAPI) to identify all nuclei. Micrographs were obtained using a LEICA DMRM microscope equipped with a Nikon digital camera, and NIS element imaging software (Nikon). Quantification of cells positive for specific marker was carried out by counting 100-300 cells, using Adobe Photoshop software version 8.0 (Adobe).

RESULTS

PBTs grown as a monolayer maintain stem cell marker expression. Three glioma cell lines were studied, one high grade glioblastoma multiforme (BT4), one well differentiated oligodendroglioma (Olig1), and one recurrent ependymoma (EPN1) (Table 1). A control cell line of mouse neural stem cells (C17.2) was also included for comparison. To determine stem cell marker expression, nestin a cytoplasmic intermediate filament protein and Sox2 a transcription factor were used. For identification of differentiated mature brain cells, antibodies raised against the following markers were used. GFAP an intracytoplasmic filamentous protein that is a constituent portion of cytoskeleton was used to detect astrocytes. CNPase a constituent of cells that elaborate myelin in the central and peripheral nervous system was used to detect oligodendrocytes. Microtubule associated proteins are known to play an important role in brain neuron microtubule assembly, so MAP2 was used to identify neurons. Ki67, a prototypic cell cycle related nuclear protein expressed by cells in all phases of the active cell cycle (G1, S, G2 and M phase), was used to detect proliferating cells. Ki67 is absent in resting (G0) cells.

Cells were scored according to specific cell marker expression together with appropriate cell structure. Immunocytochemistry (ICC) was used to compare the molecular stem cell markers expression as well as markers of the three neural lineage differentiated cells for the three PBT cell lines. Cells grown as a monolayer from all three PBT cell lines expressed nestin and Sox2 at different

level, with highest expression found in EPN1, 41% for nestin and 50% for Sox2 (Fig. 1). However, nestin and Sox2 expression was even higher in the C17.2 control cell line 74% and 60%, respectively (Figure 1). Interestingly, the percentages of CNPase positive cells was high (>40%) with little variability among the four tested cell lines (Fig. 1). The percentages of neural marker MAP2 was differential among cell lines as 24% of BT4 cells expressed MAP2, and only 5% of Olig1 showed positive expression. The percentages of GFAP positive astrocytes were low, 8% for C17.2, 9% for EPN1, 13% for BT4, and 4% for Olig1 (Figure1). These results show that cells grown as a monolayer from PBT gliomas contain populations of cells that express NSC and differentiated cell markers.

Table 1. Tumours and patients characteristics

Tumour	Age	Sex	Diagnosis	Site
Olig 1	4 years and 9 months	Female	Oligodendroglioma (grade III)	Right fronto-temporo-parietal lobe
BT4	3 years and 9 months	Female	Giant cell glioblastoma multiforme (grade IV)	Frontal lobe
EPN1	21 years 1st tumour was diagnosed at 14 years of age and treated by surgery and radiotherapy. 2nd recurrence was at 16 years of age and treated by surgery and chemotherapy.	Male	Ependymoma (grade III) (third recurrence)	Cerebral hemisphere

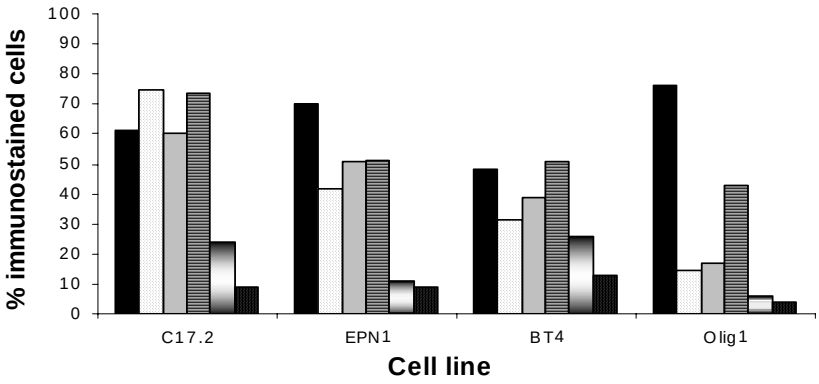


Figure 1. **The percentage of stem cell and differentiation markers detected in different cell lines when grown as a monolayers**. Cells from three PBT (EPN1, BT4, and Olig1), and one control cell line (C17.2) were cultured in serum media for 3 days, and immunostained with anti-Ki67 ( ) as a proliferation marker. nestin ( ), and Sox2 ( ) as stem cell markers. The cells were also stained for lineage-specific marker CNPase/oligodenedrocyte ( ), MAP2/neuron ( ), and GFAP/astrocytes ( ). 100-300 immunopositive cells were counted and present as percentages.

Selection and expansion of neural stem cells that can self-renew from Paediatric gliomas. To assess the presence of neural stem-like cells in human PBTs, cells grown as a monolayer (Figure 2 A, D, and G) were harvested and transferred into serum-free medium supplemented with bFGF and hEGF to promote NSC proliferation. Regardless of the pathological subtype, within three to four days after plating, neurosphere-like colonies appeared in all PBT cell lines (Figure 2 B, E, and H). One critical feature of neural and other stem cells is the ability to self-renew. The capacity of individual cells derived from neurospheres to form new neurospheres was then tested. Primary neurospheres were dissociated into single-cell suspension and reseeded into fresh proliferative medium supplemented with growth factors (bFGF and hEGF), after 24-48 hours new neurospheres were formed (Figure 2 C, F, and I).

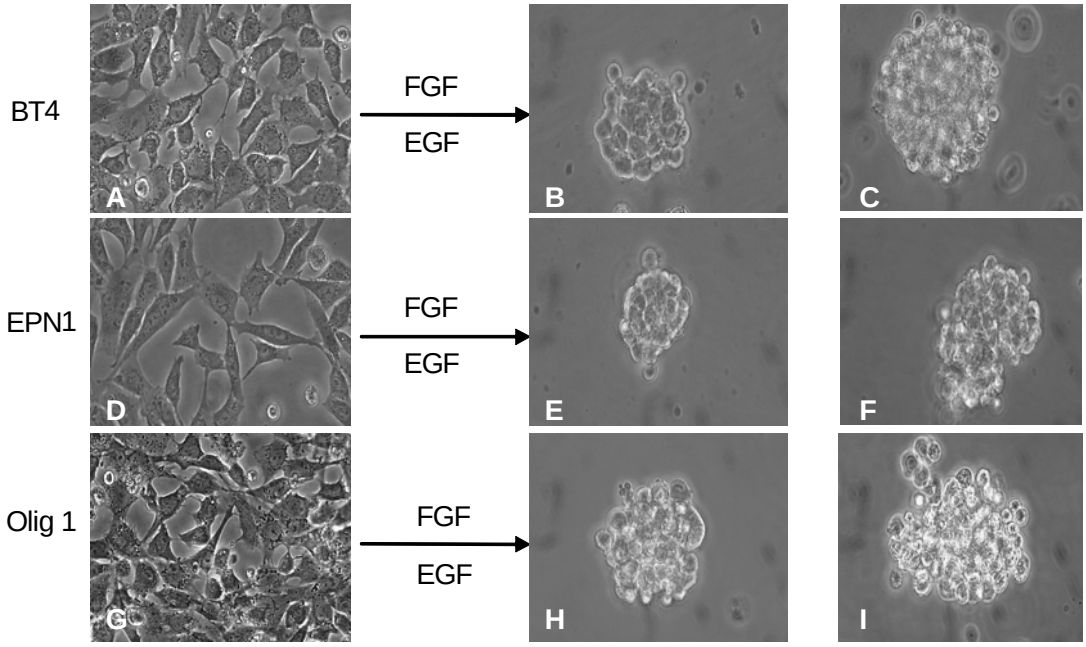


Figure 2. ***In vitro* characterisation of tumour cells.** Tumour cells were cultured in serum media and serum free SCM media supplemented with bFGF and hEGF. Phase photomicrographs of tumour cells cultured in 15% serum/DMEM grown as adherent monolayers: BT4 (A); EPN1 (D); and Olig1 (G). Cells grown as non-adherent neurospheres: BT4 (B), EPN1 (E), and Olig1 (H) in SCM with addition of growth factors. Secondary tumour neurospheres formed 24-48 hours after sub-culturing of dissociated primary neurospheres in fresh medium supplemented with growth factors: BT4 (C), EPN1 (F), and Olig1 (I).

Tumour neurospheres contain cells that express CD133. CD133 is a 120 kDa cell surface glycoprotein originally shown to be a haematopoietic stem cell marker, and recently found to be a marker of normal human neural stem cells. To test whether CD133 positive cells were present in cultured tumour neurospheres, we analysed the expression of CD133 using flow cytometry. Neurospheres were dissociated into a single cell suspension and incubated with anti-CD133, and analysis of cells expressing CD133 was carried out by flow cytometry. It was found that (mean ± SE), 48.7% ± 2.9 of Olig1, 47.2% ± 10.5 of BT4, and 40.4% ± 8.9 of EPN1 cells expressed CD133 (Figure 4A). Figures 3 B, C, and D show typical flow cytometry histograms for stained and unstained neurospheres.

Cells derived from cultured tumour neurospheres are multipotent. Another important feature of neural stem cells is multipotency i.e. the ability to differentiate into neurons, astrocytes, and oligodendrocytes. To test whether brain tumour derived neurospheres had the potential for multilineage differentiation; single cell suspensions were generated from neurospheres and cultured in 3% FBS/SCM on specific surfactants (laminin and fibronectin) or in the presence of specific factors (LIF, PDGF, RA) and incubated for 7 days to allow differentiation. A control neural stem cell line (C17.2) was used which has been previously shown to differentiate under appropriate conditions. Cells were then stained with stem cell and differentiated cell markers as well as a proliferation marker.

In the control cell line (C17.2), stem cell markers were highly expressed in cells cultured in 3%FBS/SCM, 81% for nestin and 56% for Sox2, whereas expression of differentiated cell markers were lower compared to cells growing in differentiated conditions (Figure 4). Cells growing under various differentiation conditions showed reduced expression of stem cell markers (Figure 4 A and C). The stem cell marker Sox2 expression was maintained at a significantly higher level in cells cultured with the addition of LIF (61%) (Figure 4A). Cells growing on either laminin or in the presence of PDGF showed raised expression of the neuronal cell marker MAP2 to 69% and 52%, respectively (Figure 4 A, B, and F). Additionally, the addition of PDGF increases the expression of oligodendrocyte marker CNPase (Figure 4 A and D). Increased expression of GFAP positive astrocytes was observed in both fibronectin and PDGF conditions 37% and 15% respectively (Figure 4 A, B and E). There were too few C17.2 cells (<15 cells) when cultured with the addition of RA to analyse the data (data not shown). These results show that mouse neural stem cells are multipotent, giving rise to cells with neuronal and glial characteristics.

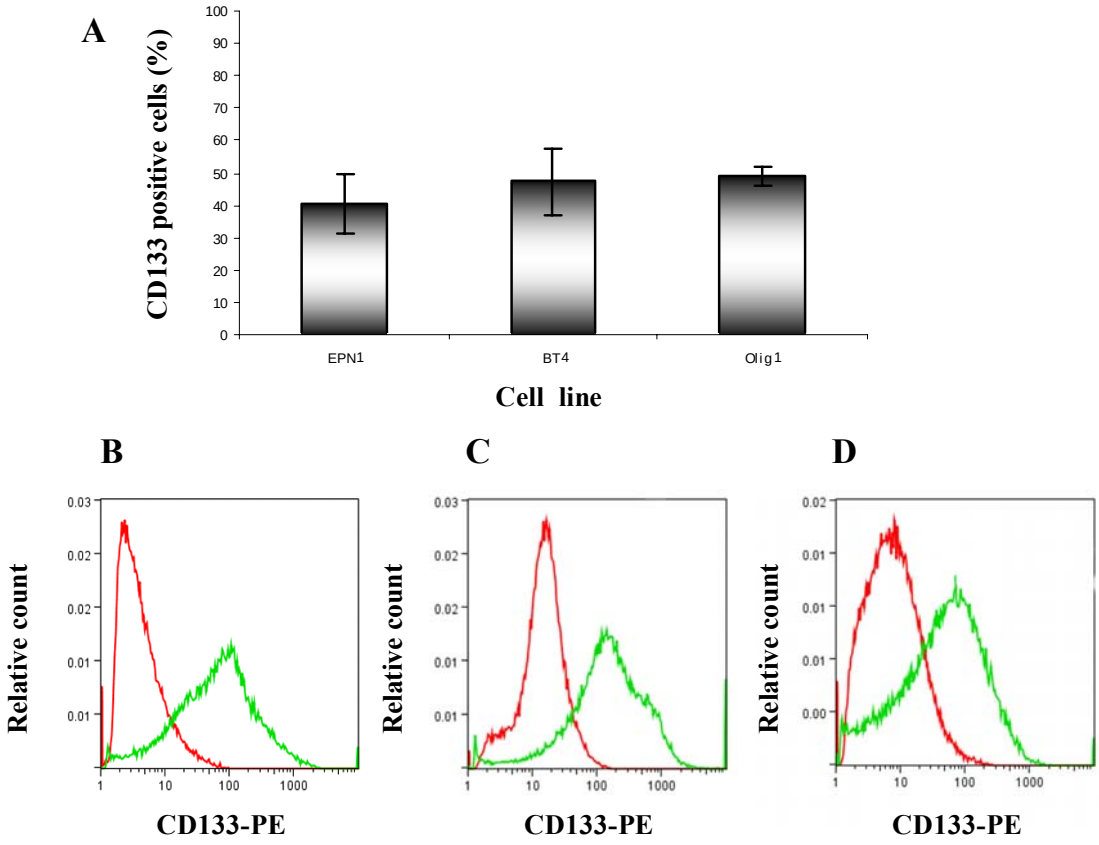


Figure 3. **CD133 protein expression on tumour neurospheres**. Neurospheres were dissociated into single cell suspensions and immunostained for CD133, then subjected to flow cytometry for quantification of CD133 expression. A, CD133 expression in different cell lines. These data represent mean and standard error of triplicate experiments. Flow cytometry histograms from single representative experiment for EPN1 (B), BT4 (C), and Olig1 (D). The green peak represents positive cell staining for CD133, and the red peak represents the unstained population of cells.

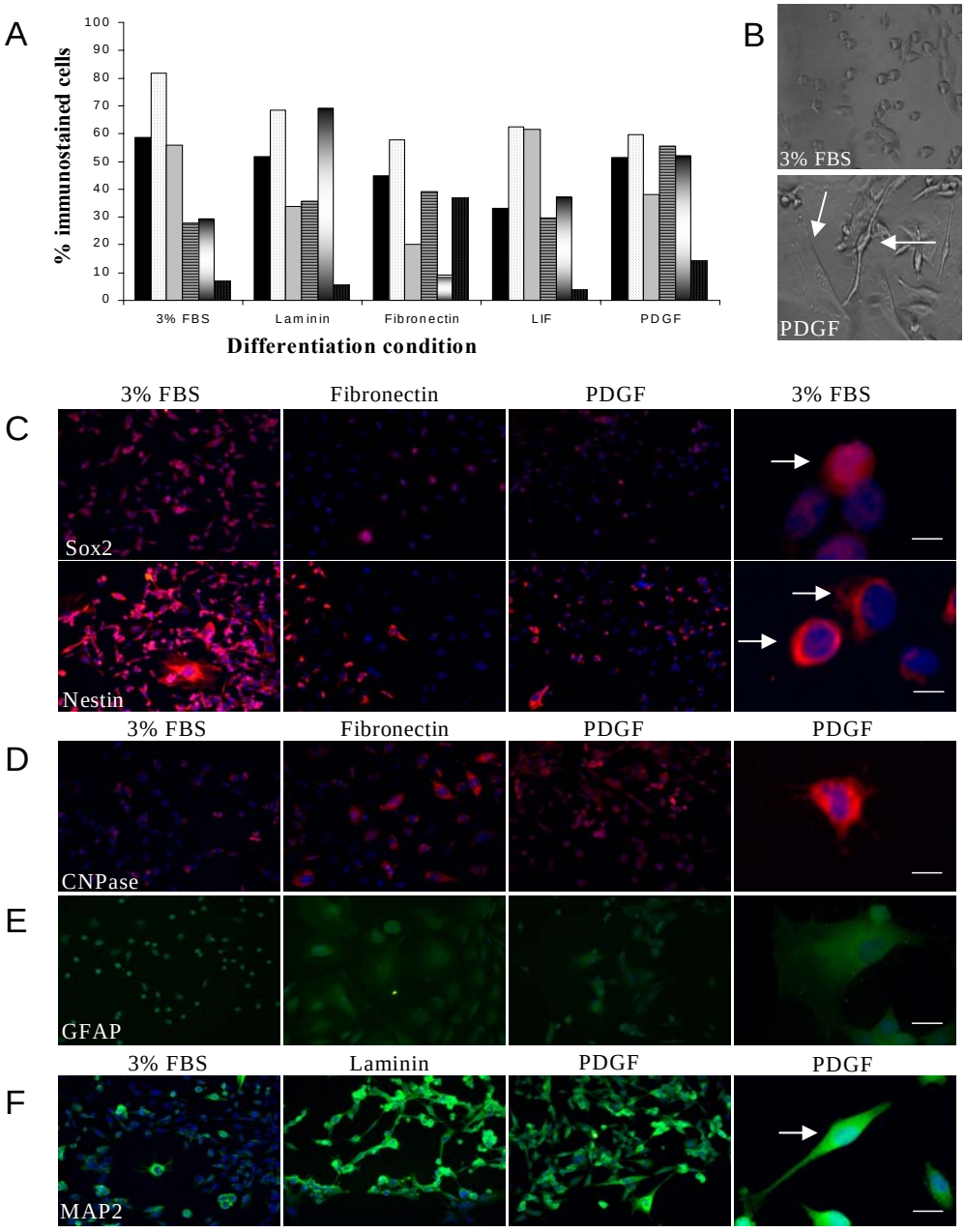


Figure 4. **Cells derived from C17.2 neurospheres are multipotent.** Neurospheres were dissociated into a single cell suspension, and cultured in various conditions that promote differentiation for 7 days. Expression of various markers detected by ICC: Ki67 ( ) a proliferation marker; nestin ( ), and Sox2 ( ) for stem cells. Cells were also stained for lineage-specific marker of oligodendrocytes/CNPase ( ), neurons/MAP2 ( ), and astrocytes/GFAP ( ). Graph (A) shows differences in stem cell and differentiation marker expression under various differentiation conditions. Data presented as percentages which were obtained by counting 100-300 cells. B, shows changes in the structure of cells plated with PDGF. C-F representative images (magnification × 20) of cells where the expression was altered under the condition indicated arrows identify positive cells. The final column in each case shows a high magnification image (magnification × 40) Scale bar 50 µm. All nuclei were counterstained with DAPI (blue) and pictures merged using Adobe Photoshop software. C, NSC markers (nestin and Sox2) are reduced in cells exposed to fibronectin or PDGF. D, plating cells on either fibronectin or with PDGF increases the expression of CNPase. E, culturing cells with fibronectin or PDGF lead to an increased number of GFAP positive astrocytes. F, culturing cells on either laminin or with the addition of PDGF increases the expression of MAP2.

.

For Olig1 derived neurospheres, NSC markers nestin and Sox2 were highly expressed in cells cultured in 3% FBS/SCM, 33% and 63%, respectively (Figure 5 A). Under differentiation conditions NSC markers expression were reduced, with lowest expression found in cells grown on fibronectin pre-coated chamber slides, 7% for nestin and 5% for Sox2 (Figure 5 A and B). Additionally, the percentages of Ki67 positive cells were reduced in cells grown under conditions promote differentiation with lowest percentage found in cells grown on fibronectin (<21%). Cells plated with the addition of LIF and RA showed reduction in stem cell markers, whilst maintaining proliferation (Figure 5A). Exposing cells to laminin, PDGF or retinoic acid, increased the number of MAP2 positive cells, 30%, 38%, and 21 % respectively (Figure 5 A and E).On the other hand exposing cells to either fibronectin or PDGF increased the expression of the oligodendrocyte marker CNPase (Figure 5 A and C). The addition of RA to cells dissociated from Olig1 neurospheres or plating them on fibronectin, enhanced the development of cells morphologically consistent with GFAP positive astrocytes (Figure 5 A and D).

Approximately 45% and 68% of BT4 neurospheres growing in 3% FBS/SCM were positive by ICC for nestin and Sox2, respectively (Figure 6 A and B). Under differentiation conditions, these cells showed reduced NSC markers expression, developed morphology and immunocytochemical staining patterns consistent with cells of glial and neuronal lineages (Figure 6). Ki67 (proliferation) levels remained remarkably high under all conditions tested (>45%) (Fig. 6A). The percentages of MAP2 positive cells increased in cells cultured on either laminin pre-coated chamber slides, or with the addition PDGF or RA, 27%, 28% and 39%, respectively (Figure 6 A and F), whilst the percentages of astrocyte and oligodendroglial cells both increased with the addition of PDGF or plating cells on fibronectin (Figure 6 A, C and D).

EPN1 neurospheres cultured in 3%FBS/SCM contained many cells expressing neural stem cell markers (70% for Sox2 and 44% for nestin) (Figure 7A and B), and relatively few cells expressing neuronal marker MAP2 (6%), oligodendroglial marker CNPase (27%), and cells with astrocytes like structures (7%) (Figure7A). Under differentiation conditions, ICC revealed an increase in the proportion of cells expressing MAP2, CNPase, and generates numerous GFAP positive astrocytes (Figure 7). The percentages of Ki67 positive cells was high (>50%) with little variability among differentiation conditions (Figure 7). Similar to the BT4 cell line, laminin, RA, and PDGF differentiate cells towards the neuronal lineage 22%, 25%, and 24%, respectively (Figure 7 A and F), while fibronectin or PDGF differentiate cells toward astrocyte and oligodendroglial lineages (Figure 7 A and C). Surprisingly, in this cell line LIF seems to maintain high NSC marker expression 71% for Sox2 and 67% for nestin (Figure 7A).

Taken together, these data revealed that PBT gliomas derived neurospheres from the three neural lineages are multipotent, but at different levels under various differentiation conditions.

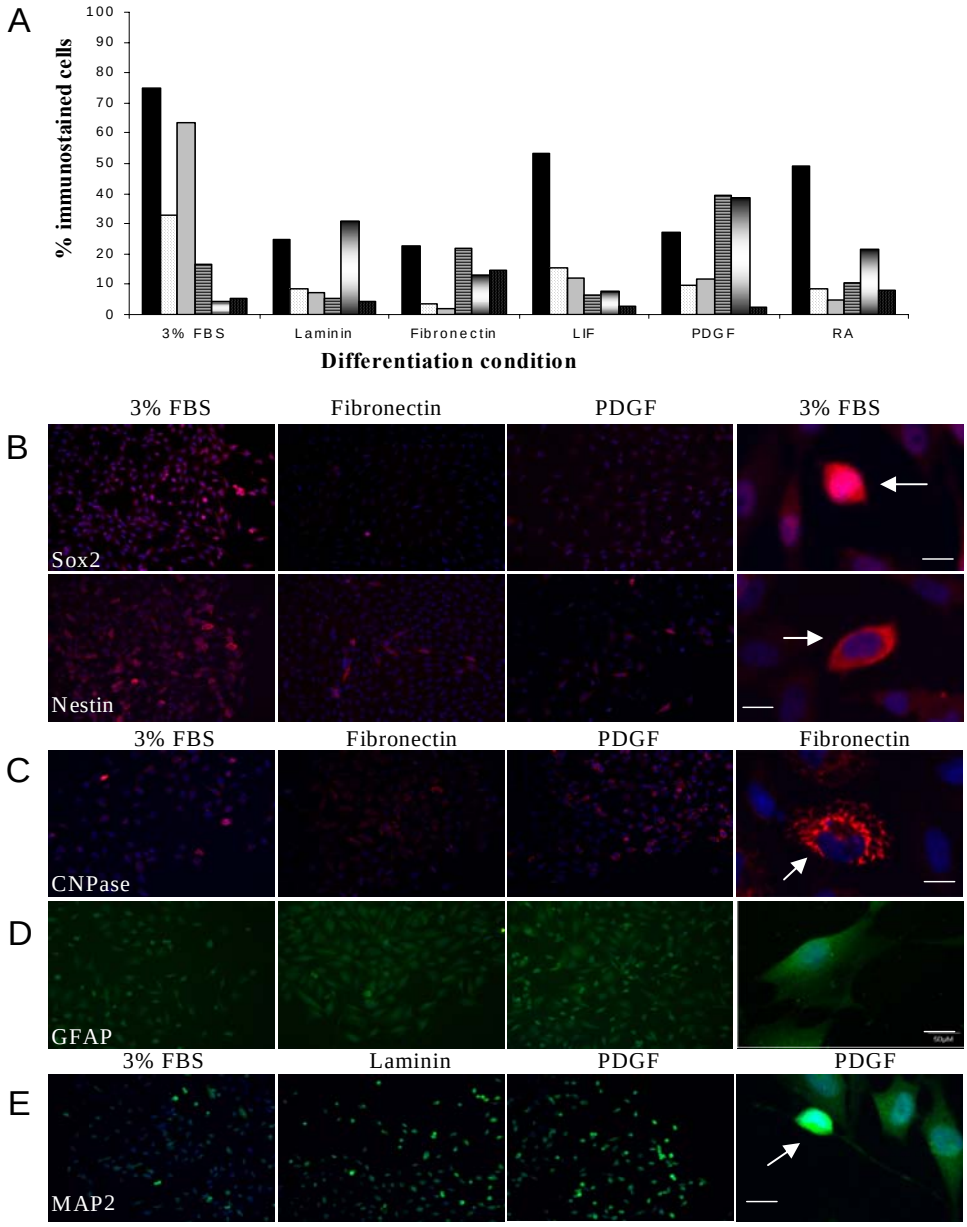


Figure 5. **Cells derived from Olig1 neurospheres are multipotent.** Neurospheres were dissociated into a single cell suspension, and cultured in various conditions that promote differentiation for 7 days. Expression of various markers detected by ICC: Ki67 (■) a proliferation marker; nestin (□), and Sox2 (▭) for stem cells. Cells were also stained for lineage-specific marker of oligodendrocytes/CNPase (▤), neurons/MAP2 (▭), and astrocytes/GFAP (■). Graph (A) shows differences in stem cell and differentiation marker expression under various differentiation conditions. Data presented as percentages which were obtained by counting 100-300 cells. B-E Representative images (magnification × 20) of cells where the expression was altered under the condition indicated, arrows identify positive cells. The final column in each case shows a high magnification image (magnification × 40). Scale bar 50 µm. All nuclei were counterstained with DAPI (blue) and pictures merged using Adobe Photoshop software. B, NSCs markers (nestin and Sox2) are reduced in cells exposed to fibronectin or PDGF. Plating cells on either fibronectin or with PDGF leads to an increased number of CNPase (C) and GFAP positive cells (D). E, culturing cells on either laminin or with addition of PDGF increases the expression of MAP2.

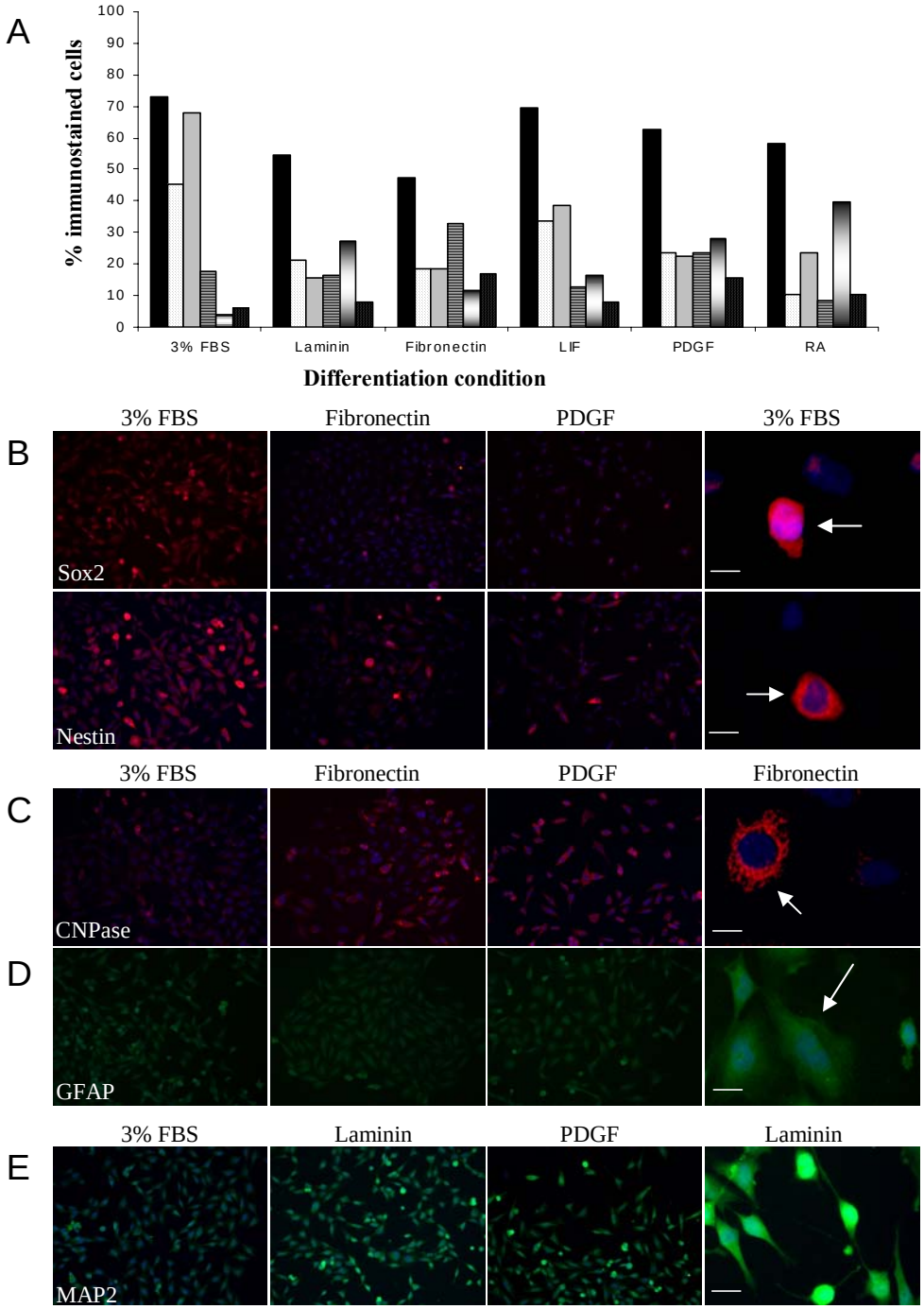


Figure 6. **Cells derived from BT4 neurospheres are multipotent.** Neurospheres were dissociated into a single cell suspension, and cultured in various conditions that promote differentiation for 7 days. Expression of various markers detected by ICC: Ki67 (■) as a proliferation marker; nestin (□), and Sox2 (▭) for stem cells. Cells were also stained for lineage-specific marker of oligodendrocytes/CNPase (▤), neurons/MAP2 (▭), and astrocytes/GFAP (■). Graph (A) shows differences in stem cell and differentiation marker expression in various differentiation conditions. Data presented as percentages which were obtained by counting 100-300 cells. B-E Representative images (magnification × 20) of cells where the expression was altered under the condition indicated, arrows identify positive cells. The final column in each case shows a high magnification image (magnification × 40) Scale bar 50 µm. All nuclei were counterstained with DAPI (blue) and pictures merged using Adobe Photoshop software. B, NSC markers nestin and Sox2 are reduced in cells exposed to fibronectin or PDGF. Plating cells on either fibronectin or with PDGF increases the expression of CNPase (C) and GFAP positive cells (D). E, Cells cultured on either laminin or with addition of PDGF increases the expression of MAP2.

DISCUSSION

Stem cells are functionally defined as self-renewing, multipotent cells that exhibit multilineage differentiation.[13] Somatic stem cells are thought to self-renew to generate all of the mature cells types of a particular tissue through differentiation, although rigorous identification and isolation of tissue specific stem cells has accomplished prospectively in only few organs. The neurosphere assay has permitted rigorous *in vitro* characterisation of the neural stem cells.[13,14]

Recently Liu and colleagues found that primary cultured cell lines established from GBM contain a subpopulation of cells that express stem cell markers.[15] However, they did not show the change in the expression of these markers when grown as neurospheres. Additionally, they demonstrated that stem cell marker CD133 expression was higher in recurrent tumours than autologous primary tumour tissue.[15] Results from our study revealed that a large population of nestin and Sox2 positive cells from PBT glioma cell lines could be maintained without differentiation in 15% FBS tumour media. Stem cell enrichment was carried out as described previously for NSCs by neurosphere assay. [8] There was about 3 fold increase in stem cell markers expression in cells from Olig1 when grown as neurospheres in serum free SCM supplemented with growth factors, whereas, cells from BT4 cell line showed only a 20% increase in stem cell markers expression when grown as neurospheres. In contrast, cells from EPN1 (recurrent tumour) showed only mild differences in stem cell markers expression when switched into SCM. Probably, this variation in stem cell markers expression found between the different tumour cell lines could be due to the differences in the level of malignancy (grade) of tumour or in the case of EPN1 selection of a stem cell population on recurrence. Furthermore, our data showed that human PBT gliomas contain cells that differentiate into three neural lineages. This was particularly obvious in the well differentiated oligodendroglioma brain tumour.

In this report, we have identified a new population of cancer stem-like cells in three paediatric gliomas that can self-renew and undergo multipotent differentiation. In spite of their all being multipotent, the percentages of differentiated cell types formed varied considerably from one tumour to another, a similar observation was previously reported by Hemmati *et al*. [9]

There are no standard differentiation conditions that can be used to induce NSCs differentiation into neuron, astrocyte or oligodendroglial cells. Many researchers have used different conditions to induce embryonic or neural stem cell differentiation: laminin and PDGFA have been used to induce neuronal differentiation of embryonic cells.[16,17] Fibronectin and PDGFA have been used to induce astrocyte differentiation of human NSCs.[18] RA the acidic form of vitamin-A has been found to induce

neuronal differentiation of pluripotent mouse embryonic cells.[19] In agreement with this, our results showed that either laminin or PDGF is the best factor for neuronal cell differentiation, whereas fibronectin or PDGF can be regarded as best route to glial cell differentiation. One exception to this was with the line Olig1 which failed to produce astrocytes in response to PDGF. Unlike cancer stem-like cells, mouse neural stem cells showed no growth when cultured with the addition of RA, suggesting that RA reduce mouse NSCs proliferation and compromised cell viability.[19]

In conclusion, it has been demonstrated that three different PBT glioma cell lines contain neurosphere-forming cells that are self-renewable and can differentiate into three neural lineages, supporting the presence of BTSCs in these cell lines. To conclusively demonstrate the presence of BTSCs in PBT gliomas, the potential of these cells for self-renewal and multipotent differentiation need to be tested *in vivo*. This could be achieved by intracranial injection of neurosphere-forming cells or CD133 positive cells (sorted by flow cytometry), into the brain of immunodeficient mice to assess their capacity to form a tumour *in vivo*.

ACKNOWLEDGEMENTS

I would like to thank and honour my supervisor, Dr B Coyle for her invaluable advice, sincere interest and enthusiasm shown throughout work. A special thanks to Dr D Hussein for her help and excellent technical support. Thanks are also due to Dr L Storer for her expert advice and support. The control cell line was a gift from Vitezslav Bryia (Sweden). I must express my genuine appreciation to the Kurdistan Regional Government (KRG) for funding me throughout my study.

REFERENCES

1. Shaw EG, Scheithauer BW, O'Fallon JR, Tazelaar HD, Davis DH. Oligodendrogliomas: the Mayo Clinic experience. J Neurosurg 1992;76(3):428-34.
2. Bleyer WA. Epidemiologic impact of children with brain tumors. Childs Nerv Syst 1999;15(11-12):758-63.
3. Kleihues P, Louis DN, Scheithauer BW, Rorke LB, Reifenberger G, Burger PC, et al. The WHO classification of tumors of the nervous system. J Neuropathol Exp Neurol 2002;61(3):215-25; discussion 26-9.
4. Kaatsch P, Rickert CH, Kuhl J, Schuz J, Michaelis J. Population-based epidemiologic data on brain tumors in German children. Cancer 2001;92(12):3155-64.
5. Sklar CA. Childhood brain tumors. J Pediatr Endocrinol Metab 2002;15 Suppl 2:669-73.
6. Tait DM, Thornton-Jones H, Bloom HJ, Lemerle J, Morris-Jones P. Adjuvant chemotherapy for medulloblastoma: the first multi-centre control trial of the International Society of Paediatric Oncology (SIOP I). Eur J Cancer 1990;26(4):464-9.
7. Taylor MD, Poppleton H, Fuller C, Su X, Liu Y, Jensen P, et al. Radial glia

cells are candidate stem cells of ependymoma. Cancer Cell 2005;8(4):323-35.

8. Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, et al. Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci U S A 2000;97(26):14720-5.
9. Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, et al. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci U S A 2003;100(25):15178-83.
10. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003;63(18):5821-8.
11. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 2004;64(19):7011-21.
12. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. Nature 2004;432(7015):396-401.
13. Dirks PB. Brain tumour stem cells: the undercurrents of human brain cancer and their relationship to neural stem cells. Philos Trans R Soc Lond B Biol Sci 2007.
14. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005;5(4):275-84.
15. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 2006;5:67.
16. Demoulin JB, Enarsson M, Larsson J, Essaghir A, Heldin CH, Forsberg-Nilsson K. The gene expression profile of PDGF-treated neural stem cells corresponds to partially differentiated neurons and glia. Growth Factors 2006;24(3):184-96.
17. Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, Martin GR. Laminin--a glycoprotein from basement membranes. J Biol Chem 1979;254(19):9933-7.
18. Flanagan LA, Rebaza LM, Derzic S, Schwartz PH, Monuki ES. Regulation of human neural precursor cells by laminin and integrins. J Neurosci Res 2006;83(5):845-56.
19. Martin-Ibanez R, Urban N, Sergent-Tanguy S, Pineda JR, Garrido-Clua N, Alberch J, et al. Interplay of leukemia inhibitory factor and retinoic acid on neural differentiation of mouse embryonic stem cells. J Neurosci Res 2007; 85:2686-701.

پوختە

نیاسینا خانێن بنەرەتی پەنجەشێرێ (stem cells) لپەنجەشێرێن مەژیێ سەری زاروکاندا

ئارمانج: دەست نیشانکرا خانێن بنەرەتی یێن پەنجەشێرێ ژ سێ پەنجەشێرێن مەژیێ سەری یێن زاروکا.

جھ: سەنتەرێ پزیشکی یێ مەلەکی / باژێرێ نوتنگھام / ئنگلترا.

ڕێکێن ڤەکولینێ: سێ جورێن خانێن پەنجەشێران ھاتنە ڤەکولین کو ئەڤێن خارێ ڤەدگرتن (high grade glioblastoma multifome (BT4), well differentiated oligodendroglioma (Olig1), and recurrent ependymoma (EPN1)) ھەروەسا جورەکی خانێن بنەرەتی یێن سیستەمێ دەمارێن مشکان بو ھەڤبەرکرنێ ھاتە بکار ئینان.

ئەنجام: ئەنجاما ڤەکولینێ نیشاندا کو نیشانگەرێن خانێن بنەرەتی، دناف وان خانێن ھاتینە چاندێن دناف 15٪ (foetal calf serum) د مینن. ئەف خانێن ھاتینە چاندن دکارن ناڤ ھندەك مادێن تایبەت زێدە بن و خانێن دەماری وەك ئیشییێن تری دروست بکەن (Neurospheres) . ڤان خانێن دەماری شیانێن خو زێدەکرنێ ھەنە. ڕێژەیا خانێن ئەرێنی کو نیشانگەرێ(CD133) دناڤدا ھاتیە دیتن بڕێکا فلوسیتومتری (flowcytometry) برەنگێ خارێ بون ± (mean (BT4 47.2 % ± 10.5, EPN1 40.4% ± 8.9 and Olig1 48.7% ± 2.9) standard error) ھەژی گوتنێ یە کو (CD133) نیشانگەرەکە بو خانێن بنەرەتی یێن دەمارا. دیف ڤەکولینێن ھاتینە ئەنجامدان دیار بو کو ئەڤ خانێن نیشانگەرا (CD133) تێدا ھەین دکارن دابەش ببن بو چەند جورێن خانێن دەماری وەکو (neurons, astrocyte and oligodendrocyte). ڕێژا دابەشبونا خانێن بنەرەتی دناڤ وان سێ پەنجەشێرێن مەژی سەری ھاتینە دیارکرن جیاواز بوو.

دەرئەنجام: دڤێ پشکنینێدا دیار بو کو پەنجەشێرێن مەژی سەرێ زاروکان ھندەك خانێن تێدا ھەین کو وەکو خانێن بنەرەتی نە کو شیانێن خو زێدەبوونێ وە دابەش بوون بو سێ جورێن خانێن دەماری ھەنە.

الخلاصة

الكشف عن خلايا عصبية سلالية في السرطانات الدماغية للاطفال

الهدف: تميز خلايا سلالية عصبية في ثلاثة أنواع من سرطانات الدماغ في الاطفال.
المكان: المجمع الملكي الطبي \ مدينة نوتنكهام \ بريطانيا.
المنهجية: شملت الدراسة ثلاثة أنواع من سرطانات الدماغ في الاطفال و شملت كل من (high grade glioblastoma multiforme BT4, one well differentiated oligodendroglioma olig1 and one recurrent ependymoma EPN1).
و قد شملت الدراسة خلايا عصبية معيارية من الفئران للمقارنة(C17.2).
النتائج: أظهرت النتائج أن الخلايا السرطانية تبقي علامات الخلايا السلالية عندما تنمو على شكل طبقة واحدة في 15% من مصل الدم للبليد الجنيني(foetal bovine serum/Dulbecco's modified Eagle's medium) هذه الخلايا لها القابلية على ان تنقسم و تتكاثر لتكوين كريات عصبية(neurosphere) عند زرعها في وسط خالي من مصل الدم و ذلك بأضافة محفزات النمو (FGF and EGF). يمكن لهذه الخلايا ان تنقسم و تعيد بناء كريات جديدة عندما يتم فصلها وزرعها في وسط مماثل مع اضافة هذه المحفزات. تم تحديد نسبة الخلايا الايجابية لعلامة خلايا السلالية العصبية (CD133) باستعمال التحليل فلوسايتوميتر (flowcytometry) في جميع الخلايا المزروعة و كانت النسب كالتالي: BT4 47.2%±10.5, EPN1 40.4%±8.9, Olig1 48.7%±2.9 (المتوسط،الخطأ المعياري). يمكن لهذه الخلايا ان تتخصص الى خلايا عصبية وخلايا مساندة neuron, astrocyte, and oligodendrocyte تحت ظروف تساعد على تخصص الخلايا السلالية. ولكن على مستويات مختلفة لكل خط خلية سرطانية.
الأستنتاجات: السرطنات الدماغية للأطفال تحتوي على خلايا مشابه للخلايا السرطانية السلالية التي لها القابلية على الانقسام و تجديد نفسها و لها القابلية ع ىالتحول الى ثلاثة انساب عصبية.

PREVALENCE OF HAEMOGLOBINOPATHIES IN SULAIMANI – IRAQ

SANA D. JALAL, MBChB, FICMS*
NASIR A. AL-ALLAWI, MBChB, MSc, PhD **
AZAD H. FARAJ, MBChB, MSc ***
NAJMALDIN H. AHMED, MBChB, MSc****

Submitted 12 January 2008; accepted 21 April 2008

ABSTRACT

Background Thalassaemia major is an important health problem in Sulaimani, a large province at Northeastern Iraq, and the need to initiate a preventive program for this potentially fatal disorder is paramount. As a prerequisite to such program this study was initiated to map the province for hemoglobinopathies.
Material and Methods A total of 1472 subjects (736 couples) attending Sulaimani premarital Health centre were screened using red cell indices and sickling test. For those who had MCV<80 fl and/or MCH < 27 pg or had a positive sickling test, this was followed by Hemoglobin HPLC and iron studies
Results Based on above investigations, 61 individuals (4.14%) were found to have ß-thalassaemia minor, 4 (0.27%) sickle cell trait, 2 (0.14%) Hb C trait, and 2 (0.14%) δß-thalassaemia minor, and one (0.07%) had Hereditary Hemoglobin F Persistence (HPFH) homozygous state. Moreover, 49 individuals (3.3%) had α- thalassaemia, including one with Hb H disease (0.07 %). The study also revealed a consanguinity rate of 24.3% among the screened couples.
Conclusions The high prevalence rate of ß- thalassaemia carrier state and consanguinity, among premarital couples should further strengthen the need for initiating a preventive program for hemoglobinopathies in this region.

DMJ 2008;2(1):71-79.

Key words: Thalassemia, Hemoglobinopathies, Sulaimani, Iraq, Prevention

Hemoglobinopathies are the most common single gene disorders worldwide with a considerable frequency in certain areas particularly Mediterranean and Middle Eastern countries, including Iraq. Hemoglobinopathies include structural variants (such as Hb S, Hb C, Hb E), and thalassaemias which are inherited defects in globin chains synthesis.[1,2] **β** – thalassaemia major is an important health problem in Sulaimani province , with more than 600 registered cases in a population of over 1.5 million (Records of the Preventive Health

* *Lecturer of Hematology, Sulaimania College of Medicine, Sulaimani, Iraq*
** *Professor of Hematology, Head of Department of Pathology, Dohuk College of Medicine, Dohuk, Iraq*
*** *Director of Public Health Laboratory, Sulaimani,Iraq*
**** *Community Medicine Physician, Directorate of Health, Sulaimani,Iraq*
Correspondence author: *Nasir A. Al-Allawi, Head of Department of Pathology, Dohuk College of Medicine, Dohuk, Iraq. E-mail : nallawi@yahoo.com*

Department- Sulaimani). Sulaimani is a large province lying in North Eastern Iraq, bordering Iran. The high carrier rate and the frequency of consanguineous marriages, necessitate establishing an effective prevention program. As a prerequisite to such a program, mapping the province for hemoglobinopathies is essential, and since such a task has not been performed previously in Sulaimani, this study was conducted to address it.

SUBJECTS AND METHODS

This study was carried out between the 23rd September 2006 to the 14th January 2007. The subjects were couples attending the premarital health centre at Sulaimani. This center is the only center in the province authorized by legal authorities to perform the mandatory premarital checks. These checks include ABO grouping and Rhesus typing, HBs Ag and VDRL (Venereal Disease Research Laboratory Test). The average number of attendees investigated by the center per day is 20-30 couples.

For the purposes of this study, alternate couples from those attending for premarital checks on alternate working days were enrolled. A total of 1472 subjects (736 couples) were thus enrolled. A short concise questionnaire including: name, age, place of birth, residence, family history of thalassaemia or sickle cell disorder and consanguinity of the couple was taken.

A 5 ml sample was aspirated from each subject by venepuncture and was divided between EDTA (2ml) and plain tubes (3ml) for the purpose of this study. The EDTA sample was first used to perform sickling test, then it was processed in Beckman-Coulter hematology analyzer (USA) (which is daily calibrated using calibrant material provided by the manufactures) to determine the red cell indices. If the MCV < 80 fl and / or MCH < 27 pg, or if the sickling test was positive for any member of the couple, then the EDTA sample was processed further for the quantitation of Hb A2 and Hb F and Hb S (if sickling is positive) or other variants, in an automated ion-exchange high performance liquid chromatography system using the β-thalassaemia short program on the Bio–Rad variant instrument (Bio-Rad Laboratories, Belgium). If the results of Hb HPLC were normal, then the iron status was estimated.[3,4]

The subject is considered as β-thalassaemia minor if he/she has hypochromia and/or microcytosis with A2 >3.5%. Reduced transferring saturation <15 % was considered as an indicator of iron deficiency. Increased Hb F of 5-20%, with no excess in Hb A2 was considered as δβ-thalassaemia trait. A presumptive diagnosis of α-thalassaemia was made if β-thalassaemia minor, δβ-thalassaemia trait and iron deficiency were excluded. The expected gene frequency of different Hb disorders calculated applying the Hardy-Weinburg equation.[4]

RESULTS

The 1472 subjects enrolled had ages ranging from 16-58 yrs. with a median of

23 yrs for males and 20 yrs for females. The consanguinity rate among these couples was (24.3%).

Out of 1472 subjects included in this study, microcytosis (MCV< 80fl) and / or hypochromia (MCH < 27 pg) was found in 173 (11.75%). **β**-thalassaemia minor was identified in 61 (4.14%), and jron deficiency in 53 cases (3.6%). δß-thalassaemia minor was documented in two individuals (0.14%), Hb H disease in one individual (0.07%), while a presumptive diagnosis of α-thalassaemia trait was made in 49 individuals (3.3%). One individual was found to be homozygous HPFH. Sickle cell trait was identified in 4 individuals (0.27%) and Hb C trait in another two (0.14%). Based on the above figures, the expected gene frequency of different Hb disorders is shown in (Table 1).

β- thalassaemia prevalence showed regional variation within Sulaimani province (Figure 1), with the highest frequency being found in Halabja (8.6%) while the lowest frequency found in Dukan (0%).The frequency was generally higher in the eastern and southern parts of the province (Table 2).

Table 1. The prevalence and gene frequency of Hemoglobinopathies in 1472 individuals from Sulaimani

Hb Type	Affected individuals		Gene Frequency
	No.	%	
ß–thalassaemia minor	61	4.14	0.0207
Hb S trait	4	0.27	0.0012
Hb C trait	2	0.14	0.0007
HPFH	1	0.07	0.00034
δß-thalassaemia minor	2	0.14	0.0007

Table 2. The prevalence of β- thalassaemia minor in different sectors of Sulaimani

Sector	No. of respondents	Frequency of ß–thalassaemia minor
Center	557	18 (3.2%)
Rania	160	4 (2.5%)
Chamchamal	140	9 (6.4%)
Germian (Kalar, Kifri)	164	13 (7.9%)
Qaladiza	88	1 (1.1%)
Sharazour	82	4 (4.9%)
Halabja	70	6 (8.6%)
Zimnako	50	2 (4%)
Darbaedikhan	46	2 (4.3%)
Dukan	45	0 (0)
Bazyian	50	1 (2%)
Sharbazher	20	1 (5%)

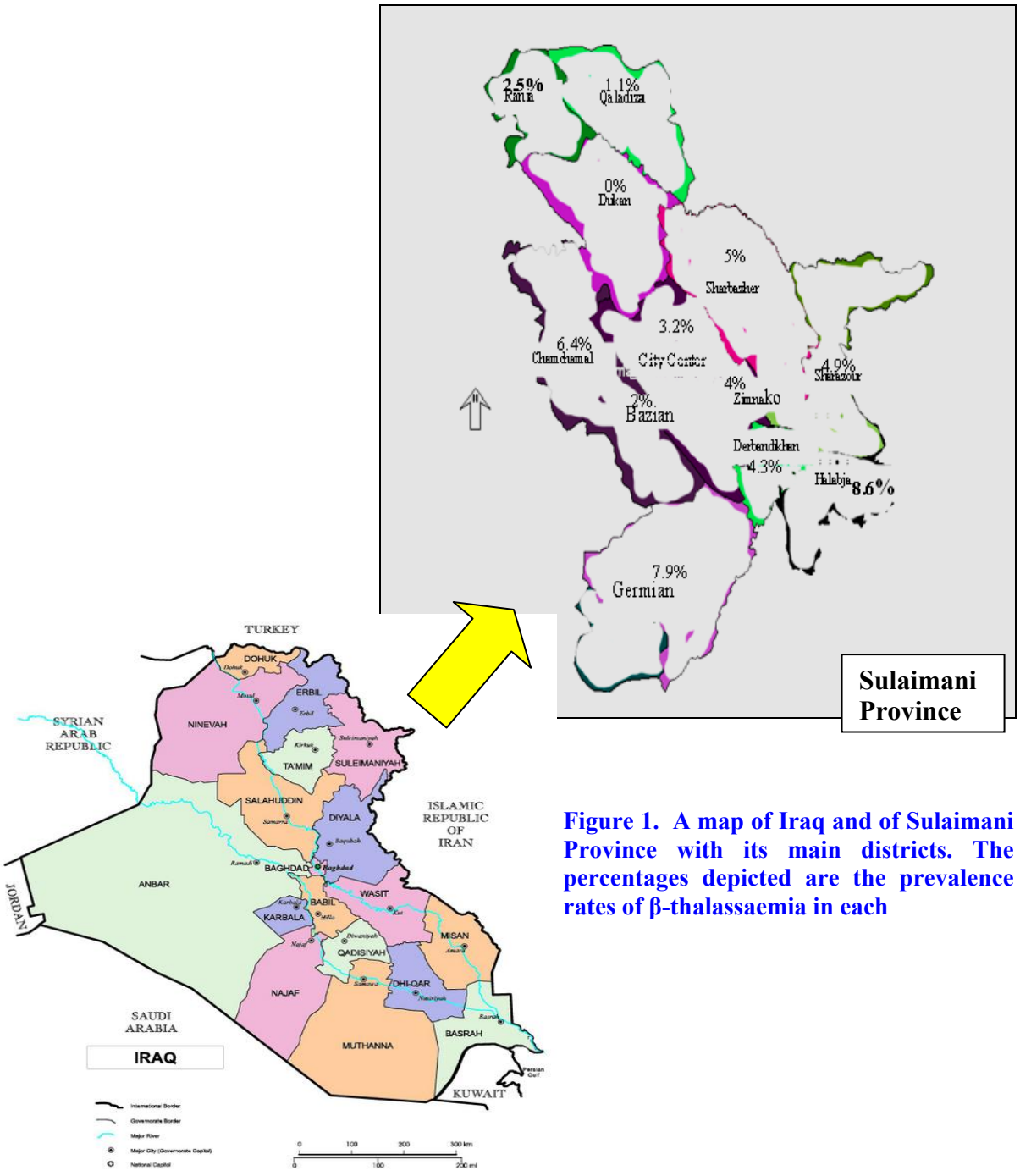


Figure 1. A map of Iraq and of Sulaimani Province with its main districts. The percentages depicted are the prevalence rates of β-thalassaemia in each

DISCUSSION

The high prevalence of β-thalassaemia as documented in this study from Sulaimani at the northeast Iraq is comparable to that reported in several Mediterranean countries.[2] Malaria was endemic throughout Sulaimani until early1990s,[5] so it would not be unexpected to find thalassaemia genes prevalent in Sulaimani or in other parts of Iraq, in view of the theory of malaria selection which offered an explanation of the high prevalence rates observed in many parts of the world.[6]

The prevalence rate of β-thalassaemia minor observed in this study (4.14%) is comparable with those reported from Baghdad (4.4%),[7] Basra (4.6%),[8] Dohuk (3.7%),[9] but higher than those reported in neighboring countries like Jordan (3-3.5%),[10,11] Lebanon (1.7-3%),[8] Saudi Arabia (3%)[12] and Turkey (2.6-3.7%).[13] The Iranian figures on the other hand is higher than ours (5-10%).[14] One notable observation in the current study, is that the distribution within the Sulaimani province varies, with the highest rates encountered in eastern districts. Because of interactions of the populations, it may not be unexpected to find higher prevalence rates in these districts of Sulaimani neighbouring Iran.

The frequency of sickle cell trait in this study was (0.27%,) which is much lower than the figures reported from Basra (6.48%)[8] Dohuk (1.2%)[9] and Saudi Arabia (reaching 17% in some areas).[15] Although the Hb S gene may have spread to our region through interaction with Arab tribes, it has been considerably diluted in the local populations.

In this study, the estimated frequency of presumed α-thalassaemia trait was (3.3%) which is approaching the figure form Jordan (2.3%),[10] but is higher than the figure reported from Baghdad (1%),[7] and much lower than Saudi Arabia figure (12-60%).[16, 17] According to the experience of Iraqi authors, α^{+}- thalassaemia trait is mostly caused by α^{+} defect, since Hb Bart's Hydrops Fetalis has not been reported , while Hb H is uncommon. The prevalence rate of α- thalassaemia trait obtained in this study may be underestimated, since its well known that a proportion α^{+}-thalassaemia trait would be missed if MCV and /or MCH used as screening tests. Reliable data on the prevalence of α thalassaemia defects in the region will require molecular characterization. The latter is currently underway.

The rate of consanguinity observed in the current study of 24.3% is high, though it is slightly less than that estimated overall in Iraq of 30%.[18] It would be expected that in a population in which consanguineous marriage is common, the frequency of homozygous births increases for a given carrier frequency.[19] Such practice in our society, as well as many other Eastern Mediterranean populations, is the rule rather than the exception, and it may socially be unacceptable for a couple to separate, based on the results of premarital tests showing that they maybe at risk of getting an affected child with a hemoglobinopathy. Such a situation could

be addressed by a well organized and targeted educational program, and would certainly become less of an issue as the living standards improve and as people change their life styles from rural to urban settings. Furthermore, basing any future preventive program on basis of premarital screening, genetic counseling and prenatal diagnosis (for those at risk), would make consanguinity less important as a cause of increased affected birth rates.

In conclusion, this study documented high prevalence rates of β-thalassaemia in Sulaimani at the Northeastern Iraq, as well as high rate of consanguineous marriages, while Hb S or other structural Hemoglobinopathies are scarce. These findings coupled with large number of thalassaemia major patients already registered in this province, support the need for initiating an effective preventive program, based on premarital screening, counseling and prenatal diagnosis. The latter however would require molecular characterization of β-thalassaemic defects in the region, which is currently underway.

REFERENCES

1. Bain BJ. Hemoglobinopathies diagnosis. 1st ed. Oxford: Blackwell Scientific Pub.; 2001.
2. Weatherall DJ .Hemoglobin and inherited Disorders of globin synthesis. In: Hoffbrand AV, Catovsky D, Tuddenham EGD. Postgraduate haematology, 5th ed. Oxford: Blackwell Scientific Pub.; 2005. p. 85-90.
3. Giardano PC. Carrier diagnosis and prevention of hemoglobinipathies using high performance liquid chromatography. BIO RAD 2006. p. 25-28
4. Galanello R, Eleftheriou A, Traaeger-Synedions J,Petrou M, Angastiniotis M. Prevention of thalassemias and other hemoglobin disorders. Vol. 1. Nicosia: TIF Publications; 2003.
5. Ahmed NH. Epidemiology of malaria in sulaimani [thesis]. Baghdad, Iraq: Baghdad Univ.; 1991.
6. Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casal –Pascual C, et al. Malaria and red cell. Hematology 2002;(1):35- 57.
7. Yahya HI, Khalel KJ, AL- Allawi NAS, Ferial Hilmi F. Thalassaemia genes in Baghdad-Iraq. East Mediterr Health J 1996;2(2):315-9.
8. Hassan MK, Taha JY, Al–Noama LM, Widad NM, Jasim SN. Frequency of hemoglobinopathies and glucose- 6-phosphate dehydrogenase Deficiency in Basra. East Mediterr Health J 2003;9(1/2):1-8.
9. Al-Allawi NAS, Jubrael JMS, Anwar A., Fariq F. Service indicators fro a regional hemoglobinopathy preventive program in Dohuk-Iraq. Proceedings of the 2nd PanaArab Human Genetics conference, Dubai, CAGS, 20-22nd Novemeber 2007. p. 78.
10. Bashir N, Barkawi M, Sharif L. Prevalence of hemoglobinopathies in school children in Jordan Valley. Ann Trop Pediatr 1991; 11: 373-6.

11. Bashir N, Barkawi M, Sharif L, Momani A, Gharaibeh N. Prevalence of hemoglobinopathies in North Jordan. Trop Geogr Med 1992; 44(1-2):122-5.
12. El- Hazami MA, Warsy AS. Appraisal of sickle cell and thalassaemia genes in Saudi Arabia. East Mediterr Health J 1999;5(6):1147-53.
13. Yildiz S, Atalay A, Baci H, Atalay EO. Beta –thalassaemia muations in Denizili Province of Turkey. Turk J Haematol 2005;22(1):181-5.
14. Karimi M, Rasekhi AR. Efficiency of premarital screening for beta –thalassaemia trait using MCH rather than MCV in the population of Faris Province, Iran. Haematologica 2002;32(2):129-33.
15. Mohammed A, Al-Hilli F, Nadkarmi KV, Bhagwat GP, Bapat JP. Hemoglobinopathies and glucose 6-phosphate deficiency in hospital births in Bahrain. Ann Saudi Med 1992;12:536-9.
16. El-Hazmi MAF. α-thalassaemia in Saudi Arabia: deletion pattern. Hum Genet 1987;76(2):196-8.
17. El-Hazmi MAF .Hemoglobinopathies, thalassaemias and enzymopathies in Saudi Arabia. Saudi Med J 1992;13: 488-99.
18. Hamamy H, Alwan A. Hereditary disorders in the Eastern Mediterranean region. Bull WHO 1994;72(1):145-54.
19. Galanello R, Eleftheriou A, Traaeger-Synedions J, Petrou M, Angastiniotis M. Prevention of thalassemias and other hemoglobin disorders. Vol. 1. Nicosia: TIF Publications; 2003.

پوختە

پلەی بوونی گرفتەکانی هیموگڵۆبین لە سلیمانی

پێشەکی: سالاسیما یەکێکە لەو کێشە تەندروستییە گرنگانەی کە لە شاری سلیمانی دا هەیە (کە ناوچەیەکی هەرێمیی گەورەیە لەباکووري رۆژهەڵاتی عێراقدا)

ئامانجی تۆژینەوە: مەبەست لەم تۆژینەوەیە, روپێوی سلێماني یە سەبارەت بە گرفتەکاني هیموگڵۆبین بە مەبەسیی دەست پێکردنی بەرنامەی خۆپاراستن لە سالاسیما و تێکچونەکاني هیموگڵۆبین

رێگەی تۆژینەوە: لە شیکاریەکانی کە ئەنجام ئەدرێت پێش هاوسەری و (1472) خوازیاری هاوسەر گیری کە شیکاریان بۆ ئەنجام دراوە تۆژینەوەیان لەسەر ئەنجام درا وپابەند بە قەبارەو رێژەی هیموگڵۆبین لە خرۆکە سورەکاندا و ئەنجامەکان شیکرانەوە وە شێوەکانی تر وەك داسە ئەنیما و رێژەی ئاسن لە خوێندا وپشت بەسترا کە قەبارەی خرۆکەي سور لە 80 فیمتولیتر ورێژەی هیموگڵوبین لە 27 پیگۆ گرام کەمتر نەبێت .

ئەنجامەکان: بەپشت بەستن بە ئەنجامەکان دەرکەوت کە 61 کەس واتە (4,14 ٪) هەڵگری خەسڵەتی سالاسیمان جۆری بێتا و 4 کەس واتە (0,27 ٪) خەسڵەتی داسە ئەنیما و 2 کەس واتە (0,14 ٪) هیموگڵۆبینی جۆری C و 2 کەس واتە (0,14 ٪) لە جۆری دلتا بێتا 49 کەس (3,03 ٪) جۆري الفا و یەك کەس لە جۆری هیموگڵۆبین H یەك کەس لە جۆري (مانەوەي هیموگڵۆبینی کۆرپەیي) رێژەي هاوسەري نێوان خزمان (3و24 ٪)

دەرئەنجام: بەربلاوی هەڵگرانی خەسڵەتی سالاسیما لە جۆری بێتا و زۆریي رێژەي هاوسەرگیری لە نێوان خزماندا , پێویستی بە دەست پێکردنی بەرنامەی خۆپاراستنە بۆ کەم کردنەوەي گرفتەکاني هیموگڵۆبین لە کۆمەڵد.

الخلاصة

درجة انتشار اضطرابات الهيموغلوبين في السليمانية / العراق

خلفية البحث: يعد مرض فقر دم البحر الابيض المتوسط مشكلة صحية مهمة في محافظة السليمانية التي هي احدى المحافظات الكردية شمال شرق العراق .

الهدف من الدراسة: للحاجة الشديدة لبدأ برنامج وقاية من هذا المرض الخطر اجريت هذه الدراسة لمسح الاقليم للوقوف عند مدى انتشار هذه الاضطرابات في الهيموكلوبين .

مواد وطرق البحث: شملت هذه الدراسه فحص 1472 شخص (736رجل و أمرأة) من المراجعين لمركز فحوصات ماقبل الزواج في السليمانية اعتمادا على اسس كريات الدم الحمراء (معدل حجم الكرية الحمراء، معدل الهيموكلوبين في الكرية الحمراء) مع فحص الكشف عن فقر الدم المنجلي متبوعة بترحيل الدم بجهازHPLC وقياس نسبة الحديد في الجسم للازواج الذين كانت قرائات معدل حجم الكرية الحمراء اقل من 80 فيمولتر مع او بدون انخفاض معدل الهيموكلوبين في الكدرية الحمراء اقل من 27 بيكوكرام او اذا كان فحص الكشف عن فقر الدم المنجلي ايجابي

النتائج: اعتمادا على الفحوصات اعلاه ، وجد ان 61 شخصا (4,14%) هم حاملين لصفة فقر دم البحر الابيض المتوسط (النوعβ) 4 اشخاص (27%) حاملين لصفة فقر الدم المنجلي ، شخصان (14%) حاملين لصفة هيموكلوبين C، شخصان لصفة فقر الدم البحر الابيض المتوسط (نوعδβ) مع وجود شخص حامل لمرض الاستمرار الوراثي لوجود الهيموكلوبين الجنيني (0.07%) بالاضافة الى 49 شخص (3.3%) حاملين لصفة فقر دم البحر الابيض المتوسط نوع (α) متضمنا حالة واحدة لمرض هيموكلوبينH (0.07%) . هذه الدراسة بينت ايضا ان نسبة زواج الاقارب كانت (24,3%)

الاستنتاجات: ان الانتشار العالي لحاملي صفة فقر دم البحر الابيض المتوسط (نوعβ) مع انتشار زواج الاقارب اكد ضرورة انشاء برنامج وقائي للحد من انتشار اظطرابات الهيموكلوبين في المنطقة .

PROSTATE-SPECIFIC ANTIGEN VERSUS DIGITAL RECTAL EXAMINATION IN THE DIAGNOSIS OF PROSTATE CANCER

SHAKER S. JABALY, MBChB, FICU*
MOHAMMAD A. MOHAMMAD, MBChB, DU(Urology)**

Submitted 13 March 2008; accepted 21 April 2008

ABSTRACT

Background Prostate cancer (CaP) is the most commonly diagnosed non cutaneous cancer and the third most common cause of death from cancer in males. PSA is considered the most useful tumor marker currently available for diagnosis and management of the CaP. The digital rectal examination is still the basis in the suspicion of CaP in males with normal or minimally high PSA levels.
Aim the aim of the study is to evaluate the effectiveness of PSA versus digital rectal examination (DRE) in identifying cases of prostate cancer among men presenting with symptoms of bladder outflow obstruction.
Patients and methods A cross sectional study was conducted at Azadi Teaching Hospital in Dohuk on 400 patients with bladder outflow obstruction above 50 years were selected between January 2005 and October 2007. DRE and serum PSA done for all patients and prostate biopsy for abnormal results.
Results The mean age 69.63±8.3 years. All cases of documented prostate cancer with positive DRE had PSA ≥ 4 ng/ml.
Conclusions In conclusion, DRE is still the basic step in the suspecting prostate cancer. Combination of DRE and PSA has the highest detection rate for CaP than each alone.

DMJ 2008;2(1): 80-90.

Key words: PSA, DRE, Prostate biopsy, Ca prostate

Prostate cancer (CaP) is the most commonly diagnosed non cutaneous cancer and the third most common cause of death from cancer in males in the US, estimated at 27,350 deaths in 2006.[1] The number of men diagnosed with CaP is increasing in many areas in the world. An increasing life expectancy in male population[2] and increasing use of prostate-specific antigen (PSA) for early detection of the disease[3] are probably the two main factors accounting for higher detection rate. Most cases of CaP diagnosed nowadays are non-metastatic disease[4,5] and thus, many patients being suitable for potentially curative therapy.

PSA is serine-like protease produced by epithelial cells of the prostate gland, releasing from prostatic epithelium, and appears in the blood. PSA is considered the most useful tumor marker currently available for diagnosis and management of the CaP.[6] However, it is not specific for CaP. Several non-malignant conditions

** Specialist Urologist, Senior consultant / lecturer, Department of Urology, Dohuk College of Medicine, Dohuk, Iraq*
*** Azadi Teaching Hospital, Dohuk, Iraq*
Correspondence author: *Shaker S. Jabaly,Department of Urology, Dohuk College of Medicine, Dohuk, Iraq. Email: balindi6@yahoo.com*

of the prostate are associated with elevated PSA levels e.g. prostatic intraepithelial neoplasia, acute prostatitis, prostatic ischemia, and nodular prostatic hyperplasia (NPH).[6-8]. Furthermore, not all CaP cause an elevated PSA concentration.[9] NPH is still the most common cause of elevated serum PSA in non-malignant causes.[10]

The DRE is still the basis in the suspicion of CaP in males with normal or minimally high PSA levels. When palpable, CaP is usually represented by induration of the prostate on DRE.[11,12]

AIM OF THE STUDY

The aim of the study is to evaluate the effectiveness of PSA versus DRE in detecting cases of CaP among men presenting with symptoms of bladder outflow obstruction.

PATIENTS AND METHODS

A cross-sectional study was conducted in the outpatient clinic of Urology at Azadi General Teaching Hospital. The Hospital is the main referral one in Dohuk Governorate. Between January 2005– October 2007, a total of 400 men aged 50 years and over with lower urinary tract symptoms (LUTS) were selected. The exclusion criteria were patients with previously diagnosed CaP and patients with lower urinary tract symptoms owing to causes other than bladder outflow obstruction.

All patients included were first examined by DRE and then sent for PSA measurement. Any asymmetry, nodularity or indurations were considered abnormal. Blood sample was taken for PSA measurement at Azadi Hospital lab. PSA level was determined by the enzyme-linked immunosorbant assay (ELISA). A PSA value of ≥ 4 ng/ml is considered abnormal. Any patient with suspicious DRE or PSA level ≥ 4ng/ml submitted to Tru-cut biopsy of the prostate using a spring- driven biopsy gun under local anesthesia & antibiotic cover. Three specimens were obtained from each side and an additional biopsy from the suspicious area. A minimum of sextant biopsies were obtained from each patient, occasionally a biopsy is obtained after simple open prostatectomy.

RESULTS

The mean age of the patients was 69.6 ± 8.3 years and ranged from 50 – 99 years. Out of the 400 symptomatic patients included in the study, 213 (53.2%) underwent histopathological examination (Tru-cut biopsy of the prostate or open prostatectomy).

Table 1 shows the clinical distribution of men who underwent histopathological examination.

Table 2shows that 55% of cases of CaP had PSA ≥ 40 ng/ml, compared to only 0.6% in other diseases of the prostate. 80.7% of patients with prostate disease other than CaP (80.7%) had PSA level < 10 ng/ml compared to only 12.5% in cases with CaP.

Table 3 shows that all cases of CaP documented by biopsy and with positive

DRE had PSA ≥ 4 ng/ml, but 83.3% of CaP cases with negative DRE had PSA ≥ 4 ng/ml.

The sensitivity (TPR), specificity (TNR), false positive rate (FPR), false negative rate (FNR), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+ve LR) and negative likelihood ratio (-ve LR) for cut-off levels of PSA were summarized (Table 3.4). By plotting sensitivity against 1-specificity, a ROC curve was constructed (Figure 1); the diagnostic accuracy of PSA was evaluated by ROC curve by SPSS program for windows version 15.0 to calculate the area under the curve and was 96.9%. This means that PSA is sensitive marker for the presence of CaP with a sensitivity of 87.8% at 10 ng/ml in men presenting with symptoms suggestive of bladder outflow obstruction, but its specificity as seen on the ROC curve is high (91%) at 10 ng/ml with reasonable specificity being achieved throughout much of the range of values shown. The sensitivity of the test is diminished at a level 40ng/ml to only 52.1%.

Table 1 Distribution of the study population underwent biopsy by DRE results

DRE*	Biopsy (N= 213)			Total No. (%)
	NPH** No. (%)	Ca Prostate No. (%)	Non-specific Granulamatous Prostate No. (%)	
Positive	5 (3.0)	35 (85.4)	1 (33.3)	41 (19.2)
Negative	164 (97.0)	6 (14.6)	2 (66.3)	172 (80.8)
Total	169 (100.0)	41 (100.0)	3 (100.0)	213 (100.0)

**DRE = digital rectal examination, ** NPH = nodular prostatic hyperplasia*

Table 2 Distribution of study population by PSA level

PSA†	Biopsy		Total No. (%)
	Ca prostate No. (%)	Other Prostatic diseases * No. (%)	
4-<10	5 (12.5)	138 (80.7)	143 (67.8)
10-<20	6 (15.0)	26 (15.2)	32 (15.2)
20-<40	7 (17.5)	6 (3.5)	13 (6.2)
40+	22 (55.0)	1 (0.6)	23 (10.9)
Total	40 (100.0)	171 (100.0)	211 (100.0)

* include NPH and Non-specific granulomatous prostatitis., † = prostatic specific antigen

Table 3 Distribution of men who underwent biopsy by DRE and PSA results

DRE*	PSA† (ng/ml)	Biopsy			Total No. (%)
		NPH˚ No. (%)	Ca Prostate No. (%)	Non-specific Granulamatous Prostate No. (%)	
Positive (N= 41)	≥4	5 (100.0%)	35 (100.0%)	1 (100.0%)	41 (100.0%)
Negative (N= 172)	≥4	163 (99.4%)	5 (83.3%)	2 (100.0%)	170 (98.8%)
	<4	1 (0.6%)	1 (16.7%)	0	2 (1.2%)
	Total	164 (100.0%)	6 (100.0%)	2 (100.0%)	172 (100.0%)

* DRE digital rectal examination, † PSA prostatic specific antigen, ˚ NPH nodular prostatic hyperplasia

Table 4 Sensitivity, Specificity, FPR, FNR, PPV, NPV, + ve LR and – ve LR of PSA test

Positive if PSA ≥	Sensitivity TPR %	Specificity TNR %	FPR %	FNR %	PPV %	NPV %	Positive LR	Negative LR
4	100	51.5	48.5	0	19	100	2.06	0
10	87.8	91	9	12.2	52.9	98.5	9.74	0.13
20	70.7	98	2	29.3	80.5	96.7	35.35	0.3
40	51.2	99.4	0.6	48.8	91.3	94.7	85.33	0.5
60	41.5	99.4	0.6	58.5	89.5	93.7	69.2	0.6
80	26.8	99.7	0.3	73.2	91.7	92.3	89.33	0.7
100	22	99.7	0.3	78	90	91.8	73.3	0.8

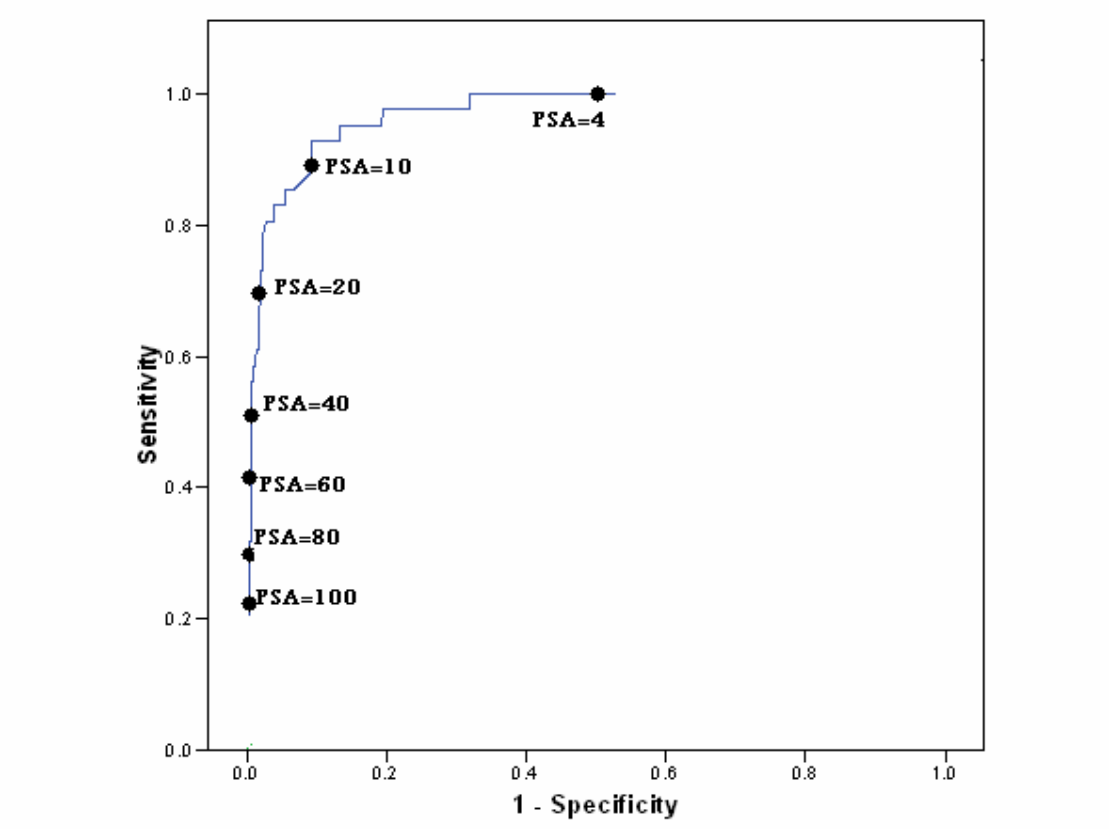


Figure 3. Receiver Operating Characteristic Curve for PSA at different cut-off levels

DISCUSSION

PSA is produced by prostatic epithelial tissue and is detected in the epithelial cells of prostate, NPH tissue, primary and metastatic CaP cells.[13] There is evidence that the rate of increase in the serum PSA is proportional to the cancer burden.[14-16] This study confirms that the sensitivity of PSA is a useful marker for detection of CaP, but shows that its specificity is poor at low cut-off levels.[17] Eighty one percent of patients with NPH had PSA level between 4-10 ng/ml, elevated PSA level (PSA > 4 ng/ml) was found in 53.8% of patients with symptomatic NPH which could be due to either urinary retention or indwelling Foley catheter.[18] In this study PSA demonstrates the specificity problems. As Oesterling had said,[19] the serum PSA concentration itself lacks sufficient sensitivity and specificity for diagnosing CaP in an ocean of NPH. Some patients with CaP have serum PSA within normal range.[14, 20] Our results showed that about 2.44% of patient with CaP have normal PSA. This limits the usefulness of PSA as a guide to the need for prostatic biopsy. In patients presenting with symptoms of bladder outflow obstruction, with a marginally elevated PSA level between 4 and 10 ng/ml and in whom non-surgical treatment is proposed one is faced with a diagnostic dilemma. Because of the poor specificity of PSA in such patients, as demonstrated from the ROC curve in this

study, many men would undergo unnecessary prostatic biopsy if PSA was used as the sole criterion for biopsy. In an attempt to improve the discriminating ability of PSA in patients with normal DRE and PSA level between 4 and 10 ng/ml, (the level at which PSA is least specific), the concept of PSA density (the PSA concentration divided by volume of the prostate) has been introduced.[21,22] However, Brawer *et al.*[23] was unable to confirm the advantage of PSA alone in identifying CaP. The concept of PSA velocity (the rate of change of PSA with time) has been advocated as a more useful test for detecting CaP than a single measurement of PSA. Carter *et al.*[24] found that a PSA velocity of 0.75 ng/ml per year had 90% specificity for CaP compared with a cut-off value for serum PSA of ≥ 4 ng/ml. Many men with NPH have high PSA levels because of large volumes of hyperplastic tissue[16] and this will tend to cause an overlap in PSA levels between patients with CaP and those with NPH. However, serum PSA provides good discrimination between patients with or without CaP. The specificity and sensitivity of PSA can be improved by excluding men with symptomatic NPH.[17]

DRE has been used in diagnosis and screening for CaP for many decades and its importance is well established.[25] The sensitivity of DRE in the diagnosis of CaP was found to be 39-45% in clinical trials.[26,27] The high percentage rate of positive DRE in the present study arises because most of the patients with CaP had abnormal DRE and thus represent a selected population in which 35 out of 41 patients had CaP proved by biopsy. However, despite all the technological developments, DRE is still the basic step in the diagnosis of CaP.

The high incidence of the CaP in the study population can be explained by late presentation combined with patients' selection, which was about 55% in patients with PSA ≥ 40 ng/mL. Granulomatous inflammation of the prostate has been reported in some patients receiving Bacillus Calmette-Guerin (BCG) therapy for bladder cancer, after TURP and in patients with systemic granulomatous disease, both infectious and non-infectious.[28-32] Most cases, however, are non-specific and resolve spontaneously with no therapy. In this study 1.23% had non-specific granulomatous prostatitis.

Results from other studies conducted on the prostate cancer have used TRUS guided biopsy, but this could not be attempted in the present study as TRUS needle applicator is not available in our hospital now, so we depend on trans-rectal digitally guided tru-cut biopsy of the prostate.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the ability of PSA to identify CaP can be improved by selecting out groups of patients and by adjusting the cut-off level of PSA to the patients under study, the normal range of this test should be adjusted according to the population under study. DRE and serum PSA provides a good discrimination between patients with and without CaP. The

sensitivity and specificity of PSA can be improved by excluding men with symptomatic NPH and patients with serum PSA level between 4-10 ng/ml. The specificity of PSA as a diagnostic test for CaP is reduced in men with symptoms of bladder outflow obstruction. No method alone reached a satisfactory diagnostic value for CaP. Only when these methods were combined (DRE & PSA level) an accuracy rate of 96.6% was achieved. This study also emphasizes that there is no single normal level for PSA. However, to determine which method is superior to predict CaP, further study needs to be done. The most effective method is to admit TRUS in addition to PSA and DRE in men with normal DRE and PSA between 4-10 ng/ml to diagnose CaP.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56(2):106-30.
2. Yancik R. Population aging and cancer: a cross-national concern. Cancer J 2005;11(6):437-41.
3. Jemal A, Ward E, Wu X, Martin HJ, McLaughlin CC, Thun MJ. Geographic patterns of prostate cancer mortality and variations in access to medical care in the United States. Cancer Epidemiol Biomarkers Prev 2005;14(3):590-5.
4. Quinn M, Babb P. Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part I: international comparisons. BJU Int 2002; 90(2):162-73.
5. Varenhorst E, Garmo H, Holmberg L, Adolfsson J, Damber JE, Hellstrom M, et al. The National Prostate Cancer Register in Sweden 1998-2002: trends in incidence, treatment and survival. Scand J Urol Nephrol 2005; 39(2):117-23.
6. Glenski WJ, Malek RS, Myrtle JF, Oesterling JE. Sustained, substantially increased concentration of prostate-specific antigen in the absence of prostatic malignant disease: an unusual clinical scenario. Mayo Clin Proc 1992; 67(3):249-52.
7. Brawer MK. Prostatic intraepithelial neoplasia and prostate-specific antigen. Urology 1989; 34(6 Suppl):62-5.
8. Oesterling JE. Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 1991;145(5):907-23.
9. Spencer JA, Alexander AA, Gomella L, Matteucci T, Goldberg BB. Clinical and US findings in prostate cancer: patients with normal prostate-specific antigen levels. Radiology 1993;189(2):389-93.
10. Smith DS, Catalona WJ, Herschman JD. Longitudinal screening for prostate cancer with prostate-specific antigen. JAMA. 1996; 276(16):1309-15.
11. Presti JC, Jr. Prostate cancer: assessment of risk using digital rectal examination, tumor grade, prostate-specific antigen, and systematic biopsy. Radiol Clin North Am 2000; 38(1):49-58.

12. Jewett HJ. The present status of radical prostatectomy for stages A and B prostatic cancer. Urol Clin North Am 1975; 2(1):105-24.
13. Rao AR, Motiwala HG, Karim OM. The discovery of prostate-specific antigen. BJU Int 2008; 101(1):5-10.
14. Stamey TA, Kabalin JN. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. Untreated patients. J Urol 1989; 141(5):1070-5.
15. Palken M, Cobb OE, Warren BH, Hoak DC. Prostate cancer: correlation of digital rectal examination, transrectal ultrasound and prostate specific antigen levels with tumor volumes in radical prostatectomy specimens. J Urol 1990;143(6):1155-62.
16. Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987; 317(15):909-16.
17. Gillatt D, Reynard JM. What is the normal range for prosate-specific antigen? use of a receiver operating characteristic curve to evaluate a serum marker. Br J Urol 1995; 75(3):341-6.
18. McNeil BJ, Keller E, Adelstein SJ. Primer on certain elements of medical decision making. N Engl J Med 1975; 293(5):211-5.
19. Oesterling JE. Prostate-specific antigen. Improving its ability to diagnose early prostate cancer. JAMA. 1992; 267(16):2236-8.
20. Hudson MA, Bahnson RR, Catalona WJ. Clinical use of prostate specific antigen in patients with prostate cancer. J Urol 1989;142(4):1011-7.
21. Benson MC, Whang IS, Pantuck A, Ring K, Kaplan SA, Olsson CA, et al. Prostate specific antigen density: a means of distinguishing benign prostatic hypertrophy and prostate cancer. J Urol 1992;147(3 Pt 2):815-6.
22. Benson MC, Whang IS, Olsson CA, McMahon DJ, Cooner WH. The use of prostate specific antigen density to enhance the predictive value of intermediate levels of serum prostate specific antigen. J Urol 1992;147(3 Pt 2):817-21.
23. Brawer MK, Aramburu EA, Chen GL, Preston SD, Ellis WJ. The inability of prostate specific antigen index to enhance the predictive the value of prostate specific antigen in the diagnosis of prostatic carcinoma. J Urol 1993;150(2 Pt 1):369-73.
24. Carter HB, Pearson JD, Waclawiw Z, Metter EJ, Chan DW, Guess HA, et al. Prostate-specific antigen variability in men without prostate cancer: effect of sampling interval on prostate-specific antigen velocity. Urology 1995;45(4):591-6.
25. Chodak GW. Early detection and screening for prostatic cancer. Urology 1989;34(4 Suppl):10-2; discussion 46-56.
26. Mueller EJ, Crain TW, Thompson IM, Rodriguez FR. An evaluation of serial digital rectal examinations in screening for prostate cancer. J Urol 1988; 140(6):1445-7.
27. Lee F, Littrup PJ, Torp-Pedersen ST, Mettlin C, McHugh TA, Gray JM, et

al. Prostate cancer: comparison of transrectal US and digital rectal examination for screening. Radiology 1988;168(2):389-94.

28. Oates RD, Stilmant MM, Freedlund MC, Siroky MB. Granulomatous prostatitis following bacillus Calmette-Guerin immunotherapy of bladder cancer. J Urol 1988;140(4):751-4.
29. Linn R, Klimberg IW, Wajsman Z. Persistent acid-fast bacilli following intravesical bacillus Calmette-Guerin. J Urol 1989;141(5):1197-8.
30. Helpap B, Vogel J. TUR-prostatitis. Histological and immunohistochemical observations on a special type of granulomatous prostatitis. Pathol Res Pract 1986;181(3):301-7.
31. Mikolich DJ, Mates SM. Granulomatous prostatitis due to Mycobacterium avium complex. Clin Infect Dis 1992;14(2):589-91.
32. Bray VJ, Hasbargen JA. Prostatic involvement in Wegener's granulomatosis. Am J Kidney Dis 1991;17(5):578-80.

پوختە

پشکنینا کماخێ و بەراوەرکرن لگەل پشکنینا PSA ژ بو دەست نیشانکرنا شێر پەنجا پروستاتی

پیشەکی: شێر پەنجا پروستاتی دهیته هژمارتن وەك مشه ترین شێر پەنجێت (ژ بلی پیستی) دهێتە دەست نیشانکرن و سێ یەمین مشه ترین ئەگر بو مرنا نەخوشا ژ بەر شێر پەنجا پروستاتی ل دەف توخمێ نێر. PSA دیار بوو کو با شترین (دیارکەرێ پەنجه شێرێ) کو تازه دیار بووی ژ بو دەست نیشانکرن و چارەسەرییا نەخوشێت شێر پەنجا پروسگتاتی. پگشکنینا کماخێ هێشتا شەنگستەیە بو دەست نیشانکرن شێر پەنجا پروستاتی ل تگوخمێ نێگر ل گگەل ئاسگتێ PSA یگێ نورمگال یگان پیچەکێ بلند ژ نورمال.

ئارمانج: ئەگەر و ئارمانجا قێ قەکولینێ ئەو بو کو هەلسەنگاندنا کارتێکرنا PSA لگەل پشکنینا کماخێ بو دەست نیشانکرنا ئێشا شێر پەنجا پروستاتێ لدەڤ نەخۆشێن تووشی نیشانێت گرتنا بوریێت میزێ بووین.

نەخوش و ڕێکێت قەکولینێ: قەکولین هاتنە کرن ل نەخوشخانا ئازادی یا فێرکرنێ یا گشتی لسەر 400 نەخوشێت تووشی گرتنا بووریێت میزێ بووین و یێت کو ژیێ وا ژ 50 سالیێ پتر، د ناڤبەرا کانونا ئێکێ 2005 ێ و چرییا دووێ یا سالا 2007ێ. پشکنینا PSA و پشکنینا کماخێ بو هەمییا هاتە کرن لگەل وەرگرتنا پارچەکا بچیک ژ پروستاتێ بو هیستوپاتولوجێ بو ئەنجامێت نەدڕوست.

ئەنجام: ژیێ باراپتری نەخوشا لنێزیکێ 69.63 سالیێ بوون و هەمی نەخوشێت کو توشی نەخوشییا شێرپەنجا پروستاتێ بووین PSAیێ وان پتر بوو ژ 4 نگ/مللتر و پشکنینا کماخێ پەسەندکر کو گرێ ژی یێت پەیدابووین دپروستاتێدا.

نیڤشک: پگشکنینا پروسگتاتێ بڕێگا کمگاخێ هێگشتا ئێگگە ژ پینگاڤێگت شنگەسگتە ژ بگوو دەسگت نیگشانکرنا شگێر پگەنجا پروستاتێ، پشکنینا پروستاتێ بڕێکا کماخێ لگەل یا PSA و لگەل وەرگرتنگا پارچگەکا پروسگتاتی بگو هیگستوپاتولوجیێ، یان بێ وەرگرتنا قێ پارچێ ژێک دیار بوو کو ڕێکەکا نمونەییە بو دەست نیشانکرنا شێر پەنجا پروستاتێ.

الخلاصة

فحص الموثة عن طريق المستقيم بالمقارنة مع PSA في تشخيص سرطان الموثة (البروستاتا)

خلفية البحث: سرطان الموثة هو اكثر الامراض الخبيثة المشخصة شيوعا (بعد استبعاد الأورام الجلدية) وتعتبر السبب الثالث للوفاة من الاورام عند الذكور. ان مولد المضاد النوعي للموثة (PSA) هو الاكثـر اعتمـادا للاسـتخدام فـي تشخيص المرضى المصابين بمرض الموثة الخبيث. فحص الموثة عن طريق المستقيم (DRE) وارتفاع مستوى مولد المضاد النوعي للموثة هي الاشارات الاولية المعتمدة لتشخيص ورم الموثة الخبيث.

الهدف: الغرض من الدراسة هو لتقييم مدى فعالية PSA مع فحص الموثة عن طريق المستقيم فى تـشخيص مـرض سرطان الموثة لدى المرضى المصابين بأعراض انسداد المجرى البولي.

المرضى و طرق البحث: اجري البحث فى مستشفى أزادي التعليمى العام على 400 مريض المصابين بأعراض انسداد المجرى البولي والذين أعمارهم تتجاوز 50 سنة خلال الفترة ما بين كـانون الأول 2005- تـشرين الثـاني 2007، وأجري فحص PSA وفحص الموثة عن طريق المستقيم لكل المرضى مع أخذ خزعة مـن الموثـة للنتـائج الغيـر الطبيعية.

النتائج: معدل العمر للمرضى 69.63 سنة، كل المرضى المصابين بسرطان الموثة والذين لديهم عقدة فى الموثة عـن طريق فحص المستقيم ثبت ان لديهم PSA أكثر من 4 ng/ml.

الأستنباطات: فحص الموثة عن طريق المستقيم لا يزال الخطوة الاساسية لتشخيص سرطان الموثة. فحص الموثة عـن طريق المستقيم مع PSA مع أخذ أو عدم أخذ خزعة من الموثة تعتبر الطريقة المثلى لتشخيص سرطان الموثة.

EARLY DETECTION OF DIABETIC NEPHROPATHY IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS

QAYSER S. AL-HABEEB, MBChB,DIM,MSc*
HANA A. TAWFIK, MBChB, DPH**
HISHAM R. AL-EIDANI, MBChB, FICMS (Med)***

Submitted 10 January 2008; accepted 10 June 2008

ABSTRACT

Background: Recent studies have demonstrated that the onset and course of diabetic nephropathy can be ameliorated to a very significant degree by several interventions but these interventions would have their greatest impact if instituted at a point very early in the course of development of this complication. This study has been designed to explore the problem of diabetic nephropathy in type 1 Diabetes Mellitus particularly its early detection in a sample of children and adolescent patients up to 18 yrs of age.

Objectives: Estimating the prevalence of microalbuminuria and hyperfiltration, and assessing the relationship between microalbuminuria and GFR in type 1 diabetic children and adolescents.

Patients and methods: A cross-sectional design and a consecutive sampling procedure were adopted to enroll 115 patients (59 males and 56 females) who met the inclusion criteria, from those attending the National Diabetic Center of Al Mustansiriyah University / Baghdad during the period from the 1st of August 2005 to the end of July 2006. Micral test II was used to screen early morning (spot) urine samples for increased albumin excretion rate while the novel use (in Iraq) of Schwartz formula made possible estimating the GFR from serum creatinine and demographic characteristics. The results were used for assessing the relationship between microalbuminuria and GFR. Important risk factors including patients age and disease duration, have also been evaluated.

Results: The mean patients age was 14.05 (±2.95) years, and the mean disease duration was 6.52 (±2.85) years.The prevalence of microalbuminuria in the study sample was (48.70%) , estimated as increased urinary albumin excretion (20-200mg/l). Statistically significant associations were found between microalbuminuria and longer duration of diabetes (p value = 0.017),and older age of diabetic patients (p value = 0.031). The overall prevalence of hyperfiltration (estimated as GFR of ≥130ml/min/1.73m2) was (16.52%), comprising (63%) normoalbuminuric and (37%) exhibiting microalbuminuria. Male preponderance was evident (89.48%). Factors showing significant association with hyperfiltration state were: male gender (p value = 0.013),and older age of diabetic patients (p value = 0.031) There was a statistically significant inverse correlation between the different levels of albumin excretion rate and the levels of estimated GFR. (r = - 0.79 P value = 0.024).

DMJ 2008;2(1): 91-106.

Key words: Diabetic nephropathy, Microalbuminuria, Glomerular filtration rate, Micral 11 test, Schwatz Formula

* *Assistant Professor, Dohuk College of Medicine, Dohuk, Iraq*
** *Colleg of Health and Medical Technology, Baghdad, Iraq*
*** *Al Mustansiriyah Medical College, Baghdad, Iraq*
Correspondence author: Qayser Sahib Al-Habeeb, *Dohuk College of Medicine, Dohuk, Iraq.*

Type one diabetes mellitus (T1DM) is a multifactorial autoimmune disease thought to arise from a complex interaction between both genetic susceptibility and environmental insult(s).[1] It is the most common endocrine metabolic disorder of childhood and adolescence and accounts for 5-10% of all diagnosed cases of diabetes.. Type 1 diabetes mellitus patients are unique diabetes subpopulation, as these individuals are young and within the productive phase of their life, and researches suggested they may have more severe disease than type two diabetes mellitus (T2DM) patients, with a greater burden from complications· Diabetic nephropathy (DN) is the leading known cause of end-stage renal disease (ESRD). Epidemiological studies have demonstrated that diabetic nephropathy occurs in approximately one-third to one half of all patients with T1DM and, today, diabetes is the most important cause of renal failure in the industrialized world[2,3] Hyperfiltration, microalbuminuria (incipient DN) and macro-proteinuria (overt DN) characterize the clinical stages of DN. Microalbuminuria (MA) is the best predictor of high risk of developing established (overt) diabetic nephropathy. [4] Thus, the detection of (MA) has played a key-role in the management of T1DM. Therefore a reliable easy test for routine screening for MA is desirable, such a test has been developed and marketed as Micral Test II. Several cross-sectional and prospective studies have demonstrated that glomerular hyperfiltration is frequently detectable in TIDM children and adolescent patients.[5,6] In all these studies persistent glomerular hyperfiltration increases the risk of developing MA (incipient DN). The increased glomerular filtration rate (GFR) is a well established feature of early uncomplicated T1DM in children and adolescent patients[4,6] It has been shown that patients with early glomerular hyperfiltration and diabetes of 3-6 yrs duration have a greater risk of subsequent nephropathy.[(5)].Once overt DN is established, current therapeutic strategies, including improved glycemic control, and effective antihypertensive therapy, tend to slow, but are unable to arrest progression of the process.[7]Recent studies have demonstrated that the onset and course of DN can be ameliorated to a very significant degree by several interventions, but these interventions would have their greatest impact if instituted at a point very early in the course of the development of this complication.[7] Among the great world-wide interest in early detection of DN in T1DM children and adolescent patients, only few Iraqi studies have been documented.[8-10] This study has been designed to explore this very little investigated problem in Iraq, aiming at estimating the prevalence of MA and hyperfiltration. and assessing the relationship between MA and GFR in type 1 diabetic children and adolescents.

PATIENTS AND METHODS

The study was conducted in the National Diabetes Center, Al-Mustansiriyah University / Baghdad during the period from the 1st of August 2005 to the end of July 2006.A cross

sectional design and consecutive sampling procedure was employed to enroll patients attending for treatment and follow up. Eligible patients should meet the following criteria:

1 - Patients up to the age of 18 years with type 1 diabetes mellitus as defined by WHO criteria.[11]

2 - The duration of the diabetic state should be 3 years or more.[12]

Exclusion Criteria[2]: Presence of overt proteinuria**,** urinary tract infection, .hematuria, ketonuria**,** pregnancy**,** acute febrile illness,. heart failure, clinical conditions causing dehydration (due to possibility of false positive results on albumin measurements), any wasting disease that could cause severe undernourishment**,** after heavy exercise or heavy meal, and short–term pronounced hyperglycemia

Data Collection:

1st visit : Potentially eligible patients (127) were interviewed for past medical history, and underwent full clinical examination including blood pressure measurement , followed by obtaining a freshly voided urine sample which was tested for the presence of protein, cells, and blood casts, using a dipstick technique (Multistix 10, SG, Bayer, Bridgend, UK), to exclude proteinuria (strip result of 30 mg/dl), UTI, haematuria, and ketonuria**.** During this visit, 12 patients were excluded from the study due to the presence of one or more of the exclusion criteria. The rest were asked to come the next visit, fasting with an early morning urine sample collected as instructed.

2nd Visit: The enrolled 115 patients (59 males, and 56 females) were studied according to a unified protocol consisting of a questionnaire especially designed to accommodate the relevant demographic and clinical data**.** During this visit, fasting blood samples were taken for the required laboratory tests .

Measurement of renal functions:

A -Micral Test: Micral Test II (Roche diagnostic GmbL , Mannheim, Germany) is an immunological strip, gold labeled, optically read test for the immunological, semi-quantitative in vitro determination of urinary albumin from 0 up to concentration of 100 mg/L. MA was considered to be present if urinary albumin excretion rate (AER) in spot (first morning urine sample) was 20-199 mg/l.[4]

B - Glomerular Filtration Rate (GFR) :

Schwartz formula was used in this study for the first time in Iraq .

Utilizing the proportionality between GFR and height/serum creatinine, Schwartz formula[13] was used to provide an estimate of GFR based on a constant multiplied by the child's height divided by serum creatinine, thus:

$$C_{Cr}\ (mL/min/1.73m^2) = \frac{K \times Height\ (cm)}{S_{Cr}\ (mg/dL)}$$

C_{Cr} : Creatinine clearance;

S_{Cr}, : Serum creatinine;

K : Constant

The constant K is directly proportional to the muscle component of body, and varies with age and sex, the value of K to be used in premature infants is 0.33, in full term

infants <1 year of age is 0.45, , in children up to 13 years old is 0.55 and also in adolescent girls and boys the value of the constant changes to 0.7. Normal GFR cutoff value ≥90 ml/min/1.73m^2. Hyperfiltration ≥130 ml/min/1.73m^2 http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm.

Grouping of the Studied Subjects:

Enrolled patients were classified into three groups according to the results of Micral test, and the estimated GFR (using Schwartz Fomula) :

Group 1:

Normoalbuminuria

Micral Test (-ve)

Normal GFR

90 -129 mls/min/1.73m^2

Below normal GFR

< 90 mls/min/1.73m^2

Group 2 :

Normoalbuminuria

{Micral Test (-ve)}

Hyperfiltration

GFR ≥130 mls/min/1.73m^2

Group 3:

Microalbuminuria

Micral test (+ve) ;

Normal GFR

90 -129 mls/min/1.73m2

Hyperfiltration

GFR ≥ 130 mls/min/1.73m2

Below normal GFR

< 90 mls/min/1.73m2

Statistical Analysis:

SPSS version 10.0 under windows XP was used to analyze data, and Excel 2003 under windows XP for figures.

Proper inferential statistics were used to analyze the results including Chi-squared test, one-way Anova and Pearson's correlation coefficient**.** A p-value less than 0.05 was considered statistically significant, and less than 0.001 considered highly significant .

RESULTS AND DISCUSSION

Microalbuminuria has been shown to be the earliest stage of DN in TIDM. Once MA appears the fatal outcome is predictable, therefore, a crucial goal in prevention would be to demonstrate factors early during the disease which may indicate the progression to macroalbuminuria and then to the ESRD.[4] Glomerular filtration rate is increased in early diabetes and considered as a significant risk factor for persistent MA and incipient nephropathy in TIDM children and adolescents as confirmed by Dahlquist et al.[14] Several studies indicated that hyperfiltration is of considerable importance, the most convincing was that done by Rudberg et al.[15] Before MA develops, GFR is elevated, indeed, both glomerular hyperfiltration and MA are early signs of development of DN.[16] The prominent finding of the current study was the detection of MA in 56 out of 115 patients (48.70 %) (Table 2). Of substantial concern is the marked variability in the occurrence of MA among T1DM patients in previous studies (range from 2.7% to 42%).[8,9] A number of complex variables could have accounted for those differences including background genetics, population, selection criteria, microalbuminuria definition,

methods of laboratory investigation, and the varied methodology used which further hinders valid comparisons. Starting with previous Iraqi studies, the lowest figure reported by Dahar,[9] 2.7% contrasted that found by Askar,[10] 40%,and Atiya[8] 42.02%. A low rate of 6% was found in prospective studies in Brazil, Canada, and Boston (USA)[17]. This discrepancy among different countries may be attributed to defective diabetic control which may be related to factors like type of insulin regime (conventional or intensive), dietary management, exercise, and the cornerstone of management "patient's education". Another reason is the fact that T1DM patients were mostly children and adolescents, and Insulin therapy mandates repeated injections on daily basis, a point which constitutes a good reason for poor compliance.

The other related finding is slight increase in the frequency of MA in females compared to males (30 vs 26 constituting 53.57% vs 46.44% respectively) (Table 2) the difference did not achieve statistical significance. Within the limits of the local available literature, none of the previous Iraqi studies made use of Schwartz formula to estimate the GFR in T1DM children and adolescents up to 18 years old. The method is easy and convenient for both the investigator, and the patient, with the advantage of rapid determination of the GFR, less invasive than other methods, and the avoidance of urine collection, especially for age groups like those enrolled in this study.[13] Several previous cross sectional and prospective studies have demonstrated that hyperfiltration is frequently detectable in type 1 diabetic children and adolescents.[5,18] In this study 16.52% of the patients had increased levels of GFR (Hyperfiltration) (Table 3) which was in accordance with the results reported by Chiarelli etal.[6] and Rajic et al.[18] In other prospective studies almost two-thirds of the patients had hyper-filtration.[15,19] Some studies reported that glomerular hyperfiltration was not a significant risk factor for DN.[20] Still, others like Yip et al.[21] claimed that they can not disregard the role of increased GFR on the development of DN because the increased level of urinary albumin excretion rate (UAER) was observed only in hyperfiltrating patients. Other studies revealed that glomerular hyperfiltration was a strong risk factor for DN.[6,15] These contradictory results are not surprising considering the multifactorial nature of diabetes and diabetic nephropathy, the different criteria used in patient's selection, and the different methods of GFR measurement..

Among the 19 hyperfiltrating patients in this study, 17 subjects were males 89.48%, while females constituted 10.52%, the M/F ratio was 8.5/1, and proved statistically highly significant (p value=0.000) , these results were in agreement with the results of the screening program done in Italy where males constituted 60.87% of the hyperfiltrating patients vs 39% of females.[6] Similar results were reported from Saudi Arabia,[22] It is not generally agreed that male gender increases DN risk in T1DM.[(18)] Jabri et al.[23] stated that males

and females are equally affected.

As regards the frequency of low GFR in female compared to male subjects (61.54 % vs 38.46 %), F / M ratio was statistically highly significant (p value=0.000), this finding was similar to the results reported by Mauer et al.[24] Out of the 19 hyperfiltrating patients, 12(63.16%) were normo-albuminuric The difference was statistically highly significant (p value = 0.000); implying a hyperfiltration process even before the appearance of MA, a finding which may indicate that elevated GFR is an early process in the pathogenesis of DN in T1DM patients. Those patients with hyperfiltration may be at particular risk of developing DN,[25] hence detecting hyperfiltration at an early stage may be of prime importance.[4] Askar[10] reported that the mean GFR was significantly higher in normoalbuinuric patients than in the control and microalbuminuric groups. The remaining seven hyperfiltrating patients 36.84% in the current study, developed MA, this result was close to that of Mogensen[5] who reported that 30% of the hyperfiltrating patients developed MA, and was higher than that of Chiarelli et al. 21.74% in Italy.[6] Rudberg et al.[15] and Dahlquist et al.[26] reported that half of the hyperfiltrating patients developed MA, which indicates that glomerular hyperfiltration increases the risk of developing MA (Incipient Diabetic Nephropathy). In a retrospective study by Mogensen,[5] and a follow-up study by Chiarelli et al,[6] they showed a strong predictive value for hyperfiltration on the occurrence of MA.. Seven out of the 56 microalbnminuric patients (12.5%) had hyperfiltration, which was in agreement with the results reported by Rajic et al.[18] and Bangstad et al.[27] The other 34 patients (64.29%) predominantly had unchanged GFR, in agreement with the results reported by Rajic et al,[18] and a further group of patients 15(26.79%) had hypofiltration, in agreement with the results reported by Rajic et al.[18] and Bangstad et al.[27]

There was a statistically significant inverse correlation between different levels of AER and GFR (r = - 0.791, p value = 0.024) a finding in agreement with the results of Mogensen.[(5)] Previous studies on T1DM patients resulted in different estimates of AER and GFR, separately, as well as regarding their relationship; this was emphasized particularly in the early stages of the DN.[18]

There was a clear preponderance of male gender in group 2 in comparison with groups 1, and 3 (Table 5). This finding was comparable to the study done by Hovid.[28] While in the MA group 3 there was a slight excess of females, the difference was not significant (p value=0.308). This finding was comparable to an Iraqi study by Atiya,[8] but disagreed with Dahar,[9] where he reported that male gender predominated in MA patients..The frequency of hyperfiltration increased significantly with increasing age of the diabetic patients in group 2 (0% in children 7-10 yrs , 25% in 11-14 yrs, and 75% in 15-18 yrs) (Table 6). The difference was statistically significant (p value = 0.031) .The same trend was found in MA group 3 (17.85% , 32.15%, 50%)

respectively. these results are in agreement with studies from Finland, UK, France, and Iraq,[9,29] which revealed that the age is a risk factor for development of MA and onset of DN. This finding was disagreed by Dahar,[9] and Luiza et al.[30] where they found no significant difference between diabetic patients with or without MA regarding age.

As for the duration of diabetes the eGFR was significantly increased in group II, 50% of the patients were hyperfiltrating within the duration (<5 yrs), 41.66% within (5-10 yrs), and 8.33% (>10yrs) (Table 7). It has been nicely demonstrated by Mogensen[5] that in the first decade after diabetes becomes manifest, the GFR is increased, while at the same time UAER is still normal or slightly increased. Similar results were reported by Rajic et al.[18] Bangstad et. al,[27] who found that their patients did hyperfiltrate after two and a half years from the onset of T1DM. These results indicate that the elevated GFR in T1DM even in the stage of normoalbuminuria can be an important risk factor for future MA and DN.Chiarelli et al.[6] followed a cohort of patients with hyperfiltration and found that the GFR was persistently elevated in the first 6 yrs, thereafter a slow decrease in GFR was observed. Atiya[8] found that the duration of T1DM is the most important risk factor for the development of MA and progression to overt nephropathy. Although MA is thought to be rare in T1DM patients of less than 5 years duration[8], the current study revealed that 17.86% of the T1DM patients had MA within 5yrs duration. This finding was in accordance with the EURODIAB IDDM Complications Study Group and WHO Multinational Study of Vascular Disease in Diabetes Study Group, where Stephenson and Fuller[12] found that raised UAE occurs before 5 years of IDDM 18% in EURODIAB and 15% in WHO studies. Atiya[8] found that 38% of Iraqi TIDM patients developed MA within 2-5 years duration, a finding which is higher than the results,of this study; the discrepancy might be attributed to the difference in duration (3-<5yrs for the current study vs 2-5 yrs).Thus the results indicate that MA does occur in TIDM patients with duration less than 5 yrs. Regarding the duration of T1DM (5-10yrs), the prevalence of MA was 68.86% which was the highest percentage of MA according to the duration of diabetes , this result was in agreement with that found by Atiya. [8] In the current study the percentage of cases with hyperfiltration showed a progressive decrease as the duration of the disease was increasing (50%, 41.66%, 8.33%) corresponding to the disease duration (less than 5years, 5-10, more than 10yrs respectively). As for the duration of >10 yrs, the percentage of MA in this study (14.29%) was lower than those reported by Atiya[8] and Dabelea et al.[31] The reason might be related to the small size of the sample (n=14/115).

Table 1. Baseline characteristics of participants

Characteristics of participants	Male N=59 (51.30%)		Female N=56 (48.7 %)		Total N=115		P value
	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range	
Age (years)	13.99 (±3.04)	7-18	14.08 (3±.07)	7.5-18	14.03 (2±.95)	7-18	0.88 NS
Disease duration (years)	7.07 (3±.21)	3-16	5.97 (±2.49)	3-16	6.52 (2±.85)	3-16	0.40 NS

Table 2: Results of Micral II test by gender

Gender	Micral Test		Total	χ^2 P. value
	- ve	+ve		
	No. (%)	No. (%)	No. (%)	
Male	33 (55.93)	26 (46.43)	59 (51.3)	0.308 NS
Female	26 (44.07)	30 (53.57)	56 (48.7)	
Total	59 (100)	56 (100)	115 (100)	

Table 3. Participants gender by the estimated Glomerular Filtration Rate

eGFR*	Male	Female	Total	χ^2 P. value
	No. (%)	No. (%)	No. (%)	
60 – 89	10 (38.46)	16 (61.54)	26 (22.61)	0.000 HS
90 – 129 normal	32 (45.71)	38 (54.29)	70 (60.87)	0.973 NS
≥ 130	17 (89.48)	2 (10.53)	19 (16.52)	0.000 HS
Total	59 (51.3)	56 (48.7)	115 (100.0)	

** The estimated GFR using Schwartz Formula (mL/min/1.73m^2)*

Table 4. Micral test results by the eGFR

eGFR* (ml / min / 1.73 m^2)	Micral (-ve)	Micral (+ve)				Total	χ^2 P-value
		20mg/L	50 mg/L	100mg/L	Total	No. (%)	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)		
≥ 130	12 (20.34)	6 (31.58)	1 (3.85)	0 (0)	7 (12.5)	19(16.52)	0.000 HS
90-129	36 (61.02)	10 (52.63)	17(65.38)	7 (63.64)	34(60.71)	70(60.87)	
<90	11 (18.64)	3 (15.79)	8 (30.77)	4 (36.36)	15(26.79)	26(22.61)	
Total	59 (100.0)	19 (100.0)	26(100.0)	11(100.0)	56(100.0)	115(100.0)	

** The estimated GFR using Schwartz Formula (mL/min/1.73m^2)*

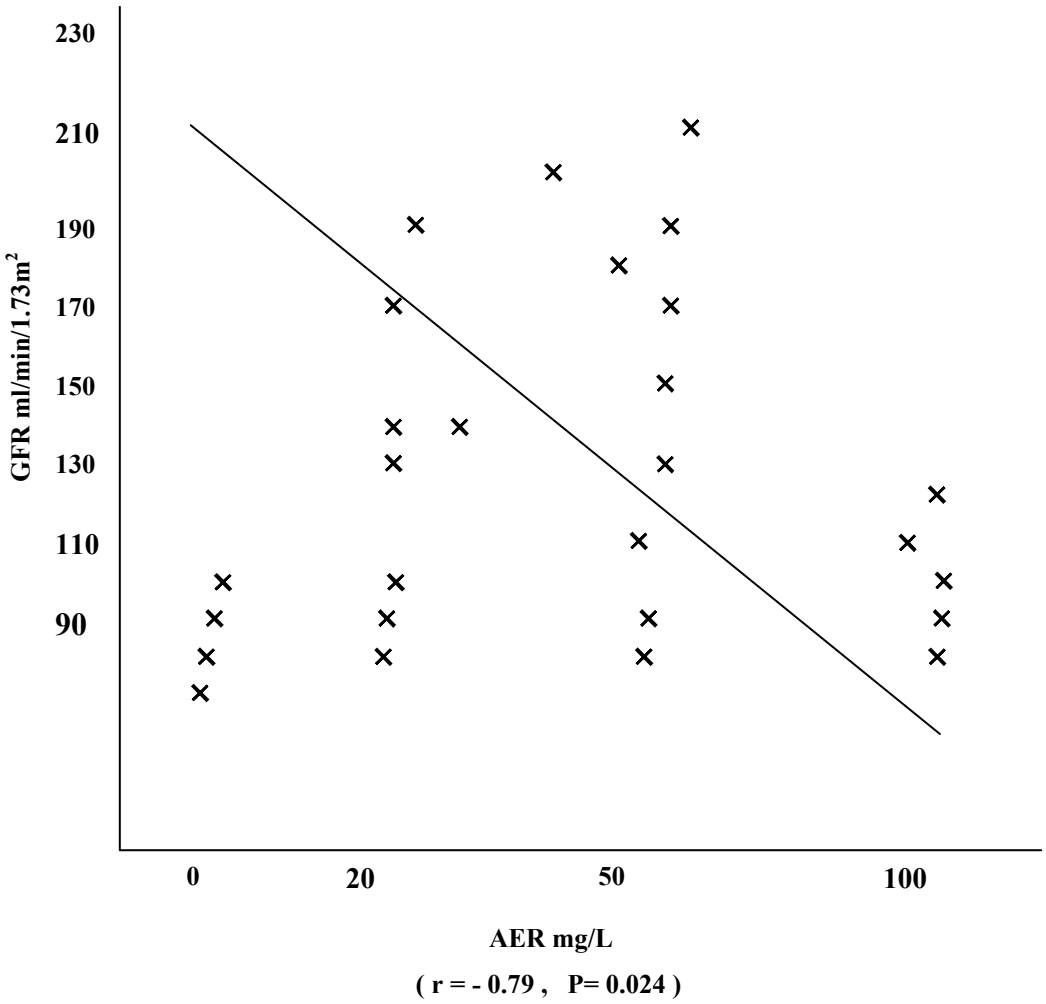


Figure 1. Scatter diagram showing the correlation between the degree of microalbuminuria (mg/L)and the eGFR (mL/min/1.73m^2)

Group (1)	Group (2)	Group (3)
n= 47 (40.87%)	n= 12 (10.43%)	n= 56 (48.70%)
Micral test (-ve)	Micral test (-ve)	Micral test (+ve)
Normal GFR=36		with or without
Below normal=11	Hyperfiltration	Hyperfiltration
Male 22 (46.81%)	Male 11 (91.66%)	Male 26 (46.43%)
Female 25 (53.19%)	Female 1 (8.33%)	Female 30(53.57%)

Figure 2. The studied subjects categorized by Micral II Test, and eGFR

Table 5. Distribution of the categorized groups by gender

Gender	Group 1 No. (%)	Group 2 No. (%)	Group 3 No. (%)	Total No. (%)	χ^2 P. value
Male	22 (46.81)	11 (91.66)	26 (46.43)	59 (51.3)	0.013 S
Female	25 (53.19)	1 (8.33)	30 (53.57)	56 (48.7)	
Total	47	12	56	115	

Table 6. Distribution of the categorized groups by age

Age (years)	Group 1 No. (%)	Group 2 No. (%)	Group 3 No. (%)	Total No. (%)	χ^2 P. value
7-10	6 (12.77)	0 (0.00)	10 (17.85)	16 (13.91)	0.031 S
11-14	19 (40.43)	3 (25.0)	18 (32.15)	40 (34.78)	
15-18	22 (46.80)	9 (75.0)	28 (50.0)	59 (51.30)	
Total	47	12	56	115	

Table 7. Distribution of the categorized groups by disease duration

Duration of DM(yr)	Group 1 No. (%)	Group 2 No. (%)	Group 3 No. (%)	Total No. (%)	χ^2 P. value
<5	17 (52%)	6 (18%)	10 (30%)	33 (28.70)	0. 017 S
5-10	25 (37%)	5 (7%)	38 (56%)	68 (59.13)	
>10	5 (36%)	1 (7%)	8 (57%)	14 (12.17)	
Total	47	12	56	115	

CONCLUSIONS

1. The estimated overall prevalence of hyperfiltration was (16.52%)with evident male preponderance (89.48%) highlighting male gender as a risk for hyperfiltration. (63%) of hyperfiltrating patients were normoalbuminuric, implying a hyperfiltration process even before the appearance of MA. The remaining (37%) exhibited MA, implying that glomerular hyperfiltration might have a role in the development of MA (incipient DN).
2. Microalbuminuria manifested by increased urinary albumin excretion was encountered in 48.70% of the patients. This was the highest documented frequency among the previous Iraqi studies. There was a statistically significant inverse correlation between the different levels of AER and the levels of eGFR .(r = -0.79 P. value 0.024).
3. The frequency of hyperfiltration increased significantly with increasing age of the diabetic patients. The same trend was found with microalbuminuric group, a setting which points to patient's age as a risk factor for the development of MA, and the onset of DN. The majority of MA cases and hyperfiltrating groups occurred at the age 15-18 yrs.
4. The duration of diabetes had a substantial role in the development of hyperfiltration. (50%) of the patients were hyperfiltrating within the duration of less than 5 yrs, and decreased thereafter to (41%)at 10 yrs. Beyond this duration no effect has been noticed on the GFR (p value= 0.017).Contrary to some previous studies, (17.86%) of the studied patients developed MA in less than 5 yrs duration, and it increased thereafter up to 10 yrs (67.86%).(p value 0.017) .

RECOMMENDATIONS

1. Systematic measurement of both GFR and UAER is recommended, since the two parameters help identifying children and adolescent patients at risk of DN and ESRD.
2. Diabetic children and adolescents, exhibiting an early increase in GFR (hyperfiltration) should be considered as a high risk group for developing persistent MA, and, consequently, incipient DN. In such cases every effort should be made to achieve the best glycemic control from the very beginning of the disease.
3. In T1DM, screening for MA might be performed in less than 5yrs after diagnosis,. If the result is negative at the initial screen, yearly screening is recommended. Also the use of Micral Test II, in an early morning (spot) urine sample is recommended as a convenient screening tool, if expense is tolerated.
4. Proper follow-up of patients with MA, including those with low level of AER, makes possible, effective modulation of the factors responsible for either progression or regression.

5. In children and adolescents repeated measurements of GFR are laborious, time consuming, and sometimes inaccurate. Moreover, annoying repeated venous sampling, and tedious urine collections are problematic when repeated evaluations are required. Therefore it is recommended that the various formulas that have been developed to allow prediction of the GFR from serum creatinine and demographic characteristics, are made use of in future research methodology.
6. To conduct more elaborate analytical studies including other regional diabetic centers in the country for more comprehensive understanding of the problem**.** Also extending the scope of the study by including adult diabetics through the application of suitable GFR estimation formula. (MDRD equation. for adults above 18 yrs old).

REFERENCES

1. Alemzadeh R, Wyatt DT. Diabetes mellitus: In: Behrman RE, Kliegman RM, Jenson HB, eds. Nelson textbook of pediatrics. 17th ed. Philadelphia: WB Saunders Co; 2004. p. 1947–72.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus (Position Statement). Diabetes Care 2006;28:S43-S8.
3. Fowler MJ. Diabetes: Magnitude and Mechanisms. Clinical Diabetes 2007;25:25-8.
4. Mogensen CE. Microalbuminuria and Hypertension with focus on type 1 and type 2 diabetes. J of Int Med 2003;254:45-66.
5. Mogensen CE. Early glomerular hyperfiltration in insulin dependent diabetics and late nephropathy. Scand J Clin Lab Invest 1986;46:201-6
6. Chiarelli F, Verrotti A, and Morgese G. Glomerular hyperfiltration increases the risk of developing microalbuminuria in diabetic children. Pediatr Nephrol 1995;9:154-8.
7. Cooper ME. Pathogenesis, prevention, and treatment of diabetic nephropathy. Lancet 1998;352:213-9.
8. Atiya JK. Microalbuminuria in children and adolescents with Type I Diabetes Mellitus [dissertation]. Baghdad, Iraq: FICMS (Pediatrics); 2002.
9. Dahar AH. Relationship between Hypertention and Micro-albuminuria in type 1 Diabetes Mellitus (Insulin dependent) in childhood and adolescence[dissertation]. Baghdad, Iraq: FICMS (Pediatrics); 2005.
10. Askar FK. Diabetic Nephropathy and its related risk factors: Biochemical and clinical evaluation of the patients [PhD thesis]. Baghdad, Iraq: Medical College, Al-Nahreen Univ.; 1996.
11. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications-Part 1: Diagnosis and classification of diabetes mellitus. Geneva: WHO; 1999.
12. Stephenson JM, Fuller JH: Microalbuminuria is not rare before 5 years of IDDM: EURODIAB IDDM Complications Study Group and the WHO Multinational Study of Vascular

Disease in Diabetes Study Group. J Diabetes Complications 1994;8:66 – 73.

13. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 1976;58:259–63.
14. Dahlquist G, Aperia A, Broberberg O, et al. Renal function in relation to metabolic control in children with diabetes of different duration. Acta Paediatr Scand 1983;72:903-9.
15. Rudberg S, Persson B, Dahlquist G. Increased glomerular filtration rate as a predictor of diabetic nephropathy An 8-year prospective study. Kidney Int 1992;41:822.
16. Viberti GC, Hill RD, Jarret RJ. Microalbuminuria as a predictor of clinical nephropathy in insulin dependent diabetes mellitus. Lancet 1982;1:1430-2.
17. Warram JH, Gearin G, Laffel L, Krolewski AS. Effect of duration of type 1 of diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinine ratio. J Am Soc Nephrol 1996;7(6):930-7.
18. Rajić M, Bogićević M, Antić S, Ilić S., Vlajković M, Avramović M, et al. The relationship between the rates of urinary albumin excretion and glomerular filtration in type 1 diabetes mellitus patients. Facta Universitatis/ Series Medicine and Biology 2003;10(1):36-40.
19. Amin R, Turner C, van Aken S. The relationship between microalbumuria and glomerular filtration rate in young type 1 diabetic subjects: The Oxford Regional Prospective Study. Kidney Int 2005; 68(4):1740-9.
20. Svensson M, Sundkvist G, Arnqvist HJ, Björk E, Blohmé G, Bolinder J et al. Signs of nephropathy may occur early in young adults with diabetes despite modern diabetes management. Diabetes Care 2003; 26: 2903-9.
21. Yip JW, Jones SL, Wiseman MJ, Hill C, Viberti G, et al.. Glomerular hyperfiltration in the prediction of nephropathy in IDDM: a 10-year follow-up study. Diabetes 1996;45:1729–33.
22. Abdullah MA. The clinical presentation of childhood diabetes mellitus in Riyadh. Saudi Med J 1989;10(6):495-7.
23. Jabri AM, Kadhem KL, AL-Aloosi HN. Diabetes mellitus in childhood. Journal of the Faculty of Medicine-Baghdad 1989;13(3):337-43.
24. Mauer M, and Drummond K. The Early natural history of nephropathy in type 1 diabetes: I. Study Design and Baseline Characteristics of the Study Participants. Diabetes 2002;51: 1572-9.
25. Rossing P, Hougaard P, Parving H-H. Risk factors for development of incipient and overt diabetic nephropathy in type -1 diabetic patients. Diabetes Care 2002;25:859-64.
26. Dahlquist G, Stattin EL, Rudberg S. Urinary albumin exertion rate and glomerular filtration rate in the prediction of diabetic nephropathy; a long-term follow-up study of

childhood onset type -1 diabetic patients. Nephrol Dial Transplant 2001;16:1382-6.

27. Bangstad HJ, Østerby R, Rudberg S, HARTMANN A, BRABRAND K, HANSSEN KF, et al. Kidney function and glomerulopathy over 8 years in young patients with Type I (insulin-dependent) diabetes mellitus and microalbuminuria. Diabetologia 2002;45:253–61.
28. Hovid P. Initation, progression and remission of diabetic nephropathy. Dan Med Bull 2005;52:119-42.
29. Levy-Marchal C, Sahler C, Cahane M. Risk factors for microalbuminuria in children and adolescents with type 1 diabetes. J Ped Endocrinol Metab 2000;13(6): 613-20.
30. Luiza M.A, Caramori, Jorge L, Gross. Glomerular filtration rate, urinary albumin excretion rate, and blood pressure changes in normoalbuminuric normotensive type 1 diabetic patients: an 8 year follow-up study. Diabetic Care 1999;22:9.
31. Dabelea D, Serban V, Bacanu GS. Determinants of microalbuminuria in diabetes. R J I M 1994; 32(4):291-7.

پوختە

زودەست نیشانکرنا تێکداچوونێن کولچیسکا لجەم زاروك و سنیلێن تووشی نەخوشیا شەکرێ جورێ ئێکێ بووین

پێشەکی: ڤەکولینەکا تازە دیارکریە کو چێبوونا باشکرنا رەوشا نەخوشیێن تووشی ئێشا شەکرێ بووین تایبەت دەربارەی تێکداچوونێن کولچیسکا و ب شێوێ باش ب رێکا چەندین دەستێوەردانا، بەلێ ئەگەر ل قوناغێن دەستپێکری یێن نەخوشیێ دەست پێکر. ئەڤ ڤەکولینە هاتە نەخشاندان ژ بو لێگەریانێ ل ڤرفتاریا تێکدانا کولچیسکا ژ ئەگەرێ نەخوشیا شەکرێ ژ جورێ ئێکێ و ب تایبەت ژ دەستنیشانکرنا وێ لجەم نموونەکێ زاروکا و سنیلا لژێر ژیێ 18 سالی دا.

ئارمانج: هژمارتنا بەلاڤبوونا (microalbuminuria) و (hyperfiltration)، هەروەسا هەلسانگاندنا گرێدانێ دناڤبەرا وان دا.

رێکا ڤەکولینێ: ڤەکولینەکا برگەیی و شێوێ ل دویف ئێک یێ وەرگرتنا نمونا هاتە ب کارئینان بو هەلبژارتنا 115 نەخوشا (59 نێر و 56 مێ) ژ وان نەخوشا ئەوێن قەستا سەنتەرێ نەخوشیا شەکرێ ئەوا گرێدای ب زانکویا موستەنسریە ڤەیە ل بەغدا ژ 2005/8/1 تا 2006/7/31. (Micral test II) هاتە ب کارئینان ژ بو دیارکرنا هاڤتنا (albumin) دناڤ نموونێن میزێ ئەوێن سپێدێ هاتینە وەرگرتن و بو جارا ئێکێ ل عیراقێ هاوکێشا شوارتز هاتە ب کارئینان بو دەرئینانا (Glomerular filtration rate) ب متمانەکرنێ لسەر رێژا کریاتینین د ناڤ خوینێ دا و چەند ساوخەتێن دیموگراڤی یێن نەخوشا. گرێدان دناڤبەرا (microalbuminuria) و (Glomerular filtration rate) هاتە هەلسانگاندن و هەروەسا چەند فاکتەرن مەترسیێ هاتنە هەلسانگاندن وەك ژیێ نەخوشی و ماوێ نەخوشیێ.

ئەنجام: تێکرایێ حسابی یێ چیێ نەخوشی 14.05±2.95 سال ویێ ماوێ نەخوشیێ 6.52±2.85 بوو. رێژا هەبوونا (microalbuminuria) (کو د هاتە پێڤان ب شێوێ زێدەبوونا د هاڤتنا البومین دا 20-200 ملغم/لتر) 48.7٪ بوو. رێژا (Glomerular filtration rate) 16.52٪ بوو و ژ وانا 37٪ (microalbuminuria) هەبوو و 63٪ (microalbuminuria) نەبوو و رێژا بلندتر لجەم رەگەزێ نێرا بوو (89.48٪). گرێدانەکا پێچەوانی هەبوو دناڤبەرا (microalbuminuria) و (Glomerular filtration rate) (r=-0.79, p value= 0.024). ئەو فاکتەرێن کو پەیوەندی ب زێدەکرنا (Glomerular filtration rate) رەگەزێ نێربوو (p value=0.013) و درێژبوونا ژیێ (p value=0.031) و فاکتەرێن کو پەیوەندی ب (microalbuminuria) هەی درێژیا ماوێ نەخوشیێ (p value=0.017) و درێژبوونا ژیێ (p value=0.031).

الخلاصة

الاكتشاف المبكر للاعتلال الكلوي لدى الأطفال والمراهقين المصابين بداء السكر / النوع الأول

الخلفية: أظهرت الدراسات الحديثة امكانية تغيير مسار الاعتلال الكلوى الناتج عن مرض السكر وتحسينه بدرجة كبيرة من خلال مداخلات عديدة الا ان تاثير هذه المداخلات لا يكون كبيرا الا اذا شرع بتطبيقها في مراحل مبكرة جدا .صممت هذه الدراسة لتقصي مشكلة الاعتلال الكلوي الناتج عن مرض السكر من النوع الاول وتحديدا اكتشافه المبكر لدى عينه من الاطفال و المراهقين لحد السنة الثامنة عشر من العمر.

ألاهداف: قياس معدلى شيوع البيلة الميكروالبومينية و ارتشاح الحافظات الكلويه اضافة الى تقييم العلاقه بينهما

ألمنهجية: أعتمد نمط الدراسة المقطعية والاعتيان المتعاقب لادخال 115 مريض (59 ذكر ،56 انثى) ممن تنطبق عليهم ضوابط الادخال من مراجعي المركز الوطني لمرض السكر التابع للجامعة المستنصرية في بغداد للفترة من (2005/8/1 – 2006/7/31).تم استخدام فحص (ميكرال II) في تحري زيادة الطرح الالبومبني في عينات الادرار الصباحية في حين استخدمت لاول مرة في القطر معادلة (شوارتز) لحساب معدل ارتشاح الحافظات الكلوية اعتمادا على مستوى الكرياتنين بالدم وبعض المواصفات الديموغرافية للمرضى. وظِفت النتائج لتقييم العلاقة بين البيلة الميكروالبومينية و ارتشاح الحافظات الكلوية فضلاً عن إجراء تقييم لبعض عوامل الاختطار المهمة وهي عمر المريض ومدة المرض .

النتائج: الوسط الحسابي لعمر المريض هو (14.05±2.95) والوسط الحسابي لمدة المرض هو (6.52±2.85). معدل شيوع البيلة الميكروالبومينية(مقاسا كزيادة في طرح الالبومين 20-200 ملغم\لتر) هو (48.7%). معدل شيوع زيادة ارتشاح الحافظات الكلوية محسوبا على اساس (GFR≥130ml/min/1.73m2) هو(16,52%) منهم(37%) لديهم البيلة الميكروالبومينية و(63%) بدونها مع غلبة واضحه في الذكور (89.48%). هناك تناسب عكسي بين المستويات المختلفة للبيلة الميكروالبومينية مع مستويات ارتشاح الحافظات الكلوية (r = -0.79, P-value = 0.024). ان العوامل التي اظهرت ارتباطا بزيادة نسبة ارتشاح الحافظات الكلوية هي الذكورية (P-value = 0.013) و تقدم العمر (P-value= 0.031) في حين ان العوامل التي اظهرت ارتباطا بالبيله الميكروالبومينيه هي طول فترة المرض (P- value= 0.017) و تقدم عمر المريض (P-value=0.031).

PREVALENCE OF METABOLIC SYNDROME IN PATIENTS WITH ISCHEMIC HEART DISEASE

QAYSER S. AL-HABEEB, MBChB,DIM,MSc*
MOHAMMED H. AL-MYAHI, MBChB, FICMS (Med), CABM, FICMS (Cardiol)**
JASSIM M. HUSSAIN, BSc (Community Health)***

Submitted 10 January 2008; accepted 10 June 2008

ABSTRACT

Background Metabolic syndrome is a combination of medical disorders that increase one's risk for cardiovascular disease and diabetes.Whereas the syndrome is under scrutiny and extensive investigations worldwide, it has been very little investigated in Iraq with a considerable lack of local pertinent data.
Objectives Estimation of the prevalence of metabolic syndrome in patients with ischemic heart disease and assessing the severity of coronary artery disease in patients who meet the criteria of metabolic syndrome.
Patients and methods The study was carried out at Ibn Albitar hospital, a tertiary center for cardiovascular surgery/Baghdad/Iraq from 1st Oct. 2005 to 30th Dec. 2006. A cross sectional design and consecutive sampling procedure were adopted to enroll 300 patients comprising 226 males and 74 females who met the eligibility criteria and were assigned to undergo coronary angiography. Documentation of data regarding medical history, the required measurements, and investigations was accomplished in accordance with a specially designed data sheet that included all relevant information.
Results The overall prevalence of metabolic syndrome in the study sample was 69.33 %. Differentially, the prevalence was very much higher among patients with ischemic heart disease 84% than those without ischemic heart disease 10%. The estimated difference was statistically highly significant (p=0.01). Only 240 patients showed angiocardiographic evidence of ischemic heart disease; (single vessel disease 24.2%, two vessels disease 35.8%, triple vessels disease 23.3%, and left main stem disease 16.7%). There was no significant difference in the prevalence of metabolic syndrome among different subgroups of patients with ischemic heart disease classified by the results of coronary angiography. There is a need for having a unified definition of the metabolic syndrome to allow for proper assessment and valid comparison between prevalence data in different populations.
Recommendations highlighted the need for wider analytical studies enrolling bigger samples with the aim of obtaining a more valid inference, in addition to community based surveys to help early recognition of metabolic syndrome, identify patients at risk of ischemic heart disease, and reduce the impact of ischemic heart disease on the community.

DMJ 2008;2(1): 107-116.

Key words: Metabolic syndrome, Ischemic heart disease, Prevalence

The concept of the metabolic syndrome, comprising central obesity, hypertension, raised triglycerides, low "High density lipoprotein-cholesterol"

* *Assistant Professor, Dohuk College of Medicine, Dohuk, Iraq*
** *Consultant Cardiologist, Ibn Albitar Hospital, Baghdad, Iraq*
*** *Ibn Albitar Hospital, Baghdad, Iraq*
Correspondence author: Qayser Sahib Al-Habeeb, *Dohuk College of Medicine, Dohuk, Iraq.*

(HDL), and raised fasting plasma glucose concentrations, is now well established. It is known under various other names, such as metabolic syndrome X, insulin resistance syndrome, and Reaven's syndrome. Three definitions of the metabolic syndrome are currently in common use: the World Health Organization (WHO) definition; the European Group for the study of Insulin Resistance (EGIR) definition; and the National Cholesterol Education Programme Expert Panel Adult Treatment Panel III (ATP III) definition.[1,2] The syndrome affects a large number of people in a clustered fashion. In some studies, the prevalence in the USA is calculated as being up to 25% of the population. An estimated 55 million US adults have metabolic syndrome. If the revised value for impaired fasting glucose is used, the estimate jumps to 64 million adults.[3] Some experts predict that at least half of persons over age 60 would meet the criteria for the metabolic syndrome. Obesity and diabetes trends seem to mirror metabolic syndrome trends. Several studies have shown an association between the metabolic syndrome and increased cardiovascular events.[4-8] Despite significant controversy, most experts appear to believe that the increased cardiovascular risk seen in these subjects is probably due to the clustering of known cardiovascular risk factors. However, even though there have been reports of an increased prevalence of coronary heart disease (CHD) in these subjects, these reports have used surrogate markers for CHD.[9,10] The incidence of CHD was also significantly greater in the metabolic syndrome positive group.[11] Country wise the data about this subject are very scanty. This study has been designed to explore this very little investigated problem and to verify its local pattern in a sample of Iraqi people.

The aim of the present study is to estimate the prevalence of metabolic syndrome in patients with ischemic heart disease and to assess the severity of coronary artery disease in patients who meet the criteria of metabolic syndrome.

PATIENTS AND METHODS

This cross-sectional study was carried out at Ibn Albitar hospital; a tertiary center for cardiac surgery/Baghdad/Iraq, from 1st October 2005 to 30th December 2006. Eligible patients were those referred to Ibn Albitar hospital during the study period, with chest pain suggestive of chronic stable angina, and were assigned to undergo coronary angiography. Consecutive sampling procedure was followed to enroll (300) patients comprising (226) males and (74) females who met the eligibility criteria.

A specially designed data sheet was used to document the relevant data regarding age, gender, blood pressure, waist circumference, height and weight for computation of the body mass index, past medical history of hypertension, diabetes mellitus and smoking, in addition to the required laboratory investigations; fasting blood sugar and serum lipid profile including: serum cholesterol, HDL, LDL and serum triglycerides.

All enrolled patients underwent coronary angiography on the assigned dates, the results of which were used to classify patients into those with and without angiocardiographic evidence of IHD. Those with IHD were further subdivided into: single vessel, two vessels, three vessels, and left main stem disease. Various subgroups were then assessed and compared with regard to their baseline characteristics and for the prevalence of metabolic syndrome.

Metabolic syndrome was diagnosed in the presence of 3 or more of the following ATP III criteria [1,2]:

1. Abdominal obesity (waist circumference):

Men > 102 cm (>40 in) and Women > 88 cm (>35 in)

2. Triglycerides ≥ 150 mg/dl (1.7 mmol/l).

3. HDL cholesterol:

Men < 40 mg/dl (1.0 mmol/l) and Women < 50 mg/dl (1.3 mmol/l)

4. Blood pressure: >130 / > 85 mmHg Or on anti-hypertensive treatment.

5. Fasting blood sugar: ≥110 mg/dl (6.1 mmol/l) Or on antidiabetic treatment.

Statistical analysis: Chi square and Z test.

RESULTS

All included patients underwent coronary angiography which yielded 240 positive cases versus 60 negative ones. These results divided the study sample into two groups, those with angiographic evidence of IHD and those without such evidence. Comparison of the two groups with regard to their baseline characteristics revealed no significant differences regarding age, gender, hypertension, fasting blood sugar, or lipid profile; however, patients with IHD showed significantly higher prevalence of smoking (p = 0.017), positive family history of IHD (p = 0.003), and BMI of 29.238 ± 3.947 (p = 0.026) (Table 1).

Table 1. Baseline characteristics of patients with and without IHD

Baseline characteristics	patients with IHD N= 240	patients without IHD N= 60	Comparison of Significance *	
			P-value	Sig.
Mean Age (Year)	56.52± 8.6	55.72±11.89	0.554	NS
Male	(185) 77.1%	(41) 68.3%	0.109	NS
Female	(55) 22.9%	(19) 31.7%		
Hypertension	(165) 68.8%	(34) 56.7%	0.054	NS
Diabetes mellitus	(148) 61.7%	(34) 56.7%	0.571	NS
Smoking	(83) 34.6%	(10) 17.0%	0.017	S
+ ve F.H. history of IHD	(142) 59.2%	(20) 33.3%	0.003	H.S
BMI (Kg/m2)	29.238 ± 3.947	27.613 ±2.336	0.026	S
S. cholesterol (mmol/l)	4.916 ± 1.206	4.636 ± 1.385	0.120	NS
HDL (mmol/l)	0.946 ± 0.199	0.98 ± 0.184	0.235	NS
LDL (mmol/l)	3.153 ± 1.2	2.973 ± 1.007	0.284	NS
S. triglycerides (mmol/l)	2.552 ± 1.035	2.333 ± 0.769	0.125	NS
F.B.S (mmol/l)	6.825 ± 3.87	6.143 ± 2.55	0.083	NS

** Based on Chi square and Z test.*

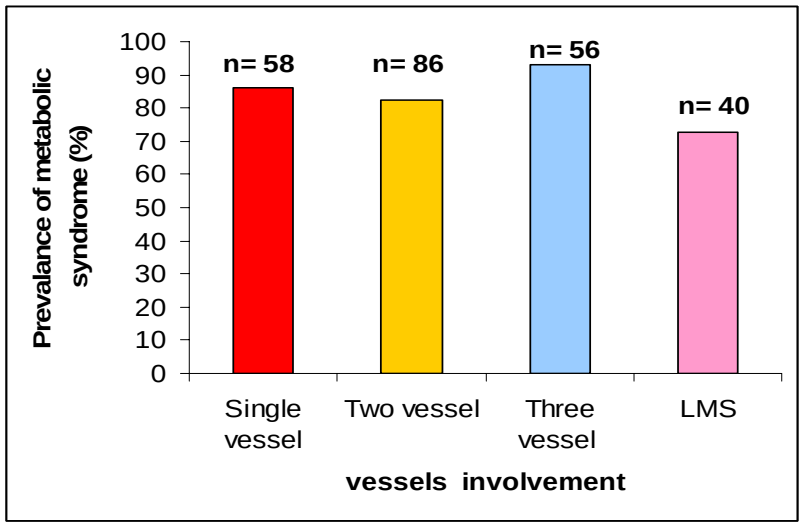
The results of coronary angiography in patients with IHD showed that most patients had two vessels disease (35.8%), followed by those with single vessel disease (24.2%), three vessels disease (23.3%) and left main stem disease (16.7%) (Table 2).

Of the 300 patients enrolled in the study only 208 patients met the adopted criteria of metabolic syndrome making an overall sample prevalence of (69.33 %). Differentially, the prevalence of metabolic syndrome was very much higher among patients with IHD (84%) than those without IHD (10%). The estimated difference was statistically highly significant (P=0.01).

There was no significant difference in the prevalence of metabolic syndrome among different subgroups of patients with IHD classified by the results of coronary angiography.

Table 2. Coronary angiographic findings in patients with IHD

Coronary angiographic findings	No. (%)
Single vessel disease	58 (24.2)
Two vessels disease	86 (35.8)
Three vessels disease	56 (23.3)
Left main stem	40 (16.7)
Total	240 (100.0)



Prevalence (%) of metabolic syndrome among different subgroups of patients with IHD

DISCUSSION

The results of coronary angiography were positive in 240 patients while the remaining 60 patients were proved to have no evidence of ischemic heart disease. Comparing the two groups with regard to their baseline characteristics revealed no significant differences in all the studied variables except for Smoking (p = 0.017), positive family history of IHD (p = 0.003), and BMI (p = 0.026). Several factors might have contributed to these results such as sample size ,sampling procedure and the state of being "under treatment" and controlled for long periods.[11,12] The prevalence of metabolic syndrome was significantly higher among patients with ischemic heart disease (84%) than those without (10%), (P = 0.01). Bela C.B. et al[13] in a similar study, enrolling Canadian population (793 men and 315 women) with coronary artery disease, and using ATP III diagnostic criteria, they found that 51% of them had metabolic syndrome. Lakka H.M et a[14] demonstrated that patients suffering from the metabolic syndrome are about three times more likely to experience cardiovascular events than those free of the syndrome and they also found a 2 – 4 fold increased risk of cardiovascular death with metabolic syndrome in a sample of 1209 Finnish men free from diabetes and cardiovascular disease at baseline. Metabolic syndrome predicted atherosclerosis progression and cardiovascular events in 888 subjects (73.4%).[13] In Framingham study, the metabolic syndrome alone predicted ≈ 25% of all new-onset cardiovascular disease. In the absence of diabetes, the metabolic syndrome generally did not raise 10-year risk for coronary artery disease to >20% .Kevin E.K. et al[14] used the WHO criteria in their study, they evaluated interrelationships between angiographic coronary artery disease, the metabolic syndrome, and incident cardiovascular events among 755 subjects who were referred for coronary angiography to evaluate suspected myocardial ischemia; 25% of the cohort had the metabolic syndrome at study entry. Compared with subjects with normal metabolic status, subjects with the metabolic syndrome had a significantly lower 4-year survival rate (94.3% versus 97.8%, p= 0.03) and event-free survival from major adverse cardiovascular events (death, nonfatal myocardial infarction, stroke, or congestive heart failure (87.8% versus 93.5% P= 0.003). When the subjects were stratified by the presence or absence of angiographically significant CAD at study entry, in subjects with angiographically significant CAD, the metabolic syndrome resulted in significantly higher risk of cardiovascular events than in subjects with normal metabolic status (hazard ratio 4.93, 95% CI 1.02 - 23.76; P= 0.05), whereas it did not result in increased 4-year cardiovascular risk in subjects without angiographically significant CAD (hazard ratio 1.41, 95% CI 0.32 - 6.32; P= 0.65).[14] In Swiss population by using ATP III criteria the metabolic syndrome was present in 18.0% of the whole population. In CAD-negative participants, the metabolic syndrome was present in 9.5%, whereas 20.4% CAD-positive

patients had the metabolic syndrome. There was an increased presence (5-fold) of CAD in metabolic syndrome positive patients, compared with those with metabolic syndrome negative patients. There was also a significantly increased presence (2.5-fold) of coronary artery disease in metabolic syndrome positive participants when compared with all participants who were metabolic syndrome negative patients.[12]

Wong J. et al[8] found that metabolic syndrome was associated with an increased prevalence of IHD (17.2% metabolic syndrome vs 11.6% no metabolic syndrome, p = 0.0001) across all age groups. Metabolic syndrome subjects had an IHD prevalence equivalent to that seen in subjects who were one decade older without metabolic syndrome. There was a strong relationship between the number of metabolic syndrome risk factors and IHD prevalence (r = 0.99, p = 0.0001).[15] The present work showed that around one fourth of patients (24.2%) with IHD had single vessel disease, while one third (35.8%) had two vessels disease and less than one fourth (23.3%) had three vessels disease and only (16.7%) had left main stem disease. W.J et al[16] in their coronary angiography study reported different results that included: (9%), (18%), (28%), and (48%) for patients with single, double, triple, and left main stem disease, respectively. In the present study, no significant difference was found in the prevalence of metabolic syndrome among different subgroups of patients with IHD classified according to the number of vessels involved which means that the presence of metabolic syndrome could not predict the severity or extent of the underlying atherosclerotic burden as manifested by CAD. On the other hand, Bela CB and Martial G[13] in a CAD study found that subjects with metabolic syndrome had more advanced coronary disease than those without the syndrome, cumulative coronary stenosis score and the frequency of patients with >50% coronary artery narrowing were higher, and there was a strong tendency for higher rates of previous myocardial infarction in metabolic syndrome positive patients, this study also followed the ATP III criteria. Another study by Blatter MC et al[9] found that the metabolic syndrome was also associated with more severe coronary disease (P = 0.01). Such controversial findings might be due to other unknown contributory factors, a setting which dictates the potential need for expanding research in this field for a better definition of the problem.

CONCLUSIONS

1. Using the ATP III criteria, the overall prevalence of metabolic syndrome was (69.33 %),being much higher among patients with IHD (84%) than those without IHD (10%), (p = 0.01).
2. There was no significant difference in the prevalence of metabolic syndrome among different angiogaphically classified subgroups indicating that the presence of metabolic syndrome could not predict the severity or extent of the underlying atherosclerotic burden as manifested by CAD.

3. Different definitions for the metabolic syndrome, various study designs, and the way of population selection, all make direct comparison between prevalence data in different populations a very problematic task. These same reasons might have affected the final results of this study, a point which highlights the necessity for having a unified definition of the metabolic syndrome and optimizing study design and population selection in any future study.

RECOMMENDATIONS

The novel results of this study have to be further assessed using a more elaborate analytical study and enrolling a bigger sample with the aim of overcoming any awkwardness in generalizability.

The high prevalence of metabolic syndrome mandates the conduct of community based surveys for early recognition of metabolic syndrome to identify patients at risk of IHD, and reduce the impact of IHD on the community.

REFERENCES

1. The IDF consensus worldwide definition of the metabolic syndrome. J Invasive Cardiol 2000;12(1):13-9.
2. Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109:433–8.
3. Duncan GE, Li SM, Zhou X-H. Age-specific prevalence of metabolic syndrome in US adolescents and adults. Diabetes Care 2004; 27(10):2438-43.
4. Lakka H-M, Laaksonen DE, Lakka TA. The metabolic syndrome and total and cardiovascular mortality in middle aged men. JAMA 2002;288:2709–16.
5. Isomaa B, Almgren P, Tuomi T. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001;24:683–9.
6. Alexander CM, Landsman PB, Teutsch SM. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants aged 50 years and older. Diabetes 2003;52:1210–4.
7. Lindbald U, Langer RD, Wingard DL. Metabolic syndrome and ischemic heart disease in elderly men and women. Am J Epidemiol 2001;153:481–9.
8. Wong ND, Sciammarella MG, Polk D. The metabolic syndrome, diabetes, and subclinical atherosclerosis. J Am Coll Cardiol 2003;41:1547–53.
9. Blatter MC, Kalix B, Morabia A, James RW. Small dense lipoprotein particles and reduced paraoxonase-1 in patients with the metabolic syndrome. The J Clin Endocrinol Metabol 2004;90(4):2264-9.
10. World Health Organization. Definition,

diagnosis and classification of diabetes mellitus and its complications: report of a WHO Consultation. Part 1: diagnosis and classification of diabetes mellitus. Geneva, Switzerland: World Health Organization; 1999. Available at: http://whqlibdoc.who.int/hq/1999/WHO_NCD_NCS_99.2.pdf

11. Heitzer T, Yla-Herttuala S, Luoma J, Kurz S, Munzel T, Just H, et al. Cigarette smoking potentates endothelial dysfunction of forearm resistance vessels in patients with hypercholesterolemia: role of oxidized LDL. Circulation 1996;9:1346–53.
12. Kaplan NM. The deadly Quartet, Upper obesity, Hypertension, Dyslipidaemia and Glucose Intolerance. Arch Intern Medicine 1989;549:1514-20.
13. Bela CB, Martial G. Incidence and clinical characteristics of the metabolic syndrome in patients with coronary artery disease. Coron Artery Dis 2003;14(3):207-12.
14. Kevin EK, David EK, Delia J, Leslee JS, Noel BM, Barry LS, et al. Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease. Circulation 2004;109:714-21.
15. Wong J, Molyneaux L, Constantino MI, Twigg SM, Yue DK. The metabolic syndrome in type 2 diabetes. Diabetes Obes Metabol 2006;8(6): 690–7.
16. Proudfit WJ, Bruschke AV, MacMillan JP, Williams GW, Sones FM. Fifteen years survival study of patients with obstructive coronary artery disease. Circulation 1983; 68: 986-97.

پوختە

بەلاڤبوونا (Metabolic Syndrome) لجەم نەخوشێن تووشی نەخوشییێن دلی ییێن تاجی بووین

پێشەکی: رامان ژ metabolic syndrome ئەوە هەبوونا چەند تێکداچوونێن پزیشکی کو دبیتە ئەگەرێ زێدەبوونا مەترسییێ ب تووشبونێ ب نەخوشییێن دلی و رەهێن خوینێ و ئێشا شەکرێ. سەرەرای کو metabolic syndrome یا لژێر ڤەکولینێن بەرفرەه دایە ل هەمی جیهانی دا بەلێ ڤەگولینێن نافخویی دکێمن.

ئارمانج: ژماردنا بەلاڤبوونا metabolic syndrome لجەم نەخوشێن تووشی نەخوشییێن دلی ییێن تاجی بووین و هەلسانگاندنا توندیا تووشبونا رەهێن خوینێ ییێن تاجی لجەم کەسێن تووشی metabolic syndrome بووین.

نەخوش و ڕێکێن ڤەکولینێ: ڤەکولین هاتە کرن ل نەخوشخانا (ابن البیطار)، سەنتەرێ تایبەت ب نشتەگەریا دلی و رەهێن خوینێ، ل بەغدا/عیراقێ ژ 2005/10/1 تا 2006/12/30. ڤەکولینەکا برگەیی و وەرگرتنا ل دویف ئێک یا نموونا بو وەرگرتنا 300 نەخوشا (226 نێر و 74 مێ) تووشێ (ذبحة صدرية) ئەوێن هاتینە رەوانەکرن بو نەخوشخانێ بو ئەنجامدانا نشتەکەریا قەستەریا رەهێن خوینێ ییێن تاجی. پێزانین هاتنە دوکیومێتکرن ل دویف میژووییا نەساخییێ و ب ڕێکا ئەنجامدانا چەند پشکنییێن پێویست و لدویف وێ پرسنامێ ئەوا هاتیە چێکرن بو ڤێ ڤەکولینێ.

ئەنجام: ڕێژا بەلاڤەبوونا metabolic syndrome ل نموونێ ڤەکولینێ 69.33٪ بوو و ئەڤ ڕێژە پتر بوو لجەم نەخوشێن تووشی نەخوشییێن دلی ییێن تاجی بووین (84٪ بەرامبەر 10٪ لجەم ل وان کەسێن نە نەخوش). پشکنینا قەستەرێ یا ئەرینی بوو لجەم 240 نەخوشا و ئەنجامێن تووندیا تووشبونا رەهێن خوینێ ییێن تاجی بڤی شێوەی بوو: ئێک رەه خوینێ 24.2٪، دوو رەه 35.8٪، سێ رەه 23.3٪،چەقێ سەرەکی یێ چەپێ 16.7٪ بەلێ چێ جیاوازیا گرنگ ژ لایێ ئاماری ڤە نەبوو. پێویستیا ئێک لاکرنا پێناسێن metabolic syndrome ژ بوو دەرئێخستنا جیاوازیا دناڤبەرا ڤەکولینا دا ب شێوەکێ دروست و ئاسان.

دەرئەنجام: بەرڤرەهکرنا ڤەکولینێ تا کو ژمارەکا پتر ژ خەلکی پشکدار بن تێدا تا کو بگەهینە ئەنجامێن باشتر و باشتر ئەوە نموونە دناڤ کومەلکەهێ دا بهێتە وەرگرتن ژ بو زوو دەستنیشانکرنا metabolic syndrome و دەستنیشانکرنا ئەوێن کەسێن د مەترسیا تووشبونێ دا ب نەخوشییێن دلی ییێن تاجی ژ بو کیمکرنا کاتیکرنا وێ لسەر کومەلگەهێ د ا.

الخلاصة

معدل شيوع المتلازمة الأيضية لدى المصابين بامراض القلب التاجية

الخلفية: المتلازمة الايضية هي تزامن لمجموعة اعتلالات طبية تتسبب في ازدياد نسبة اختطار الاصـابة بـامراض القلب والاوعيه الدمويه وداء السكر. وعلى الرغم من ان المتلازمه تخضع للتمحيص والدراسه الواسعة فـي مختلـف انحاء العالم الا ان الدراسات المحليه شحيحه وهناك ضرورة واضحة للمعلومات بهذا الصدد.

الاهداف: حساب معد ل شيوع المتلازمة الايضية لدى المرضى المصابين بامراض القلب التاجية وتقييم شدة اصـابة الشرايين التاجية لدى المرضى المصابين بالمتلازمة الايضية.

المرضى والطرق: اجريت الدراسة في مستشفى ابن البيطار المركز التخصصي لجراحة القلب والاوعية /بغداد/ العراق للفترة من 2005/10/1 الى 2006/12/30 .

اعتمد نمط الدراسة المقطعيه واسلوب الاعتيان المتعاقب لضم 300 مريض (226 ذكور و 74 اناث) مصاب بالذبحـه الصدريه المستقرة من المحالين الى المستشفى لا جراء عملية قسطرة الشرايين التاجية. تم توثيق المعلومات الخاصـه بالتاريخ المرضي والقياسات والفحوص المطلوبة وفقا للاستمارة الا ستبيانية المعدة خصيصا للدراسة والتي شملت كـل المعلومات المطلوبة للدراسة.

النتائج: معدل شيوع المتلازمة الايضية في عينة البحث كان (69,33%) وكان معدل الـشيوع اعلـى بكثيـر لـدى المصابين بامراض القلب التاجية (84% مقابل 10% لدى غيرالمصابين) و بدلالة احصائية عالية المستوى. فحـص القسطرة كان موجبا لدى 240 مريض وكانت النتائج تتراوح فى شدة اصابه الشرايين التاجيه كالاتي: شريان واحـد 24.2% شريانين 35.8% ثلاثة شرايين 23.3% الفرع الرئيسي الايسر 16.7% الا انه لم يثبت وجود فارق احصائي في معدل شيوع المتلازمة الايضية بين المجاميع المختلفه مصنفه حسب شدة الاصابه. وجـود حاجـه الـى توحيـد تعاريف المتلازمه لجعل القياس و المقارنة بين الدراسات ايسر وادق.

التوصيات: ابرزت الحاجة الى التوسع في اجراء الدراسات التحليلية على عينات اكبر للحـصول علـى نتـائج اكثـر مصداقية و القيام بالمسوح المجتمعيه الواسعة بهدف الاكتشاف المبكر للمتلازمة الايضية وتحديد المرضى المعرضين للاصابة بامراض القلب التاجية لتقليل اثارها في المجتمع.

TRAUMATIC HYPHEMA: A STUDY OF 40 CASES

ARIF Y. BALATAY, MBChB, PhD (Ophthalmology)*
HAVAL R. IBRAHIM, MBChB, D.Ophthalmology**

Submitted 28 January 2008; accepted 10 June 2008

ABSTRACT

Background Hyphema is a relatively common problem in our society with complications and risk sequels. No study has been conducted on this problem in Kurdistan region.
Objectives to detect common causes of ocular trauma in Dohuk governorate and to detect the most vulnerable age group involved with the visual acuity outcome after treatment.
Methods The study was conducted in Azadi General Teaching Hospital and the Emergency Hospital / Dohuk / Kurdistan region, from June 2006 to June 2007. A follow-up clinical study of patients presenting with traumatized eyes with hyphema was conducted. The study included 40 patients of traumatic hyphema out of 137 cases of ocular trauma. Ocular examinations (visual acuity, intra ocular pressure, fundoscopy and others) were done for all patients at presentation and subsequently during the follow-up.
Results The annual prevalence rate of traumatic hyphema in Dohuk governorate was about 5 per 100.000 individuals. The study showed a male predominance. A total of 35% of cases were encountered among children aged (6 – 10) years. Blunt trauma was observed in (60%) of patients while the other (40%) had penetrating traumas. A total of (90%) of females suffered from penetrating trauma while males were injured by blunt trauma more frequently. The left eye was relatively more frequently involved (55%) than the right. A total of 37 eyes regained acceptable final visual acuity, while two eyes progressed to no light perception, and one eye had just light perception.
Conclusions and Recommendations Tranexamic acid was found to reduce re-bleeding in our cases. Increased intraocular pressure is one of the most frequent complications of traumatic hyphema that may ultimately result in impaired vision. Complete eye examination is essential to assess concomitant injuries which reflect the severity of initial trauma.

DMJ 2008;2(1): 117-126.

Key words: Hyphemia, Eye trauma, Dohuk

Hyphema is the accumulation of blood in the anterior chamber of the eye and microhyphema is the term used for circulating red blood cells in the aqueous humor of the anterior chamber without grossly visible blood.[1-8]

Hyphemas are most frequently caused by ocular trauma; however, non-traumatic causes include iris neovascularization (associated with diabetes, intraocular tumors, or retinal vascular occlusive disease), iris tumors such as juvenile xanthogranuloma, or anterior spillover from a vitreous hemorrhage.9

Traumatic hyphema can be caused by either blunt or penetrating injury.[10] Blunt

* *Lecturer, Dohuk College of medicine, Dohuk, Iraq*
** *Directorate of Health in Dohuk, Iraq*
Correspondence author: *Arif Y. Balatay, Dohuk College of medicine, Dohuk, Iraq. Email: arifbalata2004@yahoo.com*

trauma causes posterior displacement of the lens-iris diaphragm scleral expansion in the equatorial zone, which leads to disruption of the major iris arterial circle, arterial branches of the ciliary body, and/or recurrent choroidal arteries and veins. A tear at the anterior aspect of the ciliary body is the most common site of bleeding and occurs in about 71% of cases.[11] A penetrating injury can cause hyphema by directly damaging the ocular vasculature.[12]

Seventy percent of the cases are under 20 years of age.[5,13,14] The agent producing a hyphema is usually a projectile that strikes the exposed portion of the eye. Various missiles and objects have been incriminated, including balls, rocks, projectile toys, air gun pellets, hockey pucks, bungee cords, paint balls, and the human fist.[15,16] With the increase of child abuse, fists and belts have started to play a prominent role. Males are involved in three fourths of cases.[17, 18]

Hyphema is classified by the amount of blood in the anterior chamber and the following clinical grading system for traumatic hyphemas is preferred[10]:

Grade 1 - Layered blood occupying less than one third of the anterior chamber.

Grade 2 - Blood filling one third to one half of the anterior chamber.

Grade 3 - Layered blood filling one half to less than total of the anterior chamber.

Grade 4 - Total clotted blood, often referred to as blackball or 8-ball hyphema.

The prevalence of traumatic hyphema has been estimated at 17- 20 per 100,000 per year.[19,20]

METHODS

This study aims at evaluating the management of traumatic hyphema in the Ophthalmology Department of Dohuk Medical College in the patients who are attended to Azadi Teaching General Hospital and Dohuk Emergency Hospital. This study includes 40 patients of traumatic hyphema among 137 cases of ocular trauma over the period June 2006 and June 2007. The variables studied were: demographic variables; causes; severity and type of hyphema; visual and intraocular pressure outcome and application and outcome of medical and surgical treatment.

Patients with a diagnosis of spontaneous hyphema and postoperative hyphema were excluded. All of the cases included had no history of sickle cell disease or trait. None of the females was pregnant as all were children. Data obtained from patients′ records include: Date of presentation, age, sex, race, profession, residence, admission to hospital (outpatient or inpatient), duration of hospitalization and sickle cell status. The records were also reviewed for histories of systemic and ocular diseases and drug intake, especially aspirin. Clinical data obtained at the time of initial examination include visual acuity, size of hyphema, intraocular pressure, associated ocular and adnexal injury, and general physical examination.

Follow-up examinations on each patient included visual acuity (VA),

Intraoccular pressure (IOP), slit-lamp examination and fundoscopy to determine the final VA and IOP, and the occurrence of any subsequent complications and the therapeutic interventions.

RESULTS

Table 1 shows male predominance with a male : female ratio of 3 : 1. Age distribution shows a peak between 6-20 years with an average age of 14 years and an age range of 4 -39 years.

Traumatic hyphema cases were due to blunt trauma in 24 patients (60%) and penetrating trauma in 16 (40%). Traumatic hyphema in female patients were due to penetrating trauma in 90%, while in male patients 76.7% were due to blunt trauma. The most frequent causes of blunt trauma were shown in table 2.

Table 1. Age and sex distribution of patients with traumatic hyphema

Age groups	Male	Female	Total	Percent
Up to 5 years	2	3	5	12.5
6-10 years	9	4	13	32.5
11-15 years	6	3	9	22.5
16-20 years	5	0	5	12.5
21-25 years	3	0	3	7.5
26-30 years	2	0	2	5.0
31-35 years	1	0	1	2.5%
36-40 years	2	0	2	5.0
Total	30	10	40	100.0

Table 2: Blunt and penetrating trauma by source of injury

Blunt Trauma		Penetrating Trauma	
Causes	No.	Causes	No.
Kid pistols	7	Knives	7
Stones	6	Sharp metals	4
Human fist	3	Sharp woods	2
Snow balls	2	Mine injuries	2
Road traffic accidents	2	Sharp glasses	1
Balls	1		
Blunt woods	1		
Fall from height	1		
Towel	1		
Total	24	Total	16

Twenty-two patients (55%) had left eye hyphema. The occupations or professions of patients with hyphema were as follows: Students (24), Children (8), private jobs (3), and one case each of house wife, policeman, farmer, carpenter, and driver.

At the time of presentation 24 patients (60%) had grade I hyphema, 9 patients (22.5%) with grade II, 5 patients (12.5%) with grade III and 2 patients (5%) with grade IV.

The Initial VA and final VA of the 40 patients of our study are shown in table 3:

Six patients (15%) had increased IOP during the first 24 hours, and with treatment by topical timolol maleate and systemic acetazolamide two patients (5%) remained with elevated IOP at the end of first week therefore anterior chamber (AC) washout done for them under general anesthesia, but still one patient (2.5%) had elevated IOP in spite of treatment and resolution of hyphema Gonioscopy revealed angle recession. It is generally true that the larger the hyphema volume, the greater the likelihood of increased IOP. Intravenous Mannitol 20% was used only for one patient to decrease the IOP in addition to topical timolol and oral acetazolamide

Synechia formation was noticed in three patients (7.5%), one of whom was grade I, one was grade II and one was grade IV. Two cases (5%) developed optic atrophy, one was grade III and the other grade IV. Only one case (2.5%) developed re-bleeding and was treated surgically. Corneal blood staining occurred in one patient (2.5%) due to prolonged large hyphema (grade III) with elevated IOP.

Only two patients with traumatic hyphema due to blunt trauma (8.3% of blunt trauma) needed surgical intervention as their hyphemas were occupying greater than 75% of the anterior chamber for 6 days with an IOP of 25 mmHg or more (to prevent corneal blood staining), while all the patients with penetrating trauma (16 patients (100%)) needed surgical intervention.

Table 3. Initial VA and final VA of patients with hyphema

Initial VA	Final VA							
	6/6	6/9 6/12	6/18 6/30	6/36 6/60	C.F	L.P.	N.L.P.	
≥6/12	7	2	0	0	0	0	0	9
6/18 - 6/30	4	2	0	1	0	0	0	7
6/36 - 6/60	4	3	1	1	0	0	0	9
C.F.	0	0	1	0	1	0	0	2
L.P.	5	0	4	1	0	0	1	11
N.L.P.	0	0	0	0	0	1	1	2
Total	20	7	6	3	1	1	2	40

Twenty six patients were admitted to the hospital, all patients with penetrating trauma and 10 patients with blunt trauma (41.7%) due to the following causes:

- One case of grade III hyphema with corneal laceration due to penetrating trauma who developed spontaneous corneal perforation in the second postoperative day. Suturing was done for the cornea in the Emergency Hospital and after 48 hours melting of the cornea occurred with hypopyon and the affected eye had been eviscerated.
- Extracapsular cataract extraction was done for six out of eight patients with traumatic cataract and posterior chamber.
- Implantation was done for three patients after the inflammation had subsided (4 weeks or more after the trauma) and for children older than 3 years.
- Peritomy was done for four patients searching for scleral ruptures and for the extent of scleral wounds.
- Vitrectomies were done for four patients with vitreous loss and prolepses.

DISCUSSION

In other studies, the mean annual incidence of hyphema was approximately 17 per 100,000 populations.[19,20] The population of Dohuk governorate is about 800,000 and the number of reported cases of hyphema was 40 during one year. However, the annual incidence rate of traumatic hyphema in Dohuk governorate could not be calculated as there was an unknown number of hyphemic patients who were managed in private clinics inside or outside Dohuk.

Data from the present study showed that 80% of the cases were under 20 years old with slight differences from other studies (70% according to Schein et al).[14] However sex distribution and size of hyphema (grades) were similar in both studies. Males constituted 75% in the present study similar to the results of Crouch[17] and Edwards[18] in the USA. The percentages of grades in the present study are close to those of Crouch[17] and Edwards[18].

The present study and those of Crouch and Edwards have noted that traumatic hyphema is an injury of youth, with males being at a greater risk than females. Young males are known to engage in more violent activities.

In the present study the most common causes of penetrating traumatic hyphema were due to home accidents by knives, sharp objects, and blast injuries, while blunt trauma hyphema were due to kids' pistols, stones and snow balls. In other studies[15,16] the most common causes of traumatic hyphema were balls, rocks, toys, hockey pucks, bungee cords, paint balls, belts and human fists. This variation in the causative agents between our locality and other places is related to the parents' education levels, the large number of family members (children), the lack of safety plans in house building, the deficiency of education institutes and the lack of educational TV programs

concerning safety inside houses and prevention of avoidable house accidents.

An acute rise in IOP to greater than 25 mmHg occurred in 15% of the cases, which is lower than that reported by Read[11]. This acute IOP rises is due to trabecular meshwork obstruction by red blood cells, platelet, fibrin, and direct concussive damage to outflow channels. IOP may rise in the early and late stage after hyphema.

Patients in the present series had a lower rate of re-bleeding (2.5%) in comparison to the results of Crouch,[17] Read,[11] and Spoor et al[21] who observed secondary hemorrhage in 24.2% of African American patients and in 4.5% in Caucasian patients. The reason for this is not clear but the racial background and the use of tranexamic acid could explain our lower re-bleeding rate. The incidence and severity of side effects were very low and no patients complained or had to stop treatment. Tranexamic acid has become the treatment of choice for traumatic hyphema in our locality and Europe and aminocaproic acid in North America.

In the present study three cases developed synechia formation and were from different grades. No significant correlation existed between synechia formation and severity of hyphema as other studies found.

In the present study, poor final visual outcome occurred in 17.5% (counting fingers or less), while the results of Read[11,22] showed that 14% of hyphemic patients had poor visual results from associated trauma, including such complications as glaucoma, vitreous hemorrhage, retinal detachment, choroidal rupture, or scleral rupture, and 11% of hyphemic patients have poor visual outcome directly attributed to the hyphema.

CONCLUSIONS

A number of variables may complicate the course of patients who present with traumatic hyphemas. Keeping these potential complications in mind during treatment may tip the scale toward a good clinical outcome with preservation of useful visual acuity.

The present series of patients had a low rate of re-bleeding which can be explained by the use of systemic steroids with tranexamic acid. Increased IOP is one of the frequent complications of traumatic hyphema that may ultimately result in impaired vision. IOP may rise in the early and late stage after hyphema.

Patients frequently had more than one associated injury. Thus a complete eye examination is required to assess concomitant injury which reflects the severity of initial trauma. The anterior and posterior segments injuries had significant predictive factors on a poor final visual outcome.

RECOMMENDATIONS

1. Parents' education about the preventing children from handling sharp objects especially knives.
2. Raising awareness of authorities and parents about the risks of toy guns.

3. Protection with a special eyewear made of polycarbonate lenses when there is risk of eye injury at work.
4. Close and frequent follow-up by the ophthalmologists of patients presenting with traumatic hyphema to detect and manage subsequent complications early.
5. It is recommended that studies of traumatic hyphema include those due to blunt trauma only, because the level of blood in the AC can not be estimated exactly in penetrating traumatic hyphema, as the AC may show decreases in its depth and volume (hypoyony).

REFERENCES

1. DiFiori JP. Sports-related traumatic hyphema. Am Fam Physician 1992;46:807-13.
2. Ng CS, Sparrow JM, Strong HP, Rosenthal AR. Factors related to the final Visual outcome of 425 patients with traumatic hyphema. Eye 1992; 6(pt3):305-7.
3. Berrios RR, Dreyer EB. Traumatic hyphema. Int Ophthalmology Clin 1995;35:93-103.
4. American Academy of Ophthalmology. The athlete's eye: ophthalmology and sports. 1st ed. San Francisco, CA: American Academy of Ophthalmology; 1982.
5. Shingleton BJ, Hersh PS, Kenyon KR. Eye Trauma. 1st ed. St. Louis, MO: Mosby-Year Book, Inc.; 1991.
6. Zacovic JW, McGuirk TD, Knoop KJ. Bilateral hyphemas as a result of air Bag deployment. Am J Emerg Med 1997;15:323-4.
7. Liebmann JM. Management of sickle cell disease and hyphema. J Glaucoma 1996; 5:271-5.
8. Zagelbaum BM. Sports Ophthalmology. 1st ed. Cambridge, MA: Blackwell Science Publishers; 1996:184-209.
9. Wright KW. Interactive ophthalmology textbook and review. Philadelphia: Lippincott Williams & Wilkins 1997.
10. Crouch ER Jr, Williams PB. Trauma: ruptures and bleeding. In: Tasman W, Jaeger EM, editors. Duane's Clinical Ophthalmology. Philadelphia, Pa: Lippincott Williams & Wilkins; 2001. p. 1-18.
11. Read JE, Goldberg MF: Traumatic hyphema: Comparison of medical treatment. Trans Am Acad Ophthalmol Otolaryngol 1974; 78: 799.
12. Rastogi S. Hyphema, Postoperative. [online]. 2005 [cited 17 Jun 2006]. Available from URL: http:// www.emedicine.com/oph/topic68.htm
13. Filipe JA, Barros H, Castro-Correia J. Sports-related ocular injuries: a three-year follow-up. Ophthalmology 1997;104:313-8.
14. Schein OD, Hibberd PL, Shingleton BJ, Kunzweiler T, Frambach DA, Seddon JM, et al. The spectrum and burden of ocular injury. Ophthalmology 1988;95:300-5.
15. Morris DS. Ocular blunt trauma: loss of sight from an ice hockey injury. Br J Sports Med 2006; 40(3): e5.
16. Listman DA. Paintball injuries in

children: more than meets the eye. Pediatrics 2004; 113(1 Pt 1):e15-8.
17. Crouch ER Jr, Frenkel M. Aminocaproic acid in the treatment of traumatic hyphema. Am J Ophthalmol 1976;81(3):355-60.
18. Edwards WC, Layden WE. Traumatic hyphema. A report of 184 consecutive cases. Am J Ophthalmol 1973;75(1):110-6.
19. Agapitos PJ, Noel L-P, Clarke WN. Traumatic hyphema in children. Ophthalmology 1987;94:1238-41.
20. Kennedy RH, Brubaker RF. Traumatic hyphema in a defined population. Am J Ophthalmol 1988;106:123-30.
21. Spoor TC, Kwitko GM, O'Grady JM, Ramocki JM. Traumatic hyphema in an urban population. Am J Ophthalmol 1990;109:23-7.
22. Read JE. Traumatic hyphema: comparison of medical and surgical treatment for traumatic hyphema. Ann Ophthalmol 1975;7:659–70.

پوختە

کوم بوونا خینێ لبەریکا پێشیێ یا چاقی ژبەر بریندار بوونا چاقی

پێشەکی: کوم بوونا خینێ لبەریکا پێشیێ یا چاقی ئێکە ژ دیاردێت مشە د کومەلگەها مەدا وئەۆ دیاردە چێدبیت یا تێکەل بیت لگەل هندەك سەربارکێت دی یێت ترسناك کو لدەمەکێ گیرودا پەیدا دبن .

لدیف زانینا مە هێشتا چ ڤەکولینێت ڤی بابەتی نەهاتینە کرن ل هەرێما کوردستانێ .

ئارمانجا ڤەکولینێ: ئارمانجا سەرەکی یا ڤەکولینێ ئەوە کو بشێین گرنگترین ئەگەر و هوییێت کو دبنە ئەگەرا بریندارباوونا چاقی دیارکەین وهەر وەسا پیتە وسەرنج بێتەدان لسەر ریژێ کوم بوونا خینێ لبەریکا پێشیێ یا چاقی لپاریزگەها دهوك بێتە دیارکرن وهەر وەسا دەست نیشانکرنا وی ژیێ کو پتر بەرهەۆ کو تووشی ڤان جورە بریندارێا دبن ، و پلادیتنا وان پشتی چارەسەریێ .

جهێ ڤەکولینێ: ئەۆ ڤەکولینە هاتە کرن لنەخوشخانا فێرکرنێ یا گشتی یا ئازادی و نەخوشخانا تەنگاڤیا لپاریزگەها دهوك / هەرێما کوردستانێ ژ خزیرانا (2006) ێ هەتا خزیرانا (2007) ێ .

پلان وریکێت ڤەکولینێ: ئەڤە ڤەکولینەکا دیڤ چوون و پاشە روژیە وکلینکی یە بو وان نەخوشێت کو تووشی بریندارباوونا چاڤا بووین و بریندارباوونێ ئەۆ نەخوشە تووشی کوم بوونا خینێ کرین لبەریکا پێشیێ یا چاقی . ڤێ ڤەکولینێ (40) نەخوش ڤەگرتن ژڤی رەنگێ تووش بوونێ ژ کویێ (137) نەخوشێت تووشی بریندارباوونا چاڤا بووین. هەمی پشکنین هاتنە کرن بو ڤان نەخوشا ل روژا ئێکێ یا هاتنا وان (پلا دیتنێ – پەستانا چاقی – پشکنینا تورِا چاقی و دەمارا دیتنێ) وهندەك پشکنینێن دی یێن گرنگ .

ئەنجام : ریژا توشبوویێت ڤی جورێ بریندارباوونێ لڤێ ڤەکولینێ دا نێزیکی (5) ژ (100000) کەسا بوو ، ریژێ تووشبوویێت توخمێ نێر پتربوو ژ توخمێ مێ . (35 ٪) ژ تووشبویا وی ژی بوون ییێت ناڤبەرا (6 – 10) سالییێ دا . ریژا برینیێت هەرِشینێ (60 ٪) بوو و ریژا برینیێت شەڤکرنێ ژی (40 ٪) بوو. نێزیکی (90 ٪) ژ توخمێ مێ ییێت تووشی ڤان برینا بووین ژ جورێ برینیێت شەڤکرنێ بوون ، بەلێ پا توخمێ نێر پتر تووشی برینیێت هەرشینێ بوون . بگشتی چاڤێ چەپێ پتر تووشی ڤان جورە برینا بوو (55 ٪) لبەرامبەر چاڤێ راستێ کو (45 ٪) بوو. (37) ژ توشبوویا دیتنا وان زڤری ڤە بو پلەیەکا باشا دیتنێ و دوو نەخوشا پلا دیتنا وان تێکچو بو پلا تاری بوونێ کو هەستا رووناهییێ ژی نەما و ئێک نەساخ ژیک تەنیا هەستا رووناهییێ هەبوو.

الخلاصة

التجمع الدموي في الفجوة الأمامية الناتج عن كلم العين

خلفية البحث: تجمع الدم في الفجوة الأمامية في العين هي من الحالات الشائعة نسبياً في مجتمعنا وهذه الحالة قد تكون متصاحبة مع بعض المضاعفات والمخاطر المتأخرة. في حدود علمنا لا توجد أي دراسة حول هذا الموضوع في اقليم كوردستان.

الهدف: الهدف الرئيسي لاجراء هذه الدراسة هو معرفة أهم الأسباب المؤدية الى اصابات العين مع التركيز على نسبة تجمع الدم في الفجوة الأمامية للعين الكلّمي في محافظة دهوك ودراسة نسبة انتشاره حسب الفئة العمرية، ودرج الرؤية بعد العلاج.

المنهجية: أجريت هذه الدراسة في مستشفى أزادي التعليمي العام ومستشفى الطواريء في محافظة دهوك/ اقليم كوردستان وفي الفترة الواقعة من حزيران 2006 لغاية حزيران 2007. دراسة متابعة سريرية للمرضى الذين اصيبوا في العين وأدت الاصابة الى تجمع الدم في الفجوة الأمامية للعين. شملت الدراسة (40) مريضاً من هذا النوع من ضمن (137) حالة من اصابات العين. أجريت جميع الفحوصات لهؤلاء المرضى في اليوم الاول من حصول الاصابة وأثناء المتابعة (درجة الرؤية، الضغط داخل العين، فحص الشبكية والعصب البصري وبعض الفحوصات المهمة الاخرى).

النتائج: كانت نسبة الاصابات المشمولة بهذه الدراسة هي بحدود (5) لكل 100000 شخص. كانت نسبة اصابة الذكور اكثر من الاناث، (35%) من الحالات كانت من الفئة العمرية (6 – 10) سنوات. شكلت الإصابات الراضة نسبة (60%) من الحالات والواخزة (40%) منها. في حوالي (90%) من الاناث المشمولات بهذه الاصابة كانت من الجروح الواخزة بينما اصيب الذكور أكثر بالاصابات الراضة. عموماً أصيبت العين اليسرى أكثر من اليمنى نسبياً (55%) مقابل (45%) للعين اليمنى. استعادت (37) حالة درجة رؤية مقبولة في حين اصيبت ثلاثة منها بتعذر أو عدم رؤية الضوء.

الاستنتاجات والتوصيات: أظهرت الدراسة أن عقار (Tranexamic acid) يقلل من نسبة تكرّر النزف في المصابين. كان ارتفاع ضغط العين هو واحد من أكثر المضاعفات لحدوث نزف دموي في الفجوة الامامية للعين والذي أدى بدوره الى ضعف وخلل في الرؤية. في حال حصول هذه الاصابات يجب فحص العين فحصاً شاملاً وذلك للتأكد من عدم وجود أنواع أخرى من الجروح والتي قد تؤدي الى مضاعفات أخرى.

ASSESSMENT OF INFECTIOUS DISEASES SURVEILLANCE SYSTEM IN MOSUL, IRAQ

ASMA A. AL-JAWADI*,
MAHA A. AL-NEAMI**

Submitted 12 April 2008; accepted 10 June 2008

ABSTRACT

Context There is a growing international awareness that coping with infectious diseases threat relies on effective and efficient epidemiological surveillance system.
Objective To evaluate the infectious diseases surveillance and response system in Mosul, Iraq.
Methods This study examined the structure and performance of the core activities, response and supportive functions of infectious diseases surveillance system. Data were gathered via sets of questionnaires that cover both interviews and certain observations at local, sectors and regional health levels within these institutions in Mosul city, Iraq.
Results There is an acceptable registration, reporting activities and passable supervisory visits for the disease specific surveillance systems at health facilities level, while all poor for monthly passive surveillance. Obvious lack of standardized case definitions with limited ability for laboratory diagnosis at health facilities surveyed. Feedback activities were the weakest issue in the surveillance at all levels. Nonexistence of essential activities required for the system to act as an early warning system for epidemic detection at health facilities and sectors levels. There is poor reporting facilities, although 76.5% of health facilities have computers, none of them use this equipment for compiling and reporting surveillance data.
Conclusion Special attention required for the improvements in supervision, standardized case definitions and quality of reporting, analysis and feedback of monthly passive surveillance, with a continuous support for the disease specific surveillance systems activities.

DMJ 2008;2(1): 127-140.

Key words: Evaluation, Epidemiological Surveillance, Infectious diseases

In many communities especially developing countries infectious diseases continue to be substantial causes of mortality, morbidity, and rising health-care costs, and must be carefully monitored and controlled.[1]

Infectious diseases (ID), emerging and re-emerging including the deliberate release of biological agents, and the challenges of new diseases repeatedly threaten global health security primarily because of concerns about bioterrorism, AIDS, and the spread of SARS and Avian influenza.[2,3] Besides, infectious diseases surveillance (IDS), is perhaps one of the earliest strategies adopted in the 20th century for the purpose of control of the spread of disease. It is regarded as a tool of

*Professor of public health and preventive medicine, Head of the Department of Community Medicine, Mosul College of Medicine, Mosul, Iraq
** Lecturer of Community Medicine. Department of Community Medicine, Nineveh College of Medicine, Mosul University, Mosul, Iraq
Correspondence author: Asma A. Al-Jawadi Head of the Department of Community Medicine, Mosul College of Medicine, Mosul, Iraq. E-mail: asmaa_aljawadi@yahoo.com

information to inform the public health specialist, policy makers, administrators, and health care workers about the distribution and determinants of health conditions. Further more, it can (and should) guide and measure the impact of intervention.[4]

Epidemiological surveillance provides data about incidence of disease in the community; that can help raise or lower the threshold of clinical suspicion for a particular infectious disease, encouraging early detection and appropriate treatment.[5]

The aim of the present study is to evaluate the structure, performance, epidemic preparedness and response of infectious diseases surveillance systems at all health care levels in Mosul city, according to the core and supportive functions.

METHODOLOGY

Before conducting the present evaluation, agreement was obtained from Nineveh Health Directorate (NHD) and the structure of the health care services and surveillance system levels in Mosul were delineated too.

Mosul, is the capital of Ninevah province which is the 2nd largest city in Iraq, located in the north, it's population is about 2,500,000. In Mosul city the IDS was established at 3 levels: Local level which includes 60 health facilities, i.e., 26 primary health centers (PHCs), 8 hospitals, 25 governmental general practice clinics (GGPC), network of laboratories which include laboratories at (PHCs), hospitals, and the Public Health Laboratory (PHL). Health sectors level inside Mosul City comprises the right and left health sectors, which drain the morbidity data from the whole city; and Regional level represented by Primary Health Care Department (PHCD) that is articulated to NHD. In this study 33 health facilities were surveyed (26 PHCs + 7 hospitals), excluding those which did not follow IDS.

Evaluation protocol was based on a modified version of the protocol for the assessment of national communicable diseases surveillance and response systems.[6-8] Questions were reviewed, modified, field-tested and adapted to suit the local context and depended on a framework that comprises core activities i.e. case-detection, registration, case-confirmation, reporting, analysis and feed back. The activities of associated response including epidemic type of responses (epidemiological investigation) or management type of response which occur periodically over time were also covered, in addition to other supportive functions including communication, training, supervision and resources provision.

Four sets of questionnaires were used to examine the health facilities level (PHCs and hospitals), health sectors level, regional level, and the laboratories network within these institutions. The design of the present evaluation deals with surveillance system on the basis of its components which include; structure i.e. brief description of organizations within IDS levels; surveillance process by examining the core activities and supportive functions of IDS at all levels; evaluation of epidemic preparedness and

response system of IDS at health levels; and evaluation of laboratories within these health institutions for their role in ID confirmation.

All sets of questionnaires have been filled through direct personal communication and small group discussion (about 2-3 persons per group taking in consideration the crowded and busy working time) with the head and the focal person in situ. Additionally, there were certain observations monitored by the investigator as a complementary measure for the evaluation process.

The fieldwork was carried out between 1st May 2004 and 1st May 2005. Substantially, 60 health facilities with their laboratories were visited in Mosul city through 231 visits. Certain institutions needed to be visited more than twice to have a proper look at the minute points within the daily activities that enable the investigator to give methodological description of the disease surveillance process at different levels.

The IDS in Mosul can be organized in two approaches; the integrated approach of IDS that targets diseases need immediate notifications, weekly reporting system and monthly passive reporting system (MPS); as well as the disease specific surveillance approach that constitutes; acute flaccid paralysis (AFP), cholera watch system (CWS), measles surveillance system (MSS), direct observation treatment, short course (DOTS) program for TB.

Data were summarized and frequency distribution tables were constructed. Tables were stratified according to the surveillance levels, and the findings were arranged through descriptive statistical measurements (frequency and percentage). The average percent of achievement of each core and support function is calculated by dividing the summation of achieved number of this function over the sub-systems on 165 which is the total number of assessed facilities for each sub systems.

RESULTS

Table 1 depicts the performance of core activities of IDS at health facilities surveyed. For case detection and registration of IDS, this table demonstrates that ID registries are present in all of health facilities, although there are no separate records for each disease category. There is difference among the systems in correct filling of these registries, being 100% for AFP, CWS and DOTS and it is weak in MPS (14.2%). Activities of data reporting is shown in the same table, the availability of surveillance forms all the time including time of visit was 100%, similar finding is recorded for the timely reporting at each reporting period and complete reporting of surveillance reports. There is a variation among the systems in the achievement of zero reporting which is complete in AFP, CWS, DOTS and MSS and poor (15.2%) in MPS. Inquiry about the reporting indicates that 85.4% of health facilities manpower found the reporting form easy to use while 14.5% found them time consuming with a variation among the five systems in the achievement of these two activities.

In data analysis the same table

indicates that all health facilities have appropriate denominators and described data by person. The description of data by place and time varied among the IDSS, being fully applied in all systems apart from MPS, and no description of data by time in DOTS. Almost one tenth (12.1%) have threshold level for action i.e. when the level of any disease occurrence become above the usual one, an action should be taken.

Indicators of epidemic preparedness and response evaluation of IDS are also shown in table 1. The implementation of prevention and control measures and having plan for disease epidemic and response found to be carried out more extensively in AFP than in others. DOTS and MPS had no plan for disease epidemic and response. One quarter (21.2%) of health facilities have stokes of drugs for CWS compared to zero level in other systems. Of these facilities, 66.6% were complaining from shortage in drugs and vaccine for MPS within the past year i.e. 2003.

Table 1 also portrays the feedback from higher level to health facilities, which is very limited for the MSS, MPS, and CWS (6.1%, 6.1%, and 3.0%) respectively and completely absent for both AFP and DOTS. Only 1.2% of health facilities were conducting meeting with community leaders within the last six months before the present survey.

For case confirmation of ID within PHCc, the study demonstrated that 23.8% of PHCc had standardized case definition (SCD) for the priority ID, and 12.6% of them could confirm diagnosis of these diseases while higher fractions of hospitals had SCD with moderate ability to confirm diagnosis of such diseases (37.0% and 41.6%) respectively.

The result of evaluation of supervision and training support functions is illustrated in table 2. The supervision of surveillance activities and receiving recommendations during supervisory visits from the higher levels were 100% at AFP and MSS, and less in CWS (63.6%) and DOTS (55.9%). Surveillance data was reviewed in 5% of health facilities. All the surveillance manpower within all health facilities surveyed was ready to implement any recommendation from higher level. Training of the staff in disease surveillance varied among the different subsystems, being 21.2% in MPS to 82.3% in DOTS and almost 81% in others, Training on data management had a total achievement of 21.0% while training in epidemic management varied among the five systems, where half of the staff were trained in CWS (51.5%) and MSS (45.5%) and none for DOTS (Table 2).

Concerning the tool of communication, the present study shows that hand posting was the main tool of data reporting for the five systems of IDS. Although 85.3% of health facilities have telephones, only 21.8% use this facility for emergency notification of CWS. At the time of the survey, none of these health facilities have other communication technologies as E-mail, fax or radio call.

Table 1. Performance of core activities of infectious disease surveillance at health facilities surveyed, Mosul, 2004

Core activities	Infectious disease surveillance systems					
	*AFP (n=33) (%)	CWS (n=33) (%)	DOTS (n=33) (%)	MSS (n=33) (%)	MPS (n=33) (%)	% of achievement
Detection and registration						
- Presence of local surveillance manual	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	0.0
- Existence of clinical register	(100)	(100)	(100)	(100)	(100)	100
- Correct filling of clinical register	(100)	(100)	(100)	(84.8)	14.2)	81.8
Reporting						
- Presence of surveillance forms all the time over the past 6 months	(100)	(100)	(100)	(100)	(100)	100
- Timely reporting at each reporting period	(100)	(100)	(100)	(100)	(100)	100
- Complete reporting of each report	(100)	(100)	(100)	(100)	(100)	100
- Zero reporting**	(100)	(100)	(100)	(100)	(15.2)	80.0
- Reporting form is easy to use	(100)	(69.7)	(93.9)	(100)	(36.4)	85.4
- Reporting form is time consuming	(0.0)	(12.1)	(3.0)	(0.0)	(57.6)	14.5
Data analysis						
- Description of data by person	(100)	(100)	(100)	(100)	(100)	100
- Description of data by place	(100)	(100)	(100)	(100)	(0.0)	80.0
- Description of data by time	(100)	(100)	(0.0)	(100)	(0.0)	60.0
- Performance of trend analysis	(0.0	(0.0	(2.9)	(0.0	(0.0	0.6
- Presence of appropriate denominator	(100)	(100)	(100)	(100)	(100)	100
- Presence of action threshold for action	(21.2)	(12.1)	(12.1)	(15.2)	(0.0)	12.1
Epidemic preparedness						
- Implementation of prevention and control measures based on surveillance data	(21.2)	(18.2)	(6.1)	(6.1)	(0.0)	10.3
- Having plan for disease epidemic preparedness and response	(33.3)	(39.4)	(0.0)	(27.3)	(0.0)	20.0
- Had emergency stocks of and vaccines in the past year	(0.0)	(21.2)	(0.0)	(0.0)	(0.0)	4.2
- Experienced a shortage in drug and vaccines in the past year	(0.0)	(0.0)	(6.1)	(0.0)	(66.6)	18.7
Feedback						
- Received at least one feedback report during last 6 months.	(0.0)	(3.0)	(0.0)	(6.1)	(6.1)	3.0
Conducted meeting with community leaders within the last 6 months	(6.1)	(0.0)	(3.0)	(3.0)	(0.0)	1.2

AFP (acute flaccid paralysis), CWS (cholera watch system), MSS (measles surveillance system) MPS (monthly passive surveillance).

***zero reporting: when no cases have been detected, which assure the next level that no data have been lost nor forgotten.*

Table 2. Performance of supportive functions of infectious disease surveillance at health facilities surveyed, Mosul, 2004

Supportive functions	Infectious disease surveillance systems					
	*AFP (n=33) (%)	CWS (n=33) (%)	DOTS (n=33) (%)	MSS (n=33) (%)	MPS (n=33) (%)	% of achievement
Supervision						
- Surveillance activities supervised in the last 6 month	(100)	(63.6)	(55.9)	(100)	(18.2)	67.5
- Surveillance data reviewed during the visit	(9.1)	(6.1)	(0.0)	(12.1)	(0.0)	5.5
- Receiving recommendations during visits	(87.9)	(87.9)	(15.5)	(87.9)	(12.1)	64.3
- Implementation of any recommendation given during visits	(100)	(100)	(100)	(100)	(100)	100
Training						
- Training in disease surveillance	(81.8)	(81.8)	(82.3)	(81.8)	(21.2)	69.8
- Training in epidemic management	(39.4)	(51.5)	(0.0)	(45.5)	(6.1)	28.5
- Training in data management	(18.2)	(21.2)	(39.4)	(30.3)	(6.1)	21.0

The availability of resources at health facilities surveyed was variable. Calculators were present in all of the examined health facilities, electricity generators were available in 94.1%; while 85.3% of health facilities had telephone, 88.3% had stationary, more than three-quarters (76.5%) had computers and 29.4% of these health facilities had their special vehicles. Almost one tenth (8.8%) had spray pump and disinfectants and none of them have radio call for urgent notification. These resources were used to support all surveillance systems of infectious diseases.

Table 3 summarizes the average percent of achievement of each core and supportive functions for the IDS evaluation results at health sectors and regional level. This evaluation showed that there was no real role of NHD in the disease surveillance process. For this reason the PHCD had been regarded as the regional level in Mosul City. The national surveillance manual for ID is present at the regional level and the capacity of submitting specimens to higher level (mainly polio specimen and hemorrhagic fever) was available.

For data reporting, it had an achievement rate of more than 80% at both levels, regional level is sure about the availability of surveillance forms in all levels. Reports were received from health

sectors timely and completely and submitted to higher level. Both sectors personnel found reporting forms easy and not time consuming. Zero reporting for IDS was present. Both levels analyze data by persons, by time and place; trend analysis was not performed. There were no incidence, prevalence and case fatality rates calculation, although both have appropriate denominators. Usage of surveillance data for action was present in both levels. No survey was done for the prevalence measurement of any disease during the past year (i.e. 2003) except for hepatitis B. The feedback activities within two levels were weak, but there were reports or bulletins to disseminate surveillance data of IDS from regional to health sectors level.

Both levels have a threshold of action for any epidemic prone disease under surveillance (only), implemented a prevention and control measures based on surveillance data, responded within 48 hrs of notification, and able to perform mass vaccination campaign with coverage evaluation. One sector had a previous outbreak investigation, had little emergency stocks of drugs and vaccines in the past year (for one or two of ID), had plan for epidemic preparedness and response, and able to perform a comparison between current and previous data. Both sectors had experienced a shortage of drugs and vaccines during the past year (2003), and non of them had a written case management protocol.

Both sectors were supervised by the regional level for IDS activities, with no surveillance data reviewed and no written supervision reports during the last six months. Although, the evaluation reports for all health facilities were done regularly, most of the heads of sections concerned with different surveillance systems did not perform the desired supervisory visits, nor supervised from the higher level during the past year (2003). Training on disease surveillance and data management was performed in both levels.

Electricity generators, vehicles, telephone, calculators, computers, and sanitary material all were available at both levels. Hand posting was the main mode of reporting beside telephone, but no place for E-mail, fax, satellite phone and radio call except at regional level.

Laboratories evaluation portrays that malaria is the only disease that can be diagnosed and confirmed by all assessed laboratories (at health facilities, hospitals laboratories and PHL). Dysentery is the only disease which is diagnosed by both PHCc and hospital laboratories. Widal and brucella agglutination tests were performed by PHL, 87.5% of hospital and 34.6% of PHCc laboratories. TB was confirmed by all hospital laboratories and only by 7.7% of PHCc laboratories. Meningitis was diagnosed by all hospitals laboratories, while hepatitis was diagnosed just by PHL. None of these laboratories could test for poliomyelitis, measles and hemorrhagic fever.

Table 3. Total achievement of core and supportive functions at the health sectors and regional level

Core and Supportive functions	% of achievement	
	Health sector level	regional level
- Data reporting	83.3	100.0
- Data analysis	61.1	66.7
- Feedback	25.0	50.5
- Epidemic preparedness	72.7	64.0

DISCUSSION

Conducting evaluation of disease surveillance systems in Mosul City at the time when Iraq is witnessing a various morbidity challenges is regarded as a tool of information of efficient disease control in order to accredit a solid strategies and regulations for construction of health and wellbeing.

This study revealed that the availability of clinical registers in all health facilities for all IDS was 100%. Correct filling for MPS was poor (14.2%) compared to other sub-system. This may be attributed to that each disease specific system is directed by specific manager who can focus on the correct filling for these epidemic prone diseases, since most of them need urgent notification. The overall achievement of this function was 81.8% which is good if compared to that of Gambia where clinical registers were available in 61% of the surveyed facilities.[9]

In general this study indicates that confirmation of ID diagnosis in Mosul was poor although it is better in hospitals than in PHCs. This may be due to availability of specialist physicians. Beside, SCD for the priority diseases was present in 23% of hospitals. Although the Iraqi MOH had developed SCD guideline for ID but it is still at higher level and is not distributed to the health facilities,[10] while all the public health officials insist on the adaptation of SCD for all diseases surveillance in order to ensure accurate case detection, reporting, and comparability of data. In Islamic Republic of Iran, case finding got much improved when active case finding was based on the SCD and clinical and laboratory diagnosis of 24 relevant local communicable diseases introduced into the surveillance system.[11]

On other hand, this study reveals that 41.6% of hospitals and 12.6% of PHCc can confirm cases by laboratory investigation. None of PHCs could confirm cholera cases and all stool samples should be collected and send to PHL within 3-5 days to be examined. This indicates poor quality of reported data especially for the MPS, because of the

SCD and confirmation laboratory tools shortage made the diagnosis depends mainly on doctors skills in case detection.

In the present study, all health facilities had complete compulsory reporting within a limited dead time. The same was noticed at health sectors and regional levels. Although this is a positive indicator, it may have a drawback on the accuracy of data reported. In Armenia, epidemiologists were motivated to perform actions that both pleased their supervisors and avoid punishment or demotion.[12]

Among all health facilities and sectors surveyed, zero reporting was clear in all disease specific surveillance systems except in MPS which was very poor. This may be attributed to less attention given from health authorities to this passive surveillance system. In Dohuk governorate, zero reporting was found in 21% of health facilities which is only for vaccine preventable diseases surveillance systems while it was absent in IDS.[13]

This study revealed that for the majority of IDS, almost all the personnel at health facilities, health sectors and regional levels found the ID reporting forms easy to use and not-time consuming. This may be attributed to a repeated training of the staff in health facilities on epidemiological surveillance, and to more attention given to these systems. Approximately the same figure (86%) was seen in Tanzania.[14]

The present study showed that the five ID sub-systems primarily described data by person (age, sex), 80% described data by place and 60% by time. The description of data by place and time is widely applied in AFP, CWS, DOTS, and MSS than MPS, such fact may be due to the presence of additional separate case-specific forms used for each of these system (in addition to the weekly and monthly reporting forms) that permits the description of time and place precisely. A great emphasis directed to AFP, MSS from the MOH and WHO as a disease under eradication and elimination may have played additional role.

The present study depicts poor data analysis which may be due to the absence of standardized data analysis procedures, beside the incomplete training of surveillance personnel at all levels on the analytic methods that should be performed which are very useful to recognize significant changes and support follow up action as seen in Japan, where beside routine trend monitoring of various diseases, quality control of data and integration of other sources of data would be the next goal of the surveillance system.[15]

The poor action threshold according to surveillance data noted in this study at local level (12.1%) may be attributed to the centralized strategy of action within surveillance system, in addition to the poor surveillance data analysis at health facilities that could help to raise or lower the threshold of clinical awareness for a specific condition.

Regarding the activities of IDS feedback, the reports, and conducting meeting with community leaders in general, were poor at all levels. The problem of feedback seems to be consistent throughout many countries. The

same result was seen in Armenia[13] and in Uganda.[16] This is in contrast to highly developed countries as Germany where the rate of community involvement is very high (85%) at health facilities[17] and in China where the policy of community based surveillance systems is used to enhance case detection, registration and reporting.[18]

At the regional level the presence of national plan of epidemic preparedness, written case management protocol and a rapid response team with action threshold of action can enhance their ability to respond within 48 hrs only to an epidemic prone disease after notification of an outbreak although the shortage of the drugs and supplies can limit the effectiveness of their action and response. Only AFP has better achievement than other systems and this may be due to the central and global focusing on this surveillance system that have more funds.

The differences in the achievement of the supervisory visits and the recommendation received during these visits were excellent in AFP, MSS and the CWS, but comparatively, lower in MPS and DOTS. This could be explained by more incentives paid for the AFP surveillance manpower. The incidence and prevalence of specific health problems were not calculated and not supervised at any level, and even not discussed by supervisors. This reveals the lack of one of the primary objective of the IDS. In developed countries, the supervision functions are done regularly through competent and qualified staff, with much more resources, and clear assignment of responsibility and accountability among authorized surveillance personnel.[17]

In the present study, there is difference among the systems of training in disease surveillance, with low level of training in MPS in comparison with relatively higher levels in other systems (DOTS, AFP, and MSS) where the majority of their staff has been trained. This may be explained by the fact that training courses are funded by certain organization as WHO.

In developed countries such as USA, and Germany, the training in disease surveillance, epidemiology, computerized data management and epidemic management are provided on regular basis and updated with any new threat or new communicable health events, with a high level of training offered to the staff in every aspect of disease surveillance.[19, 20]
It is worth noting that surveillance systems within developing countries share difficulty of collecting and compiling statistics without appropriate technology and training,[21,22] nevertheless the availability of generators, calculators, stationary and statisticians can help to simplify the registration and reporting procedures.

The main obstacle noticed in this evaluation is the vehicles, which were available in only 29.4% of health facilities. All of the surveyed sites depended on hand posting as a tool of communication, which may add another hindrance to the speed of reporting. Radio call, fax, and E-mail were not used for reporting surveillance data in the present evaluation (apart from regional level), this may be explained by the low

appreciation of the value of rapid notification. The same is present in many developing countries.[12,23] While In Australia, as an example of developed country, data are received from various clinical sources (hospitals, laboratories and clinics) via papers, telephone and fax.[24] Earlier, in Sweden Jansson suggested that electronic reporting is more rapid and contains more complete information.[25]

This study concluded that the registration, reporting and supervision activities were acceptable, feedback were the weakest issue for the disease specific surveillance systems, while all were poor for MPS with repeated shortage of emergency stocks. The manual reporting is the predominant communication method at all levels. Efforts are needed to augment the MPA activities, with continuous support for disease-specific surveillance systems activities.

ACKNOWLEDGMENTS

Our sincere thanks to all members of epidemiological surveillance section, PHCs.

REFERENCES

1. Kembabanova G, Ivanova R, Deschevoi S. Epidemic investigation of diphtheria, Republic of Kazakhistan 1990-1996. J Inf Dis 2000;181 Suppl 1:569-72.
2. Centers for Disease Control and Prevention. Public health surveillance: questions and answers. Atlanta: Centers for Disease Control and Prevention; 2001.
3. Kahn E. Public health surveillance data value survey: forming a standard tool to measure surveillance data use. Public Health and Environment 2004;5:23-31.
4. Hallaj Z. Constrains facing surveillance in the Eastern Meditrranean Region. Geneva: World Health Organization; 1996.
5. Koo D, Wetterhall SF. History and current status of the national notifiable diseases surveillance system. J Public Health Manag Pract 1996;2:4-10.
6. World Health Organization. Protocol for evaluation of epidemiological surveillance systems. Geneva: World Health Organization; 1997. WHO/ EMC/ DIS/ 97. 2.
7. World Health Organization. Surveillance of communicable diseases, A training manual. Cairo: Regional office for the Eastern Mediterranean; 1998. WHO-EM/ CDS/ 52/ E/ L/98.
8. World Health Organization. Protocol of the assessment of national communicable disease surveillance and response systems. Geneva: World Health Organization; 2001. WHO/CDS/CSR/ ISR.
9. World Health Organization. Assessment of national communicable disease surveillance systems and epidemic preparedness and response in Gambia. Preliminary Report. Geneva: World Health Organization: 2001.
10. Ministry of Health. Communicable disease control; guidelines of case definitions. Baghdad, Iraq: Ministry of

Health; 1999.
11. Karimi A, Kadivar MR, Fararoee M, Alborzi A. Active case finding of communicable diseases in the south of the Islamic Republic of Iran. East Mediterr Health J 2000;6(2/ 3):487-93.
12. Wuhib T, Chorba TL, Davidiants V, MacKenzie WR, McNabb SN. Assessment of infectious diseases surveillance systems of the Republic of Armenia: an example of surveillance in the Republics of the former Soviet Union. BMC Public Health 2002;2(1):3-12.
13. Rajab AJ. Assessment of communicable disease surveillance systems in Dohuk [Msc thesis]. Dohuk: Dohuk Univ.; 2003.
14. Nsubuga P, Eseko N, Wuhib T, Ndayimirije N, Chungong S, McNabb SN. Structure and performance of infectious disease surveillance and response, United Republic of Tanzania. Bull World Health Organ 2002; 80(3):196-203.
15. Oshiro H, Kawamoto K, Nose T: Surveillance system of infectious diseases in Japan [abstract]. J Epidemiol 1996;6 Suppl 3:81-5.
16. Centers for Disease Control and Prevention. Assessment of infectious disease surveillance in Uganda. MMWR 2000;49(30):687-91.
17. Rushworth RL and Bell SM. Improving infectious disease surveillance in New South Wales. Med J Aust 1999;154(12):828-31.
18. Zeng G, Zhang JK, Rou KM. Infectious disease surveillance in China. Biomed Environ Sci 1998;11(1):1-7.
19. Schmidt HJ. Epidemiology of infectious disease in the Federal Republic of Germany. Klin Pediatric 1991;203(6):433-8.
20. Doyle TJ, Glynn KM, Groseclose SL. Completeness of notifiable infectious disease reporting in the United States: an analytical litrature review. Am J Epidemiol 2000;155(9):866-74.
21. World Health Organization. Recommended surveillance standards. Geneva: World Health Organization; 1997. WHO/EMC/97.3.
22. Centers for Disease Control and Prevention. Health information system: routine, surveillance, monitoring and evaluation--- what really matters? HNP, flash issue (42). Atlanta: Centers for Disease Control and Prevention; 2001.
23. World Health Organization. Assessment of the national communicable disease surveillance and response system, Ethiopia. Wkly Epidemiol Rec 2001;2(76):9-16.
24. Miller M, Roche P, Spencer J, Deeble M. Evaluation of Australia's national notifiable disease surveillance system. Commun Dis Intell 2004;28(3):311-23.
25. Jansson A. Sensitivity and timeliness of case reporting in the Swedish statutory surveillance of communicable diseases 1998-2002 [Msc thesis]. Karolinska: Karolinska institute, Department of Public Health Sciences; 2004.

پوختە

هەلسانگاندنا سیستەمێ لێ گەریانا پەژی یا نەخوشییێن ڤەگر ل میسل، عیراقێ

پێشەکی: هشیاربوونەکا نیڤ دەولەتی یا هەی کو بەهنگاریا مەترسییێن نەخوشییێن ڤەگر متمانی یێ ددەتە لسەر بەرنامەکێ لێ گەریانا پەژی یێ کاریگەر کو پێشبینییێ یێ ددەت ب هەبوونا پەژا ل پاشەروژێ.

ئارمانج: هەلسانگاندنا بەرنامەکێ لێ گەریانا پەژی ل باژێرێ میسل، عیراقێ.

رێکا ڤەکولینێ: ڤەکولینەکا وەسفا سیستەمێ لێ گەریانێ و هەلسانگاندنا ئەرك و چالاکییێن سەرەکی و یێن پالپێشت بو بەرنامێن لێ گەریانێن یێن تایبەت ب پەژێن نەخوشییێن ڤەگر و درێکا فورمێن پرسنامی کو متمانییێ ددەتە لسەر چاڤپێکەتنێن ئێکسەر و هەروەسا زێرەڤانیا هەمی چالاکییێن بەرنامێن لێ گەریانا پەژی لسەر سێ ئاستێن دەزگەهێن ساخلەمییێ لدویف سیستەمی ساخلەمییێ یێن هەین ل پارێزگەهێ.

ئەنجام: هاتە دیارکرن کو هەمی دەزگەهێن ساخلەمییێ یێن کو بەرنامێ لێ گەریانا پەژی چێ دگریت سجلا تومارکرنا نەخوشێن سەرەدانا بنگەهی دکەن یا هەی و تا رادەکی تومارکرن تێدا یا مەقبوول بوو پێگری ب راپورتێن سفری (zero reporting) سەرەدانێن سەرپرشتییێ بو پتریا چالاکییا بو وان ئێشێن کو بەرنامێن لێ گەریانا تایبەت دگەل لاوازییێ د بجیهینانا ڤان چالاکییا بو ئێشێن نەرینی بوون د هەیڤێ دا. کیماسیا بەرجاڤ هەبوو د هەبوونا رێبەرێن پێناسەکرنا حالەتا. هەروەسا چالاکییا راپورتێن ڤەکراندنێ ژ هەمیا لاواز تر بوو. ب شیوەکێ گشتی سیستەمێ لێ گەریانا چالاك نە ل وی ئاستێ بلندبوو کو کاربکەت وەك سیستەمەك ژ بو زوو دەسنیشانکرنا پەژا. رێکێن ئاگەهدارکرنێ یێن روتینی هاتنە ب کارئینان ئەو بوون ئەوێن نە یێن کاریگەر بوون. رێکێن مودیرن یێن ئاگەهدارکرنێ نەبوون ل هەمی ئاستادا ژ بلی پشکا چاڤدیریا تەندروستی یا دەستپیکی.

دەرئەنجام: لاوازیەکا بەرڤاژ هەبوو د بجهئنانا د رەسدا نەرینی یا هەیڤانە. و دڤێت پتر پیتە بێ بهێتە دان ژ بو باشکرنا بجهینانا وێ ژ لایێ سەرپرشتێ کرنێ دا، شروڤەکرنێ دا، راپورتێن ڤەکراندنێ دا و ریبەرێ پیناسا حالەتا هەروەسا باشکرنا رێکێن ئاگەهدارکرنێ و ل هەمی ئاستادا و پشگیریا هەمی چالاکییێن لێ گەریانێ ئەوێ کار پێ دهێتە کرن.

الخلاصة

تقييم نظام رصد الامراض الانتقالية في الموصل، العراق

خلفية البحث: هناك وعي عالمي متنامي يسلم بأن مواجهة مخاطر انتشار الأمراض الانتقالية يعتمد على نظام رصـد وبائي رصين وفعال يساهم في التنبه السريع وينذر بحدوث الأوبئة المستقبلية.
هدف البحث: تقييم نظام رصد الأمراض الانتقالية في مدينة الموصل/مركز محافظة نينوى في العراق.
طريقة الدراسـة: اعتمدت الدراسة على وصف نظام الرصد وتقييم الوظائف والفعاليات الأساسية والمـساندة لأنظمـة الرصد الخاصة بوبائية الأمراض الانتقالية وذلك من خلال استمارات استبيانيه تعتمد على أجراء المقـابلات المباشـرة والمراقبة لفعاليات أنظمة الرصد الوبائي ضمن ثلاث مستويات للمؤسسات الصحية طبقا للنظام الصحي الموجود فـي المحافظة.
النتائج: أظهرت الدراسة بأن كافة المؤسسات الصحية المشمولة بالرصد تمتلك سجل للمراجعين مـع تـدوين مقبـول والتزام بفعالية التقرير الصفري والزيارات الإشرافية لمعظم الأمراض الانتقالية ذات الرصد الخاص والفعال مع ضعف منظور في أداء كل هذه الفعاليات للأمراض ذات الرصد السلبي الشهري. وهناك نقص ملحوظ في دليل تعريف الحالة لكل الأمراض، كما كانت وظيفة التغذية الأسترجاعية هي الأضعف من بقية وظائف الرصد وعلى كافـة المـستويات. وأوضحت الدراسة أن أنظمة الرصد الفعالة ضمن الرصد الوبائي لم تكن على درجة عالية من الجاهزية لكـي تعمـل كأنظمة رصد مبكر لاكتشاف الأوبئة بسرعة وفعالية، مع استخدام أساليب الإبلاغ الروتينية غير الكفوءة، أما التقنيات الحديثة في الإبلاغ فلم تكن موجودة في جميع مستويات الرصد ما عدا قسم الرعاية الصحية الأولية.
الاستنتاج: هناك ضعف واضح في أداء الرصد السلبي الشهري و حاجته إلى اهتمام أكبر لتحسين أداء فعالياته كافة من حيث الأشراف، التحليل، التغذية الأسترجاعية ودليل تعريف الحالة بالإضافة إلى ضرورة تحسين وسائل الإبلاغ لبيانات الرصد ولكافة المستويات مع دعم لجميع فعاليات التقصي المعمول بها للأمراض ذات الرصد الفعال.

SYNOVIAL SARCOMA OF THE FOOT: CASE REPORT

INTISAR S. PITY, MBChB, MSc, MIBMS Histopathology *

Submitted 8 January 2006; accepted 12 January 2008

ABSTRACT

Synovial sarcoma is a rare soft tissue sarcoma in the foot. It is commonly localized in the extremities, especially the lower thigh and knee areas. The histopathological, immunohistochemical, and cytogenetic findings of a foot synovial sarcoma are described.

DMJ 2008;2(1): 141-145.

Key words: Primary foot synovial sarcoma, Immunohistochemistry, Cytogenetic analysis

Synovial sarcoma is the fourth most common soft-tissue sarcoma, accounting for approximately 8-10% of all soft tissue sarcomas.[1,2] The neoplasm often originates in paraarticular regions of the major joints and burses of the extremities, particularly around the knee, hip, and shoulder joints. Other localizations such as the foot, intraarticular, and internal organs are unusual sites.[3-5]

Two different histological types have been identified; the classic biphasic type, composed of epithelial and spindle cell components and a monophasic type in which a single cellular component is dominant.[3,4,5] The monophasic form is more difficult for diagnosis, and represents a real diagnostic problem. The use of immunohistochemistry in such cases is a very important to confirm the diagnosis.[3,6,7] Positivity for cytokeratins in epithelial-like areas and for vimentin in mesenchyma-like areas with fused cells, is crucial for the diagnosis.[3,6,8] Recently, the expression of MIC2 (CD 99) and bcl-2 supports the diagnosis.[1,2,8] Cytogenetically, the finding of a specific translocation between X and 18 chromosomes (X;18) (p11.2;q11.2) is of a considerable help in the diagnosis, particularly in the less differentiated forms.[2,4,8]

The case reported is of an early monophasic and later biphasic SS of the left foot. The clinical and pathological features of this rare neoplasm are described.

CASE REPORT

A 32-years old male, who was known to have gout and on treament, from Dohuk city in the North of Iraq, came at May 2005 to Azadi hospital with a small painful mobile swelling in the left foot. Clinically, there was a non-ulcerated small subcutaneous nodule at the level of the second toe. Imaging techniques showed a soft tissue swelling with foci of calcification, but normal toe's bones and joints. Grossly, the lesion was a sharply circumscribed, sized 2 x 1.5 x 1 cm,

* *Assistant professor, Department of Pathology, Dohuk College of Medicine, Dohuk, Iraq*
Email "dani2000fadi@yahoo.com"

homogeneous gray-yellowish on cut section. The histopathological examination showed a dense proliferation of oval to fusiform cells arranged in a lightly fasciculated pattern interlacing with highly vascularized areas (Figure 1,2). There was a moderate mitotic activity. The tumor borders were not damaged and the surgical resection borders were free. The histological diagnosis was a spindle cell sarcoma, consistent with monophasic synovial sarcoma, but other tumors like fibrosarcoma, cellular schwannoma, and hemangiopericytoma were considered in the differential diagnosis. Although it was considered necessary, in this case, to carryout electron microscopy, immunohistochemistry, and cytogenetic studies, but unfortunately were not available in our country. After that, total body bone scan was done and failed to reveal any metastasis. The post-operative course was uneventful and so the patient didn't take any postoperative radiotherapy or chemotherapy.

One year later, the lesion recurred at the same site and the microscopic sections revealed a classical biphasic synovial sarcoma (Figures 3). In addition, a metastatic nodule was detected in the liver. The patient then went to an outside specialized center, where the immunohistochemical studies done showed positive pankeratin, vimentin, CD 99, and bcl-2, in addition to the cytogenetic analysis that confirmed the presence of t(X;18) (p11.2;q11.2) translocation.

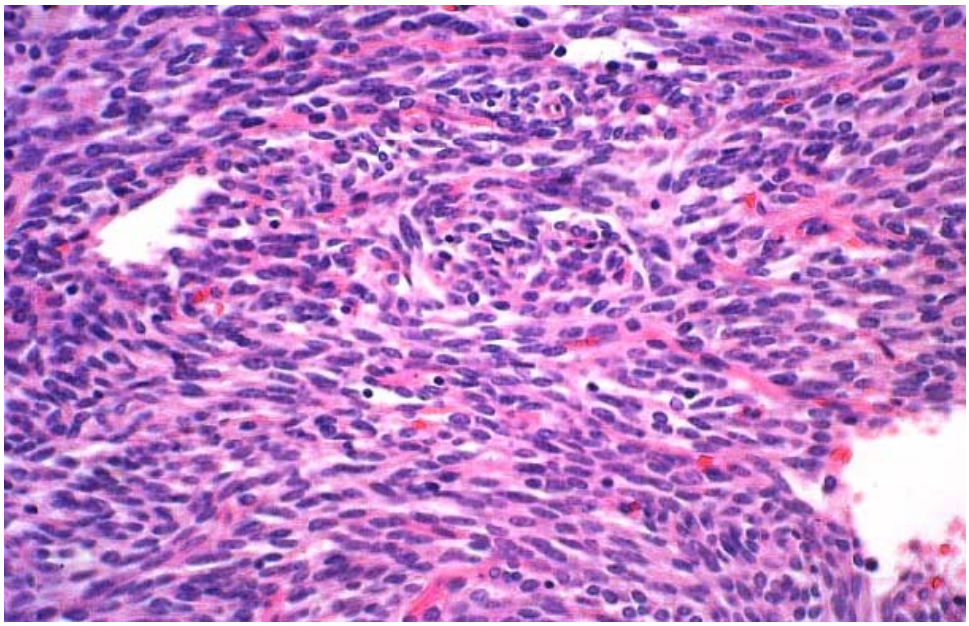


Figure 1. Monophasic synovial sarcoma showing a dense proliferation of fusiform cells exhibiting a lightly fascicular pattern of growth (X400)

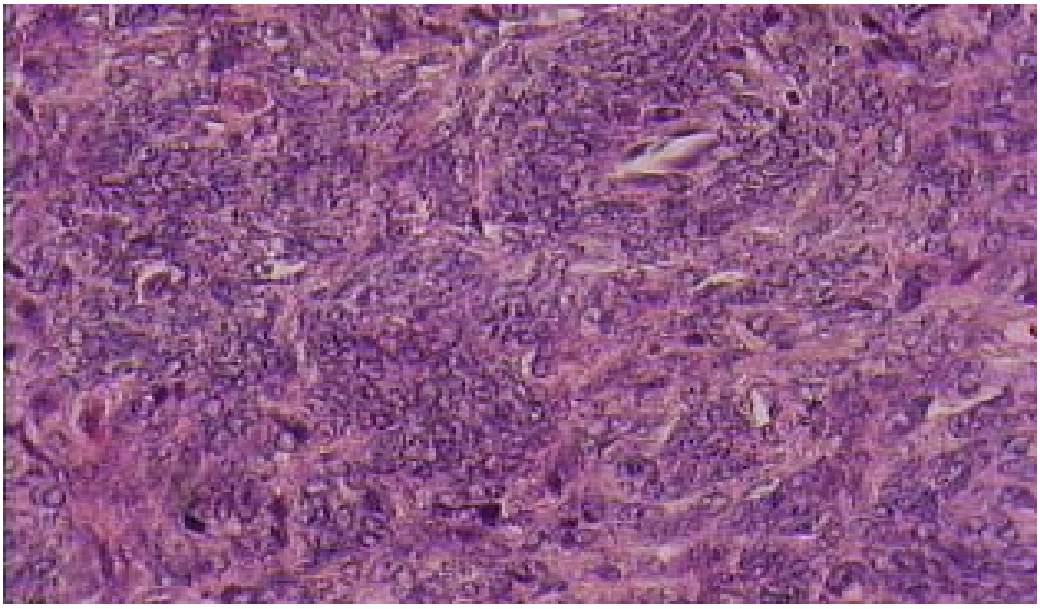


Figure 2. Biphasic synovial sarcoma showing a dense oval to spindle cell element intermingled with small gland-like structures (X400)

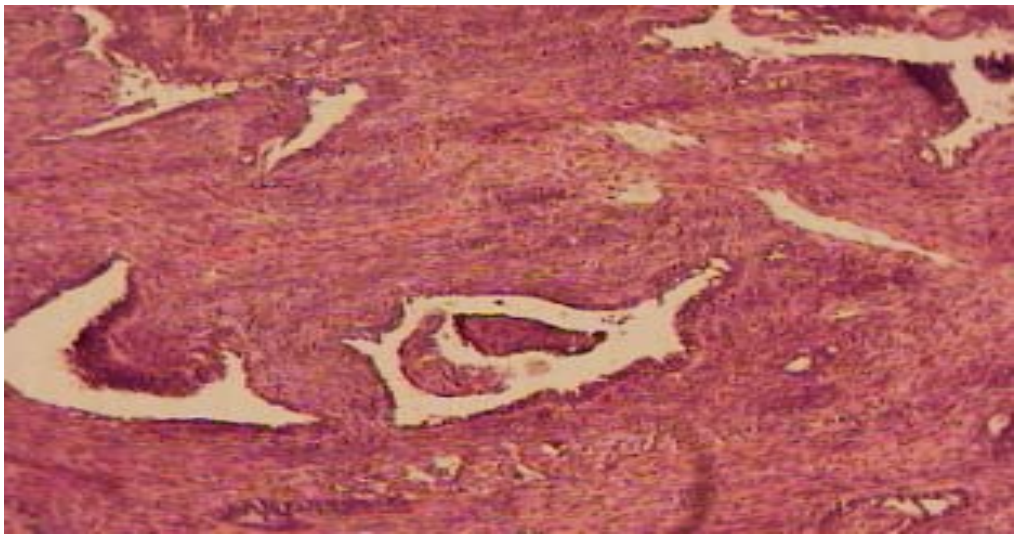


Figure 3. Biphasic synovial sarcoma containing both glandular and stromal components (X250)

DISCUSSION

Synovial sarcoma around the toes district is an unusual location (1,2). The primary delay in specification of the type of the spindle cell tumor was unfortunately due to absence of sophisticated histopathological techniques as immunohistochemistry, electron microscopy, and cytogenetic studies. However, recurrence of the tumor in the same location after one year as the

classical biphasic synovial sarcoma strengthened the diagnosis. In addition, the diagnosis was later confirmed immunohistochemically and cytogenetically in an outside specialized center. The patients age was typical for synovial sarcoma, which is a tumor of young adults (1,2). Recurrence of the tumor in the present case can be explained by the fact that the neoplasm has an aggressive biological behavior with a high probability of recurrence (1,2) in addition to the possibility of incomplete resection.

RECOMMENDATION

The entrance of more histopathological techniques, other than the conventional hematoxylin and eosin, in our labs is mandatory for proper diagnosis and patient management. In addition, opening of a well organized unit for cancer registry in Kurdistan (for both common and rare neoplasms), in collaboration with the ministry of health in Baghdad, as it will be a good reference for researches in the future.

REFERENCES

1. Enzinger FM, Weiss SW. Soft tissue tumors. 2nd ed. St. Louis: Mosby Company, 1988.
2. Perez CA. Unusual nonepithelial tumors of head and neck. Principles and practice of radiation oncology 1998;43:1125-34.
3. Hora M, Hes O, Michal M, Fisher C. Biphasic synovial sarcoma of the perineum. BJU Int 2001;87:903-4.
4. Cihak RA, Lydiatt WM, Lydiatt DD, Bridge JA. Synovial sarcoma of the head and neck: chromosomal translation (X;18) as a diagnostic aid. Head Neck 1997;19:549-53.
5. Artico R, Bison E, Brotto M. Monophasic synovial sarcoma of hypopharynx: case report and review of the literature. Acta Otorhinolaryngol Ital 2004;24:33-6.
6. Fisher C. Synovial sarcoma. Ann Diagn Pathol 1998;2: 401–21.
7. Loukopoulos P, Heng HG, Arshad H. Canine biphasic synovial sarcoma: case report and immunohistochemical characterization. J Vet Sci 2004;5:173-80.
8. Brodsky JT, Burt ME, Hadjdu SI, Casper ES, Brennan MR. Tenosynovial sarcoma. Clinicopathologic features, treatment, and prognosis. Cancer 1992;70:484-9.

پوختە

زێدە گوشتێ زلالی ل پی: وەسفا حالەتەکی

زێدە گوشتێ یێ زلالی دهێتە هژمارتن وەرەمێن گوشتی یێن دکێمن ل پی. و یا مشەیە ل رانی و ل جهی چوکی. وەسفا ئەنجاما پشکنینا شانا و پشکنینا کیمیا نەسیجی هاتە کرن دگەل پشکنینا خانا یا وراسی بو وەرما پە

الخلاصة

الورم اللحمي ألزلالي في القدم: وصف حالة

الورم اللحمي ألزلالي يعتبر ورم نسيج لحمي ناعم نادر في القدم . هو عموماً شائع في الأطراف خصوصاً الفخذ الأوطأ ومناطق الركبة . تم وصف نتائج الفحص النسيجي المرضي ، و فحص الكيمياء النسيجية المناعية مع الفحوصات الخلوية الوراثية لورم اللحمي ألزلالي في القدم.

CASE REPORT

RECURRENT BASAL GANGLIA HAEMORRHAGE: TRANSIENT ISCHAEMIC ATTACK (TIA) OR ACUTE TRANSIENT FOCAL NEUROLOGICAL DEFICIT (TFND)?

FARHAD O. HUWEZ, PhD, MRCPI, FRCP*

Submitted 28 August 2006; accepted 12 January 2008

ABSTRACT

The widely accepted definition of a transient ischaemic attack (TIA) is sudden, focal neurological deficit (cerebral or retinal deficit) lasting for less than 24 hours, which is presumed to be of vascular origin. This case demonstrates that the arbitrary time limit of 24 hours did not help the correct diagnosis and management of this patient. It supports the calls to change our approach to the definition and the management of TIA (under) towards a syndrome of acute transient focal neurological deficits (acute TFND), which could only be guided by imaging.

DMJ 2008;2(1): 146-154.

Key words: Transient Ischaemic attacks (TIA), Primary Intracranial haemorrhage, Basal ganglia haemorrhage,Transient Focal Neurological Deficits (TFND)

The widely accepted definition of a transient ischaemic attack (TIA) is sudden, focal neurological deficit (cerebral or retinal deficit) lasting for less than 24 hours, which is presumed to be of vascular origin.1,2 Here a patient is presented with sudden focal neurological symptoms and signs in the distribution of the right middle cerebral artery (MCA) lasting few hours and diagnosed as TIA treated with aspirin without prior CT scan of the head. Subsequently the CT scan of the brain revealed significant right basal ganglion haemorrhage. His symptoms resolved but recurred after one year in the same pattern and on that occasion aspirin was not started until CT scan of the brain was done revealing significant right basal ganglion haemorrhage. This case presentation supports the calls to change our approach to the definition and the management of TIA(under) towards a syndrome of acute transient focal neurological deficits (acute TFND), which could only be guided by imaging.

* *Consultant Physician and Geriatrician, Lead Physician/ Stroke Services, Basildon Hospital, Nethermayne, Basildon, Essex SS15 5NL, Telephone 01268 593416;, Facsimile 01268 598897, E-mail: farhad.huwez@btuh.nhs.uk*

CASE REPORT

A 67 years old gentleman attended the Medical assessment Unit (MAU) on the 02.10.2004 with sudden left sided facial droop, slurred speech and left sided

weakness of five hours duration. The patient's symptoms started on the 0530 hours and lasted until 1030 hours. There was no headache, vomiting or signs of meningeal irritation. He had history of systemic hypertension but there was no history of TIA or stroke. He was taking atenolol 50 mg per day, finasteride 5 mg per day. He lives with his wife and fully independent. He is an ex-smoker for the 22 years. The Glasgow Coma Scale (GCS) was 15/15, BP 150/86 mmHg, heart rate 80 bpm sinus rhythm, and the oxygen saturation was 99% on air. He had left facial droop but the swallowing was intact. The power in the left arm and leg was 4/5 but the right side was normal. The plantar reflexes were flexor. The ECG and chest X-ray were normal. The blood results including the renal functions, liver functions, full blood count, and blood glucose were all normal. The total serum cholesterol was 5.9 mmol/l. The patient was admitted to the MAU over night, and reviewed next day when it was found that he no recurrence of his symptoms. He remained orientated, able to eat and drink, and mobile. *As the symptoms lasted < than 24 hours, TIA was diagnosed and started on aspirin and atorvastatin.* CT scan of the brain, echocardiogram, and Carotid Doppler scans were arranged as outpatient. The CT scan of the Brain was done on the 03.11.2004 (Figure 1) showed right basal ganglion haemorrhage, and therefore he was called back for observation over night at the MAU. The patient did not have any focal neurological deficit. *The aspirin was discontinued.* He was commenced on ramipiril 2.5 mg per day in addition to his atenolol for the hypertension. He was discharged home on the same day. Subsequently the transthoracic echocardiogram and Carotid Doppler scans were normal. A review in March and September 2005 revealed no recurrence of his 'TIA' and his hypertension was well controlled. On 06.12.2005 he attended the clinic saying that in October 2005 (one year after the first presentation) he had another "TIA" diagnosed in the community, which presented as left facial drop, and left arm weakness. *On direct questioning, the patient and his wife confirmed that the symptoms lasted less than four hours.* A CT scan of the Brain was requested which was done on 08.02.2006 showing recurrent right basal ganglia haemorrhage (Figure 2). Since then, a repeat CT scan of the Brain on the 4th July 2006 (Figure 3) is reported to show calcification of the right basal ganglia. He has no focal weakness.

DISCUSSION

This case demonstrates that the arbitrary time limit of 24 hours did not help the correct diagnosis and management of this patient. The CT scan of the brain on both occasions was arranged as non-urgent on outpatient basis because the diagnoses made were TIA on both occasions. The first episodes of "TIA" were either an ischaemic, which became haemorrhagic while on aspirin, or it was a spontaneous primary intra-cranial haemorrhage (PICH), which was missed on presentation.

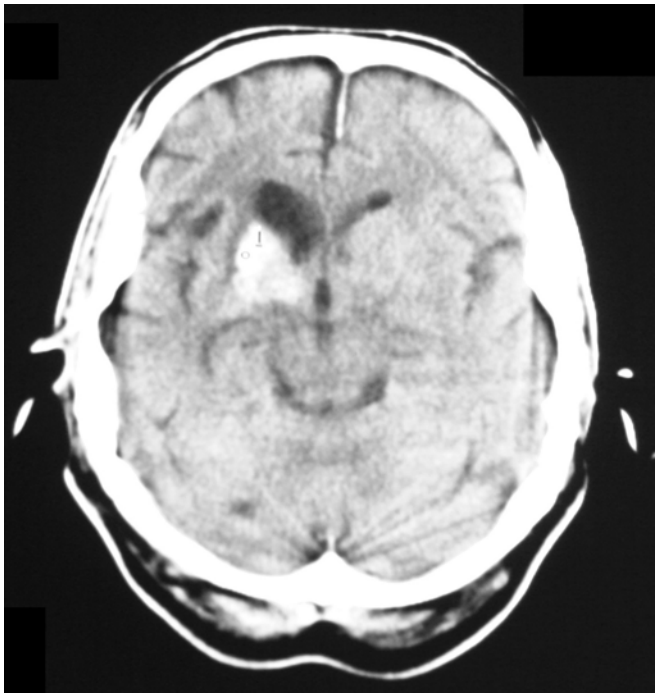


Figure 1. CT scan of the Brain on the 3rd November 2004 about a month after the initial presentation showing acute right basal ganglia bleed

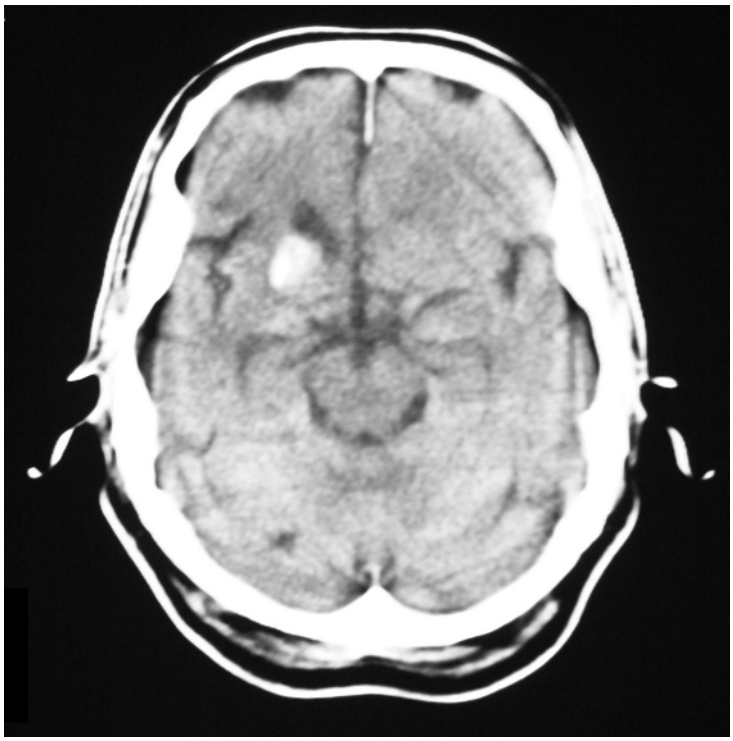


Figure 2. CT scan of the Brain on the 8th February 2006 showing the recurrence of the right basal ganglia bleed while not on antiplatelets

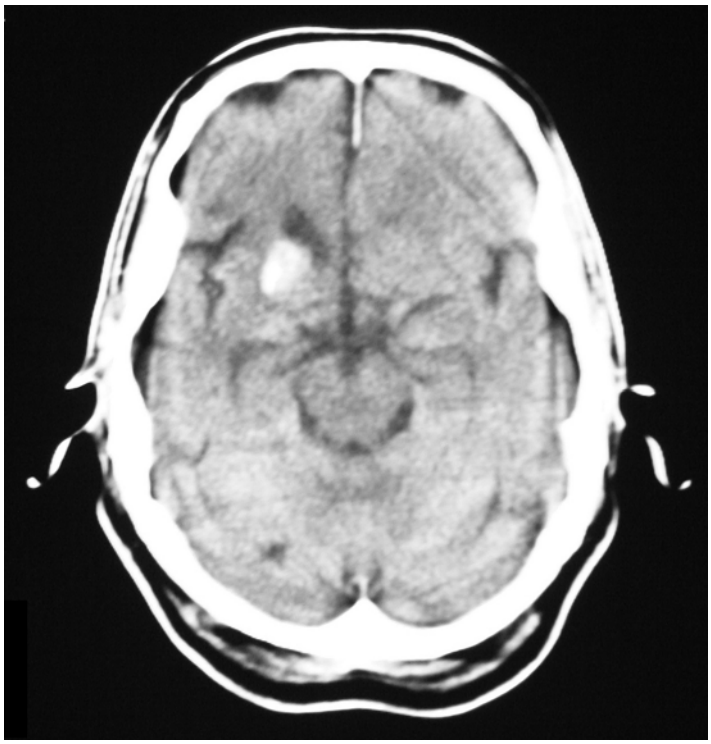


Figure 3. Follow up CT scan of the Brain on the 4th July 2006 showing calcification in the right Basal ganglia

The second episode of the "TIA" which occurred one year later happened while he was not on aspirin or other antiplatelet agents and hence it was a recurrence of the right basal ganglia haemorrhage. On both occasions the prevailing standard criteria for the diagnosis of TIA had been used. This clearly shows that the current definition of TIA did not contribute to an accurate diagnosis and appropriate management of this patient.

It is very important to reach the right diagnosis of TIA for many reasons including implementing the right therapeutic measures and future management plans. A major limitation of the prevalent definition of TIA is that if it was strictly adhered to, no patient with acute ischaemic stroke will be eligible for thrombolysis as the therapeutic window is restricted up to 3 hours.3 Furthermore, the current definition is covering patients who have cerebral infarctions in 15-20% of the patients.4-6 This means that the current definition covers patients who have actually cerebral infarctions rather than cerebral ischaemia. Another limitation of the current definition of TIA is the variety of neurological conditions that might mimic or masquerade as TIA. A sudden onset of focal neurological deficit lasting less than 24 hours had been reported in patients with chronic subdural haematoma who had been prescribed antiplatelets without having prior CT scan of the brain.7 In the latter case report, an elderly man presented with intermittent numbness and weakness of his left upper extremity

typical of symptoms arising from a right sensori-motor cortex TIA. He was treated empirically with antiplatelets for several days before cerebral imaging, which showed chronic subdural haematoma with an acute component. A similar patient was reported to have symptoms suggestive of recurrent TIA, which subsequently shown to be due to chronic subdural haematoma.8 Furthermore, primary intracranial haemorrhage has been reported to cause sudden brief transient neurological deficits of less than 24 hours duration, which means that such cases might be diagnosed on clinical grounds as TIA according to the existing definition.9-11 This makes a strong case early CT scanning in acute stroke and suspected TIA to exclude intracranial haemorrhage.12 In addition, it is widely acknowledged that brain tumours may present as transient neurological deficits such as meningioma.13 In the latter report, a patient was reported who had a sphenoid wing meningioma presenting with transient symptoms mimicking the presentation of a transient ischaemic attack (TIA).

The time-based definition of TIA emerged in the 1950s and 19601,2 long before brain imaging was available. Indeed the 24-hour criterion for the definition of TIA was introduced completely arbitrary and it was based on the assumption that if the syndrome persists for 24 hours or longer, an injury to the brain parenchyma should be detectable by microscopy. i.e. the definition was proposed to enable microscopic visualization of brain injury. Attempts to resist the current definition of TIA were made in 1964 by Acheson and Hutchinson who suggested duration of one hour as the maximal duration for diagnosing TIA,14 but it was the Marshall's proposal in 1964 of the maximum 24-hour duration for diagnosing TIA prevailed.1 This is interesting because about three fourths of the patients in Marshall's data had symptoms less than one hour. Subsequently in 1975, the revision of the NIH classification document, *the 24-hour limit was adopted*.[15]

The abovementioned limitations of the classical definition of TIA have been fully discussed by Albers et al.[16] in their proposal for a new definition on behalf of the TIA Working Group, who had called to abandoning the 24-hour limit to differentiate TIA from acute ischaemic infarction. They suggested that TIA should be defined as a "brief episode of neurological dysfunction by focal brain or retinal ischaemia, with clinical symptoms lasting less than one hour and without evidence of acute infarction". However the latter proposed definition, has not been widely accepted, and being criticised. Ideally TIA is to be used to reflect patients who have ischaemia without infarction and / or occasionally haemorrhage as in this case presented here. In the absence of biochemical markers for diagnosing cerebral infarction or injury from haemorrhage or another pathology, *the only solution is cerebral imaging, which should be done before anti-platelet therapy*. Moreover, the latest guidelines from the Royal College of Physicians (2004) had taken on the classical definition with a recommendation that aspirin should

be started as soon as the diagnosis of TIA is made.[17] However the real problem is in the accurate diagnosis of the TIA from other causes of acute transient focal neurological deficits (acute TFND), which may include PICH as in this case report, subdural haematoma, or brain tumour (Box1) and for the latter the management of the patient requires early cerebral imaging.

TIA should be considered as an acute medical emergency. The lack of resources in the district general hospitals (DGH) is the most obvious reason for the inadequate access to *early CT scanning of the brain in many of the DGHs.* Although this is widely true, there must room to alter *some of this* practice because eventually almost all the patients will have scans *but on the expense of two significant problems*; firstly they are done late, and secondly antiplatelets are usually started before the diagnosis is established. The only way forward is to look again at the definition of transient ischaemic attacks in a way that reflects the underlying pathology, and helps to manage individual patients appropriately. The main two descriptive words of the attack are transient, and ischaemia. The New Shorter Oxford English Dictionary provides many definitions for the word transient including passing away with time; not durable or permanent; temporary, or staying for a short time.[18] *However, none of those meanings is bound by a time limit of 24 hours.* The diagnosis of cerebral ischaemia without infarction should be equivalent to that of unstable angina of the acute coronary syndromes (ACS) where patients have manifestations of myocardial ischemia i.e. pain and / or ECG abnormalities but without biochemical and/ or electrocardiographic (ECG) evidence of myocardial infarction. The differentiation of TIA from cerebral infarction and excluding TIA mimics deserves the same importance if not more. If these concepts become generally acceptable, TIA should only be diagnosed in patients with acute transient focal neurological deficits (acute TFND) with cerebral imaging. Until prospective evidence becomes available, one option to minimize errors in the management of patients with TIA would be to lower the arbitrary duration of TIA to those suggested by Albers and colleagues.[16] to one hour or less. *If the symptoms last more than one hour, antiplatelets should not be initiated without cerebral imaging, which should be done as soon as possible.* Furthermore, the initiation of aspirin will hinder thrombolytic therapy where this modality of treatment is available. The fact that a patient presenting with a TIA is at high risk of subsequent adverse events indicates urgent need for more aggressive approaches to this clinical condition. It is reported that the 90-day risk of stroke after a TIA is greater than 10%, with the highest risk occurring in the first 2 days.[19]

To facilitate the appropriate management of patients with TIA, the use of a clinical syndrome of acute transient focal neurological deficits (acute TFND) will covers TIA which will necessitate cerebral imaging to exclude TIA mimics such as shown in this case. In addition, it seems that prescribing antiplatelets for acute

TFND lasting few hours but less than the 24 hours used for the definition of TIA, may put patients into risk. An important limitation of these statements is that they are based on a case report but in future when imaging resources become more available, it will direct clinicians for more accurate management.

Box 1: Some common causes of acute Transient focal Neurological Deficits (acute TFND)*

- TIA
- Primary intra-cranial haemorrhage (PICH)
- Sub-dural haematoma
- Brain tumours

** Obviously there are many other causes that could be incorporated into this list*

ACKNOWLEDGMENTS

I express my thanks to the Basildon Medical Illustrations Department for the work on the slides and to Dr Udayraj Umasankar (SpR at University Hospital Lewisham Hospital / London) for reading the article. My greatest appreciation is to the patient and his wife to allow me to use this case history and the scans for the teaching and publication

REFERENCES

1. Marshall J. The natural history of transient ischaemic cerebro-vascular attacks. QJM 1964;33:309–24.
2. Fisher CM. Intermittent cerebral ischemia. In: Wright IS, Millikan CH, editors. Cerebral Vascular Disease. New York: Grune & Stratton; 1958. p. 81–97.
3. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue Plasminogen activator for acute ischemic stroke. N Engl Med J 1995;333: 1581-7.
4. Davalos A, Matias-Guiu J, Torrent O, Vilaseca J, Codina A. Computed tomography in reversible ischemic attacks: clinical and prognostic correlations in a prospective study. J Neurol 1988;235:155-8.
5. Dennis M, Bamford J, Sandercock P, Molyneux A, Warlow A. Computed tomography in patients with transient ischaemic attacks: when is a transient ischaemic attack is not a transient ischaemic attack but a stroke? J Neurolo 1990;237:257-61.
6. Evans GW, Howard G, Murros KE, Rose LA, Toole JF. Cerebral infarction verified by cranial computed tomography and prognosis for survival following transient ischaemic attack. Stroke 1991;22:431-6.
7. Wilkinson CC, Multani J, Bailes JE. Chronic Subdural haematoma presenting with symptoms of transient ischaemic attack (TIA): a case report. W V Med J 2001;94: 194-6.
8. Friedrich I, Mader R, Kaplan CB, Schonfeld S. Chronic subdural haematoma simulating transient ischaemic attack. Harefuah 1989;116: 413-4.
9. Gunatilake SB. Rapid resolution of symptoms and signs of intracranial haemorrhage: case report. BMJ 1998;316:1495-96.
10. Ivo L. CT scanning can differentiate

between ischaemic attack and haemorrhage. BMJ 1999;319:1197.
11. Joseph F, Owens V, Weir P, Little V, Barrett JA. Intracranial haemorrhage masquerading as a transient ischemic attack (TIA) in a patient with atrial fibrillation- case report. CME Geriatric Medicine 2003;5(3): 124-6.
12. MacWalter RS, Dutta D, Fraser HW, Nimmo MJ. The importance of identifying intracranial haemorrhage as a cause of transient focal neurological symptoms. Scott Med J 2000;45:117-8.
13. Oluigbo CO, Choudhari KA, Flynn P, McConnell RS. Meningioma presenting as transient ischemic attacks. British journal of Neurosurgery 2004;18:635-7.
14. Acheson J, Hutchinson EC. Observations on the natural history of transient cerebral ischaemia. Lancet 1964;2:871-4.
15. A classification and outline of cerebrovascular diseases. Stroke 1975; 6:564-616.
16. Albers GW, Caplan LR, Easton JD, Fayad PB, Mohr JP, Saver JL, Sherman DG . Transient ischaemic attack proposal for a new definition. N Engl J Med 2002; 347:1713–6.
17. The Intercollegiate Working Party for Stroke, Royal College of Physicians. National Guidelines for Stroke. 2nd ed. London: Royal College of Physicians; 2004.
18. The New Shorter Oxford English Dictionary. Volume 2. Oxford University Press., pp 3369.
19. Johnston SC, Gress DR, Browner WS, Sidney S. Short-term prognosis after emergency department diagnosis of TIA. JAMA 2000;284(22):2901-6.

پوختە

خوینرشتنا بەردەوام ژ (basal ganglia): TIA یان TFND ؟

ئەڤ حالەتە دیاركەت كو داناڤا دەمێ 24 سەعەتا هاریكاری نەدكر د تشخیسكرنا و چارەسەریا دروست یا نەخوشا و ئەڤ حالەتە پشتەڤانینا كارێ دهێتە كرن بو گوهورینا پێناسا و چارەسەریا TIA بەرەف TFND كو بتنێ دهێتە دەستنیشانكرن ب رێكا وێنێن تیشكێ.

الخلاصة

النزف المتكرر من (basal ganglia): TIA أو TFND ؟

هذه الحالة تبين بأن تحديد فترة زمنية ب 24 ساعة لا يساعد على تشخيص و معالجة المرضى و يدعم هذه الحالة المحاولات الجارية وضع تعريف و معالجة المرضى المصابين ب TIA نحو متلازمة TFND و التي يمكن تحديدها فقط عن طريق التصوير الشعاعي.

CONGENITAL PHLEBECTASIA OF THE INTERNAL JUGULAR VEIN (CASE REPORT)

ABDULLA S. ABDULLA, MD, Board in general surgey*
MOHMMED H. ALDABAGH, MBChB, FIBMS**

Submitted 4 October 2007; accepted 21 April 2008

ABSTRACT

Congenital phlebectasia of the jugular vein is a rare entity and there are few reports from the world about this subject .Here we present a child with congenital phlebectasia of the right internal jugular vein, which appeared as a compressible mass in the neck during straining and coughing. It is important to differentiate it from other neck masses .Colored Doppler is a simple non invasive procedure for diagnosis. The treatment is conservative for asymptomatic patients.

DMJ 2008;2(1): 155-160.

Key words: Phlebectasia, Jugular vein

A mass that appears in the neck upon straining (Valsalva maneuver), coughing, sneezing or crying may be the result of a laryngocele, jugular phlebectasia or superior mediastinal tumor. Jugular phlebectasia (also known as venous congenital cyst, venous aneurysm, venous ectasia or essential venous dilatation) refers to an isolated abnormal fusiform or saccular dilatation of the internal jugular vein and it usually present with a swelling in the right side of the neck. Most patients are children, boys being more twice as often affected as girls. Phlebectasia may affect any vein in the neck, especially in this sequence: internal jugular, external jugular, anterior jugular and the superficial communicants. Jugular phlebectasia is an asymptomatic benign condition whose etiology is unknown.[1] Histological examination has failed to clarify the etiology of the venous ectasia. Histologically, diffuse fibrosis and disrupted architecture of the elastic tissue suggest the results of a mechanical effect .[3] Absence of a wide mediastinum or air in the mass on simple chest films eliminates a mediastinal tumor or laryngocele respectively. Non-invasive diagnosis of jugular phlebectasia can be achieved using ultrasonography combined with Doppler flow imaging and spiral computerized tomography scan with contrast. No treatment is indicated for this benign self-limiting condition, except for the few patients who complain of symptoms (feeling of constriction, choking, bluish discoloration, thrombosis, and discomfort during physical activity or tongue pain) and require surgical removal of the

* *Consultant general surgeon, Azadi teaching Hospital, Dohuk and Director General, Directorate of Health, Dohuk, Kurdistan region, Iraq.*
** *Consultant pediatric surgeon, Department of surgery, Dohuk College of Medicine, Kurdistan region, Iraq*
Correspondence author: *Mohmmed H. Aldabagh, Department of surgery, Dohuk College of Medicine, Kurdistan region, Iraq. Email: m_h_ald@yahoo.com*

affected vein. Surgical removal for cosmetic purposes alone consists of a unilateral excision of the internal or external jugular vein, a procedure that produces no gross side-effects .[1]

CASE REPORT

We present a 6 years old boy presented with a soft compressible mass in the neck. The mass appears only on crying or coughing and totally disappear at rest (Figure 1). The child is otherwise healthy and he has no other complain. But the swelling made the parents worried. On physical examination the child looks healthy. A mass appear on his right side of the neck upon straining (Valsalva maneuver), the mass emerges from bellow the right sternocleidomastoid muscle extends up to the right anterior triangle of the neck as shown in (Figure 2). The mass can also made prominent by pressing on the lower neck making the vein engorged (Figure 3). The skin is normal in color and the mass is soft compressible not tender, no temperature variations, no palpable nearby lymph nodes and no bruit. Examination of other systems was normal. Chest X-ray was normal with no mediastinal widening. The diagnosis was settled by colored Doppler study which revealed internal jugular vein dilatation on straining. No other investigations required.

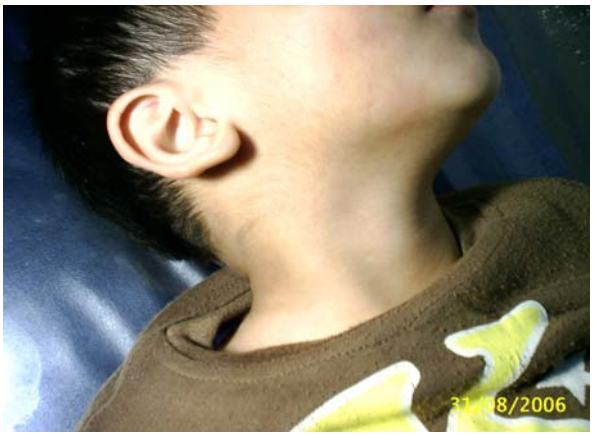


Figure 1. Lesion not visible at rest

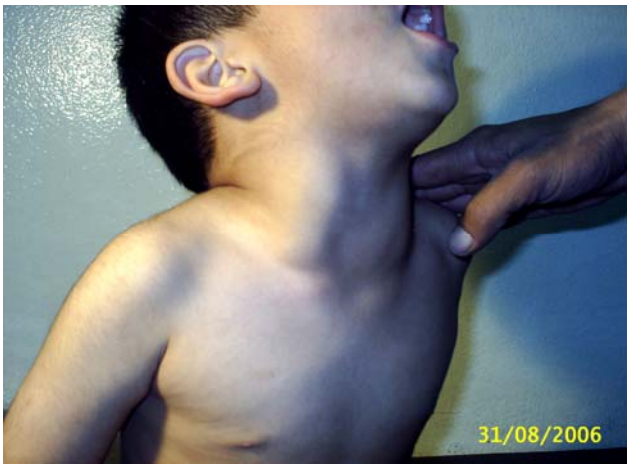


Figure 2. Lesion visible at straining

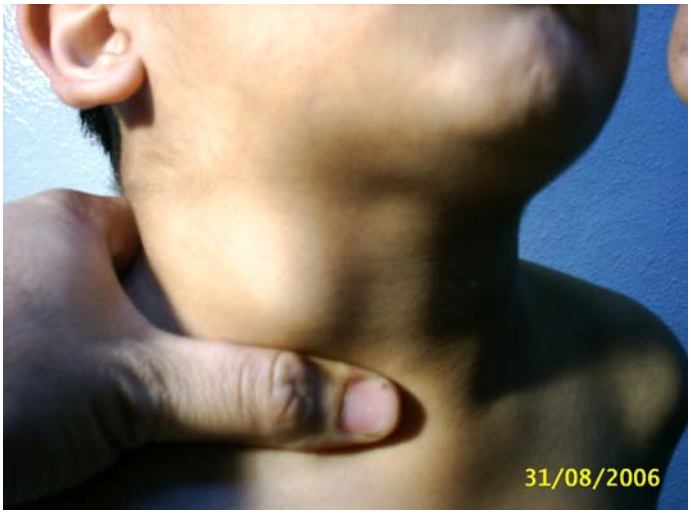


Figure 3. Lesion made prominent by pressure on the lower neck

DISCUSSION

Venous ectasia in the neck is a rare entity, especially in children. The internal and external jugular veins are generally affected.[2,3] however, there are reports of anterior jugular vein ectasia.[4] In our case The internal jugular vein was affected which is more common.[1] Several factors have been suggested in the etiology of internal jugular phlebectasia including congenital muscle defects in the vein wall and increased scalenus muscle tone due to compression of an unusually laterally placed vein .[4]

Regarding the sex incidence it is reported that males are more commonly affected than females .[1] Other authors reported equal sex incidence. Our patient was a 6 years old boy. Clinically the mass appears in the neck as a fusiform, soft, cystic mass manifested by straining, coughing, crying, or sneezing .[3,5]

Neck lesions in children are not uncommon and accurate diagnosis of the mass is important to differentiate it from other types of masses, as there are reports of patients underwent risky surgery based on wrong diagnosis. [4]

Three types of swelling distend on Valsalva and disappear completely at rest: (a) tumors or cysts of the superior mediastinum, (b) external laryngeal diverticulum and laryngocele, and (c) venous enlargement of the superior vena caval system. [3] Other differential diagnosis for the swelling could include a branchial cyst, cystic hygroma,and cavernous haemangioma.[6,7]

To exclude mediastinal mass we took chest X-ray which revealed no widening and also showed no air at the region of the mass that exclude laryngeocele .Colored Doppler was used to confirm the diagnosis which revealed internal jugular vein dilatation upon Valsalva maneuver. Colored-doppler ultrasonography is a noninvasive accurate imaging technique to distinguish the jugular venous enlargement, and it defines the extent of the lesion and its relationship with surrounding structures in the neck.[3] Two

comparable examination on Doppler should be made for differential diagnosis; first, when the child is on Valsalva maneuver, and second at rest, because it is reported that the diameter of the affected vein at rest is not statistically different from the normal one.[6] Computerized tomography, or percutaneous venography may show dilatation, but they are considered unnecessary. [3]

Surgery is indicated for cosmetic reasons and in symptomatic patients.[1] The swelling is not known to progress rapidly and there have been no instances of spontaneous rupture of the swelling or other serious complications.[6,8,9] Balik et al reported a case who had jugular phlebectasia with thrombosis, and suggested surgical removal of the involved segment without delay because of thrombosis and some other unknown potential complications. [10] However we did not find other reports supporting that.

Surgical procedures include ligation of the affected vein which is the standard procedure and usually has no unwanted sequels. Some authors believe that ligation of the jugular vein may produce effects of venous congestion in a small subset of patients resulting in cerebral oedema. Jugular vein ligation is too radical procedure for such a benign condition, and this definitely can not be applied in cases with bilateral affliction.[11]

Our patient was asymptomatic apart from the swelling and the family had no major concern about the sight, so there were no indications for surgery. We reassured the family of the good prognosis and no further measures were taken.

We concluded that internal jugular phlebectasia is benign condition need no intervention unless symptomatic, but it is important to differentiate it from other neck masses. Colored Doppler is simple reliable non invasive diagnostic procedure.

REFERENCES

1. Pediatric surgery update .Jugular Phlebectasia. [online] Vol 17 (2); 2001. Accessed 10/6/2007. Available from http://home.coqui.net/titolugo/PSU17201.pdf.
2. Rajendran VR,Vasu CK, Anjay MA, Anoop P . Unilateral internal jugular phlebectasia. Indian J Pediatr 2004;71(8):751-3.
3. Mehmet M, Harun G, Mustafa A,Bahar M . Jugular Phlebectasia in Children. Journal of Turgut Özal Medical Center 1997; 4(1):107-8.
4. Chatrath P,Mitchell TM, Jani P. Essential venous dilatation of the anterior jugular vein presenting as a neck lump. CME bulletin Otorhinolaryngology, Head & Neck Surgery 2002;6(2):70 – 1.
5. Indudharan R,Quah BS,Shuaib IL. Internal jugular phlebectasia. An unusual cause of neck swelling.Ann Trop Paediatr1999;19(1):105-8.
6. LaMonte SJ, Walker EA, Moran WB. Internal jugular phlebectasia. clinicoroengentographic diagnosis. Arch Otolaryngol 1976;102(11):706 – 8.
7. Ordon DH, Rose JS, Kottmeier P. Jugular vein ectasia in children. A

report of 3 cases and review of the literature. Radiology 1976;118(1): 47 - 9.

8. Uzun C, Taskinalp O, Koten M, Adali MK, Karasalihoglu AR, Pekindil G. Phlebectasia of left anterior jugular vein. J Laryngol Otol 1999;113 (9):858 – 60.
9. Gordon DH, Rose JS, Kottmeier P, et al. Jugular vein ectasia in children. A report of 3 cases and review of the literature. Radiology 1976; 18 (1):147 – 9.
10. Balik E, Erdener A, Taneli C, Mevsim A, Sayan A, Yuce G. Jugular phlebectasia in children. Eur J Pediatr Surg 1993;3:46-7.
11. Walsh RM, Murty GE, Bradley PJ. Bilateral internal jugular phlebectasia. J Laryngol Otol 1992; 106 (8):753 – 4.

پوخته

فرەهبوونا خوین ڤەگێرا گەردەنێ د زکماك دا (راپورتا حالەتەکێ)

حالەتێ فرەهبوونا خوین ڤەـێرا ـەردەنێ د زکماك دا ئێک ژ حالەتێن دکێمن، ل جیهانێ ـەلەك کێم حالـەتێن نێزیکی وی حاتینە تومارکرن و بەلافکرن، ئەڤ حالەتە زارویەکە کو حالەتێ فرەهبوونا خوین ڤەـێرا ـەردەنا وی لـدەڤ هـەبوو و ب شـێوێ وەرومەکا فەشارـرتی و ل دەمێ کوخکێ و بیهنیژینـێ دا دیـاربوو. ـەلـەك ـرنکـە ئـەڤ حالەتـە ژ نەخوشـیێن دیترێـن کـو ل ـەردەنێ پەیدابن بهێنە جوداکرن. ئامیرێ دوبلەرا رەنگاو رەنگ دهێـە ب کارئینـان ژ بـو دەستنیـشانکرنا ڤـێ نەخوشـیێ. د پرانیا دەمان دا چێ چارەسەری پیدڤی نینە و ب تایبەت ل دەمێ چێ نیشان دـەل دا نەبن.

الخلاصة

توسع الوريد الوداجي الغائر الخلقي (اشهار حالة)

تعتبر حالة توسع الوريد الوداجي الخلقي من الحالات النادرة. هناك تقارير منشورة حول العالم حول بعض الحالات الموثقة المشابهة. في هذا التقرير نقدم طفلا مصابا بحالة توسع الوريد الوداجي الغائر الايمن الذي ظهر على شكل كتلة قابلة للانضغاط في العنق عند السعال او الحزق. من المهم تفريق هذة الحالة عن غيرها من الاورام التي تصيب العنق. يعتبر الفحص بجهاز الدوبلر الملون من الاستقصاءات غير الباضعة المهمه و البسيطة لتشخيص المرض. العلاج في اغلب الاحيان تحفظي خصوصا للحالات غير المصاحبة لاعراض.

SNAKE BITE- A RARE AND UNUSUAL CAUSE OF MYOSITIS OSSIFICANS

ISMAIL M. ALI, MBChB, DMRD*

Submitted 20 November 2007; accepted 21 April 2008

ABSTRACT

Calcification in the muscles designated in the term of (myositis ossificans) constitutes special group. This case report represent a rare case of snake bite at the leg of old female patient which is complicated later on with discharging sinus at the site of the bite with excursion of whitish pieces resembling sequestrated bone piece. The X-ray film showed extensive subcutaneous calcification with intact bones. Calcification in the soft tissue secondary to snake bite should be kept in mind in studying any soft tissue calcification due to any cause.

DMJ 2008;2(1): 161-165.

Key words: Snake bite, Myositis ossificans

Calcification in the muscles designated in the term of (Myositis ossificans) constitute special group. In the localized form of this condition, the ossification is usually end result of of the trauma; several conditions other than trauma may cause calcification in the muscles,[1] snake bite complicated with myositis ossificans is not reported to the best of our knowledge as the cause of that condition.I'm reporting in the case report the radiological appearance as complication of the snake bite.

CASE REPORT

73 years old female patient was referred to the x-ray clinic for x-ray of her leg for exclusion of chronic osteomylitis. Clinically there was a discharging sinus in the anterior aspect of the distal part of her right leg; this was present for the last 6 years. With continuous discharging and excursion of whitish pieces of variable sizes having the appearance similar to that of the sequestrated bone pieces she has no fever.the affected area of the leg was hard on palpation with tenderness with mild swelling.

Radiological appearance:

Extensive subcutaneous calcific plaques are noted in figure 1 both tibia and fibula were intact and no bony changes of osteomylitis noted. Marked thinning of subcutaneous soft tissue noted mainly at the site of the sinus with small piece of bony like structure seen through the sinus.
At the start, the diagnosis was straight and the orthopaedition was informed of the changes and that no changes of the osteomylitis are present. We had the experience of having similar changes of radiological picture were noted before years at the leg of a Turkish patient for

*Assistant lecturer, Department of Radiology, Dohuk College of Medicine, Dohuk, Iraq.
E-mail: Ismail_19452002@ yahoo.com

snake bite affection as well.

So our patient was asked in detailed history for any past history of snake bite. The patient then remembered that 23 years ago she had the snake bite at the same area at a village in Kurdistan and the sinus was discharging on and off.

The cause was labeled so and symptomatic treatment with antibiotic and dressing was given.

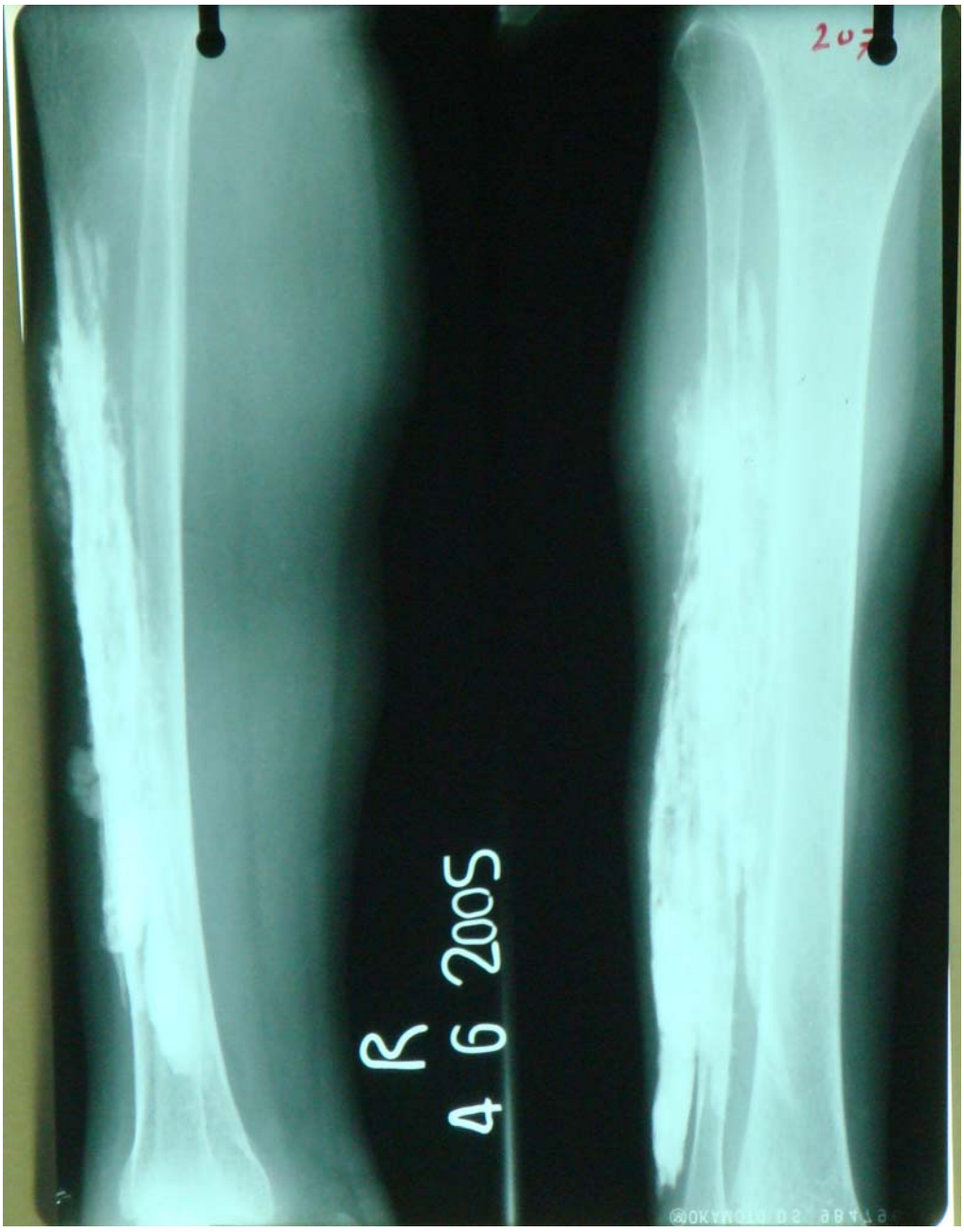


Figure 1. Extensive soft tissue calcifications with intact tibia and fibula

DISCUSSION

The deposition of calcium in the soft tissue appears in various conditions, most frequently in the vessels of elderly patients affected by atherosclerosis.[1,2,3]

Hilbash[1] classify calcification within the soft tissue into six groups:

1-dystrophic calcification
2-metastatic calcification
3-calcinosis
4-myositis ossificans
5-calcification in the vessels
6-calculi

The classification alone include several conditions in which calcification is found in the vicinity of joint such as calcaneous bursitis,of shoulder joint,gout and other rare forms of calcinosis interstitalis

According to the Shinz[1] calcinosis interstitalis is classified into three groups:

1-calicium metastasis
2- Calcinosis interstitalis uinversalis
3-strictly local calcific deposit

Calcification in the muscle, designated as ((myositis ossificans)) contribute special group.

In the localizedform of this condition, the calcification is the result of the trauma; ususally these deposits first become recognizable on a radiograph as pale shadows of calcium which easily escape the notice of inexperienced observer.

The generalized form of myositis ossificans may be demonstrated even in the developmental age.

Large ossifications around joint may occur in individuals taking large quantities of milk.[1,2] Calcification may follow hemorrhage in the soft tissues.[2]

Snake bite is believed to be associated with local soft tissue necrosis; victims may suffer extensive muscle damage.

Muscle necrosis is produced by the snake venom causing vaculation, lysis, and cell necrosis of the muscle affected.[4-7]

It has been found that the loss of the muscle mass consequent to poor muscle regeneration is a common sequel following snake bite,[6,8] it appears that muscle necrosis after attempts of regenerations of muscle fibers end with calcification of both soft tissues and muscle affected.

CONCLUSION

Reviewing the literature extensively showed that snake bite is not included as a cause of such calcification and has not been reported to the best of my knowledge as a cause of soft tissues calcification as local form of myositis ossificans and this make reporting such appearance very necessary and adding a cause to the list of calcification to be considered always.

REFERENCES

1. Kohler, Zinner EZ. Boderlines of the normal and pathology in skeletal roentgenology. 11th ed. New york: Grune and Stratton; 1968.
2. Sutton D. Text book of radiology and imaging. 7th ed. Vol2. China: Churchill Livingstone; 2003.
3. Goldman L, Bennett J. Cecil textbook of medicine. 21st ed Philadelphia: WB

Saunders Company; 2000.

4. Neto HS, Marques MJ. Microvessel damage by B. Jararacussu snake venom: pathogenesis and influence on muscle regeneration. Toxicon 2005;46(7):814-9. Epub 2005 sep 29.
5. Mebs D, Ownby CL. Myotoxic component of snake venoms: their biochemical and histological activities. Pharmacol Ther 1990;48(2)-36.
6. Harris JB, Cullen MJ. Muscle necrosis caused by snake venoms and toxins. Electron Microsc Rev1990;3(2):183-211.
7. Evans J, Ownby CL. Neutralization of odema, hemorrhage and myonecrosis induced by North American crotalid venoms in stimulated first-aid treatments. Toxicon 1999; 37(4):633-50.
8. Gutierrez JM. Understanding snake venoms: 50 years of research in Latin America. Rev Biol Trop 2002;50(2):377-94.

پوخته

گرتنا کلسی ل ماسولک و شانا ژ ئەگەرێ پێڤەدانا ماریٚ ژەهراوی

ـرتنا کلسی ل ماسولک و شانێن دەست و پیا دبیت چێ دبیت وەك درژانکەکێ پێڤەدانا ماری ژەهراوی ژ بەر مرن و حەلاندنا خانە و شانێن ماسولکا و نیشانێن دبیت دیاربن پشتی ماوەیەکێ درێژ ژ پێڤەدانا ماری. ئەڤ حالەتە هاتە راپورت کرن پشتی کو نەخوشەکا 73 سالی سازندە کرن ژ وەرمبوون و ئێشانێ ل پیێ خو یێ راستێ بو ماوەکێ درێژ و هەبوونا برینەکێ ل جهێ پیشیا پی و دەرکرنا چەند پارچێن کلسی ژ برینێ کو نیشانێن وێ نێزیک بوون بو کولبوونا هەستی یا دوم درێژ پشتی تووشی پێڤەدانا ماریٚ ژەهراوی بووی پێش 23 سالا ل کوردستانێ. ئەڤ درژانکە دڤێت نەهێت ژبیرکرن هەر ل نەخوشەکی تەکلوساتێن شانا هەبوون دەمێ کرنا پشکنینا تیشکێ.

الخلا صة

تكلسات عضلية و نسيجية بسبب لدغ أفعى سامة

إن التكلس العضلي والنسيجي في الأطراف قد يحصل كإحدى المضاعفات لسم الثعبان لما يسببه مـن مـوت وتحلـل الخلايا والنسيج العضلي وقد تظهر أعراضه وعلاماته بعد مدة طويلة من حادث اللدغ. تبين هذه الحالة النادرة لمريضة عمرها 73 سنة تعاني من وجود تورم بسيط والآم في ساقها الأيمن لمدة طويلة مع وجود جرح في الجهـة الأماميـة للساق وخروج قطع كلسية بيضاء من الجرح وعلامات يشبه التهاب العظم المزمن نتيجة لتعرضها للدغ أفعى سامة قبل ما يقرب من 23 سنة في إحدى قرى كردستان. إن لدغ الحية السامة تسبب مضاعفات جسدية متنوعة وان إحدى هـذه المضاعفات هي حصول تكلسات عضلية ونسيجية بعد مدة طويلة من اللدغ، وأن هذا النوع من التكلسات يجب وضعها في وضع عين الاعتبار عند التشخيص التفريقي لحالات التكلسات النسيجية الحاصلة لأسـباب أخـرى عنـد إجـراء الفحوصات الشعاعية.