Volume 11 (4) 2013 ISSN 1681-6579

IRAQI JOURNAL OF MEDICAL SCIENCES

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Iraqi Journal of Medical Sciences

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

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

Manuscripts:

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

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Examples

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

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

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

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

- **Tables**: Each table should be typed on a separate page double-spaced, including all headings, number all tables with English numerals and include a short title. Vertical lines between columns are to be avoided.
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CONTENTS

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Published by Al-Nahrain College of Medicine
ISSN 1681-6579
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Guidelines, Calculators and Clinical Judgement

Khalid Abdulla *FRCPE, FRCP*

A guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk with a risk calculator was published by the American College of Cardiology and the American Heart Association on 12th November 2013 [(1)]. It generated a controversy in the medical [(2)] and lay [(3)] press and was thought by many to overestimate cardiovascular risk and result, if implemented, in massive over prescription of statin drugs.

The event raises questions on **whether guidelines are flawless and whether doctors are bound to abide by them!**

Throughout the history of medicine, great physicians have written instructions to guide their colleagues in their practice. Those *guidelines* reflected the personal experience and judgment of their writers and the knowledge they inherited from their predecessors. With the advent of clinical **randomized controlled trials (RCTs)** in the second half of the previous century, guidelines took a different shape: more accurate, organized and **evidence based**. They came to be written by committees of experts who study the clinical trials done in a particular subject and write instructions based on the results of these trials. The instructions are intended to help doctors in assessing the condition of their patients, estimating their risk and advising about management. They are frequently accompanied by tables, algorithms, calculators etc. With the widespread use of computers nowadays, these guidelines are frequently aided by software applications, which facilitate their use and make them available on computers, tablets and mobile phones which can be carried in the pocket of the user or accessed on the internet anywhere.

Back to the questions:

Are guidelines flawless? Are doctors bound to abide by them?

They are to start with *guidelines* and not *laws*. They are not legally binding. They are meant to help the doctor make informed decisions, not to provide ready made decisions.

Guidelines are based on the results of randomized controlled trials and *on the assumption that your patient is similar to the patients studied or to a group within them to a sufficient degree that justifies treating him in a similar manner and expecting similar results.* But, every individual patient is in fact unique and cannot be precisely represented by patients included in the trials.

Trials are done in different countries (*mostly developed countries, an important point to remember for a doctor practising in a developing country*). They lump together patients of different ethnicities, nationalities, environment, cultures, education, economic level, etc. The studies may have been done ten or twenty years ago and things may have changed since. The results of trials are based on statistical analysis of large numbers of patients who are divided into groups by artificial boundaries according to various parameters (e.g. age, number of cigarettes smoked, etc). The groups are represented by their averages like the mean or median. Your patient is not necessarily represented well by the average used. If he is 60

year old, you may have an instruction, which treat him similar to a 69 year old because he is in the same age group. The risk of a certain condition or the outcome of a certain intervention depends on many factors in each patient. The clinical study cannot take into account all factors that may affect the result. It is almost certain that there are factors in your patient which have not been included in the clinical trials studied. There is not enough trials and guidelines to have a specific guideline for each ethnicity, nationality, culture, level of education, intelligence, economic state, etc.
The following may serve as explanatory examples:

- **CHADS2 score** [4]: It is a guideline on anticoagulation to prevent stroke in patients with nonvalvular atrial fibrillation according to the presence of other risk factors (congestive heart failure, hypertension, age 75 y or above, diabetes and previous stroke or transient ischemic attack). It treats all patients with nonvalvular atrial fibrillation, permanent and paroxysmal, in the same way based on evidence from clinical trials showing similar risk of stroke in permanent and paroxysmal atrial fibrillation [5,6]. But *are patients with paroxysmal atrial fibrillation a homogeneous group?* Is it logical to think that patients who experience one attack every several months, which lasts few minutes carry the same risk of stroke as those who have daily or every few days attacks that last hours [7]? Do we have to treat our patients with paroxysmal atrial fibrillation in the same way regardless of the frequency or the duration of their attacks?
- **Osteoporosis fracture risk calculators and treatment guidelines:** The calculator estimates the 10 year risk of developing a fracture depending on the result of bone mineral density assessment and some other risk factors. There are more than one calculator developed in various places. The WHO one (FRAX) [8] is probably the most widely used. Another calculator (QFracture [9,10]), developed in the United Kingdom, assesses fracture risk regardless of bone mineral density relying on a larger number of other risk factors that are more readily available. Its developers justified this by evidences that most fragility fractures occur in women with normal bone mineral density [11] and that risk prediction algorithms that do not include bone mineral density are almost as good as those that do [12]. However no calculator can be perfect enough to consider all possible factors that affect the probability of developing fragility fractures like balance and coordination, presence of other diseases, drugs, alcohol consumption, smoking, body weight, type of work, home atmosphere, intelligence and education, using a car or public transport and so on. No calculator can take all these into account but you should in your particular patient, especially if you are contemplating long term treatment with potentially hazardous drugs like bisphosphanates.

Guidelines are no doubt very useful. They put you in a better position by giving you an idea on the risks of your patient and the possible benefit of various interventions based on the outcome of randomized controlled trials on thousands of patients. However, it is you who should decide whether your patient could reasonably be considered similar to the average patient included in such trials. Certain characteristics in your patient that may affect his risk or his management and are not included in the guideline or the calculator should be taken into account to decide whether to apply, modify or abandon an instruction, especially if your patient falls near a border between various groups. It is your **clinical judgement** that makes the final decision. The committee who wrote the guideline on the treatment of blood cholesterol that I mentioned in the opening of this article has in fact defended its position by pointing out (among other things) that the guideline included a statement that it *should not be implemented blindly and that doctors should apply the art of the practice in dealing with individual patients*.

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Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
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Effects of Tranexamic Acid Addition on Elasticity and Tension of the Fibrin Glue

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Abstract

Background Fibrin glue is a natural, biocompatible and biodegradable topical tissue adhesive that initiates and duplicates the final stages of coagulation cascade. To prevent early fibrinolysis, antifibrinolytic agent may be added to the components of the glue. Tranexamic acid is a synthetic antifibrinolytic lysine analogue that competitively inhibits the activation of plasminogen to plasmin, hence, delays the fibrinolysis activation.

Objective To synthesize of the fibrin glue with and without tranexamic acid addition and explore the biomechanical behavior of both formulae with regard to stretching (elasticity) and tension.

Methods Using thrombin and cryoprecipitate (as a source of fibrinogen) for synthesis of the "ordinary fibrin glue". In another preparation; Tranexamic acid was added to both components (thrombin and cryoprecipitate) for synthesis of the "tranexamic acid added fibrin glue". Then, by using displacement and force transducers we measure elasticity and tension of the synthesized fibrin glue (both ordinary and tranexamic acid added fibrin glue) at different durations.

Results Tranexamic acid addition to the fibrin glue causes significantly higher elasticity results at 1 hour, 1 week durations. Significant lower tension results are witnessed at 1 hour duration, while at 1 week duration, comparison of the tension results of both ordinary and tranexamic acid added fibrin glue show no significant difference.

Conclusion Tranexamic acid addition led to change in biological behavior of the glue presents as increase in its elasticity and decreased tension. This change should be taken into consideration when the applicator needs to use this formula in the management of different areas of human body.

Keywords Fibrin glue, tranexamic acid, elasticity, tension.

Introduction

Fibrin glue is a natural, biocompatible and biodegradable topical tissue adhesive which initiates and duplicates the final stages of coagulation cascade [1-3]. This glue, besides many indications, is helpful to prevent and stop development of some complications that occur when using surgical sutures, both with the traditional threads and with the modern mechanical staplers. In the “classic” method for wound repair, there are many complications; such as major inflammation, fistulae, tissue ischemia, extensive fibrosis and hematomas, which may cause impairment of tissue healing. All these possible complications may be minimized by using fibrin glue [4-7]. Hence, uses of human fibrin glue have become quite common in different types of surgeries [8]. The efficacy and safety of fibrin glue was evaluated in conjunctival autograft fixation in primary pterygium [9-11], also, fibrin glue is frequently used in neurosurgery for dural

sealing, hemostasis, cranial nerve coating, and wrapping of non-clippable cerebral aneurysms [12].

Fibrin glue is polymerized mainly from two main components; first one -typically- contains concentrated human fibrinogen; factor XIII, and the plasma proteins. Second component usually consists of bovine thrombin, calcium chloride, and anti- fibrinolytic agent [2,4]. The two components are mixed together at the moment of application either sequentially or simultaneously [13-16], a mechanism which can be performed by using two syringes with tips forming either a common port or two ways for perfect application. When injected, the two components meet at the point of delivery, both extrinsic and intrinsic mechanisms of blood coagulation are bypassed, but the physiological final stages of coagulation cascade is faithfully replicated [17,18].

After several days, the final fibrin glue is subject to fibrinolysis by both endogenous and exogenous plasmin. Antifibrinolytics such as aprotinin, tranexamic acid, and aminocaproic acid can be added to the mixture to reduce the rate of fibrinolysis and creation of fibrin degradation products [5,16,19,20].

Tranexamic acid [4-(aminomethyl) cyclohexane carboxylic acid is a synthetic lysine analog that competitively inhibits the activation of plasminogen to plasmin - the major fibrinolytic protease- via reversible binding to the lysine binding site on plasminogen. As such, tranexamic acid shows strong antifibrinolytic activity *in vitro* and *in vivo* by preventing the interaction of tissue plasminogen activator, plasminogen and plasmin with lysine residues on the surface of fibrin [12,21-23].

The objective the study is to synthesize the fibrin glue (with and without tranexamic acid addition) and to explore the effect of tranexamic acid addition on the fibrin glue elasticity and tension.

Methods

This work was performed at the laboratories of Department of Physiology, College of Medicine, Al-Nahrain University, during the period from November 2011 to May 2012. The materials used for synthesis of the fibrin glue are:

1. Thrombin: It is related to BIOLABO CO., these thrombin vials are lyophilized, bovine source.
2. Cryoprecipitate: Obtained from the national blood bank.
3. Tranexamic acid (EXACYL™): Ampules of tranexamic acid (0.5 g/5 ml, sanofiaventis-France).
4. Calcium Chloride ($CaCl_2$): Calcium Chloride Dihydrate (BDH chemicals LTD pool, England).

Synthesis of the fibrin glue

During the experiment, the fibrin glue preparation and storage was kept under full humidified condition and constant temperature (37^{o}C) by using an incubator (vortex™). Cryoprecipitate was thawed to 37 ^{o}C temperature before used. Thrombin vial was dissolved by addition of 4 ml of 50 mM $CaCl_2$ [16]. In all experiments of the fibrin glue synthesis 100 µl of thrombin and 50 µl of tranexamic acid (= 5 mg) were used.

Procedure

800 µL of cryoprecipitate was mixed thoroughly with 100 µL of thrombin (8:1 fibrin glue). 800 µL of cryoprecipitate was mixed thoroughly with 50 µL of tranexamic acid, then 100 µL of thrombin was added (8:1:50 fibrin glue).

The elasticity tests

The test of the elasticity was to measure the elongation of the experimental glue by different weights used.

Procedure

The lever of the displacement transducer was fixed in horizontal position by equilibrium weight applied on the other side, while the clot was clipped between the lever and the micro-manipulator, this weight was just to keep the lever horizontal without increased the length of the glue (just for calibration). The weights were increased gradually by sequential addition of known weights thereby increasing the length of the clot. The elongation of the clot was recorded

by the displacement transducer which was drawn by a polygraph (Harvard apparatus limited, Universal Oscillograph, USA, 1979) on a chart paper. The elasticity test was repeated 10 times at 1 hour, 1 week and 2 weeks duration of the glue synthesis. The mean values were used for statistical analysis.

The tension tests

This test was to measure the tension that developed in the fibrin glue by pulling of the clot using the micromanibulator.

Procedure

The clot clipped between the force transducer and the micromanipulator, then, the latter was moved in such a way that it will pull the fibrin glue downward step by step, each step increased the displacement by one millimeter. During that, the increased tension of the clot was measured by the force transducer and recorded by a polygraph (lctromed limited, multitrace 2, USA, 1979), which draw this signal on a chart paper. The tension test was repeated 10 times at 1 hour, 1 week and 2 weeks duration of glue synthesis. The mean values were used for statistical analysis.

Statistical analysis

The obtained data were presented as mean ± standard deviation of mean. In graphic presentation, the means of the data were used alone (i.e., without standard deviation). Student T- test (paired and unpaired) was used for comparison between two groups for different ratios and models. *P* value less than 0.05 was considered significant.

Results

The elasticity tests

Results show an increased length of the glue with an increased weights used for both formulae at 1 hour, 1 week and 14 days durations of glue synthesis with fibrinolysed 8:1 fibrin glue after 10 days duration. Statistical analysis presents significant higher results of 8:1:50 in comparison to that of 8:1fibrin glue at 1 hour, 1 week durations (*p* values= 0.04 and 0.04, respectively). All clarified significant higher elasticity results of 8:1:50 at 1 hour duration in comparison to lower results at 2 weeks duration (*P* value= 0.03) as seen in fig. 1-3.

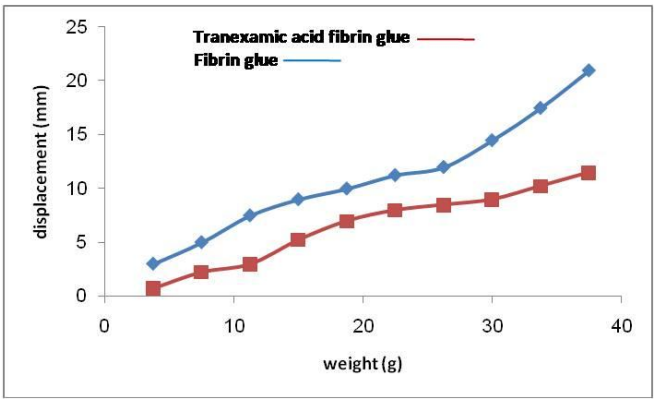

Fig. 1. The elasticity results of the 8:1 and 8:1:50 fibrin glues at 1 hour duration

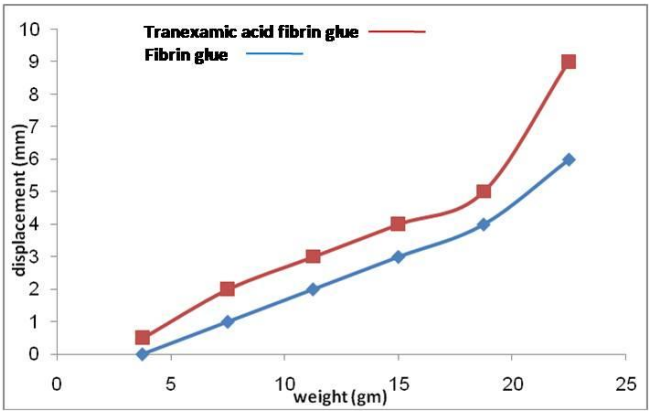

Fig. 2. The elasticity results of 8:1 and 8:1:50 fibrin glue after 1 week of glue synthesis

The tension tests

Results show clearly the increased tension of both formulae with gradual increased displacements at 1 hour, 1 week and 14 days durations. Statistical analysis presents significant higher tension results of 8:1 than that of 8:1:50 at 1 hour duration (*P* = 0.02), while at 1 week duration, tension results of both formulae shows no significant difference (*P* = 0.1). The comparison of the tension results of the 8:1:50 fibrin glue at different durations of times shows that the highest tension results were at 1 hour duration and the lowest tension results were at

14 days duration (*P* = 0.0008) as shown in fig. 4-6.

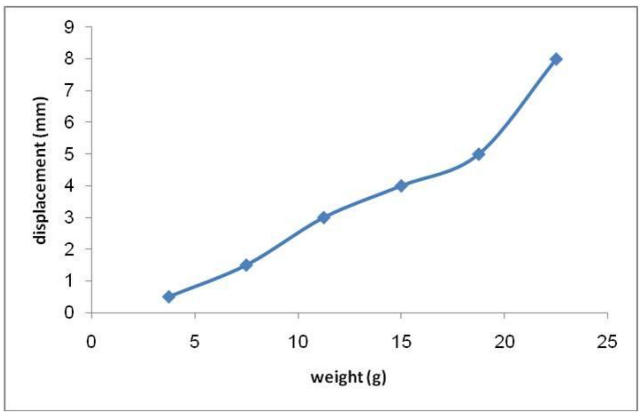

Fig. 3. The elasticity results of tranexamic acid fibrin glue (8:1:50) fibrin glue at 14 days duration.

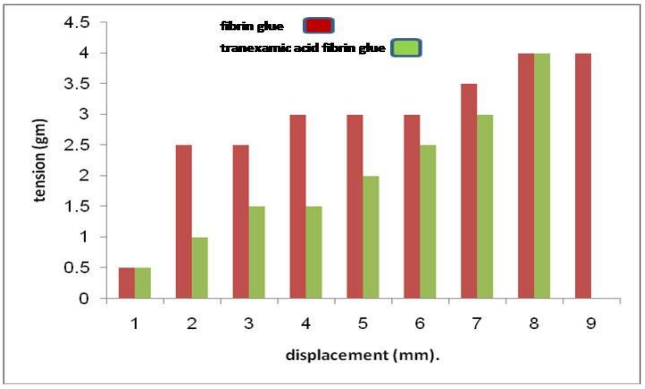

Fig. 4. Histogram of the tension results of 8:1 and 8:1:50 fibrin glue at 1 hour duration

Discussion

The higher fibrin glue elasticity with tranexamic acid addition in comparison to that without tranexamic acid addition may be attributed to change from physiological course to non physiological fine clots, which microscopically are characterized by thinner fibrin fibers [24,25], and so presented as a higher elasticity of tranexamic acid added fibrin glue. Elasticity results of the tranexamic acid added fibrin glue was significantly reduced time dependent. This gradual diminished result may be related to gradual decreased biological activity of the fibrin threads.

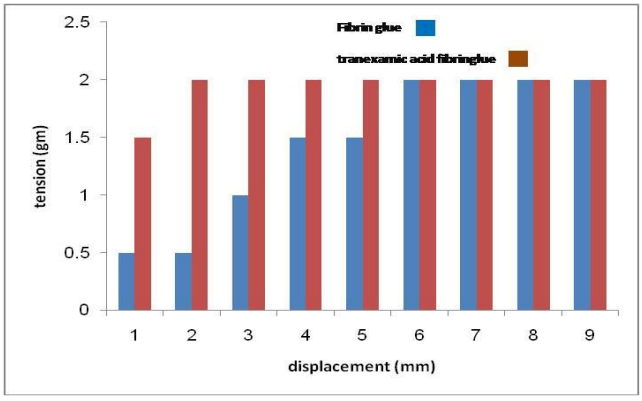

Fig. 5. Histogram of the tension results of 8:1 and 8:1:50 fibrin glues at 1 week duration

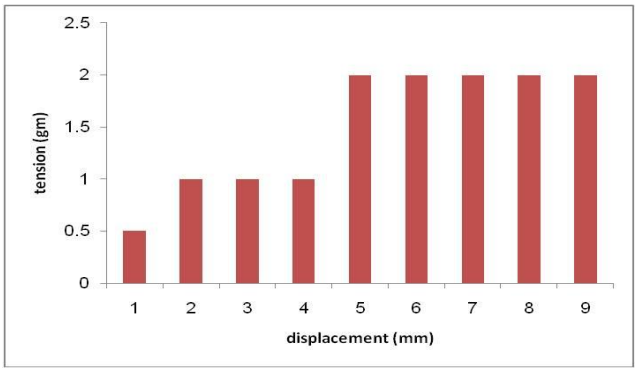

Fig. 6. Histogram of the tension results of tranexamic acid fibrin glue (8:1:50) fibrin glue at 14 days duration.

The lower tension of tranexamic acid added fibrin glue at 1hour may be explain by the change from physiological course to non-physiological fine clots, which microscopically are characterized by thinner fibrin fibers, more branching points, and a reduced pore size. These structural changes may be the reason for reduced tensile strength [24,25].

At 1 week duration, the tranexamic acid added high concentration fibrin glue showed non-significant difference in tension than the high concentration fibrin glue without addition; a result may be attributed to digestion of the fibrin threads by plasmin in non-added tranexamic acid fibrin glue, that caused reduced tension, while the fibrinolysis was delayed in tranexamic acid added fibrin glue [16,21] leading at

last to non-significant difference in tension between both formulae at 1 week duration. Tension results of the tranexamic acid added fibrin glue was significantly reduced time dependent, this gradual diminished result may be related to gradual decreased biological activity of the fibrin threads.

In conclusion, tranexamic acid addition to the components of the fibrin glue is used for delaying or prevents early fibrinolysis of the glue. This work reveals that this addition lead to change in biological behavior of the glue presents as increased elasticity and decreased tension. This change should be taken into consideration when the applicator needs to use this formula in the management of different areas of human body.

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Received 28th Aug. 2012: Accepted 29th Oct. 2013

Iraqi JMS

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

Prevalence and Risk Factors Associated with Overweight and Obesity among under than 5 Years Children

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Abstract

Background Childhood obesity is a serious public health problem with increasing prevalence worldwide.

Objectives To estimate the prevalence of obesity and overweight among under 5 years children and to clarify some factors associated with them like socioeconomic status, sedentary behavior, eating habits etc.

Methods A total of 606 children from those visiting health care center for vaccination or routine care aged 2 months up to < 5 years. The parent (or caretaker) of each child was interviewed using a special questionnaire form provides information about sociodemographic characteristics, inclusion and exclusion criteria, delivery and pregnancy, feeding and nutrition, physical activity, and measurement of weight and height.

Results The prevalence of overweight was 16.8%; of those at risk of obesity were 18.15 %. No significant association found between obesity, age, gender of the children, occupation of the parents, type and size of the family, obstetrical characteristics, eating habits. A significant association between obesity and residency of the children, educational level of parents, added food given to the child and age started to give this food, and physical activity.

Conclusion The study shows that the prevalence of overweight and those at risk of obesity are increased in Iraq in the last decade.

Keywords Overweight, obesity, under 5 years' children, BMI.

Introduction

Obesity is defined as excessive body fat that results when intake and consumption of energy are not balanced [(1-3)] and from the functional point of view can be defined as maladaptive increase in the mass of somatic fat store. An ideal definition of obesity in children will reflect both the possibility of the child to become an obese adult, as well as present and future risk of adiposity related complication [(4,5)]. Again it also defined as an excess of fat over that needed to maintain health [(6)].

Obesity is rapidly increasing in children and adolescents. Researchers reported that childhood obesity (at risk for overweight or overweight) doubled over the last three decades in the United States [(7)].

According to WHO available sources, the prevalence of children less than 5 years overweight and obesity in Iraq was 15% in the year 2006, while prevalence of underweight was 7.1% [(8-9)]; while local available sources from the Ministry of Health in our country, the prevalence of children under 5 years overweight and obesity in Iraq was 13.9% in the year 2010 (11.5 % in male, 9.5 % in female), while prevalence of underweight was 6.4% [(10)].

The intension of the study is to estimate the prevalence of obesity and overweight and those at risk among under 5 years children and to clarify some factors associated with overweight and obesity among them.

Methods

A cross sectional study was carried out during the period from 3rd January to 31st March, 2012 (a month for each center) in Baghdad Al-Karkh (Al-Rashid, Al-Shabab and Al-Washash primary health care centers).

A total of 606 children aged 2 months up to < 5 years from those visiting health care center for vaccination or routine care were included in this study. The parent (or care taker) of each child (mother, father or other member of the family) was interviewed by the researcher using a special questionnaire form constructed for this study. The questionnaire form consists of five sections provides information about socio-demographic characteristics of the children, obstetrical characteristics, feeding and nutrition of the child, physical activity, and measurement of weight and height to calculate WHZ (weight for height z score). Weight was measured with a well-calibrated digital scale. All boys and girls were barefooted with minimal cloths. Weight was measured in kilograms with an accepted error of 0.1 kg. Height was measured in centimeters with tape measures in standing position for children 2 years and more and in supine for those less than 2 years.

Weight for height and WHZ score indicators were used to estimate whether the child is obese or over weighted or not using the NCHS/WHO method. Children categorized according to their weight for height and WHZ scores into:

1. Underweight and wasted with z score below -2
2. Normal with z score -2 to +1
3. At risk of obesity with z score +1 to +2
4. Overweight and obese with z score above +2 (WHO, March 2011).

Weight for height and WHZ scores was calculated using *WHOAnthro* computer program available at WHO website.

The children included in the study sample were those aged two months to five years of both genders including those attending the health centers for vaccination, apparently healthy and those with acute mild illnesses which not affect the outcome of the study (like flu).

Children aged less than two months and five years and more, those with chronic illnesses (like respiratory disease, diabetes mellitus.. etc), those with a known syndrome causing obesity like Prader–Willi syndrome were excluded from the study.

Data of the study was analyzed using available statistical computer program of SPSS-16 (statistical packages for social sciences).

Results

The prevalence of overweight in the sample was 16.8% and of those at risk of obesity was 18.15 %. Highest proportion of overweight children was found in those aged 36-47 months, while highest proportion of children at risk was noticed in those aged 12-23 and 24-35 months. No significant association was found between WHZ and age. The proportion of overweight and obesity was found to be higher among male children. No Significant association was found between WHZ and gender. Highest proportion of overweight and at risk of obesity was found among children living in rural area. Significant association was found between WHZ and residency as show in table 1.

Highest frequency of obesity was noticed among children whose fathers and mothers had lower level of education. A significant association was found between WHZ and educational level of educational level of both parents as show in table 2.

Highest frequency of obesity was noticed among children whose fathers and mothers were workers. No significant associations were found between WHZ and occupation of both parents as show in table 3.

The proportion of overweight and obesity was found to be higher among children with extended family of two children and when the children rank fourth in the family. No Significant association was found between WHZ and Type of family, family size and rank of the child as show in table 4.

Highest proportion of overweight was noticed among children who had history of breast feeding during infancy, children with no added food, when added food started 6 months age and older. No Significant association was found between WHZ and type of feeding during infancy, breast feeding period. Significant association was found between WHZ and added food for child and age of starting added food as show in table 5.

Table 1. Distribution of the studied sample by age, gender and residency and WHZ

Parameter		Normal		At risk		Overweight		Total	
		No.	%	No.	%	No.	%	No.	%
Age (months)	3-11	160	61.3	49	18.8	52	19.9	261	100
	12-23	109	66.1	31	18.8	25	15.2	165	100
	24-35	44	59.5	17	23.0	13	17.6	74	100
	36-47	27	61.4	8	18.2	9	20.5	44	100
	48-60	16	66.7	5	20.8	3	12.5	24	100
	χ^2 = 3.049, d.f = 8, *P* = 0.931								
Gender	Female	173	64.3	51	19.0	45	16.7	269	100
	Male	183	61.2	59	19.7	57	19.1	299	100
	χ^2 = 0.692, d.f = 2, *P* = 0.708								
Residency	Rural	61	55.0	22	19.8	28	25.2	111	100
	Urban	295	64.6	88	19.3	74	16.2	457	100
	χ^2 = 5.385, d.f = 2, *P* = 0.048								
Total		356	62.7	110	19.4	102	18.0	568	100

Table 2. Distribution of the studied sample by Educational levels of the parents and WHZ

Educational Level		Normal		At risk		Overweight		Total	
		No.	%	No.	%	No.	%	No.	%
Educational level of father	Illiterate	51	63.0	14	17.3	16	19.8	81	100
	Read & write	43	51.8	22	26.5	18	21.7	83	100
	Primary school	50	56.2	18	20.2	21	23.6	89	100
	Secondary school	113	70.6	21	13.1	26	16.2	160	100
	College & above	99	63.9	35	22.6	21	13.5	155	100
	χ^2 = 14.560, d.f = 8, *P* = 0.0480								
Educational level of mother	Illiterate	41	53.9	14	18.4	21	27.6	76	100
	Read & write	52	58.4	19	21.3	18	20.2	89	100
	Primary school	62	66.0	16	17.0	16	17.0	94	100
	Secondary school	126	65.3	37	19.2	30	15.5	193	100
	College & above	75	64.7	24	20.7	17	14.7	116	100
	χ^2 = 7.807, d.f = 8, *P* = 0.0450								
Total		356	62.7	110	19.4	102	18.0	568	100

Table 3 : Distribution of the studied sample by occupations of the parents and WHZ

Occupation		Normal		At risk		Overweight		Total	
		No.	%	No.	%	No.	%	No.	%
Occupation of father	Worker	198	58.2	74	21.8	68	20.0	340	100
	Employed	15	69.3	36	16.0	33	14.7	225	100
	Others	62	66.7	0	0.0	1	33.3	3	100
	χ^2 = 8. 118, d.f = 4 *P* = 0.087								
Occupation of mother	Unemployed	224	63.3	65	18.4	65	18.4	354	100
	Employed	124	61.1	42	20.7	37	18.2	203	100
	Other	8	72.7	3	27.3	0	0.0	11	100
	χ^2 = 3.019, d.f = 4, *P* = 0.555								
Total		356	62.7	110	19.4	102	18.0	568	100

Table 4. Distribution of the studied sample by Type of family, family size and rank of the child and WHZ

Family Parameter		Normal		At risk		Overweight		Total	
		No.	%	No.	%	No.	%	No.	%
Type of family	Nuclear	269	61.7	93	21.3	74	17.0	436	100
	Extended	87	65.9	17	12.9	28	21.2	132	100
	χ^2 =5.038, d.f = 2, *P* = 0.081								
Family size	≤4	283	62.3	89	19.6	82	18.1	454	100
	>4	73	64.0	21	18.4	20	17.5	114	100
	χ^2 = 0.121, d.f = 2, *P* = 0.941								
Rank of the Child in the Family	1	130	62.8	37	17.9	40	19.3	207	100
	2	129	59.4	51	23.5	37	17.1	217	100
	3	57	67.9	12	14.3	15	17.9	84	100
	4	28	70.0	5	12.5	7	17.5	40	100
	5	7	63.6	4	36.4	0	0.0	11	100
	≥6	5	55.6	1	11.1	3	33.3	9	100
	χ^2 = 10.824, d.f = 10, *P* = 0.371								
	Total	356	62.7	110	19.4	102	18.0	568	100

Highest frequencies of overweight and obesity were found among children who had consumed <3 meals/day, child not eats with family, sometimes eating at night and not eating outside house. No Significant association was noticed between WHZ and number of meals consumed per day or habit of eating outside home, eating with the family and eating at night as show in table 6.

The proportion of overweight and obesity was found to be higher among children who sleep 13 hours and more per day and those who watch television four hours and more and who play 4 hours and more per day. A significant association was found between WHZ and the watching of television, child play hours and the sleeping hours per day as show in table 7.

Table 5. Distribution of the studied sample by Feeding during first six months, breast feeding period and added food and WHZ

Family Parameter		Normal		At risk		Overweight		Total	
		No.	%	No.	%	No.	%	No.	%
Type of family	Nuclear	269	61.7	93	21.3	74	17.0	436	100
	Extended	87	65.9	17	12.9	28	21.2	132	100
	χ^2 =5.038, d.f = 2, *P* = 0.081								
Family size	≤4	283	62.3	89	19.6	82	18.1	454	100
	>4	73	64.0	21	18.4	20	17.5	114	100
	χ^2 = 0.121, d.f = 2, *P* = 0.941								
Rank of the Child in the Family	1	130	62.8	37	17.9	40	19.3	207	100
	2	129	59.4	51	23.5	37	17.1	217	100
	3	57	67.9	12	14.3	15	17.9	84	100
	4	28	70.0	5	12.5	7	17.5	40	100
	5	7	63.6	4	36.4	0	0.0	11	100
	≥6	5	55.6	1	11.1	3	33.3	9	100
	χ^2 = 10.824, d.f = 10, *P* = 0.371								
	Total	356	62.7	110	19.4	102	18.0	568	100

Table 6. Distribution of the studied sample by Total number of meals/day and eating habits and WHZ

Eating habits		Normal		At risk		Overweight		Total	
		No.	%	No.	%	No.	%	No.	%
Meals / Day	<3	180	61.4	54	18.4	59	20.1	293	100
	≥3	176	64.0	56	20.4	43	15.6	275	100
	χ^2 = 2.023, d.f = 2, *P* = 0.364								
Child eat with family	Yes	132	61.4	46	21.4	37	17.2	215	100
	No	224	63.5	64	18.1	65	18.4	353	100
	χ^2 = 0.934, d.f = 2, *P* = 0.627								
Eating at night (extra meals)	Always	4	80.0	1	20.0	0	0.0	5	100
	Sometimes	22	52.4	12	28.6	8	19.0	42	100
	No	330	63.3	97	18.6	94	18.0	521	100
	χ^2 = 3.903, d.f = 4, *P* = 0.419								
Eating outside house	Sometimes	13	76.5	3	17.6	1	5.9	17	100
	No	343	62.3	107	19.4	101	18.3	551	100
	χ^2 = 1.982, d.f = 2, *P* = 0.371								
Total		356	62.7	110	19.4	102	18.0	568	100

Table 7: Distribution of the studied sample by Sleeping hours per day, Watching TV, Child play hours and WHZ

Sleeping habits		Normal		At risk		Overweight		Total	
		No.	%	No.	%	No.	%	No.	%
Sleeping Hours/Day	<8	85	63.4	24	17.9	25	18.7	134	100
	9-12	254	64.0	77	19.4	66	16.6	397	100
	>13	17	45.9	9	24.3	11	29.7	37	100
	χ^2 = 5.673, d.f = 4, *P* = 0.025								
Watching TV (hr)	None	255	61.7	79	19.1	79	19.1	413	100
	<4	52	70.3	14	18.9	8	10.8	74	100
	≥4	49	60.5	17	21.0	15	18.5	81	100
	χ^2 = 3.364, d.f = 4, *P* = 0.049								
Child play (hrs)	<4	287	62.9	89	19.5	80	17.5	456	100
	≥4	69	61.6	21	18.8	22	19.6	112	100
	χ^2 = 0.273, d.f = 2, *P* = 0.028								
Total		356	62.7	110	19.4	102	18.0	568	100

Discussion

Childhood obesity is a serious public health problem; the prevalence of childhood obesity is increasing rapidly worldwide [8]. There were few available national studies about the prevalence of overweight and obesity of children in Iraq.

In this study, the prevalence of overweight was higher than rates from assessment of nutritional status of children done by Iraqi Ministry of Health which found that 10.5% of less than five years children was over weighted [10]. The prevalence of obesity as reported from the Islamic Republic of Iran was (2-3%), Lebanon (3.2% among 3-19 year-old girls) [9]. On the other hand higher prevalence was estimated in Bahrain (38.5%), Kuwait (31.8%) and in Taipei, Taiwan (30%) [9].

The high prevalence of overweight and obesity in this study was mostly due to improvement in economical condition of people and incorrect feeding habits and physical inactivity.

The prevalence of overweight and obesity in the studied children were higher among those less than 36 months of age similar to assessment of nutritional status of children by Iraqi ministry of health [10] and a study done in Greece [11]. Other

studies in USA in 1997 and Canada in 2004 reported higher rates than this study [12,13].

The reason for such high rates of obesity among this age group may be due to low physical activity, early age of starting added food and the unhealthy type of added food.

The prevalence of overweight and obesity in the studied children were higher in males compared to females similar finding were found by Iraqi ministry of health in 2010 and study done in Greece in 2007 [10,11] and the opposite was found in Canada in 2004 [13].

This may be explained by the fact of eating habits, which differ between males and females.

Children living in rural areas had higher rates of overweight obesity compared to urban areas which was in agreement with a study done in Cyprus in 2005 [14]. While a study done in Greece in 2007 for preschool children found that the prevalence was higher in children living in urban area [11]. This may be due to unhealthy dietary habits among children of rural area.

Higher percentage of overweight was noticed among children whose parents had a lower level of education, mother was unemployed and father was worker. Higher rates of overweight were estimated among children with extended type of family, family size of four or less members and the rank of the child was four or less. The associations between weight for height z score (WHZ) and socioeconomic indicators used in this study were not significant (except for educational levels of both parents), which indicate that the effect of socioeconomic factors on the prevalence of obesity and overweight was not so strong. This finding is similar to study done in Greece in 2007 [11]. A study done in united states, new Mexico in 1991 did not find any associations between obesity in preschool children and parameters such as maternal education level and household size [15] while a significant relation was reported in other study in Germany in 1999 [16].

The obesity is higher due to poor knowledge about healthy habits of feeding, high number of children living in the same place and short time available to care for children.

The current study found that children who were breastfed during first six months of their life had higher prevalence of overweight, also those who breast fed less than 6 months, those children with no added food and those started added food at age of 6 month and older.

No Significant association was found between WHZ and type of feeding during infancy, breast-feeding period. Significant association was found between WHZ and added food for child and age of starting added food.

Higher percentage of overweight was noticed among children who had less than 3 meals per day, not eat with their family, eat at night extra meals sometimes, not eat outside house and not eating sweets, fruits, vegetables and drinking water. No Significant association was found between WHZ and number of meals per day, eat with their family, eat at night extra meals, eat outside house and not eating sweets, fruits, vegetables and drinking water.

The prevalence of overweight and obesity in the studied children were higher in children whose sleeping hours more than 13 hours per day, watching TV and playing video games 4 and more hours per day and children who play for 4 hours and more per day. A Significant association was found between WHZ and sleeping hours per day, watching TV hours and child play hours. This is due to the fact that physical inactivity leads to overweight and obesity.

Nationwide analytical studies are needed to assess the problem of obesity among under 5 years children and to evaluate the risk factors behind it and Health education for parents and others who can influence children life style habits.

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Received 4th Feb. 2013: Accepted 4th Nov. 2013

Iraqi JMS

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

The Effect of L-carnitine on Improving Seminal Fluid Parameters in Male Infertility

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Abstract

Background L-carnitine is an important antioxidant present in a high concentration in epididymal fluid. It supplies energy for sperms to acquire their motility in the epididymis.

Objective To evaluate the effect of L-carnitine on improving some of the seminal fluid parameters in infertile males.

Methods Ninety-six infertile males were divided into two groups, a group received L-carnitine and the other group received multivitamin. Seminal fluid analysis performed before and after treatment in both groups. Analysis and comparison of the results were carried out between both groups for all patients then for patients with primary and secondary infertility in each group.

Results The patients received L-carnitine showed no improvement in semen volume but a significant raise in sperm concentration observed in all patients. A highly significant raise of sperm motility was observed especially in patients with primary infertility.

Conclusion L-carnitine significantly improves sperm concentration and sperm motility especially in patients with primary infertility. It is recommended for oligoasthenozoopermic infertile men.

Key words Male infertility, antioxidants, L-carnitine, azoospermia.

Introduction

Infertility is defined as the failure to achieve pregnancy within one year of regular unprotected intercourse. It affects approximately 15% of sexually active couples; however, male factor contributes to about 40% of the cases [(1)]. It is characterized by low sperm concentration and/or sperm motility and/or increased abnormal sperm morphology. These changes are collectively named oligoasthenoteratozoospermia (OAT) and they are considered significant only if observed in two semen analyses collected between one to four weeks apart. This complies with the WHO guidelines (2010) [(2)]. About 30% of these cases are idiopathic (iOAT) [(3-5)]. iOAT may be related to increasing age or to non inflammatory functional alterations in epididymis or to some viral and chlamydial infections [(6-8)].

The treatment of male infertility especially iOAT can be problematic. Different drugs, dietary supplements and antioxidants are used but still

lack evidence supporting their efficacy [9]. L-carnitine is one of the important antioxidants in male fertility. It is a water-soluble amino acid that was first isolated from bovine muscle in 1905. Its structure was definitively established in 1927 [10]. In human, 75% of carnitine derives from diet while 25% is synthesized from lysine and methionine [11]. Its concentration in the epididymal fluid is 2000 folds higher than in plasma [12]. It has a role in mitochondrial β-oxidation of long chain fatty acids to provide energy for sperms and it acts also to protect the cell membrane and DNA against damage induced by free oxygen radicles [13], therefore, it has been used in the recent years to treat iOAT [14].

The aim of this study is to evaluate the effect of L-carnitine on improving seminal fluid parameters of infertile males in Mosul province and to assess its effect in patients with primary and secondary infertility.

Methods

This is a prospective randomized controlled clinical study. The study was approved by the scientific and ethical committee of the Department of Surgery at Mosul College of Medicine. Written agreements were obtained from all participants. The study, initially, included 104 infertile male patients but during the study period, 8 patients were excluded because of using herbals and medicines other than the standard drugs of our study, therefore, 96 infertile patients completed the study. The period of the study started from January 2012 to February 2013. Patient assessment and data collection were conducted in Al-Salam Teaching Hospital in Mosul. All patients underwent complete history, physical examination and investigations, including seminal analysis (SA), semen culture, hormonal studies (Follicular stimulating hormone (FSH), luteinizing hormone (LH), prolactin and testosterone) and transrectal ultrasound (TRUS) for suspected obstructive azospermia. Patients were selected according to inclusion and exclusion criteria. The inclusion criteria, according to WHO guidelines [2], include one or more of the following:

a. Sperm count < 15 million/ml.
b. Sperm total motility (A+B) < 40%.
c. Sperm of rapid progressive motility (A) < 32%.

(The grades of sperm motility are: Grade A: rapidly progressive. Grade B: slowly progressive)

The exclusion criteria are:

1. Recent febrile illnesses, taking herbals or medications that might affect seminal parameters in the last 3 months prior to the study.
2. Presence of obvious causes of male infertility (varicocele, cryptorchidism, orchitis and epididymitis with pyospermia, radiation or chemotherapy, any scrotal, inguinal or pelvic surgery, clinical hormonal abnormalities in FSH, LH, testosterone or prolactin and patients with obstructive azoospermia (diagnosed by acidic, low volume semen with low seminal fructose level and confirmed by TRUS).

The patients were categorized into two groups, the control group (48 patients) kept on multivitamin treatment containing group B vitamins (1 tablet/day) and the carnitine group (48 patients) received L-carnitine (1 g tablet twice daily). The treatment period in both groups was 2.5 months. This dose was selected because it is the most commonly used dosage in past trials on this subject [15]. SA was done prior to the onset of the study and 2 weeks after the end of the treatment course. Semen samples were collected after 3-5 days of sexual abstinence and the standard manual semen analysis was performed according to the WHO guidelines [2]. During the study period, the patients were interviewed monthly to assess their compliance with treatment and if they took other medicines.

In each group, the patients were further subcategorized, according to the type of infertility, into primary infertility group (the couples who never had history of conception) and secondary infertility group (couples with previous history of conception but now are infertile).

The aim of this subgrouping was to assess the effect of L-carnitine in primary and secondary infertility by comparing each with their control group.

Statistical analysis was performed using SPSS software 2009. The differences between carnitine group and control group were assessed using the unpaired student's t-test. The results are given as mean ± standard deviation and *P* value ≤ 0.05 was considered statistically significant.

Results

The seminal fluid parameters to be assessed in the carnitine group and the control group are shown in table 1. The age in both groups shows no statistical difference. The mean age of patients in the carnitine group is 32±7.5 years. The seminal fluid parameters in both groups are comparable and show no statistical difference. The mean durations of infertility in patients with primary and secondary infertility are comparable (3.5±1.5 vs 4±2 years). These results are important prior to the onset of treatment to avoid significant variations in the seminal fluid parameters in both groups.

Table 1. Comparison of pretreatment seminal fluid parameters and age in the studied groups

Parameters (mean±SD)	Carnitine group	Control group	P value
Age	32.35 ± 7.51	31.35 ± 7.78	0.562
Semen volume (ml)	2.767 ± 1.223	2.798 ± 0.965	0.891
Sperm concentration (million/ml)	12.85 ± 8.88	12.99 ± 7.77	0.943
Total sperm motility (%)	35.88 ± 20.16	37.13 ± 22.18	0.211
Progressive sperm motility (%)	18.25 ± 12.78	20.85 ± 18.37	0.104

Table 2 shows the comparison of seminal fluid parameters in carnitine group and the control group before and after treatment. In carnitine group, the seminal volume didn't show significant increase (*P* = 0.57) but the sperm concentration increased significantly (*P* = 0.001). There was a highly significant increase in total sperm motility (grade A+B) and rapid progressive motility (grade A) after carnitine treatment (*P* = 0.0001). In the control group, no significant increase noticed in any of the seminal fluid parameters, however, a significant drop of total and progressive sperm motility observed (*P* = 0.014, 0.011, respectively).

Table 2. Comparison of pre and post treatment seminal fluid parameters in the carnitine and control groups

Semen Parameters	Carnitine group			Control group		
	Pre treatment	Post treatment	P value	Pre treatment	Post treatment	P value
Volume (ml)	2.77 ± 1.22	2.88 ± 1.22	0.57	2.8 ± 0.97	2.66 ± 1.01	0.42
Concentration (million/m)	12.85 ± 8.88	17.55 ± 11.97	0.001	12.99 ± 7.8	13.36 ± 14.9	0.83
Motility (%)	35.88 ± 20.1	47.39 ± 23.06	0.0001	47.13 ±22.2	40.43 ± 32.2	0.014
Progressive Motility (%)	18.25 ±12.78	29.56 ± 20.15	0.0001	27.85 ±18.4	23.1 ± 18.2	0.011

A comparison of seminal fluid parameters was made between carnitine group and the control group before and after treatment in patients with primary infertility, as shown in table 3. Those patients who received carnitine showed a highly significant increase of sperm concentration (*P* = 0.013) with a similar increase in total and progressive sperm motility (*P* =

0.001 and 0.0001, respectively). Seminal parameters in the control group of primary infertility didn't show significant changes.

Table 3. Comparison of pre and post treatment seminal fluid parameters in patients with primary infertility (carnitine and control groups)

Semen Parameters	Carnitine group			Control group		
	Pre treatment	Post treatment	*P* value	Pre treatment	Post treatment	*P* value
Volume (ml)	2.54 ± 1.14	2.6 ± 0.92	0.76	2.6 ± 0.77	2.5 ± 0.9	0.58
Concentration (million/m)	12.3 ± 7.95	17.4 ±12.1	0.013	13.6 ± 6.57	11.8 ± 7.3	0.186
Motility (%)	33.8 ± 21.3	48.1 ± 25.3	0.001	44.4 ± 21.9	39.6 ± 25.1	0.187
Progressive Motility (%)	17.6 ± 13.7	31.1 ± 22.1	0.0001	24.1 ± 17.34	20.9 ± 19.4	0.212

Table 4 shows comparison of seminal parameters in patients with secondary infertility in carnitine group and the control group before and after treatment. Patients on carnitine therapy showed significant increase in sperm concentration only (*P* = 0.034). Seminal parameters didn't improve in the control group after treatment, however, significant drop noticed in total and progressive sperm motility.

Table 4. Comparison of pre and post treatment seminal fluid parameters in patients with secondary infertility (carnitine and control groups)

Semen Parameters	Carnitine group			Control group		
	Pre treatment	Post treatment	*P* value	Pre treatment	Post treatment	*P* value
Volume (ml)	3.1 ± 1.3	3.2 ± 1.5	0.620	3.1 ± 1.14	2.9 ± 1.12	0.576
Concentration (million/ml)	13.5 ± 10.1	17.75 ± 12.1	0.034	12.2 ± 9.2	15.4 ± 21.1	0.378
Motility (%)	38.5 ± 18.6	46.4 ± 20.34	0.126	50.6 ± 22.54	41.3 ± 21.1	0.032
Progressive Motility (%)	19.1 ± 11.8	27.5 ± 17.6	0.100	32.7 ± 18.9	25.8 ± 16.6	0.016

Semen volume was included in the analysis of results because a significant number of patients with primary infertility had their semen volume within the lower normal level (WHO guidelines) [2] prior to the onset of the study (equal or less than 1.7 ml), therefore, we aimed to test the antioxidant ability of L-carnitine to improve the functions of the seminal vesicle and prostate gland which contribute to the bulk of ejaculated volume of semen. Also we aimed to compare the results of the effect of L-carnitine on semen volume in this study with the results of past studies [16].

The effect of L-carnitine on improving sperm morphology was not included in this study because all the patients had their normal sperm morphology within the accepted levels prior to the onset of the study. Also the previous studies didn't showed any effect of L-carnitine on sperm morphology [16,17].

Pregnancy was not a target point in this study but we observed conception in the partners of two (out of 48) patients after L-carnitine therapy (one patient has primary infertility and the other has secondary infertility) but this didn't occur with the 48 patients in the control group.

Discussion

Human semen quality and fertility rates have been declining during the last decades [18-20]. This might be related to environmental and occupational pollutants, lifestyle changes, exposure to toxins and changes in dietary habits [21]. The dietary factors are of concern, they

include a lower intake of antioxidant nutrients such ascarnitine, vitamin A, E and C, folate, zinc and selenium [22,23]. Reactive oxygen species (ROS), at physiologic levels, are essential for normal reproductive function, however, at higher levels, they exert a negative effect [24]. The seminal plasma has a high concentration of antioxidants, which protect gametes from ROS. Depending on these facts, oxidative stress occurs when there is an excess of ROS or decrease of antioxidant levels or both which is found in most patients with iOAT [25,26]. Oxidative stress can impair sperm motility and morphology and may lead to sperm cell death [27,28]. The dose of L-carnitine used in this study is similar to that used in past trials on this subject [15], however, other studies used higher doses for longer periods with no superior results [15,17]. Up to date, it is unknown if higher doses can yield a difference in outcome.

This study is a randomized controlled clinical study in which two groups of infertile males received either multivitamin or carnitine but not both drugs. This is similar to the study of Sigman et al [29] but unlike the study of Lenzi et al [16] who used a placebo-controlled double-blind cross over design in which two groups of patients received both placebo and carnitine at intervals separated by washout periods to observe the effect of both carnitine and placebo. This design was not applied in our study because the length of any residual drug effect is unknown, therefore, the needed length of a washout period can't be predicted.

In this study, the seminal volume showed no significant increase after carnitine therapy. This is comparable to the results of Lenzi et al [16]. The sperm concentration showed a highly significant increase after carnitine therapy). This was also observed in both groups of primary and secondary infertility. This was also comparable to the results of past studies [15-17] but the later results of Sigman et al [29] and Lenzi et al [30] showed no significant changes in sperm concentration. The effect of carnitine on sperm concentration was unexpected because the intracellular metabolic action of carnitine and its posttesticular (epididymal) effect might contribute to the effect on sperm motility, however, Lenzi et al [16] suggested that this might be due to an improvement in the epididymal microenvironment which reduces gamates phagocytosis and increases ejaculated sperms.

This study included four azoospermic patients (zero sperm concentration) who received carnitine, one of them showed increased sperm concentration. The effect of L-carnitine on azoospermia had not been studied in the past trials; therefore, further studies are needed on a larger number of azoospermic patients.

The most prominent effect of L-carnitine was on total sperm motility and progressive sperm motility. Both of these parameters increased to a highly significant value after carnitine therapy but on reviewing the results of those patients, we observed that only patients with primary infertility showed this significant raise while patients with secondary infertility didn't show significant raise of these two parameters. These results are more significant than the corresponding results of Lenzi et al [16] but comparable to the results of Costa et al [15], however, these studies used higher doses of L-carnitine for a longer period. The study of Sigman et al [29] used a similar higher dose of carnitine for a long period but showed no significant improvement in sperm motility; however, only 12 patients were included in this study.

In our study, the effect of L-carnitine on sperm concentration and sperm motility was assessed in patients with primary and secondary infertility, however, this was not observed in the past studies. Although conception was not a target point in this study because of the many possible interfering factors , we observed two pregnancies in the partners of two infertile males who received carnitine (out of 48 patients) while no conception observed in those who received multivitamin. The effect of carnitine on improving the conception rate needs a larger sample of patients, a longer follow up period and possibly larger doses of L-carnitine.

In conclusion, L-carnitine is an effective therapy for infertile males to improve sperm concentration and more importantly, sperm motility, which improves significantly in patients with primary infertility, therefore, we recommend its use for infertile men with oligoasthenozoospermia. Further studies are needed to assess the efficacy of L-carnitne in patients with non-obstructive azoospermia and patients with very low sperm motility. The conception rate is another target, which needs further evaluation in a larger number of infertile men using L-carnitine.

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Received 3rd Jul. 2013: Accepted 6th Nov. 2013

Iraqi JMS

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

Efficacy of Vagal Nerve Stimulation in Iraqi Patients with Refractory Epilepsy: Two-Year Experience

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Abstract

Background Refractory epilepsy patients who fail to respond to two antiepileptic drugs used appropriately are likely to have medically refractory seizure disorder and should be investigated for alternative forms of treatments like experimental drug trial, surgical treatment, electrical stimulation and combination of these. Vagal nerve stimulation is an adjunctive treatment for certain types of epilepsy.

Objectives To evaluate the efficacy of vagal nerve stimulation in refractory epilepsy, as an adjunctive therapy to antiepileptic drugs in Iraqi patients.

Methods A retrospective study recruiting 34 patients at Neurosciences Hospital in Iraq between Feb. 2008 and Jan. 2011. Diagnosed as refractory epilepsy according to International League Against Epilepsy criteria; the epilepsy state, number of the anti-epileptic drugs, frequency and severity of the attacks (using Chalfont scale) was assessed before and after the vagal nerve stimulation implantation. Programming was done every two weeks depending on clinical assessment.

Results Severity of the attacks was reduced totally 100% in 26.5% of the patients and 50-99% in 26.5% of patients. The number of attacks per month was decreased by 100% in 26.5% of patients and showed more than 50% improvement in 38% of patients. The number of the drugs used after the implantation decreased by 17.6% (P = 0.007). The most common side effects were hoarseness of voice 55.8% and dysphagia 41% only during the on time of the device.

Conclusion Vagal nerve stimulation is effective safe and well tolerated in Iraqi patients.

Key words Vagus nerve stimulation, Refractory epilepsy, Anti-epileptic drugs, Iraqis.

Introduction

According to International League against Epilepsy (ILAE) criteria "Patients who fail two antiepileptic drug (AED) medications used appropriately are likely to have a medically refractory seizure disorder and should be investigated for alternative forms of treatment [1,2]. Potential treatment options include experimental drug trials, surgical treatment, electrical stimulation and combination of the above [1].

Vagus nerve stimulation (VNS) is approved by Food and Drug Administration (FDA) for management of intractable epilepsy in 1997 [3]. It is possible that VNS may interrupt the spread of epileptiform activity if delivered at a theoretically critical time; it is possible that VNS causes small changes in brain dynamics resulting in larger effects that inhibit the brain from becoming dynamically entrained, thus interrupting any progression towards a clinical seizure [4].

Two published retrospective case series discussed the long-term outcome of patients receiving VNS therapy [5,6]. A series reporting five-year or greater outcomes of 26 patients from the University of Wisconsin noted that the median frequency of seizures reported after one year of VNS therapy was decreased from baseline (-28%), but had decreased even more by the long-term follow-up (-72%) [7].

The aim of the present study is to evaluate our first experience in Iraq and to assess the results of vagal nerve stimulation in refractory epilepsy in Iraqi patients as an adjunctive therapy to antiepileptic drugs.

Methods

A retrospective study evaluating thirty-four patients diagnosed as refractory epilepsy according to ILAE criteria who had been operated for VNS implantation by a team of neurologists and neurosurgeons in Neurosciences Hospital in Baghdad for the period between Feb. 2008 and Jan. 2011, they were interviewed at epilepsy clinic, by taking thorough history and revaluation of the epilepsy state, frequency and severity of the attacks, number of the anti-epileptic drugs (AEDs), duration of the epilepsy before and after the implantation of the device was assessed. Seizure severity was assessed by using Chalfont seizure severity scale [8].

All the patients with implanted devices from the first case which is carried out in 24th Feb. 2008 till the last one at 5th Jan. 2009 were included in the study. All subjects were consented to participate in the research prior to their inclusion in the study, and the local ethics committee approved the study protocol.

Ten patients (29.4%) out of the tested were on full doses of two AEDs (tried before with three drugs with failure due to side effects); seventeen patients (50%) were on three drugs, and seven patients (20%) on four drugs. The age of the patients ranges between 12 years and 35 years. Six (17.6%) patients were having generalized tonic clonic epilepsy, fifteen (44%) with focal and secondary generalized epilepsy, thirteen (38%) with multiple types (generalized tonic clonic, myoclonic and atonic).

The devices (Cyberonic, Houston, Texas, model 102, and 102R) were implanted by neurosurgeons with the assistance of a biomedical engineer. First programming was done two weeks after implantation and then periodic programming every two weeks depending on the clinical assessment.

Statistical Analysis

Statistical Package of Social Sciences (SPSS) version 18 was used for the purpose of data entry and data analysis. Paired t test was used to compare between numerical variables before and after the implantation of the VNS device. A *P* value of ≤ 0.05 was considered as statistically significant.

Results

Age, sex, family history of epilepsy, age of seizure onset, and seizure types before and after the implantation of the device is shown in the table 1.

Severity of the attacks:

The severity of the attacks was decreased to 54 mean score as compared to 118.9 mean score prior to device implantation ($P < 0.005$) as seen in table 2.

The severity of the seizure attacks improved totally (100%) in 26.5% of the patients and about 50%-99% in 26.5% of the patients. In the rest 47% of the patients, the improvement was less than 50% as demonstrated in table 3.

Frequency of the attacks:

The number of the attacks after the implantation of the device is decreased from 225 attacks per month to 50.6 per month after the implantation of the device ($P = 0.022$) as noticed in table 2.

The frequency of the seizure attacks per month showed total improvement (100%) in 26.5% of the patients, 50% in 38% of the patients and less than 50% in 14.7% of the patients while 20.5% of the patients showed no improvement in the frequency of the attack (Table 3).

Table 1. Demographic feature of epileptic patients

	Age (year)	Gender	Age of seizure onset/year	Family history	Seizure type/before	Seizure type/after
1	8.5	F	0.33	No	PSG	PSG
2	12	M	0.5	No	PC, AS, MS	PC, AS, MS
3	15	M	6	No	PSG	PSG
4	16	M	4	YES	PSG	PSG
5	19	M	4	No	PSG	PSG
6	20	M	7	No	GTC, MS, AS	GTC, MS, AS
7	20	M	0.5	No	PSG	PSG
8	20	M	10	No	PSG	PSG
9	21	F	10	No	PSG	PSG
10	21	M	19	No	GTC	GTC
11	21	M	0.08	No	AS, MS, GTC, PCS, MR	AS, MS, GTC, PCS, MR
12	21	M	0.75	No	GTC	GTC
13	22	F	7	No	GTC, SE	GTC, SE
14	22	M	13	No	PSG, EPC	PSG, EPC
15	22	F	21	No	GTC, AS	GTC, AS
16	25	M	13	No	GTC, AS, PSG	GTC, AS, PSG
17	26	M	11	No	GTC	GTC
18	27	M	3	No	PSG	PSG
19	30	F	20	No	GTC, MS	GTC, MS
20	32	M	21	No	GTC	GTC
21	35	M	20	No	GTC, MS	GTC, MS
22	8.5	F	1	No	PSG	PSG
23	14	M	5	No	GTC	GTC
24	16	F	6	No	GTC, MS, AS	AS
25	25	M	16	YES	GTC, MS	Free
26	16	F	12	YES	GTC, MS	Free
27	17	F	7	No	GTC	GTC
28	30	M	17	No	PSG	PSG
29	25	F	10	No	GTC	Free
30	17	F	7	No	PSG	PSG
31	19	M	8	No	PSG	PSG
32	11	F	4	No	PSG	PSG
33	21	F	11	No	PSG	PSG
34	21	M	9	No	EPC	PSG

PSG = partial seizure with secondary generalization, PC = partial seizure, AS = absence seizure, MS = myoclonic seizure, GTC = generalized tonic clonic, EPC = epilepsia partialis continua, SE = status epilepticus.

Number of the drugs:

The number of the AED used by the patients after the implantation of the device is decreased by17.6% as compared to number of drugs at the end of the study ($P = 0.007$) (Table 2).

Adverse effects of the device implantation:

Hoarseness of the voice was the most common side effect in 55.8% of patients followed by dysphagia in 41%, cough, dyspnea, palpitation in 14.7%, and headache in 5.8%.

The implanted device was removed from 4 patients due to local infection in one, aspiration pneumonia in one, suicidal attempt in one and loss of contact in another patient.

Table 2. Severity of the attacks before and after the device implantation

Parameter	No.	Before implantation of device	End of the study	t	P value
Severity of the attacks	34	118.95 ± 40.36	54 ± 38.53	9.37	≤0.005
Frequency of the attacks/month	34	225.04 ± 244.2	50.65 ± 74.35	3.49	0.0022
No. of the drugs	34	2.95 ± 0.67	2.34 ± 0.81	2.95	0.007

Table 3. Improvement in the severity of the attacks

Parameter	100% improvement		50-99% improvement		<50% Improvement		No improvement	
	No.	%	No.	%	No.	%	No.	%
Severity of the attacks	9 (34)	26.5	9 (34)	26.5	16 (34)	47	0 (34)	0
Frequency of seizure attacks	9 (34)	26.5	13 (34)	38	5 (34)	14.7	7 (34)	20.5

Discussion

This study describes 34 patients, with refractory epilepsy who did not become seizure-free despite treatment with various combinations of AEDs. All of them were unsuitable candidates for resective surgery, there were subsequently treated with VNS.

VNS is a simple surgical therapy of choice because it need a routine electro-encephalography EEG, brain computed tomographic (CT) scan or MRI that can be easily done in comparison to surgical resection which need more sophisticated investigations like functional magnetic resonance imaging (FMRI), video EEG monitoring, intraoperative EEG, positron emission tomography (PET) scan and Single photon emission computerized tomography (SPECT) which are not available now in Iraq.

In the present study, the severity of the attacks was decreased in our patients, which agree with other international studies [6,9-11]. Moreover, in 26.5% of cases the severity of the attacks improved by more than 50% and totally in 26.5%, a finding that is also reported by Cramer [12].

The attacks frequencies per month were decreased. The mean duration from the time of implantation of the device till the end of the study was 1.8 year. This finding was very compatible with those reported by DeGiorgio and coworkers [13]. Moreover, the frequency of the attacks were improved by more than 50% in 38% of the patients and totally in 26.5% of the patients improved, which agree with the results of DeGiorgio and associates [6].

In present study, the VNS were well tolerated by the patients apart from some side effects of the device that was also reported by others [14,15].

Four patients not included in the statistical analysis of the study, because of the following reasons: Patient with myoclonic epilepsy with generalized tonic clonic seizure came for follow up only four visits then lost contact till now. The second patient with multiple types of epilepsy, died from aspiration pneumonia. The third patient with partial complex seizure, the device is removed because of infection of the device the patient used to scratch the area. The last patient also the device was removed because of infection and unfortunately died after removal of the device (suicide).

In conclusion, vagal nerve stimulation is a good adjunctive tool of therapy added on drug therapy for patient with refractory epilepsy. It is effective safe and well tolerated in our patient.

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Received: 10th January 2013: Accepted 3-11-2013

Iraqi JMS

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

Analysis of N-RAS Gene Mutations and P21N-RAS Protein Expression in Iraqi Patients with in AML

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Abstract

Background N-RAS mutations are the most commonly detected molecular abnormalities in hematologic malignancies, especially in those of myeloid origin.

Objective Current study aimed to determine the frequency of N-RAS mutation and its correlation with P21N-RAS protein expression in patients with acute myelogenous leukemia (AML) in Iraq.

Methods Peripheral blood, bone marrow aspirate and biopsy samples were taken from 58 newly diagnosed AML patients (57 de novo and 1 therapy related AML) and 30 individuals with reactive bone marrow conditions were selected as a control group. Samples screened for N-RAS gene mutations using nested PCR followed by mutation sensitive digestion analysis (MSDA), and immunohistochemical analysis of P21N-RAS protein expression by using anti N-RAS monoclonal antibody.

Results N-RAS mutations at the time of diagnosis were found in 10/58 (17.24%) and P21N-RAS expression found in 5/58 (8.62%) patients with AML. There was a significant difference (P = 0.001) in P21N-RAS expression between mutant and wild type N-RAS patients with AML. No N-RAS mutations or P21N-RAS expression detected in the control group individuals.

Conclusion It can be suggested that there is activation of RAS-signaling cascade in AML patients, this is may support their role in molecular pathogenesis of acute leukemia. Also, there was a significant difference between N-RAS gene status and P21N-RAS protein expression in patients with AML.

Keyword AML, N-RAS Mutation, MSDA, P21N-RAS expression, Digital analysis.

Introduction

RAS proteins are small GTPases that act as molecular switches, transducing extra-cellular signals from activated receptors at the cell surface to the nucleus, thus, regulating cell proliferation, survival, and differentiation. Three RAS genes encode four widely expressed isoforms: H-RAS, N-RAS, and the splice variants K-RAS4A and K-RAS4B [(1)]. The N-terminal region of the RAS proteins has a common structure which comprises a highly conserved G domain but RAS proteins differ substantially at the C-terminal end (C-terminal 40 amino acids), which is known as the hyper-variable region [(2)].

The RAS proteins possess intrinsic GTPase activity (induced hydrolysis of GTP to GDP), which normally leads to their inactivation and the control signal transduction. In tumors, a point mutation resulting in loss of the intrinsic GTPase activity and RAS proteins lock in an active state, thus, does not stop anymore to send signal stimulating cell proliferation and appears to be associated with the transforming

activity of the protein. All RAS mutations were missense point mutations occur at codons 12, 13 (exon 1) and 61 (exon 2) [3,4]. Activating mutations of N-RAS are most common among myeloid malignancies, found in approximately 20% to 40% of myelogenous leukemia (AML), myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CML) and juvenile myelomonocytic leukemia (JMML) [1].

AML is characterized by a maturation block and accumulation of myeloid progenitor cells. Clinically, cytogenetically, and molecularly it has been recognized as a heterogeneous disorder [5]. Current study aimed to determine the frequency of N-RAS mutations and its correlation with P21N-RAS protein expression in patients with acute myelogenous leukemia (AML) in Iraq.

Methods

Fifty eight newly diagnosed untreated AML patients (57 de novo and 1 therapy related AML) and thirty individuals with reactive bone marrow (including 19 individuals presented with pyrexia of unknown origin (PUO) and 11 presented with idiopathic thrombocytopenic purpura (ITP)) served as control group were enrolled in this study at Department of Hematology, Baghdad Hospital at Baghdad Medical City for the period April 2011 to July 2012.

Current study was approved by the Local Ethics Committee of College of Medicine, Al-Nahrain University and informed consent in accordance with the Declaration of Helsinki was obtained from patients, control individuals or their legal guardians prior to the collection of samples and data.

Out of the total number of patients, 58 patients were diagnosed as having AML according to modified FAB classification system including; 6 M0, 11 M1, 21 M2, 10 M3, 4 M4, 5 M5 and 1 M6. Criteria's of selection for AML patients enrolled in current study was newly diagnosed, didn't receive treatment and were randomly collected in relation to age and gender.

Genomic DNA was extracted from peripheral blood specimens of patients and control individuals, N-RAS gene amplification was performed; briefly 1 µL of the extracted DNA was added to a 20-µL PCR reaction mixture containing 5 µL of AccuPower TLA PCR Premix, 10 pmol of each forward and reverse primer (Table 1) and 13µL of nuclease free water. The first round of PCR consisted of 30 cycles (denaturation at 94^{o}C for 30 seconds, annealing at 55^{o}C for 30 seconds, and extension at 72^{o}C for 30 seconds). 1 µL of the amplified product of the first round was then added to a second 20-µL PCR reaction mixture using 2nd set of primers for a further 30 cycles under identical conditions to the first round. Each round was preceded by heating at 95^{o}C for 10 minutes. Negative control (no DNA template) tube was included with each batch of samples analyzed. Beta globulin gene also amplified as control for amplification[6].

Table 1. Sequences of DNA primers[6]

First Round N-RAS Gene Primers:
• RS 12 (Forward) 5' GCTCGCAATTAACCCTGATTAC
• RS7 (Reverse) 5' ATTCCTTTAATACAGAATATGG
Second Round N-RAS Gene Primers:
• RS6 (Forward) 5'ACTGAGTACAAACTGGTGGTGGTTGGACCA
• RS5 (Reverse) 5' GGTCAGCGGGCTACCCCTGGACCA

Mutation sensitive digestion analysis (MSDA) was used for the detection of mutations at codon 12 and codon 13. The second round PCR primers (RS6 and RS5) are both mismatched at a single base from their target sequence. This creates a 5' BstNI restriction site at codon 12 and 3' restriction site within sequence at the downstream end of the amplified DNA. If the

amplified DNA has normal sequence at the first two bases of codon 12, it is cleaved at both the 5' and 3' sites by BstNI to produce an 87 bp fragment, whereas mutant DNA with a substitution affecting either of the first two bases at codon 12 results in loss of this restriction site and thus cleaves only at the 3' site to produce a 116 bp fragment. A codon 13 mutation creates an HphI recognition site. Digestion of the 135 bp amplified fragment with this enzyme thus leads to cleavage of mutant DNA at a 5' and a 3' site to produce a 75 bp fragment, while normal sequence is digested at only the 3' position to produce a fragment of 117 bp. For both enzymes, the 3' site is always cleaved and serves as a control for the digestion [6] (Fig. 1).

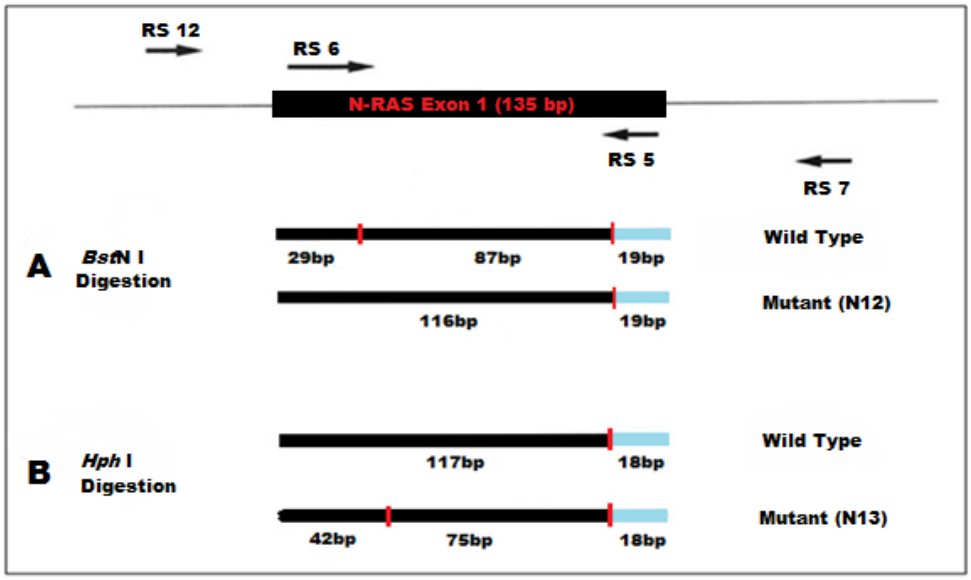

Fig. 1. Schematic illustration of the PCR-based MSDA used for the detection of codon 12 and 13 mutations. The positions of the first round primers for exon 1 (RS12 and RS7) and the second round nested primers (RS6 and RS5) are shown. (A) *Bst*NI digestion of amplified sequence for codon 12 mutations. (B) *Hph*I digestion of amplified DNA for codon 13 asp mutations [6].

PCR products were digested directly after 2nd round amplification, for codon N12 detection, 10 µl of PCR reaction mixture (about 0.1-0.5 µg of DNA), 7 µl of nuclease free water, 2 µl of NEBuffer 2 (10X) and 1µl of BstNI were mixed gently for a few seconds, then incubated at 60°C for 2 hours. The mixture was subjected to electrophoresis in 2% agarose gels containing 0.01% ethidium bromide. For codon N13 detection, 10 µl of PCR reaction mixture, 7 µl of nuclease free water, 2 µl of NEBuffer 4 (10X) and 1µl of Hph I were mixed gently for a few seconds, then incubated at 37°C for 2 hours. The mixture was subjected to electrophoresis in 2% agarose gels containing ethidium bromide [6,7] (Fig. 2-4).

Immunohistochemical staining was performed on 4 mm, formalin fixed, paraffin embedded bone marrow biopsy sections (patients and control individuals) mounted on electrostatic charged, poly-L-lysine-coated slides (Fisher Scientific, USA). Sections were deparaffinized at 60ºC overnight, rehydrated. Heat induced antigen retrieval was performed with citrate buffer pH 9. Exogenous peroxidase activity was quenched in 5 minute incubation with 2% H_2O_2, sections stained with N-RAS specific antibody (dilution: 1/1500, clone: F155, Santa Cruz, USA) and incubated overnight at 4°C. Sections stained with Biotin and Streptavidin-HRP reagent. Streptavidin-biotin complex was incubated with DAB Substrate buffer. Counterstaining was performed with hematoxylin, dehydration and

mounting processes were performed and completed [8]. immunohistochemistry staining procedure was

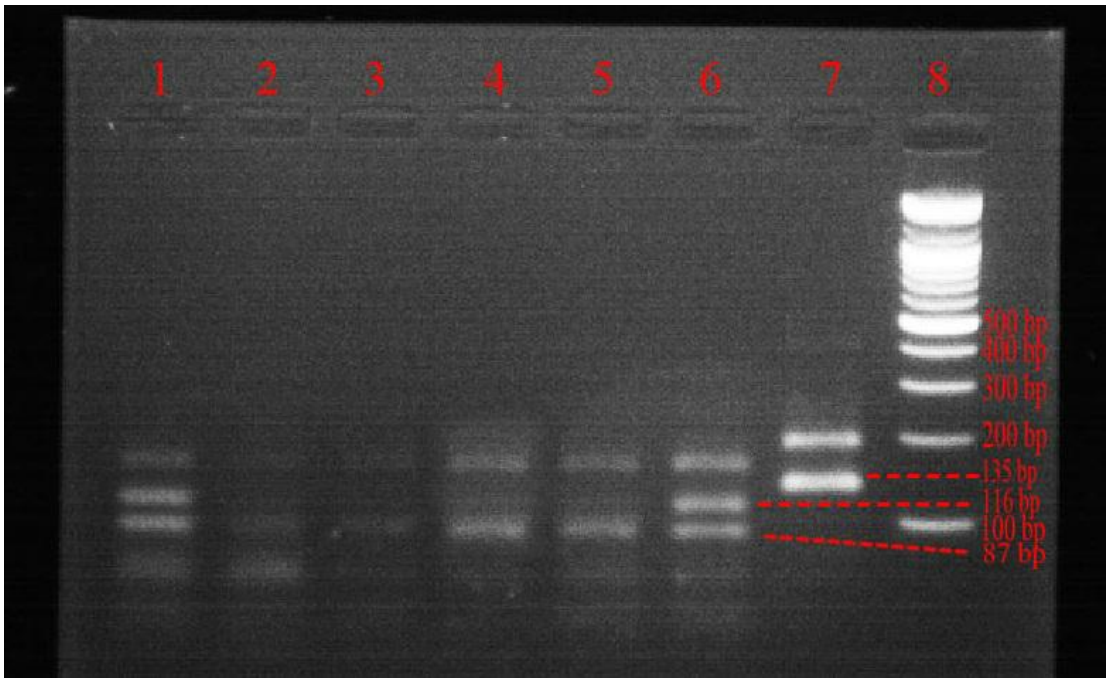

Fig. 2. Mutation sensitive digestion analysis (MSDA) from AML patients. PCR amplified DNA digested with BstNI for N-RAS codon 12 mutation. Lane 7, undigested control; lane 2, 3, 4 and 5 were wild N-RAS AML patients; Lanes 1 and 6 show AML cases with mutant N-RAS AML patents (116-bp band in lane 1, 6 was a result of N-RAS N12 mutation); lane 8, DNA size markers. Electrophoresis was done in 2% agarose gel containing ethidium bromide (final concentration 0.5 µg/ml) at (4V/cm) for 60 min.

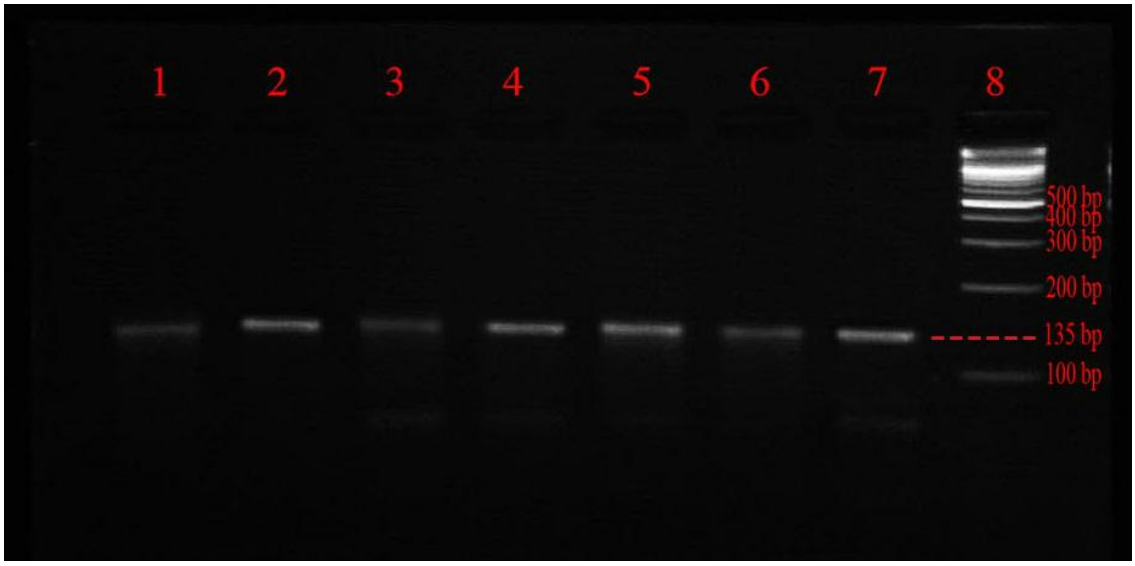

Fig. 3. Mutation sensitive digestion analysis (MSDA) from AML patients. PCR amplified DNA digested with *Hph*I for N-RAS codon 13 mutation. Lane 7, undigested control; lane 1, 2, 3, 4, 5 and 6 were wild N-RAS AML patients; Lane 8, DNA size markers.Electrophoresis was done in 2% agarose gel containing ethidium bromide (final concentration 0.5 µg/ml) at (4V/cm)for 60 min.

The cellular staining pattern for P21N-RAS was dark to light brown nuclear stain of blast cells in AML (Fig. 5). Scoring of the immune-histochemical expression was performed using specialized automated cellular image analysis system (Digimizer software v3.7.0.0 - 2010). The digital analysis software describe the expression with three parameters; which are: Intensity (intensity of staining which is negatively proportional to strength of staining), Fractional Area percentage FAP (area stained per high power field); and Digital Labeling Index DLI (calculated by multiplying the fractional area percentage by the reverse intensity, reflecting the integration of percentage of positive cells and intensity of expression) (Fig. 6).

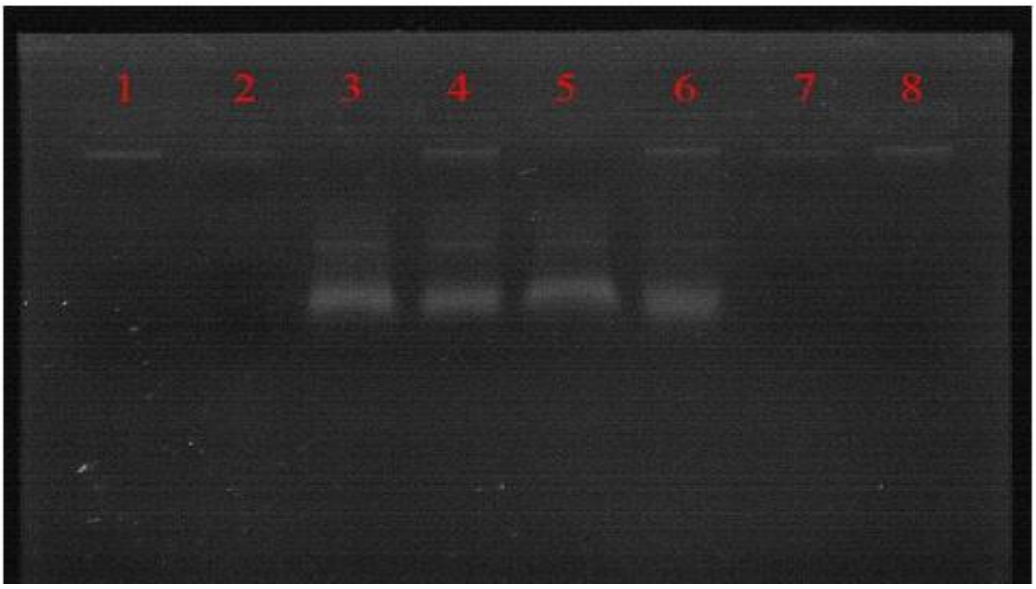

Fig. 4.BstNI and HphI enzymes digestion control (lanes 1, 3, 5 and 7 were unamplified DNA from AML patients while lanes 2, 4, 6 and 8 were unamplified DNA from control individuals). Lanes 3 and 4 contain DNA digested with BstNI. Lanes 5 and 6 contain DNA digested with HphI. Digested lanes show smear in comparison with undigested lanes, which show single bands. Electrophoresis was done in 2% agarose gel containing ethidium bromide (final concentration 0.5 µg/ml) at (4V/cm) for 60 min.

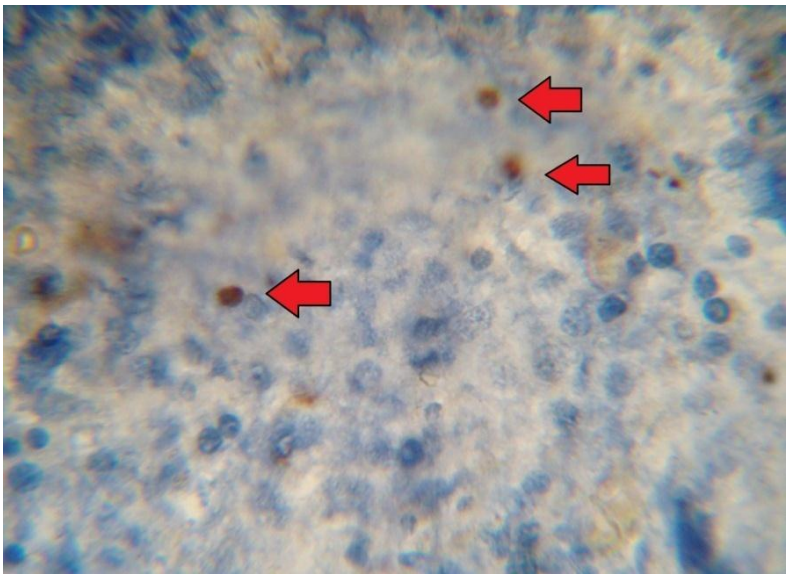

Fig. 5. Bone marrow biopsy of AML patient show positive immunohistochemical expression of P21 N-RAS (arrows refer to brownish coloration of the nucleus) (40X)

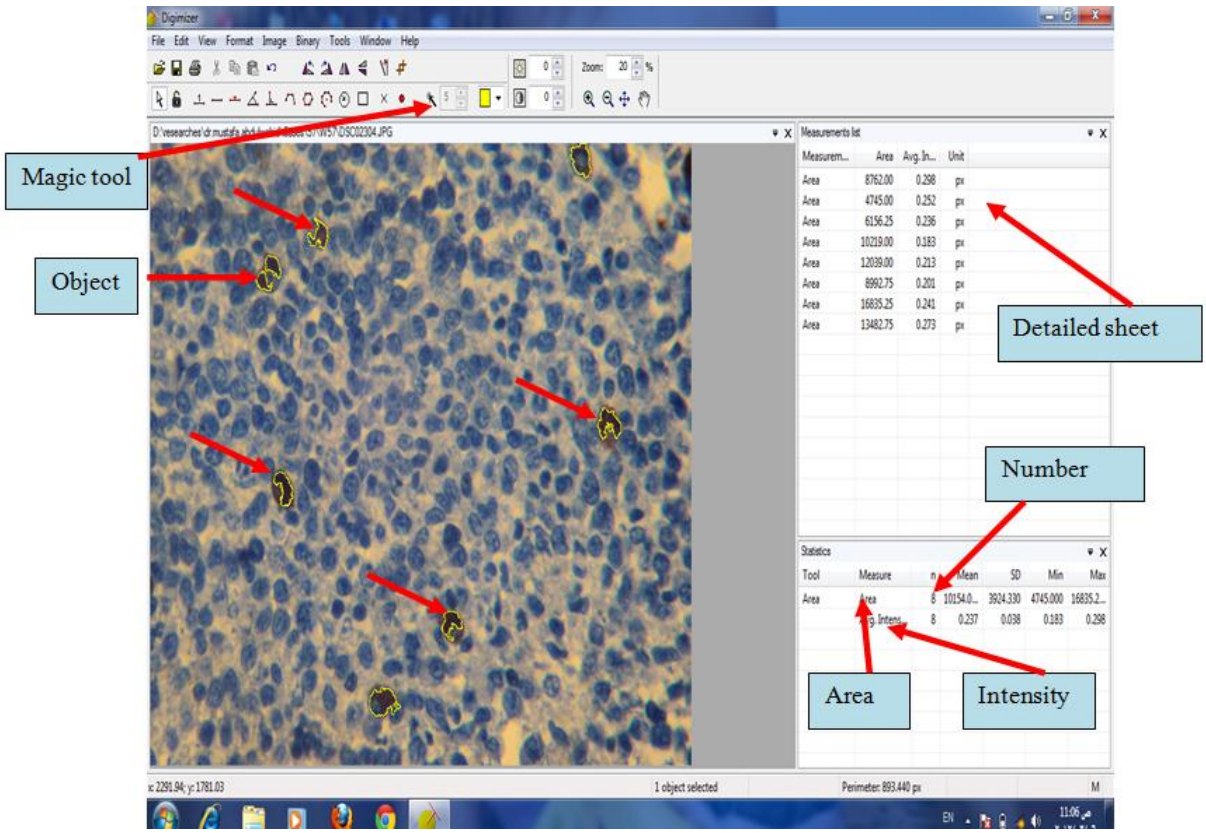

Fig. 6. Image analysis in Digimizer software v3.7.0.0

Data were analyzed using SPSS program (Statistical Package for Social Sciences) version 16 and Microsoft Office Excel 2007. Numeric data were expressed as (mean ± SE) and frequency was used to express discrete data. Student T-test was used to analyze numeric data while Chi-square and test was used to analyze discrete data. Values were considered statically significant when ($P < 0.05$).

Receiver operator characteristic (ROC) analysis was performed to determine the cut-off value for P21N-RAS protein expression at the time of diagnosis, anything above it was considered positive, and anything below it, was considered as negative (no expression).

Results

Out of 58 patients, There were 33 (56.89%) male patients and 25 (43.10%) female patients with a M:F ratio 1.3:1, mean age was 41.57 ± 2.53 year (age range was 13-75). Out of 30 individuals in control group, 18 (60%) individuals were males and 12 (40%) were females with a M:F ratio 1.5:1. The mean age was (38.77 ± 2.93) year (age range was 16-70 year).N-RAS mutations were found in 10 out of 58 (17.24%) of AML patients ($P = 0.091$). All mutations were in codon 12 and no mutation in codon 13. No mutations were detected in control group individuals. In AML patients, the mean P21N-RAS expression intensity was (0.637 ± 0.098), FAP was (4.904 ± 0.830) and DLI (10.714 ± 1.758). No P21N-RAS expression detected in control individual (0 out of 30) (Table 2).

There was no significant difference in patient's gender ($P = 0.855$) and mean age between mutant and wild type N-RAS AML patients (40.20 vs. 41.85, $P = 0.407$). The mean WBC count was significantly higher (54.33 vs. 31.25 x 10^9/L, $P = 0.033$) and the mean bone marrow blast percentage was significantly lower (56.50 vs. 69.31%, $P = 0.025$) in patients with mutated N-RAS than that of patients with wild type N-RAS (Table 3).

Table 2. Characteristics of patients and control individuals enrolled in present study

Characteristic		AML Patients (N = 58)	Control Individuals (N = 30)
Age (Year)	Mean Range	41.57 ± 2.53 13-75	38.77 ± 2.93 16-70
Gender	Male Female M:F ratio	33 25 1.3:1	18 12 1.5:1
Diagnosis		AML-M0 = 6 AML-M1 = 11 AML-M2 = 21 AML-M3 = 10 AML-M4 = 4 AML-M5 = 5 AML-M6 = 1	PUO = 19 ITP = 11
N-RAS Gene Status		Mutant = 10 Wild Type = 48	Mutant = 0 Wild Type = 30
WBC Count (10^9/L)		35.23 ± 6.60	6.34 ± 0.53
Platelets Count (10^9/L)		47.14 ± 7.22	183.73 ± 31.90
PCV percentage (%)		25.76 ± 0.82	38.40 ± 0.28
Peripheral Blood Blast percentage (%)		49.03 ± 4.26	0.00 ± 0.00
Bone Marrow Blast percentage (%)		67.10 ± 3.22	1.53 ± 0.09
Anemia		62.07	0.00
Fever (%)		44.83	63.33
Bleeding Tendency (%)		41.38	36.67
Weight Loss (%)		12.07	0.00
Splenomegaly (%)		36.21	0.00
Hepatomegaly (%)		25.86	0.00
Lymphadenopathy (%)		18.97	0.00
Intensity		0.637 ± 0.098	0.00 ± 0.00
FAP		4.904 ± 0.830	0.00 ± 0.00
DLI		10.714 ± 1.758	0.00 ± 0.00

Table 3. Clinical and hematological parameters in patients with mutant and wild type N-RAS gene

Parameter	Mutant N-RAS (N=48)	Wild Type N-RAS (N=10)	P
Gender (%)	Male 18.18 Female 16	Male 81.82 Female 84	0.828
Age (year)	40.20 ± 6.27	41.85 ± 2.80	0.407
WBC (10^9/L)	54.33 ± 9.19	31.25 ± 7.64	**0.033**
Hematocrit Percentage (%)	24.50 ± 1.36	26.02 ± 0.95	0.185
Platelets Count (10^9/L)	45.80 ± 15.24	47.42 ± 8.12	0.463
Peripheral Blood Blast Percentage (%)	38.50 ± 3.74	51.23 ± 5.10	**0.028**
Bone Marrow Blast Percentage (%)	56.50 ± 5.12	69.31 ± 3.68	**0.025**
Anemia	80%	58.33%	0.199
Bleeding Tendency	40%	41.67%	0.922
Fever	40%	45.83%	0.736
Weight Loss	30%	8.33%	0.056
Splenomegaly	50%	33.33%	0.318
Hepatomegaly	40%	22.92%	0.262
Lymphadenopathy	20%	18.75%	0.927

Regarding the distribution of N-RAS mutations within AML subtype according to FAB Classification; the mutations were detected in 1 out of 4 (25.00%) patients with M4 (*P* = 0.670), followed by M5, 1 out of 5 (20.00%) patients (*P* = 0.864), then M2, 4 out of 21 (19.05%) patients (*P* = 0.784), M1, 2 out of 11 (18.18%) patients (*P* = 0.926), M0, 1 out of 6 (16.67%) patients (*P* = 0.969), M3, 1 out of 10 (10.00%) patients (*P* = 0.837) and no mutations were detected in M6 patient(0 out of 1) (*P* = 0.605). There was no significant difference in N-RAS mutation among different AML FAB subtype (*P* = 0.105).

There was significant difference between patients with mutant and wild type N-RAS in Intensity (0.210 vs. 0.726, *P* = 0.044), FAP (6.785 vs. 4.512, P = 0.012) and DLI (32.310 vs. 6.215, *P* = < 0.001)(Table 4).

Table 4. Correlations between N-RAS mutations and Immunohistochemical expression in AML patients

Digital Parameter	Wild type N-RAS			Mutated N-RAS			*P* value
	N	Mean	SE	N	Mean	SE	
Intensity	48	0.726	0.113	10	0.210	0.051	**x 0.044**
FAP	48	4.512	0.350	10	6.785	0.952	**x 0.012**
DLI	48	6.215	0.964	10	32.310	7.687	**< 0.001**

By applying ROC curve analysis between mutant and wild type N-RAS in AML patients, DLI cut off value were 21.414 with acceptable sensitivity (71.4%) and acceptable specificity (100%)(Table 5 and Fig. 7).

Table 5. Receiver operator characteristic curve analysis between AML patients and control group

	Intensity	FAP	DLI
Cut Off Value	0.45	14.16	31.47
Sensitivity (%)	71.4	71.4	71.4
Specificity (%)	81.6	100	100

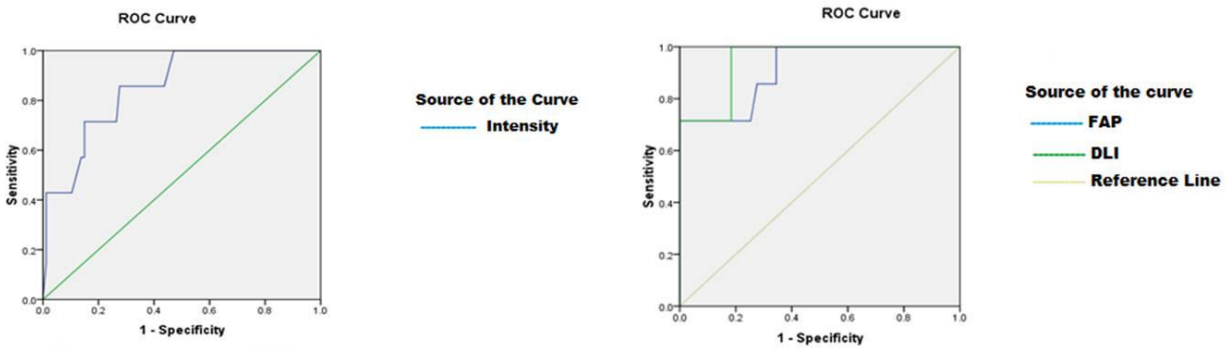

Fig. 7. Sensitivity and specificity of FAP, DLI and intensity between mutant and wild type N-RAS

By applying cut-off value, P21N-RAS expression found in 5 (8.62%) out of 58 AML patients, positive expression was found in 4 (40%) out of 10 in mutant N-RAS and in 1 (2.08%) out of 48 wild N-RAS, there was significant difference (*P* = 0.001) in P21N-RAS expression between mutant and wild type N-RAS AML patients (Table 6).

Table 6. P21N-RAS expression in AML patients

Parameter	P21N-RAS Expression		*P* value
	Positive	Negative	
Mutant N-RAS (N = 10)	4	6	**0.001**
Wild N-RAS (N = 48)	1	47	

Discussion

The clinical significance of RAS mutations has not been uniformly established, current study screened of 58 newly diagnosed AML patients with PCR-MSDA.Mutations in the N-RAS gene were found in 10 out of 58 (17.24%). This result confirms previous investigations that reported a prevalence of N-RAS mutations of (4 - 21%) in AML patients [3,9-12]. Discrepancy in RAS mutation frequency among various reports result from fact that criteria for selection of AML patients differ between various studies. N-RAS frequency in studies analyzed only de novo AML was lower than studies select AML that arose from proven MDS which is more frequently associated with N-RAS mutations [10]. Also the difference in RAS mutation frequency may explained by number of cases involved, method of screening, number of exon examined (codons 12, 13 in exon 1, codon 61 in exon 2) and type of RAS mutation (N, K and H-RAS) analyzed [12].

All N-RAS mutation detected in codon 12 (100%) and no mutation detected in codon 13, these finding were in agreement with previous studies [10,13]. Primary analyses revealed a statistically significant differencein the peripheral, bone marrow blast counts, WBC count between mutant and wild type N-RAS mutations (*P* = 0.028, *P* = 0.025, *P* = 0.033 respectively), however no significant differences had been found between the two groups with respect to age, gender, platelet count, hematocrit percentage and clinical outcomes. These findings are in agreement with those reported in literatures [3,9,10,13-15]. Mutation of the N-RAS gene affects the biology of AML. Transfection of various cell types with mutant RAS genes has been shown to stimulate secretion of interleukin-3, granulocyte, and granulocyte macrophage colony stimulating factors, leading to autonomous growth through an autocrine mechanism, increasing peripheral WBC count [16].

The highest frequency of N-RAS mutation in M4 in current study corresponded with most of the previously published studies [9,10,12]. N-RAS mutation is most likely a one event contributing to the progression/proliferation of sub-clones in AML, selection and expansion of RAS mutant clones may provide a differentiative stimulus toward the monocytic lineage [3], Van Kamp study also suggested that N-RAS mutation preferentially influences hematopoiesis to myelomonocytic differentiation or myelo-monocytic cells are more susceptible for acquiring an N-RAS mutation since N-RAS mutations are more likely to develop in cells of myelomonocytic differentiation [17]. This may be consistent with the overrepresentation of RAS mutation in M4/M5 subtypes.The low frequency of N-RAS mutation in M3 (10%) in current study corresponded with Bowen study, N-RAS mutation is relatively underrepresented in M3 where FLT3 ITD is overrepresented, both RAS mutation and FLT3 ITD are rarely present in the same tumor [3]. In the present study, P21N-RAS expression was found in 5 (8.62%) out of 58 AML patients, 4 (40%) out of 10 patients with mutant N-RAS and 1 (2.08%) out of 48 patients with wild type N-RAS show positive P21N-RAS expression. There was a significant difference (*P* = 0.001) in P21N-RAS expression between mutant and wild type N-RAS patients with AML. That is to say, there is a correlation between N-RAS gene mutations and protein expression. This finding was in agreement with previous studies [18-20].

This discrepancy between the N-RAS immuno-histochemical analysis results in different studies

might be explained by difference in the fixative used in bone marrow biopsies processing (formalin was used in current study while Bouin's solution and B5 was used in other studies). Positive P21 expression in one AML patient with wild type N-RAS may explain by the fact presence of other mutated codons that had not been screened in current study (e.g. codon 61).

Although that HphI enzyme digested the unamplified DNA, it failed to digest a 3' end of the amplified DNA (that served as a control for enzyme function) (Figure 4). Current study suggested that this negative result is not due to failure of the primer system to detect mutations in the digested PCR product but the predominance of digestion resistant band as mentioned in previous report.

Bashey and Todd studies describe an overrepresentation of the singly digested band, which is caused by the formation of restriction enzyme resistant hetero-duplexes between mutant and normal strands which are mismatched at a single base only [6,15]. In addition to that, the reverse Allele specific restriction analysis (ASRA) method described by Todd and Iland fails to demonstrate the presence or absence of wild type alleles, since a digestion resistant band merely indicates the lack of a specific mutation rather than the presence of wild type sequences [21].

In conclusion, previous results provide clues for activation of RAS-signaling cascade in AML patients, supporting their role in molecular pathogenesis of leukemia. Also, there was a significant correlation between P21N-RAS protein expression and N-RAS gene status. Thus, Immunohistochemical analysis of the P21N-RAS in blast cells of patients with AML may demonstrate the N-RAS gene expression. More studies on larger scale are required to explore P21N-RAS expression as a prognostic marker in myeloid malignancies.

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Received 25th Mar. 2013: Accepted 24th Nov. 2013

Iraqi JMS

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

Ultrasound versus Magnetic Resonance Cholangio-Pancreatography in the Diagnosis of Suspected Extra-hepatic Intrinsic Biliary Obstruction

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Abstract

Background Evaluation of obstructive jaundice is a common clinical problem.

Objective To compare diagnostic accuracy, sensitivity and specificity of ultrasound and Magnetic Resonance Cholangio-Pancreatography in the diagnosis of intrinsic obstructive biliary disease.

Methods One hundred and sixty patients with an obstructive jaundice for whom ultrasound (U/S), magnetic resonance imaging (MRI) and Magnetic Resonance Cholangio-Pancreatography (MRCP) were performed**.** The final diagnosis was confirmed by surgery, tissue biopsy and/or Endoscopic Retrograde Cholangio-Pancreatography (ERCP) in some cases.

Results Of the 164 patients, 102 (62.2%) were found to have choledocholithiasis, 42 patients (25.6%) with benign stenosis and 20 patients (12.2%) had cholangiocarcinomas. Regarding choledocholithiasis, U/S examination showed a diagnostic accuracy of 80.15% with sensitivity of 71.08% and a specificity of 95.83 %. Conversely, MRCP showed a diagnostic accuracy of 93.89%, sensitivity of 93.97% and a specificity of 93.75 %. Regarding benign stenosis: U/S showed a diagnostic accuracy of 78.62% with a sensitivity of 16.67 % and a specificity of 97.29%. The diagnostic accuracy of MRCP was 93.13%, with a sensitivity of 90% and a specificity of 94.05%. In malignant stenosis: of the 20 patients with cholangiocarcinomas, 6 were localized in the upper third or hilar biliary tract (Klatskin tumor), 4 in the mid third and 10 in the distal third of the common bile duct (CBD). The diagnostic accuracy of US in malignant stenosis was 93.13%, with a sensitivity of 61.12% and a specificity of 98.23%. For MRCP, the diagnostic accuracy in detecting malignant stenosis was 93.89%, with a sensitivity of 72.23% and specificity of 97.34%. In all cases, no difference was noticed when comparing US and MRCP.

Conclusion Lithiasis was the most common cause of extra-hepatic intrinsic biliary obstructions. U/S is considered the first choice option in the diagnostic imaging of obstructive disease. If laboratory and clinical findings are supported by U/S, ERCP is required for therapeutic purposes, or if necessary surgery is performed. Ultrasound is highly reliable for ruling out benign stenosis, though not for demonstrating their presence. MRCP is required only for staging of malignant stenosis, or if the suspicion posed by clinical and laboratory findings is not confirmed at U/S.

Key words Ultrasound, MRCP, extra-hepatic intrinsic biliary obstruction.

Introduction

Evaluation of obstructive jaundice is a common clinical problem. Often, the initial problem is to distinguish between intrahepatic and extrahepatic biliary obstruction [(1)]. Extra-hepatic Biliary obstruction may be due to a variety of causes including choledocho-lithiasis, tumors (pancreatic head cancer, ampullary cancer, and cholangiocarcinoma), and trauma, including injury after gall bladder surgery, with choledocolithiasis being the most common cause [(2)]. Many studies have shown that clinical data such as history, physical examination, and laboratory tests can accurately identify up to 90% of patients whose jaundice is caused by extrahepatic obstruction [(3-11)].

Although history, laboratory investigations and imaging techniques may help to differentiate benign from malignant biliary strictures, it remains a clinical challenge [12].

The assessment of extra-hepatic obstruction often require the use of various imaging modalities to confirm the presence, level and cause of obstruction and aid in treatment planning. The various imaging modalities can be classified into direct and indirect techniques [13]. The former are more invasive, and include ERCP and Percutaneous Transhepatic Cholangiography (PTC). They carry a higher associated risk, but have the added ability to sample tissue and perform therapeutic maneuvers, such as biliary drainage with stenting or stone removal [14-16], but with risk of morbidity 1-7% such as pancreatitis, cholangitis, perforation, bleeding and biliary leak, and mortality rate of 0.2-1.0 % and unsuccessful cannulation of the ducts in 3-9% [17-20]. Also, direct techniques are limited to the evaluation of the intrinsic biliary tract and cannot define the presence of extrinsic compression of the biliary tree by surrounding structures. Indirect techniques such as Ultrasound, CT scan and MRCP improve image quality while at same time maintain a low risk profile [1].

Trans-abdominal ultrasound is the first-line imaging investigation in patients with jaundice or right upper-quadrant pain [21-23]. Dilated ducts are usually taken as indirect evidence of biliary obstruction. The presence of normal ducts, however, does not exclude obstruction [23]; this is mainly because biliary obstruction may not be accompanied by dilatation of the CBD, conversely, the CBD increases in diameter in response to cholecystectomy and aging [24-27]. Despite these exceptions, ductal dilatation remains an excellent clue to biliary obstruction. Specifically, ultrasound has been shown to be highly accurate (78-98%) for detecting extra-hepatic biliary obstruction [1].

Magnetic Resonance Cholangio-Pancreato-graphy (MRCP) is widely performed as a primary imaging modality for the assessment of obstructive jaundice and other benign or malignant bilio-pancreatic ducts abnormalities. The primary MRCP application is the evaluation of biliary obstructions due to choledocho-lithiasis, iatrogenic strictures, cholangio-carcinoma or pancreatic carcinoma [28].

The objective of the study is to compare the diagnostic accuracy, sensitivity and specificity of ultrasound and MRCP in patients with suspected intrinsic biliary obstruction.

Methods

This prospective study was done on 164 patients (76 males and 88 females) with an age range 24-70 years (mean age is 56 years) suffering from obstructive jaundice in Al-Imamian Al-Kadhimyian Medical City from June 2010 to October 2012.

All the patients included in this study had clinical and laboratory findings suggestive of obstructive jaundice (biliary colic, jaundice, increase of serum bilirubin level above the normal limit of 2 mg/dl), all the patients being referred to radiology department by general surgeon or gastroenterologist after full clinical and laboratory examination. All the patients underwent US and MRCP examination. Any patients with positive clinical and laboratory findings of obstructive jaundice and negative ultrasound examination were excluded from the study.

Ultrasound examination: All the examinations were performed with a convex multi-frequency probe of 3-5 MHz (HD 11XE, Philips medical system). The US study was done in the supine position after adequate period of fasting for at least 6-8 hours. The ultrasound findings were classified into 3 categories: Biliary stones, benign stenosis (revealed as smooth tapering of the biliary ducts) and malignant stenosis (Irregular or eccentric wall thickening or intraluminal vegetations or isoechogenic mass, associated with an abrupt interruptions and dilatation of the biliary tree).

MRI examination: All MRI examinations were performed with 1.5 Tesla system (MAGNETOM Avanto, Siemens medical system) also after enough period of fasting for at least 6 hours.

MRCP protocol consisted of axial and coronal images (source images), and post processing of these images by means of MIP reconstruction, so as to obtain optimal visualization of the biliary tree. The mean performance time was 20-25 minutes. MRCP findings were also classified into 3 categories: biliary stones (seen as endoluminal round or oval-shaped filling defects with low intensity signal, surrounded by the high signal intensity of the bile), benign stenosis (smooth and concentric or showed distal convexity and gradual and symmetric caliber restriction) and (malignant stenosis (characterized by an abrupt irregular and eccentric interruption of the biliary tract with upper abnormal dilatation and lower regular caliber were considered malignant).

The final diagnosis of all the patients was proved by ERCP, surgery and histopathology and the results of ultrasound and MRCP were compared.

Test performance characteristics: Statistical analysis was performed using the program SPSS (version 11 for Microsoft window). Statistical significance was assumed at level of ($P < 0.05$). The sensitivity is the conditional probability that a diseased person has a positive test results. Its value can be changed by changing the cutoff point for positive test results. The specificity is the conditional probability that a disease free person has a negative test results. Positive predictive value (PPV) is the conditional probability that a person with a positive test results is truly diseased. Its value depends on the cutoff for positive test result and the prevalence of the disease in the screened population. Negative predictive value (NPV), is the conditional probability that a person with a negative test results is truly free of the disease.

Results

Of the 164 patients 102 (62.2%) were found to have choledocholithiasis, 42 patients (25.6%) with benign stenosis and 20 patients (12.2%) had cholangiocarcinomas

Regarding choledocholithiasis, ultrasound examination showed a diagnostic accuracy of 80.15% with sensitivity of 71.08% and a specificity of 95.83 % (Table 1). Four false positives were due to abnormal refraction of the wall. The 24 false negative were related to calculi of less than 2mm in size located in the distal area and/or to the patients' morphological type. Conversely, MRCP showed a diagnostic accuracy of 93.89%, sensitivity of 93.97% and a specificity of 93.75 % (Table 1). The 6 false positive were due to a small cholangiocarcinoma in the distal area in 1 patient and 1 inflammatory stenosis in the ampulla in the other 2 patients, which were considered calculi. The 4 false negatives were due to pneumobilia (intra-ductal air bubbles) disguising a stone in 2 patients, and in the other 2 due to calculi of less than 3mm. There is statistically insignificant difference between U/S and MRCP in choledocholithiasis ($P = 0.286$). Fig. 1 shows U/S and MRCP images in 37 years old female with CBD stone.

Benign stenosis: of the 42 patients with benign stenoses, 22 showed an iatrogenic stenosis as a result of laparoscopic cholecystectomy (in 14 patients), gastric resection (in 2 patients), bilio-enteric anastomosis (in 2 patients), and after ERCP (in 4 patients). Six had an obstructive cholangitis, and 14 patients had stenosis in the ampullary region resulting from inflammation and benign tumors. Ultrasound showed a diagnostic accuracy of 78.62% with a sensitivity of 16.67 % and a specificity of 97.29% (Table 1). The 4 false positive were recorded due to misdiagnosis of a malignant stenosis. On the 24 false negative were due to difficulties of the ultrasound in the visualization of distal CBD. The diagnostic accuracy of MRCP was 93.13%, with a sensitivity of 90% and a specificity of 94.05% (Table 1). The 6 false positive were due to misdiagnosis of a malignant stenosis in 2 patients and microlithiasis and dilatation of main pancreatic duct in the other 4 patients. The 4 false negative were due to artifacts from magnetic susceptibility, overlapping surgical clips and bowel gas, which prevent identification of benign stenosis. In benign stenosis comparing ultrasound and MRCP a statistically significant difference was not obtained ($P = 0.999$). Fig. 2 shows ultrasound and MRCP images in 42 years old female with benign stricture in the CBD.

In malignant stenoses: of the 20 patients with cholangiocarcinomas, 6 were localized in the upper third or hilar biliary tract (Klatskin tumor), 4 in the mid-third and 10 in the distal third of the CBD. The diagnostic accuracy of ultrasound in malignant stenosis was 93.13%, with a sensitivity of 61.12% and a specificity of 98.23% (Table 1). The 4 false positive were due to benign stenosis considered malignant, while 8 false negatives were due to a malignant stenosis misinterpreted as extrinsic compression. For MRCP, the diagnostic accuracy in detecting malignant stenosis was 93.89%, with a sensitivity of 72.23% and specificity of 97.34% (Table 1). The 4 false positives were due to misdiagnosis of benign stenosis considered malignant, whereas the 4 false negative were due to a small stenosis in 2 patients, and in the other 2 due to overlap artifacts (surgical clips and bowel gas). Comparison of ultrasound and MRCP, the analysis of data did not show a statistically significant difference (P = 0.635). Fig. 3 shows 2 MRCP images in 2 different patients with malignant stricture in the distal and proximal CBD.

Table 1. Diagnostic accuracy, sensitivity and specificity of MRCP and US in Cholelithiasis, benign stenosis and malignant stenosis

Feature	Stone		Benign stenosis		Malignant stenosis	
	U/S	MRCP	U/S	MRCP	U/S	MRCP
Number	102	102	42	42	20	20
True +ve	74	92	14	32	8	12
False –ve	24	4	24	4	8	4
False +ve	4	6	4	6	4	4
Diagnostic accuracy (%)	80.1	93.89	78.62	93.13	93.13	93.89
Sensitivity (%)	71.08	93.97	16.67	90	61.12	72.23
Specificity (%)	95.83	93.75	97.29	94.05	98.23	97.34
PPV (%)	94.87	93.88	77.78	84.21	66.67	75
NPV (%)	72.09	93.93	83.56	96.83	94.73	97.3
P value	0.286		0.999		0.635	

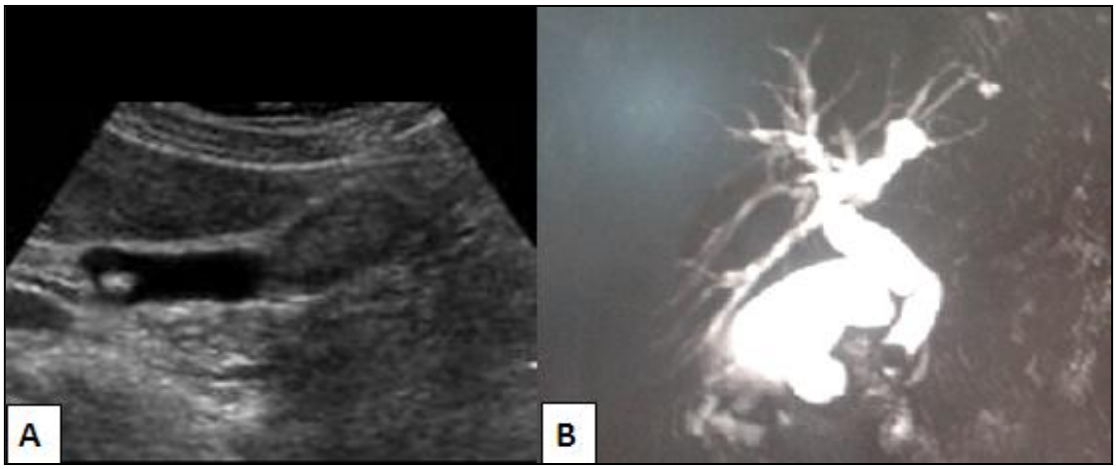

Fig. 1. 37 years old female presented with obstructive jaundice caused by stone in the CBD. A: U/S image and B: MRCP image

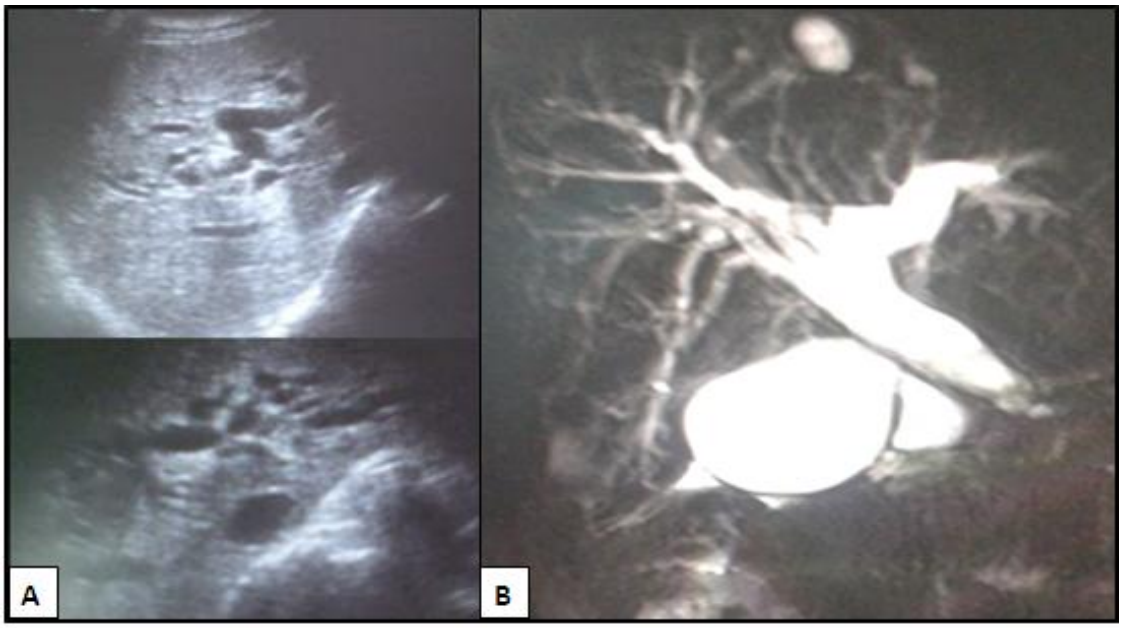

Fig. 2. 42 years old female presented with obstructive jaundice caused A: U/S image show dilated intra-hepatic & extra-hepatic bile ducts. B: MRCP image show dilated intra-hepatic & extra-hepatic bile ducts with smooth tapering at the distal part of the CBD. This case is proved to be benign stricture in CBD

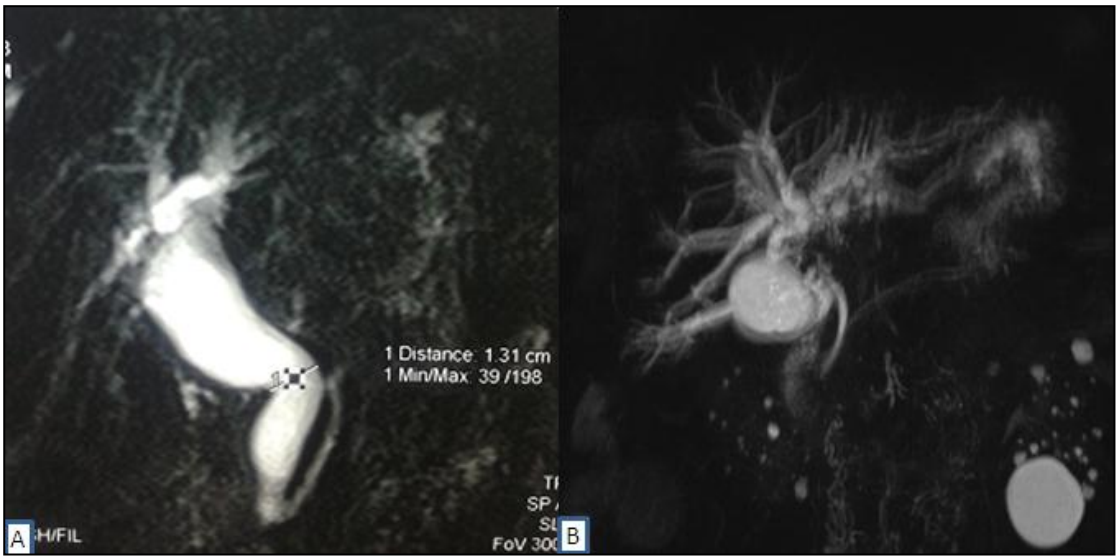

Fig. 3. MRCP in 2 different patients with malignant stricture due to cholangiocarcinoma. A: 54 years old male with distal CBD stricture. B: 62 years old male with proximal CBD stricture

Discussion

Jaundice is a common problem in medical and surgical gastroenterological practice. The surgical jaundice can be caused by the obstruction of the bile duct as with gall stones, strictures, malignancy, such as cholangio-carcinoma (in which the jaundice is persistent and progressive), periampullary carcinoma, carcinoma gall bladder and carcinoma head of pancreas. Obstructive jaundice is common amongst females and Choledocholithiasis is the commonest benign cause [(29)].

Ultrasound is the imaging tool of choice for evaluation of the biliary system and is accurate

in diagnosing cholelithiasis in 97% of cases [30]. Previously reported studies showed a sensitivity range of 20-80% [31-34]; these wide ranges of differences in sensitivity among various case series are partially attributable to the impossibility of approaching the distal CBD and ampullary region in obese patients and patients with abdominal meteorism, as well as to the variability of the US technique applied. The high sensitivity in our case series presumably derived from the change in patient's morphology and quality of the device. Since our data show that U/S and MRCP have the same diagnostic potential in choledocholithiasis, the purpose of US is to select candidates for therapeutic ERCP without proceeding to MRCP [33]. In our opinion, however, MRCP should only be applied for the correction of possible false negative cases from ultrasound. MRCP, for its high diagnostic value, is necessary in patients showing equivocal clinical and laboratory findings and negative U/S prior to performing ERCP, which is invasive procedure. Our data on MRCP diagnostic accuracy, sensitivity and specificity are comparable to the previously reported literature [32,35-41], the lowest figures for sensitivity, reported by Little et al [32] and Stiris et al [35] were due to their initial inexperience in accurately detecting small calculi in the distal CBD, and not to the limitations of MRCP.

MRCP and ultrasound virtually resolve almost all diagnostic problems, and have therefore considerably restricted the role of diagnostic ERCP. Although most authors make no distinction among the types of biliary stenosis, we subdivided them into malignant and benign stenosis, and the diagnostic value of the imaging techniques was assessed for each type, also considering that clinical and laboratory findings frequently overlap at disease onset.

The analysis of data in benign stenosis shows how diagnostic result for MRCP overlap, whereas US is highly reliable for ruling out benign stenosis, though not for demonstrating their presence. In our U/S study of benign stenosis, we obtained fair accuracy, high specificity and low sensitivity. The high specificity was attributable to the capability of U/S to detect true negatives in benign stenosis, thus showing the cause of the obstruction by calculi or malignant stenosis. The low sensitivity figures are to be related to intrinsic limitations of the methodology, which, though showing the indirect signs of stenosis [38,42], do not allow for optimal visualization of the distal CBD and the ampullary region, which is where benign stenosis are often localized. However, our comparison of U/S and MRCP reveals overlapping performance, though MRCP performed better in detecting true positives. MRCP's diagnostic accuracy, sensitivity and specificity are always high and higher than those reported by Arslan et al [43], who compare MRCP and ERCP in 78 patients with obstruction and reported a sensitivity and specificity of 86.4% and 82.4% respectively for benign stenosis.

In our study, MRCP and U/S performed on an equal level in detecting true negatives and positives in malignant stenosis. In the literature, the majority of U/S studies on malignant stenosis demonstrate a high sensitivity, and specificity and diagnostic accuracy, though-in contrast to our study- extrinsic and intrinsic causes of obstruction are examined together. Sharma et al [44] obtained a sensitivity and specificity for U/S of 94% and 96% respectively; these high figures are attributed to the prevalence of patients with GB carcinoma and pancreatic head carcinoma. Chamberlain et al [45] reported a sensitivity of 93% and specificity of 99% for U/S in the identification of the obstruction site and portal involvement when occurring. Bloom et al [46] reported a sensitivity and specificity of 98% and these high figure because both intra- and extra-hepatic cholangiocarcinomas are included in this study. In our study, US showed high diagnostic accuracy and specificity and relatively low sensitivity. These low values were presumably related to the small dimension of some cholangiocarcinomas (only showed by dilatation of the biliary ducts at US) and /or there localization in the distal CBD, a region of difficult approach for US. Our data on MRCP can be

compared with those reported on malignant stenosis by Arslan [43] (sensitivity of 88.6% and a specificity of 94.1%) and slightly lower than those by Little et al [32] (diagnostic accuracy, sensitivity and specificity of 97%,93% and 100%), Lomas et al [36] (sensitivity of 100% and specificity of 98%) and Hussein et al [38] (sensitivity and specificity of 100%); it must be born in mind that Lomas and Hussein do not make a distinction between benign and malignant stenosis. Our low sensitivity figures (72%) were due to the presence of small cholangiocarcinomas. Liang et al [47] showed the diagnostic accuracy of MRCP in malignant obstruction was 82.9%, and MRCP was found to have high diagnostic specificity for determining the location and extent of obstruction.

In conclusion, the most common cause of extra-hepatic intrinsic biliary obstruction was choledocholithiasis. Ultrasound is still the first choice imaging procedure of biliary obstructive disease, as it can shape the subsequent diagnostic and therapeutic approach, its high specificity, above MRCP in cases of lithiasis, allows patients to be referred directly for either ERCP or surgery. However, owing to its low sensitivity in most of the benign stenoses and distal CBD disease, where the clinical and laboratory suspicion is strong and unsupported by ultrasound and/or in the presence of conditions affecting ultrasound performance, and for a thorough staging evaluation of malignancy, MRCP is required.

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Received 25th Jun. 2013: Accepted 25th Nov. 2013

Iraqi JMS

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

Zinc Therapy in Treatment of Acute Diarrhea in Children Less Than Two Years

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Abstract

Background Diarrhea is the leading cause of morbidity and mortality in children in developing countries, intervention trials showed that using zinc in acute diarrhea decrease morbidity and mortality.

Objective To evaluate the role of zinc supplement in the recovery of hospitalized children with acute diarrhea.

Method The study included one hundred patients presented with acute diarrhea (age 3 months -2 years), forty eight of them were males and fifty two were females, sixty of them were infants and forty were above twelve months. Fifty patients were treated with intravenous fluid only and the other fifty patients were treated with zinc in addition to intravenous fluid.

Results The study showed that the percentage of improvement within the first three days in patients treated with zinc was 80% compared to 44% of control group with *P* value 0.001. Zn therapy reduce the severity of diarrhea, it is effective in infants but more effective in toddlers, it is similarly effective in both males and females, it is more effective in mixed fed babies than in those on breast or bottle feeding .Using low dose reduce the incidence of vomiting.

Conclusion Zinc therapy is useful in decreasing the duration and severity of acute diarrhea in children.

Keywords Zn (zinc), acute diarrhea.

Introduction

Diarrhea is one of the most important causes of morbidity and mortality in children [(1)]. The World Health Organization (WHO) suspects that there are > 700 million episodes of diarrhea annually in children < 5 yr of age in developing countries. While global mortality may be declining, the overall incidence of diarrhea remains unchanged at about 3.2 episodes per child per year. The decline in diarrheal mortality, despite the lack of significant changes in incidence, is the result of improved case management of diarrhea, as well as improved nutrition of infants and children [(2)]. Major risk factor includes environmental contamination and increased exposure to enteropathogens. Additional risks include young age, immune deficiency, measles, malnutrition, and lack of exclusive or predominant breast-feeding. In children with vitamin A deficiency, the risk of dying from diarrhea, measles, and malaria is increased by 20-24% [(3,4)]. Zinc is the second most abundant trace mineral and is important in protein metabolism and synthesis, in nucleic acid metabolism, and in stabilization of cell membranes. Zinc function as a cofactor for more than 200 enzymes and is essential to numerous cellular metabolic function [(5)]. It also improves the absorption of water and electrolytes, improves regeneration of the

intestinal epithelium, increases the levels of brush border enzymes, and enhances the immune response, allowing for a better clearance of the pathogens [6]. Shellfish, beef, and other red meats are rich sources of zinc, nuts and legumes are relatively good plant sources of zinc [7]. Dietary Zn, which is ingested with food such as meat, grains, and fruits, is absorbed at a rate of 20-80% in the duodenum and proximal small intestine. Histidine and cysteine facilitate absorption, where as phytate and fiber inhibit it. Excess dietary copper, iron or cadmium decrease Zn absorption by competing for cellular uptake and metallothion binding. After cellular uptake the metal is secreted into the portal circulation where it binds primarily to albumin. Zn absorbed from the intestine is taken up rapidly by the liver, kidney, pancreas, and spleen. Excretion occurs through fecal loses. In the presence of ongoing loses such as diarrhea requirement can dramatically increase [8].

The objective of this study was to evaluate the role of zinc supplement in the recovery of hospitalized children with acute diarrhea.

Method

This was a prospective clinical study in Al-Kadhimiya Teaching Hospital designed to evaluate the effectiveness of supplying daily zinc during acute diarrhea (without vomiting) episodes on the course and outcome of the illness. One hundred patients aged 3 months-2 years were, with a presenting symptom of acute diarrhea for less than 72 hours admitted between the 1st of June 2012 till 31 October 2012, they were divided into two groups, control group include 50 children treated with intravenous fluid (IVF) only, and other 50 treated with IVF plus zinc therapy (zinc group). Zinc sulfate tablet available in forms of 20 mg given to the patient (according to WHO guideline) 10 mg for less than 6 months age and 20 mg for more than 6 months age for 14 days; if vomiting or regurgitation occurs within 1 hr after zinc administration another dose given by dividing dose regimen. The improvement of the patient was considered when there is decrease in frequency and volume of bowel motions with correction of dehydration within three days of treatment.

Statistical analysis

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Nominal variables were expressed as number and percent. Chi-square test was used to study association between nominal variable, *P* value <0.05 was considered as statistically significant. Relative risk was calculated as number and 95% confidence interval.

Results

One hundred cases presented with acute diarrhea were admitted to Al-Kadhimiya Teaching Hospital. Fifty of them were treated with Zn therapy and IVF while other fifty cases were treated with IVF only. Table (1) shows that the percentage of improvement after 72 hours in cases who were treated with Zn and IVF was 40 patients (80%) while for those treated with IVF only was 22 patients (44%), the relative risk was (1.81) and (*P* < 0.001) which statically significant value.

Table 1. Effect of Zn on treatment of acute diarrhea

Type of treatment	No. of improved children	No. of non-improved children	Total	% of improvement	P value	Relative risk (95%CI)
IVF+Zn	40	10	50	80	< 0.001	1.81 (1.29-2.56)
IVF only	22	28	55	44		
Total	62	38	100	62		

Regarding the age of patients, in infants (aged less or equal to 12 months) the improvement was more evident in patients who receive Zn therapy and IVF 23 (76.67%) patients while for those treated with IVF only was 14 patients (46.67%), the relative risk was 1.64 (*P* = 0.017).

Children aged more than 12 months the improvement was more evident in patients who receive Zn therapy and IVF, 17 patients (85%) while for those treated with IVF only was 8 patients (40%), the relative risk was 2.12 (*P* = 0.003) as shown in table 2.

Table 2. Effect of Zn therapy in relation to age

Age (Months)	Type of treatment	No. of improved children	No. of non-improved children	Total	% of improvement	*P* value	Relative risk (95%CI)
> 12 (n = 40)	IVF+Zn	17	3	20	85	0.003	2.12 (1.20-3.74)
	IVF only	8	12	20	40		
	Total	25	15	40	62.5		
≤ 12 (n = 60)	IVF+Zn	23	7	30	76.67	0.017	1.64 (1.06-2.52)
	IVF only	14	16	30	46.67		
	Total	37	23	60	61.67		

Regarding sex of patients, in males the improvement was more evident in patients who receive Zn therapy and IVF 21 patients, (84%) while for those treated with IVF only was 11 patients, (47.82%), the relative risk was 1.75 (*P* = 0.008).

In females, the improvement was more evident in patients who receive Zn therapy and IVF 19 patients (76%) while for those treated with IVF only was 11 patients (40.74%), the relative risk was 1.86 (*P* = 0.01) as shown in table 3.

Table 3. Effect of Zn therapy in relation to gender

Gender	Type of treatment	No. of improved children	No. of non-improved children	Total	% of improvement	*P* value	Relative risk (95%CI)
Males (n = 48)	IVF+Zn	21	4	25	84	0.008	1.75 (1.10-2.78)
	IVF only	11	12	23	47.82		
	Total	32	16	48	66.67		
Females (n = 52)	IVF+Zn	19	6	25	76	0.01	1.86 (1.12-3.09)
	IVF only	11	16	27	40.74		
	Total	30	22	52	57.69		

with IVF only was 8 (40%), the relative risk was 1.75 (*P* = 0.057).

In mixed feeding, the improvement was more evident in patients who receive Zn therapy and IVF 13 (92.85%) patients while for those treated with IVF only was 6 patients (42.85%), the relative risk was 1.71 (*P* = 0.013) as shown in table 4.

Regarding feeding pattern, in the breast fed babies, the improvement was more evident in patients who receive Zn therapy and IVF 14 (87.5 %) patients while for those treated with IVF only was 7 patients (43.75%), the relative risk was 2.00 (*P* = 0.009).

In bottle fed babies, the improvement was more evident in patients who receive Zn therapy and IVF 14 (70%) patients while for those treated

Table 4. Relationship between Zn therapy and type of feeding

Feeding	Type of treatment	No. of improved children	No. of non-improved children	Total	% of improvement	*P* value	Relative risk (95%CI)
Breast (n = 32)	IVF+Zn	14	2	16	87.5	0.009	2.00 (1.11-3.59)
	IVF only	7	9	16	43.75		
	Total	21	11	32	65.62		
Bottle (n = 40)	IVF+Zn	14	6	20	70	0.057	1.75 (0.95-3.21)
	IVF only	8	12	20	40		
	Total	22	18	40	55		
Mixed (n = 28)	IVF+Zn	13	1	14	92.85	0.013	2.16 (1.16-4.04)
	IVF only	6	8	14	42.85		
	Total	19	9	28	67.85		

Vomiting occur in 5 (10 %) patients who were treated by Zn therapy and IVF and in 4 (8%) patients who were treated by IVF alone and the relative risk was 1.25% (*P* = 0.1000) as shown in table 5.

Table 5. Occurrence of vomiting in both groups (Zn and control group)

Type of treatment	Occurrence of vomiting	No. of vomiting	Total	% of vomiting	*P* value	Relative risk (95%CI)
IVF+Zn	5	45	50	10	1.000	1.25 (0.35-4.38)
IVF only	4	46	50	8		
Total	9	91	100	9		

Discussion

This study showed that zinc supplementation is effective in decreasing the frequency and volume of stool in acute diarrhea, which is similar to many other studies such as (65%, 39%) obtained in Nepal [9], and (70%, 42%) in West Bengal [10], and (66%, 38%) in New Delhi study [11], other studies showed zinc has no effect on frequency or volume of stool but it decrease duration of diarrhea as obtained by Khan AM, Larson [12]. In this study, toddlers showed better response than infants to zinc therapy (85%, 76%, respectively). A similar difference was also found in a study performed in North India [13].

This difference in improvement in relation to age may be explained by the following factors:

1. Milk contains small amount of Zn, and infants whose diet is mainly milk are expected to have Zn deficiency more readily than toddlers whose diet may include meat, nuts, and other rich sources of Zn, also mothers of breast fed infants may have zinc deficiency [14], both these facts cause serum level of zinc in infants at lower values than in toddlers, so they need longer duration of treatment to show improvement,
2. The calcium which is present in milk in high concentrations is known to decrease Zn absorption from intestine by competition [15].

According to gender, males group treated with zinc and IVF showed much better improvement than those treated by IVF only. A study performed in Guatemala, showed similar improvement in males compared to females [16].

According to types of feeding, our study shows that there is a good response in breast-fed zinc group in comparison to breast-feed control group. There is better response for bottle fed zinc group in comparison to bottle feed control group.

In addition, there was a better response for mixed fed zinc group in comparison to mixed fed control group. The impact of adding Zn in the management of acute diarrhea was more impressive in mixed fed infants compared to both breast and bottle fed ones, this may be attributable to the fact that [15] this effect of breast milk on Zn absorption, may be also the cause beyond the occurrence of the manifestation of acrodermatiris enteropatheca after weaning of breast to cow milk [11]. These results are supported by Al-Zubiady study [16]. In our study the percentage of vomiting was (10%) in Zn plus IVF group, and (8%) in IVF only group. These results were better than those obtained from Nepal study (14.2 % in Zn plus IVF group, and 8.1% in IVF only group), this difference is probably resulted from using small divided doses in our study, while in Nepal study higher dose of oral zinc were used 15 mg/day as a single dose for infant, and 30 mg/day as a single oral dose for toddlers which was associated with increased risk of vomiting [9].

In conclusion, addition of zinc to the management of acute diarrhea is useful in decreasing severity of diarrhea in form of decreasing the volume and the frequency of stool.

- Zinc therapy is effective in both infant and older age group.
- Zinc therapy is more effective in mixed fed babies than in those on breast or bottle feeding.
- Zinc therapy is effective in both males and female.
- Using of divided dose regimen decreases the frequency of vomiting and regurgitation.

We recommend encouraging the use of zinc therapy in primary health care centers and educate the mothers about the importance of Zn in treatment of diarrhea.

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E-mail: k.albahadle@Yahoo.com
Received 9th Jun. 2013: Accepted 2nd Dec. 3013

Iraqi JMS

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

Job Satisfaction among Al-Kadhimiya Teaching Hospital's Medical Doctors

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Abstract

Background Physicians have a crucial role in health service delivery and therefore their job satisfaction may lead to improved quality in patient care and may cut down costs of care by reducing patient stay in the hospital.

Objectives To determine the level of job satisfaction among medical doctors who work at Al-Kadhimiya Teaching Hospital in Baghdad, Iraq.

Methods Cross sectional study was conducted at Al-Kadhimiya Teaching Hospital. The research questionnaires distribution time was from January 9th 2013- March 30th 2013.

Results Three hundred and twenty seven medical doctors participated in this study. Age mean was (35.8±8 years). Only (12.5%) of the participating medical doctors were satisfied with their job.

Conclusion The majority of medical doctors were not satisfied with their job at Al Kadhimiya Teaching Hospital.

Key words Job satisfaction, Teaching hospital, medical doctors.

Introduction

Job satisfaction and motivation are key organizational elements that ensure quality work, promote personal growth, maintain physical and psychological health, and decrease attrition [1,2]. One of the primary reasons for evaluating employee satisfaction is to identify problems and try to resolve them before they impact on patient care and treatment [3].

Management needs information on employee job satisfaction in order to make sound decisions, both in preventing and solving employee problems [4]. Employees who have higher job satisfaction are usually less absent, less likely to leave, more productive, more likely to display organizational commitment, and more likely to be satisfied with their lives [5]. Hospitals are the key element in any health care system. Job satisfaction can be a powerful determinant of patient satisfaction, and patient satisfaction in turn is related to compliance with medical regimens, improved patient care and better outcomes [6,7]. Job satisfaction is important to the future recruitment of new doctors and retention of the existing doctors, in addition to the productivity and quality of the services provided by the doctors, who are an essential and integral component of medical care system [8].

Understanding trends in physician career satisfaction and how changes in the practice environments of physicians affect their career satisfaction is important for several reasons. First, physician satisfaction is associated with quality of care, particularly as measured by patient satisfaction. Second, dissatisfied physicians are more likely to leave the profession and discourage others from entering

[9]. Physician satisfaction is a critical topic not only for physicians but also for patients and health care administrators. When physicians are satisfied, they are significantly more likely to stay in a given practice and as a result, plan administrators are saved the financial costs associated with high turnover, as well as the decline in patient satisfaction that often accompanies high turnover [10].

The objectives of this study were:

1. To determine the level of job satisfaction among medical doctors who work at Al-Kadhimiya teaching hospital in Baghdad, Iraq.
2. To study the association between job satisfaction, the respondents' socio-demographic variables and professional characteristics such as: age, sex, marital status, highest academic or vocational degree, occupational title, total years of service at Al-kadhimiya Teaching Hospital, total years of service since employment, average weekly working hours, average monthly night shifts, monthly salary, doctor's housing evaluation, hospital's food service evaluation, choosing to be a medical doctor again and private practice.
3. To study the association between job satisfaction and different job's related factors such as: promotion, supervision, benefits, contingent rewards, operating procedures, co-workers, work and communication.

Methods

A cross-sectional study was conducted at Al-Kadhimiya Teaching Hospital. All medical doctors who work at Al-Kadhimiya Teaching Hospital at the time of conducting this research and met the inclusion criteria were invited to participate. The inclusion criteria were all medical doctors who were employed at Al-Kadhimiya Teaching Hospital at time of conducting this research with at least three months of professional service at the hospital were included in the study. Specialist doctors who were employees of the Ministry of Higher education and scientific research (Faculty member of Al-Nahrain Medical College) with a part-time work at the Ministry of Health (at Al-Kadhimiya Teaching Hospital) were not included in this research.

The research questionnaires distribution time was from January 9th 2013- March 30th 2013. A pilot study was conducted on 10 medical doctors who were working at Al-Kadhimiya Teaching Hospital, their feedbacks made the researcher translate fourteen question of the job satisfaction survey to Arabic language either totally or a single word only in addition to its original English language (based on the pilot study; some doctors did not understood a particular word in a certain questions; while others did not understood a whole questions). Those ten medical doctors were not included later in the study. Participation in this research was completely voluntary.

The participants gave their verbal consent to participate after being briefed about the research objectives. The participants were advised to return the completed questionnaire within a month enclosed in a special envelope (size of A4 paper) which was provided by the researcher to each participant in addition to the research questionnaire. A structured self-administered questionnaire was used to collect data from the participants. It consisted of Section A: Socio-demographic and professional characteristics form consisting of 15 questions (10 closed- ended and 5 fill in the blank items) developed by the researcher plus two open ended questions.

Section B: consisted of Job Satisfaction Survey (JSS) developed by Spector (1985) [11], JSS which is a 36 items, nine facet scales to assess employee attitudes about the job and aspects of the job. Each facet is assessed with four items, and a total score is computed from all items. A summated rating scale format is used, with six choices per item ranging from "strongly disagree" to "strongly agree". Items are written in both directions, so about half must be scored reversely. The nine facets are Pay, Promotion, Supervision, Fringe Benefits, Contingent Rewards (performance-based rewards), Operating Procedures (required rules and procedures), Coworkers, Nature of Work, and Communication.

JSS was developed, normed, and validated on human service personnel, making it of specific applicability to human services. The JSS seems to be a reasonable satisfaction scale for human service employees. Reliability data suggest that the total scale and subscale have reasonable internal consistency, and the limited test – retest data indicate good reliability over time [11]. Data entry and analysis was done by SPSS software (version 16). Level of significance was set at 0.05.Cronbach's Alpha was computed to ensure internal consistency of the measuring instrument. Inferential methods included Pearson correlation test, independent t test and one way ANOVA test.

Results

A total of 540 questionnaires were distributed. Out of these, 327 medical doctors from Al-Kadhimiya Teaching Hospital participated and successfully returned the completed questionnaire; the response rate was 60.55%. As shown in table 1; the age mean was 35.8±7.9 years. 43.4% of the sample was between 30-39 years old. The mean of total years of service in Al-Kadhimiya Teaching Hospital was 3.56±3.48 years. The mean total years of service since employment were 11.34±7.72 years. The mean of approximate weekly working hours was 54±22 hours. The mean monthly night shifts were 5±4 month.

Table 1. Job related factors of the study population

Variables	Mean±SD	Median
Age (years)	35.80 ±7.905	34.00
Total years or service in Al-Kadhimiya Teaching Hospital	3.5606 ± 3.48189	2.0000
Total years of service since employment	11.3416 ± 7.71984	10.0000
Average Weekly working Hours	53.75 ± 22.199	50.00
Average monthly night shifts	4.78 ± 3.949	4.00

As shown in Table 2; 60.2% of the sample was male, 75.2% were married, 2.8% divorced, 20.2% single. 72.2% of them held MBChB degree, 72.2% earn 1-2 million Iraqi Dinar monthly, only 1.5% evaluated their residential housing as average, no one evaluated their residential housing as excellent, 3.4% evaluated their food service as good, no one evaluated it as excellent, 55.7% will choose to be medical doctors again, 59.6% did not have private practice, 53.8% were Permanent resident doctors (medical board or medical diploma trainee), 4.3% were junior resident doctors, 19.9% were permanent resident doctors, 22 % were specialist doctors. The results showed that 3.9% of the sample was dissatisfied, 53.5% of the sample was ambivalent and 12.5% of the sample was satisfied. The average overall job satisfaction score was found to be 116.72 (ambivalent), indicating neutral level of satisfaction among medical doctors at Al-Kadhimiya Teaching Hospital.

It has been found that the lowest average satisfaction factor was pay 10.39±4.03 and the highest average satisfaction factor was nature of work 16.6±4.45, which is also the only factor that the respondents were found to be satisfied with. Cronbach's alpha reliability for this study was determined by including all the nine factors of job satisfaction survey questionnaire (Pay, Promotion, Supervision, Benefits, Contingent rewards, Operating procedures, Coworkers, Nature of work, and Communication). Reliability analysis showed that the Cronbach alpha coefficient of the questionnaire was 0.839, which is considered relatively high and internally consistent [12].

The results showed (Table 3) a significant positive relationship between overall job satisfaction score and age in years (r = 0.229, *P* = 0.000), total years or service in Al-Kadhimiya Teaching Hospital, which was statistically significant (*r* = 0.177, *P* = 0.01) and total years of service since employment, which was statistically significant (*r* = 0.215, *P* = 0.000).

There was a weak, negative correlation (Table 3) between overall job satisfaction score and

average weekly working hours, which was statistically significant (r = - 0.194, P = 0.000), average monthly night shifts, which was statistically significant (r = - 0.136, P = 0.014).

As shown in table 4, this study found that medical doctors who are males and medical doctors who are females had no statistically significant difference in comparison to the overall mean job satisfaction score (t (2.14), P = 0.145). Choosing to be a medical doctor again or not and having private practice or not had statistically significant difference in comparison to the overall job satisfaction score (t (13.34), P = 0.0003) and (t (25.13), P = 0.00001) respectively. There was a statistically significant difference between groups as determined by one-way ANOVA based on (Table 5). Highest academic or vocational degree attained F (4.145), P = 0.007), the hospital food service evaluation (F (4.172), P = 0.003), occupational title (F (3.975), P = 0.008), monthly salary categories (F (8.161), P = 0.000), weekly working hours category (F (4.587), P = 0.000), Al- Kadhimiya Teaching Hospital total years of service category (F (4.585), P = 0.001), total years of service since employment category (F (2.615), P = 0.012), participants' age category (F (3.598), P = 0.004), and night shifts categories (F (3.009), P = 0.030).

There was a no statistically significant difference between groups as determined by one-way ANOVA based on their housing evaluation (F (1.9), P = 0.110) and marital status (F (.812), P = 0.488).

Table 2. Distribution of the socio demographic characteristics of the study population

Variable		Frequency	%
Sex	Female	130	39.8
	Male	197	60.2
Marital Status	Divorced	9	2.8
	married	246	75.2
	single	66	20.2
	separated	0	0
	widowed/widower	5	1.5
Highest academic degree	MBChB	236	72.2
	Diploma	28	8.6
	MSc	7	2.1
	PhD	0	0
	Medical board or equivalent	56	17.1
Monthly salary	< 1 million ID	32	9.8
	1-2 million ID	236	72.2
	> 2 million ID	59	18.0
Hospital-Doctor's housing Evaluation	Not using the doctor's housing building	73	22.3
	Poor	82	25.1
	Below average	118	36.1
	Average	49	15.0
	Good	5	1.5
	Excellent	0	0
The hospital food service evaluation	Not using the Hospital's food service	70	21.4
	Poor	78	23.9
	Below average	99	30.3
	Average	69	21.1
	Good	11	3.4
	Excellent	0	0
Being a medical doctor again	No	131	40.1
	Yes	182	55.7
Non- Ministry of health Private practice *	No	195	59.6
	Yes	125	38.2
Occupational title	Junior RD	14	4.3
	Permanent RD	65	19.9
	Permanent RD (Medical board or diploma trainee)	176	53.8
	Practitioner doctor	0	0
	Specialist doctor	72	22.0

* = missing values existed, ID = Iraqi Dinars, RD = resident doctor

Table 3. Pearson correlation test of overall job satisfaction score in comparison to various continuous characteristics

	Variables	Pearson Correlation Coefficient	P Value
Overall Job satisfaction score	Age (Years)	0.229	0.000
	Total years or service in Al-Kadhimiya Teaching hospital	0.177	0.001
	Total years of service since employment Average	0.215	0.000
	Weekly working Hours	-0.194	0.000
	Average monthly night shifts	-0.136	0.014

Table 4. Independent t test of overall job satisfaction score in comparison to sex of the participants, Choosing to be a medical doctor again or not and having Private practice or not

	Parameter		No.	Mean±SD	t	df	P value
Overall Job satisfaction score	Sex	Female	130	114.27±24.345	2.14	325	0.145
		Male	179	118.35±24.925			
	Choosing to be a medical doctor again or not	No	131	110.48±25.458	13.34	311	0.0003
		Yes	182	120.58±23.132			
	Private practice	No	195	110.98±25.646	25.13	318	0.00001
		Yes	125	124.69±20.781			

Table 5. ANOVA results between overall job satisfaction score, socio demographic and Professional characteristics

	Variables	No.	df1	df2	F	P Value
Overall Job satisfaction score	Highest academic or vocational degree attained	327	3	323	4.145	0.007
	Hospital-doctor's housing evaluation	327	4	322	1.900	0.110
	The hospital food service evaluation	327	4	322	4.172	0.003
	Occupational title	327	3	323	3.975	0.008
	Monthly salary	327	2	324	8.161	0.000
	Weekly working hours categories	326	5	320	4.587	0.000
	Weekly working hours categories	326	5	320	4.587	0.000
	Al-Kadhimiya Teaching Hospital total years of service category	327	4	322	4.585	0.001
	Total years of service since employment category	327	7	319	2.615	0.012
	Participants' age category	327	5	321	3.598	0.04
	Night shifts category	327	3	323	3.009	0.30
	Marital status	326	3	322	0.812	0.488

Discussion

The response rate was 60.5%. This response rate level could be attributed to their heavy workload, lack of motivation or both. A study from Northern Iraq in 2006 in the cities of Erbil and Kirkuk had shown that 75.8% responded as always or usually, satisfied with their career as a physician. In this study, only 8.6% of the male doctors were found to be satisfied with their job. This big difference may be attributed to differences in methodology of research (for example only male physicians were included), or

to real difference in the level of job satisfaction. The northern part of Iraq has different health system administration [13]. A study from Egypt has shown that the satisfaction level among medical doctors was 38.7% [14]. A study from Kuwait (N = 60) has shown that 50% of the participating physicians were generally satisfied with their job [15]. A study from Norway (N = 1174) in 2002 has shown an average of 52.0 on the job satisfaction scale (range 10 to 70) [16]. A study from Pakistan (N = 99) has shown that overall job satisfaction was found to be 61.9% [17]. A study from Japan (N = 698) has shown that 60% of the Japanese doctors were satisfied with their job [18]. A study from India (N = 100) has shown that the proportion of doctors satisfied with their jobs in the teaching tertiary health care center were 69.5% [19].

This big difference in the satisfaction level among medical doctors from Iraq and medical doctors from the above-mentioned countries could be attributed to the current situation in Iraq in terms of lacking security and political instability. The more years the doctors spend in a hospital the more they accustom themselves to the work environment and the better co-workers relationship they can achieve. More total years of service mean more salary, maybe less working hours and to some extent more autonomy at work. Autonomy means freedom to make decisions without referring to a supervisor; autonomy to medical doctors came with more years of service; for example; a medical board trainee will became a specialist doctor when he/she finished their training period; once they became specialists, they are free to treat their patients without referring to their supervisors. More working hours mean more doctor-patient interaction, more psychologically demanding clinical decisions, more physical demand and may lead to job dissatisfaction in the end.

More night shifts mean more working hours in unsociable time, more days away from home and family, more doctors-patients interactions, more usage of the hospital services like housing and food services, which was not evaluated as good by many of the doctors; all the aforementioned factors may solely or combined lead to job dissatisfaction. A study from India [20] has revealed that increasing dissatisfaction with the number of night duties per month.

Although the current study has found no statistically significant association between overall Job satisfaction score and sex, doctor's gender and job satisfaction may be related to each genders' "suitable to do" in oriental community such as in Baghdad. For instance, a male doctors may have no or very few limitations on spending overnight shifts at the hospital, while a female doctor may find it a no easy task to do the same job; a night shift in a ward may be easier for a male doctor to stay in the resident room, while it may be a very difficult for a female doctor to do the same due to religious beliefs and traditions of the Iraqi society. A study from Egypt [14] has found that neither age nor gender was significantly associated with the degree of job satisfaction. Doctors who have private practice have more income, more money at hand can simply lead to better living standards, better living facilities, and that may lead to job satisfaction. Doctors' academic or vocational degrees and their occupational titles are much related, as most doctors who have only Bachelor degree in medicine are most likely not specialists and their work burden are expected to be heavier, which might be a source of their dissatisfaction. Occupational title and job satisfaction can be understood as the higher hierarchy the doctor might reach the more autonomous, the more salary he/she gets, and possibly less night shifts. The researcher believed that salary by itself can't simply lead to satisfaction or dissatisfaction because as the results have shown that pay was the least satisfying factor that medical doctors evaluated. Weekly working hours and job satisfaction is an intricate issue, as there are no time limits for the work of resident doctors and their salary is never increased on a working hour basis, as a matter of fact, it may decrease, if a doctor gets punished. Residents are not treated as government employees; they are treated as

under training personnel on contract basis. Pathman reported that physicians in the oldest age group indicated greater satisfaction than younger physicians [21]. The Norwegian study [16] has found that there was a moderate positive correlation with age (job satisfaction increased with increasing age). A study from India has found no statistically significant association between age of the doctors and job satisfaction [20]. The relationship between total years of service in Al-Kadhimiya Teaching Hospital and the job satisfaction may be related to better use of the work environment and better co-workers relationship over newly employed. For instance, a newly specialist doctors might not find an office to him or herself which could be a source of their dissatisfaction, while a nearly retired doctor has his/her own office in the hospital for many years ago, and he or she is not ready to leave his office until retirement.

The researcher believed that the real reason behind satisfaction is not years by itself, but the lesser workload, the higher wages, the more preferential treatment by the administration that usually came with more years of service in a particular place of work. More working hours means more doctors-patients interactions at most in a heavy workload environment it may lead to physical and psychological exhaustion, which might be the reason of dissatisfaction. More night shifts simply means more hospital works, more doctor-patient interactions, more decisions at work to be made; All combined can lead to dissatisfaction and may be even confrontation at work. A study from India [20] has found that dissatisfaction was significantly greater among doctors who had an average of ≥8 night shifts per month (i.e., twice a week).

We can conclude that the majority of medical doctors were not satisfied with their job at Al-Kadhimiya Teaching Hospital.

Acknowledgements

We would like to express my gratitude to all the medical doctors who participated in this research. My thanks also go to Dr. Emad Mahmud, the head of the community department at Al-Kadhimiya Teaching Hospital for his help in making this study completed.

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Received 7th Oct. 2013: Accepted 4th Dec. 2013

Iraqi JMS

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

Body Mass Index and Total Serum Leptin Level in Abortion

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Abstract

Background Adipose tissue secretes variety of adipokines, including leptin, which is involved in endocrine processes regulating reproduction and plays an important role in energy metabolism and fetal development during pregnancy.

Objectives To investigate the relationship between total serum leptin concentrations and anthropometric parameters including body mass index (BMI) in aborted women at the second trimester.

Methods A case control study was carried out from November 2011 to April 2012. The patients' group includes 30 aborted women at the second trimester. They were collected from Al-Elweyia, Al-Hakeem and Al-Khadhemiyia Teaching Hospital in Baghdad. Thirty healthy pregnant women (at their second trimester of gestation) were used as control. Patients and control were comparable in age. Blood HbA_1c and serum concentrations of total leptin, lipid profile, and glucose were measured in both groups.

Results Total serum leptin concentration were significantly lower in those with abortion at the second trimester compared with healthy pregnant control (3.5±0.8 pg/ml *vs.* 24.4±0.7 pg/ml, $P = 0.0001$) and leptin/BMI ratio *vs.* control (0.1±0.02 vs. 0.9±0.01 ml, $P=0.0001$). No correlation was found between leptin level and patient's age or gestational age in the case group. Highly significant correlation was found between patient's total serum leptin and their body mass index, HDL-C, total cholesterol/HDL-C ratio and atherogenic index ($P < 0.0001$).

Conclusion The significant correlation between patient's leptin and their BMI in addition to Leptin/BMI ratio even in non obese patient supports our objective that the unexplained abortion at second trimester is due to abnormality in their metabolic hormone action and reflect leptin resistance condition.

Keywords Second trimester abortion, Leptin, BMI, Gestational age.

Introduction

Leptin is the hormone product of the LEP gene and was originally thought to be produced only by adipocytes to aid in modulating satiety and energy homeostasis [(1)]. It is a polypeptide of 16 Kilo Dalton consisting of 167 amino acids, encoded by obese gene and is located on chromosome 7 in humans [(2)]. It is produced in large amounts by adipocytes and human placental trophoblasts. Its concentration is related to the mass of adipose tissue, and appears to be involved in modulating satiety and regulation of body weight homeostasis [(3)]. Moreover, leptin plays an important role in the reproductive system. It is involved in early embryogenesis, fat metabolism during pregnancy, and puberty [(4,5)].

Recent reports have demonstrated that leptin levels are elevated in serum during human and rodent gestations. Although increased adiposity during pregnancy might (as in obesity) be expected to underlie the hyperleptinemia of

pregnancy, leptin levels are elevated to an extent that cannot be attributed to the increased basal metabolic index (BMI), suggesting that during this state there is an additional source of leptin [6].

During pregnancy, plasma level of leptin starts to increase at the first trimester of gestation and then remarkably elevated during the second and third trimesters. These latter values are comparable to those found in obese humans. Within 24 h of delivery, the maternal plasma levels decline to normal values [7].

This study was designed to determine whether there is a relationship between total serum leptin level and BMI in women with unexplained abortion between 14-24 weeks and to estimate their leptin/BMI ratio and study its usage as an early predictive marker for abortion, which is due to abnormality in their metabolic hormone action.

Methods

A case control study was carried out from November 2011 to April 2012. The study included 30 women who had abortion at second trimester. They were collected from Al-Elweyia, Al-Hakeem, and Al-Khademiyia Teaching Hospital in Baghdad. Thirty healthy pregnant women (at their second trimester of gestation) were used as a control group. Patients' group and control were of a comparable age.

All patients who had hypertension, thyroid disease, diabetes mellitus, smoking, evidence of active infective, fever, chronic inflammatory diseases (including rheumatoid arthritis, joint pain, osteoarthritis, abdominal complain, inflammatory bowel disease); currently taking any medication, or having a positive test for cytomegalovirus (CMV) or toxoplasmosis were excluded from the study.

Blood samples were taken from patients during their admission to hospitals. Ten milliliters of venous blood were withdrawn from controls and all patients at the time of their abortion. One milliliter of it was transferred into Ethylene Diamine Tetra Acetic Acid (EDTA) tube for measuring glycosylated haemoglobin HbA_1c% by colorimetric method at 415 nm using (Bio-stand, France Kit) and the rest was transferred into a plain tube, allowed to clot, and then centrifuged for 10 min at 3000 rpm to collect serum.

Serum was used to determine, glucose, lipid profile including total cholesterol, triglyceride (TG), VLDL-C, and HDL-C [measured by the precipitation of chylomicrons] using colorimetric enzymatic method [8] (Biomaghreb, Sa, France kit). LDL-C was calculated if TG < 400 mg/dl by the formula of Friedewald *et al* [9]. Body mass index (BMI) was calculated by dividing study subjects weight (Kg) on their height (m^2).

Serum leptin concentrations were measured using an Enzyme linked immunosorbant assay (ELISA) method by using; Human Leptin (LEP) ELISA Kit (Catalog No. CSB-E04649h, CUSABIO BIOTECH CO., LTD, China). The minimum detectable concentration of human serum leptin is typically less than 1.56 pg/ml. Expected normal concentrations are between (3.5 - 12.5 pg/ml) at 450 nm.

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

Statistical analysis

Data were statistically analyzed by SPSS version 17. All data were presented as a mean± SE. Statistical differences between value of patients and control groups were determined by student *t*-test. Correlation between the variables was performed by spearman correlation coefficient. *P* value < 0.05 was considered as significant.

Results

Sixty pregnant women at their second trimester, between 14-24 weeks of gestation, were divided into 2 groups: the first one included 30 second trimester abortion women and the second includes 30 normal healthy pregnant women.

It was conducted to determine the level of total leptin and other biochemical parameters in patient's sera and compared it with normal healthy pregnant at the same trimester.

According to analysis of clinical and anthropometric data, table 1 shows no

significant difference between study groups in regard to their age, gestational age (GA) and BMI.

A high percentage was found among aborted women aged between 20-24 years old (40%), while the lowest percentage was found among those aged < 20 years old.

Regarding BMI, the higher percentage of abortion was 63.3% found among women with normal BMI (18.5-24.9 Kg/m^2) compared with 50% healthy normal pregnant with overweight BMI (25-29.9 Kg/m^2, and no significance differences was found between BMI ranges among all study groups.

Table 1. Anthropometric characteristics and clinical criteria of study subjects

Parameter	Patient (N = 30) Mean±SE		Control (N = 30) Mean±SE		*P* value
	No	Range	No	Range	
Age (years)	26.2 ± 5.8	17.0 - 37.0	25.6 ± 6.7	16.0 - 42.0	NS
GA (weeks)	19.3 ± 0.7	14 - 24	19.9 ± 0.5	14 - 24	NS
BMI (Kg/m^2)	24.60 ± 3.14	18.8 - 30.85	25.94 ± 3.59	17.36 - 33.9	NS

SE = standard error, GA = gestational age, BMI = body mass index

Women with second trimester abortion showed significantly lower serum concentration of total leptin (mean 3.5 ± 0.8 pg/ml) *vs.* healthy pregnant control at same trimester (24.4±0.7 pg/ml, *P* = 0.0001), and leptin/BMI ratio (mean 0.1 ± 0.02) *vs.* control (mean 0.9 ± 0.01 ml, *P* = 0.001 (Table 2).

Table 2. Comparison of biochemical indices between patients and control group

Biochemical parameter		Patient (N = 30) Mean ± SE	Control (N = 30) Mean ± SE
Serum lipid profile(mg/dl)	S. Total Cholesterol	173.4 ± 6.5	181.3 ± 7.9
	S. Triglyceride	128.4 ± 6.2	127.8 ± 10.8
	S. HDL-C	52.1 ± 3.8	55.4 ± 2.3
	S. LDL-C	98.1 ± 8.3	103.3 ± 6.4
	S. VLDL-C	25.7 ± 1.2	28.3 ± 2.6
	Total Chol/HDL-C ratio	3.9 ± 0.3	3.4 ± 0.2
	AI = (LDL-C/HDL-C) ratio	2.4 ± 0.3	2.1 ± 0.3
Serum FBG (mg/dl)		82.1 ± 7.7	80.5 ± 2.6
HbA_1c %		5.3 ± 0.1	5.2 ± 0.1
Serum leptin (pg/ml)		3.5 ± 0.8	24.4 ± 0.7*
Leptin/BMI ratio		0.1 ± 0.02	0.9 ± 0.01*

SE= standard error, BMI= body mass index, HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, VLDL-C=very low density lipoprotein cholesterol, AI= atherogenic index, S = serum, * *P*= < 0.001.

In table 3, highly significant differences (*P* < 0.001) were found between all patient's total leptin concentration and control sera when ranged according to subdivided ranges of age and BMI except no significant correlation was found in obese women with BMI ≥ 30 Kg/m^2 and this may be due to the small sample size of distributed subjects.

Correlations between total serum leptin and anthropometric and biochemical parameters in study subject are shown in table 4. Regarding leptin correlation among women with abortion

at second trimester group; a highly significant positive correlation was found between total serum leptin concentration and BMI (P = 0.0001, r = 0.682) as well as HDL-C (P = 0.008, r = 0.478) while a highly negative significant correlation was found between leptin and T.Chol/HDL-C ratio (P = 0.007, r =-0.479) as well as AI ratio (P = 0.007, r = -0.484).

Table 3. Distribution of serum leptin concentration according to subdivided ranges of age and BMI in the studied subjects

Parameter		Leptin (pg/ml)				*P* value
		Patient (N = 30)		Control (N = 30)		
		No	Mean±SE	No	Mean±SE	
Age (years)	< 20	3	5.0 ± 3.9	5	21.8 ± 1.6	0.003
	20-24	12	3.7 ± 1.1	10	22.8 ± 1.2	0.0001
	25-29	6	4.6 ± 2.0	6	25.1 ± 1.4	0.0001
	30-34	5	0.9 ± 0.2	5	27.3 ± 0.9	0.0001
	≥ 35	4	3.5 ± 2.2	4	26.5 ± 1.2	0.0001
P value		ns		0.050		
BMI (Kg/m^2)	< 18.5	-	-	-	-	-
	18.5-24.9	19	1.5 ± 0.2	13	20.8 ± 0.5	0.0001
	25-29.9	9	6.7 ± 1.5	15	26.9 ± 0.5	0.0001
	≥ 30	2	8.9 ± 5.7	2	28.1 ± 0.2	NS
P value		0.0001		0.0001		

SE= standard error, BMI= body mass index, ns = non significant

Table 4. Correlation between anthropometric and biochemical parameters with total serum leptin concentration in studied subjects

Parameters		Serum leptin (pg/ml)			
		Patient (N = 30)		Control (N = 30)	
		P	*r*	*P*	*r*
Age (years)		0.329	-0.184	0.005	0.500**
GA (weeks)		0.583	0.143	0.021	0.156*
BMI (Kg/m^2)		0.0001	0.682**	0.0001	0.897**
FBG (mg/dl)		0.682	0.078	0.133	0.281
HbA_1c %		0.213	0.234	0.392	0.162
Lipid profile	S. Total Cholesterol (mg/dl)	0.535	0.118	0.367	0.171
	S. Triglyceride (mg/dl)	0.721	0.068	0.089	0.316
	S. HDL-C (mg/dl)	0.008	0.478**	0.237	0.222
	S. LDL-C (mg/dl)	0.053	0.357	0.378	0.167
	S. VLDL-C (mg/dl)	0.744	0.062	0.270	0.208
	Total Cholesterol/HDL-C ratio	0.007	-0.479**	0.046	0.367*
	AI ratio	0.007	-0.484**	0.961	0.009

SE = standard error, GA = gestational age, BMI = body mass index, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, VLDL-C = very low density lipoprotein cholesterol, AI = atherogenic index, S = serum, * = $P < 0.05$, ** = $P < 0.001$.

Discussion

A striking change was noticed in the total leptin level in women who had abortion in their second trimester. Leptin level seems to be significantly decreased compared to pregnant women at the same gestational age. During pregnancy, total leptin levels are substantially elevated [10,11] and serum leptin levels are significantly higher than levels in non-pregnant women, and increases from the first to the third trimester [12].

The increase in leptin level is due to the additional production of leptin by syncytiotrophoblast in the placenta and thus leptin synthesis is elevated with the increase of the placental mass with advancing gestation [13]. Yang (2005) reported that the elevation in serum total leptin level in the second trimester is not due to placenta and adipose tissue only, but also to mammary epithelial cells, fetal tissue, gastric mucosa, and hepatic stellate cells, and the production by these organs leads to additional increase in leptin concentration resulted in a significant change in its level related to GA [13].

Žaneta *et al.* also found an elevation in maternal serum total leptin levels during pregnancy with alterations particularly during the second and third trimesters of pregnancy. They mentioned that the occurring physiological hyperleptinemia is not associated with decreased food intake or reduced metabolic activity in pregnant women [14]. Al-Atawi *et al.* (2004) and Augustine *et al.* (2008) have found that maternal leptin concentrations increase during pregnancy, but the increase seems to occur during the first two trimesters and then leptin levels decrease slightly during the third trimester [15,16]. Whereas, Grattan *et al.* (2007) and Ladyman *et al.* (2010) suggested that a lack of increase in leptin level in the 3rd trimester is reflective of late pregnancy being a leptin-resistant stage [17, 18]. In addition, Grathar *et al* (2007) reported that during the third trimester, leptin levels do not rise although body weight increases, indicative of pregnancy induced leptin resistance, and this contributes to reduce insulin sensitivity seen during pregnancy [19].

Regarding the relation between leptin level in second trimester abortion group with their BMI *vs*. same parameters in control, lower significant correlation was found between patient's leptin and BMI with positive correlation. The higher percentage was found among patients with normal and overweight BMI.

In normal pregnancy, Kim *et al.* (2008) explained that the leptin elevation is due to an increase in maternal body weight, as the serum leptin level is dependent on body weight [20]. More explanation was done by Delia *et al.* who reported that leptin elevation in maternal serum is due to the gradual increase of BMI throughout gestation, which combines, with an increase in estradiol levels that stimulate leptin production from adipocytes [11]. On the other hand ,Sagawa *et al.* (2002) and Masayo *et al*. in 2003 mentioned that BMI did not necessarily reflect body fat mass in pregnant women, and the remarkable elevation serum leptin level during pregnancy was not explicable by an increase of fat mass alone. They suggested that the rise of leptin during pregnancy is caused by the placenta production and that maternal plasma leptin levels are not correlated with body mass index [21,22].

In this study, patient's and control BMI was nearly comparable but their leptin level was lower than control and was more obvious when patient's leptin distributed according to subdivided ranges of BMI as mention in table 3. Non-significant difference between patients and controls in leptin levels was found among obese women with BMI ≥ 30 Kg/m^2. This result may be due to the small sample size.

Regarding the ratio of total serum leptin to BMI, a significant difference was found between abortion L/BMI ratio *vs.* normal pregnant ratio. This ratio was first used in 2001 by Brannian *et al* [23] as a predictive marker of outcomes in women undergoing in vitro fertilization (IVF). They grouped the ratio into three categories, low (0.1-0.3), moderate (0.4-0.6), and high (≥ 0.7). They reported that, very few patients became pregnant when their leptin was ≥ 3×10^4 pg/ml, even if their BMI was relatively low. Finally, they

concluded that this relationship might assist clinicians in counseling patients and improving the success of assisted reproduction.

Abortion L/BMI ratio in this study was found ± 0.1 which is in agreement those reported Brannian *et al.* (2001). This due to their low leptin levels despite the BMI classifications (normal, overweight and obese). Normal pregnant women L/BMI ratio shows ± 0.9 which is above the high ratio recorded by Brannian *et al* (2001). This study is the first one that determines the serum total leptin levels in women with 2nd trimester abortion.

Although age of both studied groups were comparable, a highly significant correlation was found between serum leptin levels *vs*. aborted maternal age *vs*. control age even when subdivided into ranges. Although leptin level was found higher among patients age range 20-24 years old, no correlation was found between GA and leptin level. On the contrary, other research has found a significant correlation between GA and maternal serum leptin levels [13,14]. They concluded that maternal serum leptin concentration throughout the pregnancy course correlates not only to body weight and BMI but also to GA. Furthermore, they didn't found a significant relationship between maternal serum leptin and GA in the first trimester but serum leptin was correlated to GA in the second trimester and is inversely related to GA in the third trimester. Also, maternal BMI is related to GA in the second trimester and the whole pregnancy, but not in the first and third trimesters [13].

In conclusion, obviously at second trimester abortion, maternal serum leptin level was found within the normal range and not elevated as expected during second trimester of healthy pregnancy. No correlation between patient's GA and leptin level compared with positive correlation in control, which might be due to lack of leptin production by the placenta. Finally, the significant correlation between patient's leptin and their BMI, even if they were not obese, support our objective that the unexplained abortion at second trimester is due to abnormality in their metabolic hormone action. Also, the low level of L/BMI ratio in patients group compared with high ratio found in normal pregnant group confirm the usage of this ratio as an early predictive marker for 2nd trimester abortion especially when it becomes ≤ 0.1.

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Received 11th Mar. 2013: Accepted 19th Nov. 2013

Iraqi JMS

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

Infusion Pump Surgery: Achievements and Limitation

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Abstract

Background Infusion pump is a special pump machine, with two different types, programmable and fixed flow pumps. These pumps used for administration of drugs through lumbar intrathecal route.

Objectives To evaluate the infusion pump surgery as a new surgical technique in Iraq used for treatment of pain and spasticity and to discuss the limitations and achievements regarding surgical techniques, the cases that to be selected for surgery and general conditions of selected cases pre and post surgery .

Methods Five patients were collected from Al-Kadhimiya Teaching Hospital complaining of either spastic lower limb due to dorsal spinal tumor, radiculopathy due to bullet injury not relieved by other techniques, or generalized pain due to tumors with different locations and liver metastases. Surgery done for all patients using (Isomed/Medtronic) fixed flow infusion pump.

Results Four patients have pain relief with different percentage when using morphine and one patient with spasticity showed mild improvement after baclofen pump injection.

Conclusion Infusion pump surgery is a new technique in Iraq, which is effective in the management of intractable pain and spasticity not relieved by medications.

Keyword Morphine, baclofen pumps, spasticity, isomed.

Introduction

Infusion pump is a drug infusion system includes a drug reservoir and a pump/controller assembly. The drug reservoir consists of a drug chamber and a propellant gas chamber. The pump/controller includes a bacterial filter, a controller circuit board, a battery in programmable types, and a micropump [(1)].

Implantation of an intrathecal drug delivery system is indicated for:

1. Chronic, intractable pain of malignant and/or benign origin that responds to opioids.
2. Long-term infusion of baclofen for severe spasticity of spinal or cerebral origin that responds to baclofen.
3. Long-term intravascular infusion of antibiotics or drugs for chemotherapy [(2)].

Pain management:

Approximately 10% of end-stage cancer patients undergo unavoidable pain in spite of medical treatment on WHO's advice. The intrathecal drug infusion pump is for pain control of the end-stage cancer patients by intrathecal analgesic infusion. The drug delivery efficiency is approximately 300 times as high as conventional oral administration and can be obtained with significantly less systemic side effect [(1)]. With the improvements in implantable materials and the miniaturization of computing systems, it has become a realistic possibility to implant devices for the continuous or intermittent injection of drugs into the cerebrospinal fluid (CSF) [(3)].

Neuraxial drug infusion has become a popular interventional treatment for intractable pain, especially for pain with a significant nociceptive component. Thus, the use of intrathecal analgesics for the treatment of cancer pain is well accepted. In contrast, the use of this therapy for chronic nonmalignant pain has been controversial reflecting concern that neuropathic pain (common in chronic nonmalignant pain syndromes) does not respond adequately to opioids and that the efficacy and cost effectiveness of neuraxial drug infusion for neuropathic pain have not been determined in controlled trials. Despite these concerns, intrathecal analgesic therapy has been used to treat neuropathic pain conditions with favorable results and the most common indication for intrathecal analgesic administration is failed back surgery syndrome, which typically includes components of nociceptive (low back) and neuropathic (extremity) pain[4].

Once the pump has been implanted, a catheter attached to the pump is guided to the lumbar spinal canal. The pump delivers pain relieving medication directly to the spinal canal. Any number of pain medications can be used with the pump. The advantages of the pump are to reduce the side effects of medications used for pain, enhance the quality of life, and reduce the overall cost of treatment. The clinical application range is targeted to the chronic pain patients (chronic pancreatitis, failed back surgery syndrome, reflex sympathetic dystrophy syndrome..etc.) and others who need continuous medication. The implantable intrathecal drug infusion system is known to have broad application range, excellent effect, and minimum side effect [1].

Spasticity affects about 500,000 people in the USA and more than 12 million people worldwide [5] is defined as hypertonia in which one or both of the following signs are present (a) resistance to externally imposed movement that increases with increasing speed of stretch and varies with the direction of joint movement, and/or (b) resistance to externally imposed movement rises rapidly above a threshold speed or joint angle. It is a sensorimotor phenomenon related to the integration of the nervous system motor responses to sensory inputs.

Although most commonly considered as a velocity-dependent increase to tonic stretch, it is related to hypersensitivity of the reflex arc and changes that occur within the central nervous system (CNS), most notably, the spinal cord. Injury to CNS results in loss of descending inhibition, allowing for the clinical manifestation of abnormal impulses. Muscle activity becomes overactive. This is mediated at several areas of the stretch reflex pathway. Although spasticity is part of the upper motor neuron syndrome, it is frequently tied to the other presentations of the said syndrome.

Contracture, hypertonia, weakness, and movement disorders can all coexist as a result of the upper motor neuron syndrome. Spasticity is a common phenomenon in patients with a wide variety of neurological disorders like cerebral palsy, multiple sclerosis, cardiovascular accidents, strokes, traumatic brain, and spinal cord injury. These patients not only suffer from severe contractures and deformities but also severe pain that incapacitates them [6]. Baclofen stimulates the presynaptic gamma-aminobutyric-acid B (GABAb) receptor, which inhibits sensory input to spinal neurons, but may also act post-synaptically [7].

Intrathecal baclofen (ITB) systems have been effectively used since the mid-1980s for treating patients with severe spasticity. The therapeutic advantage of ITB systems has especially been seen in cases with medically refractory spasticity. Common indications for ITB therapy include cerebral palsy, traumatic brain injury, spinal cord injury, diffuse anoxic brain injury, hereditary diseases such as Rett syndrome, and other etiologies leading to severe and unmanageable spasticity. Several studies have supported the use of ITB systems for the treatment of dystonia too. Although some patients may not regain complete functionality or remission of pain, ITB therapy has shown to positively affect the quality of life and ease

patient daily treatment by their caregivers. While ITB is an effective treatment option, there are still significant complications associated with the intricate surgical technique and prolonged duration of treatment [8].

Intrathecal administration of baclofen offers the benefit of delivering the medication directly into the central nervous system (CNS) without systemic side effects. Chronic intrathecal baclofen infusion (CIBI) with a surgically implanted pump device (Infusion System, Medtronic, Inc., Minneapolis, MN) has been shown to reduce spasticity and improve function, and its use has been indicated in nonambulatory or minimally ambulatory patients with spastic quadriparesis. The potential adverse effects with CIBI are significant and include infection, pump malfunction, and life-threatening withdrawal or overdose[9]. ITB is also used particularly if the oral form is poorly tolerated or ineffective [6,10].

ITB improves both spasticity and spasms. As a result of reduced spasticity and spasms, patients will be able to sleep better, become more independent with mobility, and their ability to do self-care helps improve urinary function. A decrease in muscle pain and fatigue that accompany spasm may also be seen. Thus, effective Baclofen therapy can be delivered using ITB pump, where effects of baclofen are maximized, while its side effects are minimized [6]. ITB pump implantation was routinely preceded by an ITB trial, and only patients responding favorably to the trial were offered a permanent pump [8].

ITB is a technique in which a very low dose of baclofen is delivered into the intrathecal space by way of a catheter attached to an implantable pump. The dose is less than 1% of that delivered orally because of the direct delivery system to the central nervous system; such delivery reduces the principal side effect of sedation [11].

After implantation of the pump, ITB doses are titrated over the first few months, regardless of the movement disorder being treated. When treating spasticity, infusions often begin at 100 µg/ day and are increased by 10% to 20% daily until spasticity is perceptibly reduced to the patient and physician; doses are then adjusted at less frequent intervals on an outpatient basis [12].

Complications:

1. Infections occur in 5% to 10% of patients and are caused most frequently by *Staphylococcus aureus*.
2. CSF leaks occur after baclofen catheter insertion in 5% to 15% of patients with CP (most of whom are children), in contrastto the 3% leak rate reported in adults
3. As catheter technology has improved, the frequency of catheter malfunctions has decreased from 25% to about 5% to 10%.
4. Although several small series reported previously that ITB caused more rapid progression of scoliosis than was observed before pump implantation, those series had no controls.
5. The effect of ITB on seizures has also been clarified. Authors of small series had reported either increased seizure frequency or no change in frequency.
6. Most ITB overdoses are iatrogenic and related to programming errors, which can occur when initially filling the baclofen catheter or when changing baclofen concentrations. Mild overdoses cause only listlessness and do not need to be treated. Moderate overdoses cause lethargy and hypotonia and are treated by monitoring pulse oximetry while waiting for the baclofen to metabolize. Some authors have recommended intravenous injection of 1 to 2 mg of physostigmine to treat overdoses, but the effects of such injections are minimal and brief. Severe overdoses cause bradypnea and coma and are treated by assisted ventilation [6,12].

Contraindications to implantation of an intrathecal drug delivery system were:

1. Systemic infection
2. Pump cannot be implanted less than 2.5 cm from the surface of the skin
3. Known allergies to the materials or medications
4. Drug abuse [2].

Method

Prospective case series study included five patients suffering from pain and spasticity of different pathology attending Al-Kadhimiya Teaching Hospital; one patient has spastic lower limbs due to dorsal spinal tumor, 1patient has radiculopathy due to bullet injury not relieved by laminectomy and bullet extraction or sympathectomy, and 3 patients had generalized pain due to tumors with different locations and with liver metastases. Baclofen and morphine injected to the pump with different concentrations. Isomed fixed flow pump from medtronics used in all cases. Intrathecal Baclofen used for spasticity, and morphine sulphate for pain. Daily drug flow is 0.5 ml/day, Pump chamber=35 ml.

Surgical procedure:

For all patients same procedure done, under general anesthesia, patient positioned in full lateral position with flexion of both knees to abdomen to enhance better flexion of the back, midline incision at level of third and fourth lumbar spine depending on surface landmarks(anterior superior iliac spine),then after skin and subcutaneous incision, Touhaee needle directed at that level (L3-L4) toward intrathecal route (Fig. 1),when CSF drops out then a special catheter with stylet directed intrathecally through Touhaee needle reaching the level D12-L1, by inserting about 28 cm from the catheter, fluoroscopic guidance was not a routine job.

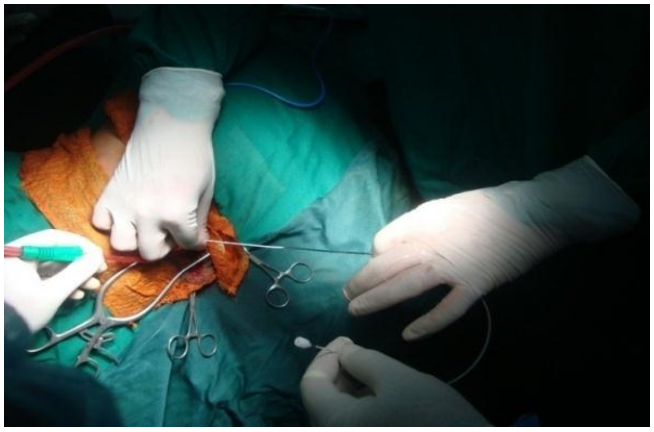

Fig. 1. Intradural insertion of lumbar catheter

After fixation of catheter to prevent slipping or kincking, subcutaneous tunneling from the back to the anterior abdominal wall (right hypochondrial region) was made and a para median incision to the anterior abdominal wall, either longitudinal or horizontal to make a pocket for the pump just above the rectus abdominis (Fig. 2).

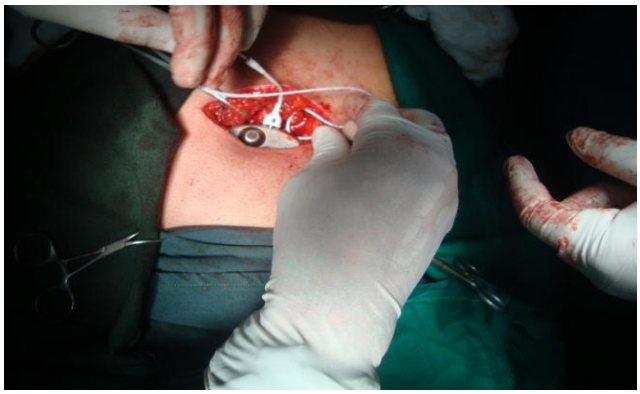

Fig. 2. Anterior abdominal pocket for the pump

Checking the action of pump before insertion to the abdomen about 5-10 ml of fluid aspirated from the chamber, then the chamber filled with drug using special draining system with filter (Fig. 3).

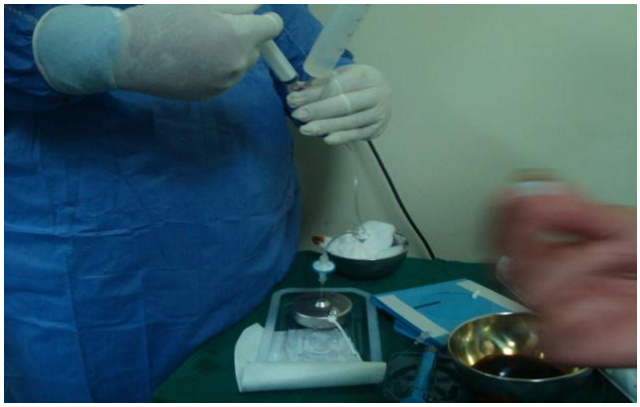

Fig. 3. Filling the pump using special system

The chamber can be filled with 35 ml of the drug. Isomed/medtronics, constant flow pump was used is that pumps 0.5 ml /day (Fig. 4).

Patient # 1

Forty six years lady with bilateral lower limb spastic weakness following spinal dorsal tumor operated upon, infusion pump done for her using baclofen to decrease spasticity of lower limbs and improving the functional status of the limbs. Bacolfen ampoule (10mg/20ml) injected to the pump.

Fig. 4. Isomed/medtronics infusion pump

Patient # 2

Sixty years old man referred for morphine pump insertion due to severe backpain associated with epigastric pain, the patient is a known case of end stage pancreatic tumor,5ml of morphine (10mg/ml) diluted with 25 ml of normal saline 0.9% injected to the pump (0.8mg of morphine intrathecal/day).

Patient # 3

Forty three middle aged man with liver and cevical metastatic tumor and complaining of severe epigastric pain associated with bilateral upper limb pain and spasticity, 4ml of morphine (10mg/ml) diluted with 26ml normal saline 0.9% injected to the pump (0.7mg of morphine intrathecal/day).

Patient # 4

Seventy years old man with pancreatic adenocarcinoma associated with liver metastases leading to severe backpain and epigasric pain, the patient addict on 70mg /day of morphine i.v preoperatively. 5ml of morphine (10mg/ml) diluted with 25 ml of normal saline 0.9% injected to the pump (0.8mg of morphine intrathecal/day).

Patient # 5

Twenty five years young aged man with bullet injury to the back leading to root injury, laminectomy at level of trauma to decompress the root but pain still present then sympathectomy done and pain still present. Morphine pump decided and inserted to him, 0.3 mg/day intrathecal morphine calculated to keep the patient under low comfortable dose.

Results

The mean age was 47.5yr (range from 25-70yr) and male to female ratio was 4:1. Pathology that indicate surgery, 40 % (2 cases) spinal cord tumor, 40% (2 cases) pancreatic tumor, (1 case) 20% root injury and liver metastases in 2 cases (40%) Baclofen used for 1 patient (20%), and morphine for 4 patients (80%).

The first patient with spastic painful weakness due to previous spinal dorsal tumor, baclofen used, as it's the first surgery for pump implantation there is some technical difficulties during operation that consisting of difficult removal of stylet from intrathecal catheter leading to small perforation in the catheter managed by using connector and bypass the perforation. There was mild improvement in spasticity and pain after surgery, but during the following refilling sessions every 2months, the patient felt no difference in spasticity as before surgery.

The second, and fourth patients, complained from end stage pancreatic tumors with liver metastasis in the fourth patient, morphine used in different doses depending on the severity of pain, there is dramatic response and pain diminished just after surgery, the fourth patient complained from morphine addiction equal to 70 mg /day, reduced to 0.8 mg /day intrathecally, with comfortable effect. The third and fourth patients, complained from liver metastases.

The fifth patient complained from root injury due to bullet injury to the back, decompressive laminectomy for pain management followed by sympathectomy without diminished pain sensation,

Low intrathecal dose of morphine decided to keep the patient under minimal comfortable dose, slightly increasing depending on patient condition (Table 1).

Table1. Characteristics of patients' data

Patient	Age (yr)	Sex	Pathology	Complaint	Type of drug
# 1	46	F	Spinal cord tumor	Spastic pain	Baclofen
# 2	60	M	Pancreatic tumor	Epigastric pain+backpain Spastic	morphine
# 3	43	M	Cervical secondary with liver metastasis	painful limbs +epigastic pain	morphine
# 4	70	M	Pancreatic tumor+liver metastasis	Backpain+ epigastric pain	morphine
# 5	25	M	Root injury	radiculopathy	morphine

Discussion

Intrathecal therapy has been proven to be very effective in the treatment of chronic therapy-resistant pain and spasticity resulting from damage of central nervous system of various etiologies [13-15]. Regarding the first patient, there was mild improvement in spasticity and pain after surgery but unfortunately presurgical complain returned back after 3rd refilling session and this is due to constant flow of baclofen without ability to increase the dose/day that meet the patient need after third session. The pumps, which was available, an Isomed type that flow 0.5 ml/day, while in other study a programmable synchromed pump used [7] that the dose can be increased or even stopped according to the patient need and general condition.

For treatment of intractable pain in patients with end stage secondary metastases, morphine used in different doses depending on patient condition, as in other studies that [1,2,4]. The drug delivery efficiency of approximately 300 times as high as conventional oral administration can be obtained with significantly less systemic side effect [1] as in our study,the daily comfortable dose after surgery for the fourth patient was 0.8mg/day, which was 70 mg/day i.m before surgery.

In conclusion, infusion pump surgery is a new minimal invasive surgical procedure in Iraq for treatment of spasticity and pain in different pathologies, with comfortable results. Programmable type of pump is better when baclofen decided while in cases that need morphine there is no difference in using constant flow or programmable pump due to the ability of changing morphine concentration.

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Received 23rd Jul. 2013: Accepted 12th Dec. 2013

Iraqi JMS

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

Spectrophotometric Changes in Glycogen Content of Gastrocnemius and Soleus Muscles in Response to Achilles Tenotomy in Rat

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Abstract

Background The glycogen content of individual muscle fibers varies according to their types as well as in response to workload changes. The effects of tenotomy on this glycogen content in skeletal muscles of the rat leg have long been studied, however, such effects on the contralateral limb was not detailed in most of these studies.

Objective To investigate the effect of tenotomy of the Achilles tendon on the glycogen content of gastrocnemius and soleus muscles fibers in both the tenotomized and contralateral limbs.

Methods Thirty adult male rats (*Rattus rattus norvegicus albinus*) divided into non-tenotomized (control) and tenotomized (experimental) groups. The Achilles tendon of the right hind limb was incised and left to heal for 2 weeks. Animals then were sacrificed and cross sections of the right and left gastrocnemius and soleus muscles were examined spectrophotometrically for the mean optical density of PAS stain.

Results A significant decrease in the glycogen content of the tenotomized and contralateral limbs of gastrocnemius muscle compared with the control group. Similar significant decrease was found for soleus muscle in tenotomized but not the contralateral limb of the experimental group compared with the control group.

Conclusions The disuse atrophy in the tenotomized limb causes decrease in the glycogen content of gastrocnemius and soleus muscle fibers. However, the change in glycogen content seems to affect the fast gastrocnemius rather than the slow soleus musclein the contralateral limb probably because the mechanical load on gastrocnemius during muscle contraction following tenotomy is higher than that exerted on soleus muscle.

Key words Skeletal muscle, Gastrocnemius, Soleus, Glycogen, Spectrophotometry.

Introduction

Skeletal muscle is made up of different fiber types, in which glycolysis for energy production takes place. Three types of skeletal muscle fibers can be identified by color: red, white, and intermediate. Red fibers are small and contain large amounts of myoglobin. Intermediate fibers are of medium size with high myoglobin content and large amounts of glycogen. White fibers are large and contain less myoglobin and they store a considerable amount of glycogen [(1)]. Skeletal muscle fibers specialize in the transformation of chemical energy into mechanical events, i.e. force production. Chemical energy is stored in glycogen particles, which constitute about 0.5-7 % of muscle weight [(2)].

The proportion of each type of skeletal muscle fibers varies between muscles, and the classification of fiber types in muscle biopsies has clinical significance for the diagnosis of muscle diseases or myopathies [(3)]. The same

fiber type with identical metabolic properties may have different size in different muscles. Accordingly, slow muscle fibers, for example, in the rat soleus are bigger than slow fibers present in rat fast muscles. Thus both fiber type and muscle of origin are relevant for fiber size [4].

The relative glycogen content of muscle fibers can be estimated histochemically with the periodic acid Schiff's (PAS) reaction [5]. In 1979, Halkjaer and Ingemann described identical concentrations of PAS stain (color) in histological sections of the same human muscle in both cryostat and paraffin-embedded sections [6]. The histochemical assessment of the glycogen content in various types of muscle fibers is usually carried out by subjective rating of the PAS staining intensity in animals [7]; in addition, this subjective rating has been also used for human muscles [8]. However, a good linear relationship was found between computerized image analysis of the optical density of PAS stained sections and biochemical assessment of glycogen content of single fibers [9].

The rat soleus, slow-twitch muscle involved in maintaining posture, contains a high percentage of slow type I fibers [10]. In contrast, both heads of gastrocnemius muscle have a majority of fast type II fibers [11].Studies investigating the effect of tenotomy on glycogen content of slow and fast skeletal muscle fibers indicate that glycogen content shows no changes within the first three hours [12], then it significantly increases 12 hours after tenotomy, with the contralateral limb serving as a control [13]. Glycogen has also been shown to increase when the tenotomized limb was compared to a non-tenotomy control. After that peak, glycogen drops to lower levels and maintains this drop [14]. To present, changes in glycogen content of the contralateral limb in comparison to the non-tenotomy control have not been investigatedyet.

The purpose of this workis to use the PAS stain for studying the effect of sharp cut injury (tenotomy) of the Achilles tendon on the glycogen content of gastrocnemius and soleus muscles fibers in both the tenotomized and contralateral limbs.

Methods

Animal preparation and sampling

The work was done during the period from January to March 2012; at the Anatomy Department of Al-Nahrain University / College of Medicine.A sample of 30 adult male rats (*Rattus rattus norvegicus albinus*) aged 3-6 months with 300±50 g body weight were chosen. Animals were divided into two groups: non-tenotomized (control) and tenotomized (experimental) groups (15 rats in each group). Animals were housed 1 per cage, given access to drinking water, and fed standard diet pellets. Cages were 60 cm length by 30 cm width and 30 cm height to ensure providing an environment suitable for reasonable activity.

Open ether anesthesia was used in all surgical procedures. Animals were anesthetized with ether-impregnated cotton-wool in airtight jar for 2-3 minutes prior to tenotomy. The animal was put in prone position and the right foot was dorsi-flexed for the Achilles tendon to be prominent. A transverse incision was made at the lower end of the posterior aspect of the leg just above the calcaneus to cut the Achilles tendon [15]. The gastrocnemius and soleus muscles got contracted up the leg. Skin wound was sprayed by Inibsaplastospray and the cut tendon was left to heal spontaneously. Upon recovery from anesthesia, the right foot was in dorsiflexion.

The control and experimental groups were sacrificed after ether-induced deep anesthesia at the end of the experiment (2 weeks after tenotomy). Both right and left gastrocnemius and soleus muscles from the control group, and the right (tenotomized), and the left (contralateral) gastrocnemius and soleus muscles from the experimental group were excised. Whole muscles were fixed immediately in 10% formalin for further tissue processing. Paraffin blocks were prepared and sectioned at 10µm thickness.

Staining

After slides dewaxing and hydration, sections were oxidized in 1% periodic acid for 10 minutes and then washed well in distilled water for about

30 seconds. Slides were then treated with Schiff reagent for 20 minutes at 38°C, dehydrated in graded alcohol (50%, 70%, 80%, 90% and 99%) 1 minute for each, cleared in xylene (2-3 minutes), and mounted in Eukitt. Control sections were pre-incubated in1% amylase (30 minutes, at 37°C) before oxidation in periodic acid [16].

Quantification of PAS staining intensity

A Poly specmicro spectrophotometer system supplied from Reichert-Jung was used in this study. Muscle samples from soleus and gastrocnemius were labeledas control and experimental groups, the latter was subdivided into tenotomized and contralateral limbs. Using a systemic random selection of 5 fields per section, ten muscle fibers from each field were selected; they were identified subjectively asType I, Intermediate and Type II in order of increasing size (perimeter) for gastrocnemius muscle,and Type I and Intermediate for soleus muscle, which is in agreement withthe previous results of Al-Kaabi [17].

In order to measure the mean optical density of muscle fibers stained with PAS stain, the value of the isobestic wave length or wavelength of maximum absorbance was entered as 510 nm [18]. The measuring diaphragm was fitted so that to include most of the inside of the selected fibers. The average absorbance of each muscle fiber type was calculated, representing the optical density of PAS stain in each type. ANOVA and paired T-tests between the mean optical density values of muscle fiber types in the control group, tenotomizedand contralateral limb of the experimental groupwere performed using Microsoft Excel 2010 statistical software.

Results

Cross-sections of controlgroup of gastrocnemius and soleus muscles, tenotomy and contralateral limbs of the experimental group of both muscles are shown in figure 1. The control group showed the mosaic pattern of staining intensity resulting from the different content of glycogen within the different muscle fiber types. Both gastrocnemius and soleus muscles in the tenotomy group revealed decreased colorintensity with almost homogeneous staining pattern. In the contralateral group, only gastrocnemius muscle showed marked decrease in the staining intensity as compared to that of the control group.

Spectrophotometric measurements of the mean optical density of PAS stain in gastrocnemius and soleus muscles are shown in figures2 and 3. For gastrocnemius muscle, ANOVA showed that there was significant decrease (P <0.05) in the mean optical density of the tenotomy and contralateral limbs of the experimental groupversus the control group. The decrease was also significant (P <0.05) when comparing the contralateral limb versus the tenotomy limb of the experimental group.

For soleus muscle, ANOVA revealed significant decrease (P <0.05) in the mean optical density between the tenotomy limb and the control group, along with that between the tenotomy limb and the contralateral one. However, the decrease was not significant when comparing the contralateral limb of the experimental group versus thecontrol group.

Discussion

When the tenotomy limb was compared with the control group, the mean optical density of PAS stain decreased significantly for gastrocnemius and soleus muscles, reflecting the decrease in glycogen content of both muscles. This decrease may be due to the effect of tenotomy itself [14] or can be due to a higher workload thrust upon both muscles as a result of muscle shortening. Studies suggest that the effect of tenotomy or disuse atrophy is a more acceptable explanation [20] since the variability of glycogen stores in response to muscle contractions is at its minimum in the second week after tenotomy.

Results of the mean optical density of PAS stain in the contralateral limb of the experimental group were different. The significant decrease in the optical density of the contralateral limb compared with control group in gastrocnemius muscle suggests that mechanical load on this muscle during muscle contraction (weight

bearing activity and locomotion) following tenotomy is higher than that exerted on soleus muscle. Tenotomy causes limping and changes the weight bearing activity and locomotion in both limbs. The change in these parameters seems to affect the fast gastrocnemius muscle rather than the slow soleus muscle.

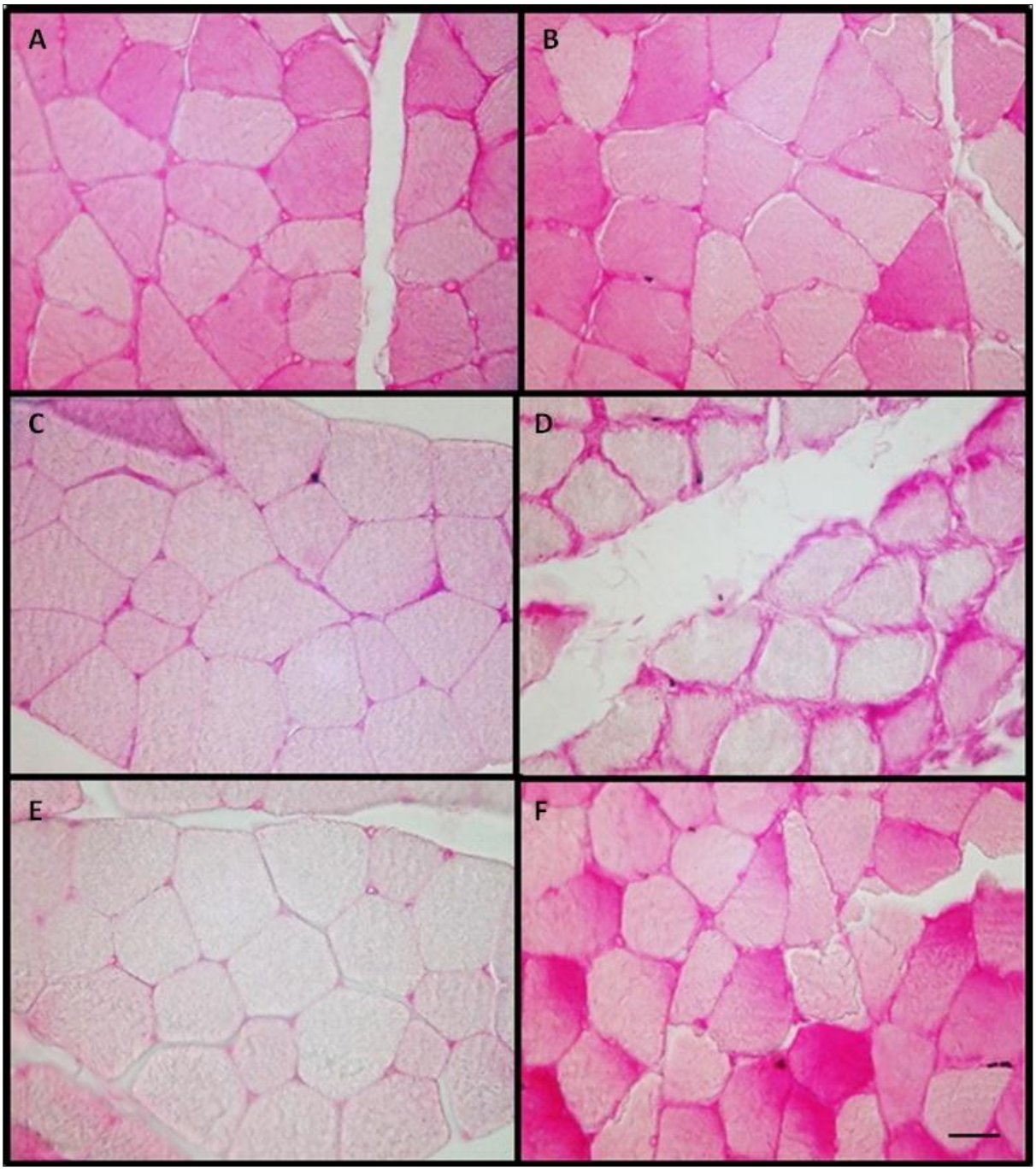

Fig. 1. Cross-sections of rat muscles stained with PAS stain. (A) Gastrocnemius and (B) soleus of the control group. (C) Gastrocnemius and (D) soleus of the tenotomy limbs. (E) Gastrocnemius and (F) soleus of the contralateral limbs in the experimental group. Both C and D show clear decrease in the intensity of PAS stain. A similar observation is evident in E, however, the PAS staining intensity seems to be unaffected in F. 400 X. Bar = 50 µm

These results are in agreement with studies on workload changes [21]. It seems thattenotomy caused the contralateral limb to depend on gastrocnemius to a greater extent than soleus muscle for its weight bearing activity as well as locomotion, an assumption that might be supported by the fact that gastrocnemius contains abundance of fast type II fibers while soleus contains predominantly intermediate fibers with absence of type II fibers [17]. These data suggest that the contralateral limb cannot be used as a control, a trend which can be seen in many previous studies [12].

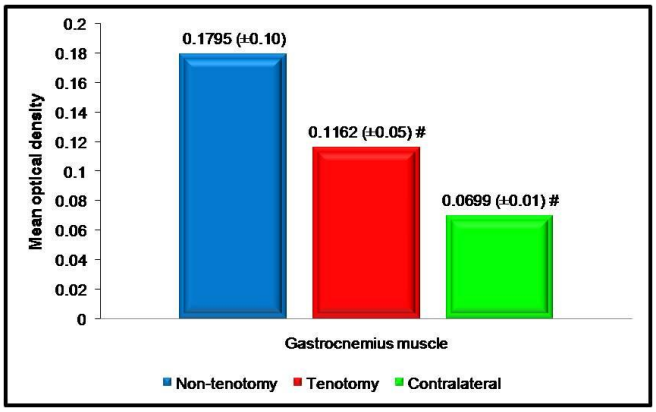

Fig. 2. The values of mean optical density (±SD) of gastrocnemius muscle for control group, tenotomy and contralateral limbs of experimental group.

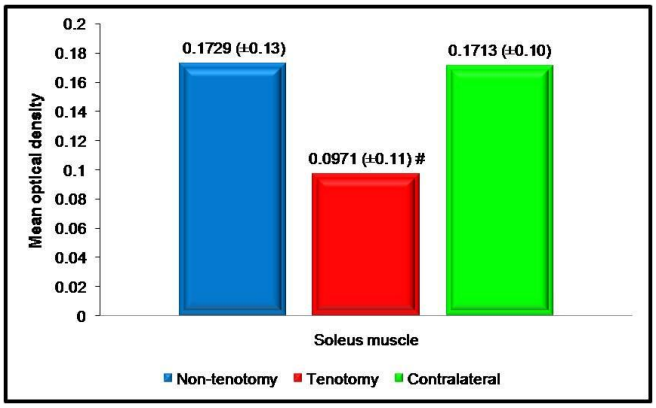

Fig. 3. The values of mean optical density (±SD) of soleus muscle for control group, tenotomy and contralateral limbs of experimental group.

A change in the workload on muscles of both sides will always ensue after tenotomytill complete healing of the tendon [21]. Limpingcauses redistribution of the workload exerted on the contralateral limb, a change that is expected to vary with time in accordance with the healing state of the tendon and the condition of the affected muscles. Therefore, it is recommended to follow up the changes in the workload exerted on the contralateral muscles at different times after tenotomy.

So we conclude from this study that thedisuse atrophy in the tenotomized limb causes decrease in the glycogen content of gastrocnemius and soleus muscle fibers. However, the change in glycogen content seems to affect the fast gastrocnemius rather than the slow soleus muscle in the contralateral limb probably because the mechanical load on gastrocnemius during muscle contraction following tenotomy is higher than that exerted on soleus muscle.

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Received 13th May 2013: Accepted 17th Dec. 2013

Iraqi JMS

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

Clinico-Epidemiological Study of Peptic Ulcer Disease among Children in Three Tertiary Health Care Centres in Baghdad

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Abstract

Background Peptic ulcer is diagnosed at endoscopy where there is a mucosal break of 5mm or larger covered with fibrin. Mucosal breaks smaller than 5 mm are called erosions that do not penetrate the muscularis mucosa whereas an ulcer extends through the muscularis mucosa in the sub-mucosa.

Objective To determine the clinical picture, epidemiological aspectof peptic ulcer in children from birth to sixteen years of age and determine the relationship of the clinico-epidemiological aspect of the disease and the socio-demographic features.

Methods Fifty-three patients with an age ranged from birth to 16 years presented with gastrointestinal tract complaint and diagnosed to have peptic ulcer by endoscopic findings. The data was collected by viewing the case sheet of every child included in the study using special questionnaire to obtain socio-demographic information.

Results The mean age was (10.73±5.2) and the majority of the patients (54.72%) were in the age range (11-16) years old, with a male to female ratio of 2.5:1. Duodenal ulcer (66.04%) is more common than gastric ulcer (33.96%). There is significant association between age groups and type of peptic ulcer. Negative family history was found in (71.7%) of patients. Hematemesis is the most common clinical feature present in (69.81%).

Conclusions It is important to investigate children with recurrent abdominal pain, nocturnal pain and positive family history of the disease. Further studies for longer duration are required to follow up complications and healing or chronicity to the adult life.

Key words Peptic ulcer, children, clinico-epidemiological view.

Introduction

Peptic ulcer disease is uncommon in children. Understanding of the etiology, the investigation and treatment of this condition has changed markedly in recent years. The advent of pediatric endoscopy in the mid-1970s allowed visualization of peptic ulcers, whereas previously they had only been seen indirectly on barium contrast studies.

Similarly, the advent of H2 receptor blockers also in the mid-1970s and of proton-pumps inhibitors in the late 1980s revolutionized treatment. The discovery of *Helicopecter pylori* (*H. pylori*) switched the understanding of the etiology of peptic ulcer disease from that of an acid driven disease to an infectious disease [(1)]. Peptic ulcer disease has changed profoundly in the last decades in Western countries in both children and adults [(2,3)]. Indeed, the prevalence of *H. pylori*-positive ulcers has declined, and a new disease has emerged: *H.pylori*-negative gastric or duodenal ulcers (DU) [(4)].

In adults, most cases of the peptic ulcer are due to non-steroidal anti-inflammatory drugs (NSAIDs) and/or alcohol, and tobacco use can be associated, whereas in children they are not, and their etiology is mostly unknown as are their prevalence and long-term history *H.pylori*-

positive ulcers in children share some features with those in adults; they occur morefrequently inolder children and in males and recurrence rate is low if the infection is eradicated.

H. pylori-negative ulcers, due to unknown causes, are more frequent in younger children, do not have a gender preference, and tend to have a higher recurrence rate, particularly in Chinese children [5].

The natural history of peptic ulcers changed again after the discovery of *H. pylori*, when even in children, eradication of the infection was associated with a cure of theulcer without long-term recurrence [6].

A Canadian study has estimated an incidenceof 1 case per 2500 hospital admissions to a university hospital. Peptic ulcers in children can be classified into primary and secondary ulcers [7].

Secondary peptic ulcer disease develops as a result of the acute stress of a severe systemic illness such as head trauma or overwhelming sepsis, or as sequel to ingestion of ulcerogenic drugs such as NSAIDs or steroids. Excluding those secondary peptic ulcers, primary peptic ulcers are even less commonly seen in pediatric practice.

Single-center series from different parts of the world showed that primary peptic ulcer disease was diagnosed in only 1.8% to 3.6% of the total number of upper endoscopies performed to investigate gastrointestinal (GI) symptoms in children [8,9].

H. pylori infection is considered to be the most important cause of primary DU in children, and eradication of the bacteria is effective in preventing ulcer relapse [2,10].

The purpose of this study is to determine the clinical features like (epigastric pain, vomiting, hematemesis, etc.) and the epidemiological aspects like (age, gender, associated factors, etc.) of peptic ulcer disease in children from birth till 16 yrs old and to find out the association between the clinical aspects of the disease and socio-demographic characteristics of the children.

Methods

Arecord-based cross-sectional study was conducted in three hospitals in Baghdad capital; Children Welfare Teaching hospital, Gastro-intestinal and Liver diseases Center, Central Teaching Hospital of Paediatrics and Endoscopy Department in Baghdad Teaching Hospital from Dec. 2011 to the first of May 2012 and kept in the hospitals from 1-3 weeks for diagnosis and treatment. The study population included 53 children aged 0-16 year old who attended the selected hospitals and were diagnosed to have peptic ulcer by endoscopic findings. The data was collected by reviewing the case sheet of every child included in the study using special questionnaire form to obtain socio-Demographic information, clinical features and outcome at discharge.

Statistical analysis: Analysis of data was carried out using (Statistical Packages for Social Sciences- version 20). Standard Chi-square test (χ^2) was used to determine the associations between two categorical variables. Yates correction formula and fishers exact test were applied for chi-square test whenever it was needed. *P* value of less than 0.05 was considered as statistically significant.

Results

The classification of the patients of the study according to the type of ulcer showed that about two thirds of the sample was suffering from DU (66.04%) and the other one third was having gastric ulcer (GU) (33.96%).

The more prevalent age group was 11-16 years old (54.72%) while (24.53%) of the sample were between 6-10 years andonly 20.75% were at or below 5 years old (Table-1). Duodenal ulcer more common in age group (11-16) years old in 68.57% of cases, in 17.14% of cases the involved age group was (6-10) years, while in 14.29% of cases it is present in patients at or below 5 years. For GU 27.78% of cases at (11-16) years old, while 38.89% at (6-10) years old and only 33.33% at 5 years or below. The association between age groups and type of peptic ulcer were statistically of high significance (*P* = 0.018).

Regarding residency, about 73.58% of the study sample was living in urban areas; 35.9% of them were having GU.

This current study founded that 71.70% of the total number were male and 28.30% were female with a male to female ratio 2.5:1, when 65.71% of DU were in males and 34.29% were infemales. In GU, 83.33% were males and 16.67% were females. About 71.70% of the patients were having negative family history; while 28.30% had positive family history, only 31.43% of DU had positive family history compared to 22.2% with gastric ulcer (Table1).

Table 1. Distribution of the study group according to the relationship between type of ulcer and demographic characteristics

Demographic variations		Type of ulcer						*P* value
		Duodenal		Gastric		Total		
		No.	%	No.	%	No.	%	
Age (Yrs)	≤5	5	14.29	6	33.33	11	20.75	0.018
	6-10	6	17.14	7	38.89	13	24.53	
	11-16	24	68.57	5	27.78	29	54.72	
	Total	35	100	18	100	53	100	
Residency	Urban	25	71.43	14	77.78	39	73.58	>0.05
	Rural	10	28.57	4	22.22	14	26.42	
	Total	35	100	18	100	53	100	
Gender	Males	23	65.71	15	83.33	38	71.70	>0.05
	Females	12	34.29	3	16.67	15	28.30	
	Total	35	100	18	100	53	100	
Family history	Positive	11	31.43	4	22.22	15	28.30	>0.05
	Negative	24	68.57	14	77.78	38	71.70	
	Total	35	100	18	100	53	100	

Regarding the associated factors of the disease, the available information in the case sheets was used and this include: history of drug intake especially NSAID and the result was that only 14.29% of patients with DU and 16,67% of patients with GU have such a history, while 71.43% didn't report any drug intake. Regarding type of water given to the child 71.43% of cases of DU used tap water, 22.86% used bottled water and 5.71% used others, while for GU, 66.67% used tap water, 22.22% bottled water and 11.11% used others (Table 2).

About the clinical presentation of the disease, according to type of ulcer, the present study founded that the most common presentation was hematemesis in both types of ulcer, in DU it was found in 65.71% of cases while 77.78% of GU had hematemesis, the second most common presentation was epigastric pain that was found in 60% ofcases of DU and in 55.56% of cases of GU, malena was found in 51.43% of patients with DU, while in 55.56% with GU, crying episodes found in 11.43% of DU and in 33.33% of GU, finally, bleeding per rectum which is found in 14.29% of cases of DU and no such complaint in GU (Table 3).

Regarding the condition of the patients at time of discharge; in cases of DU, 65.71% had complete healing, while 34.29% still had the ulcer at discharge. In cases of GU, 66.67% had healing and 33.33% had ulcer at discharge, and the current study founded no death cases from the disease (Table 4).

Discussion

In the past two decades, primary peptic ulcer disease has been more widely recognized as a diagnosis worthy of consideration in the

pediatric age group [11]. In the present study, the most common age group at time of diagnosis were [11-16] years old, they represent 54.72% of the total number, while children at and below 5 years old representonly 20.75%, the mean for age was (10.73± 5.2) and this goes with Murphy et al, in Newcastle [12], who founded that the mean age at diagnosis was (11.2) years, and the children ranged from 4 to 15 years, and symptoms present in 46% before the age of 10and 15% before 6 years of age. Also it was found that in Chiang Bor-Luen, Taiwan [13], the mean age of 33 children with duodenal ulcer was 12.1±1.6 years that range (8-15 years), and in Goggin, Ireland [14], founded that the age range was 9.8-14.25 years and thatwas near to the results of the current study, also goes with Drumm et al, in Toronto [7], in which the mean age was 10 years.

Table2. Distribution of the study group according to the type of ulcer and different factors of the disease

Associated factors		Type of ulcer			
		Duodenal		Gastric	
		N	%	N	%
Drug Intake	NSIAD	5	14.29	3	16.67
	Others	5	14.29	4	22.22
	No drug intake	25	71.43	11	61.11
	Total	35	100	18	100
Stress factors	Sepsis	0	0	1	5.56
	Shock	0	0	0	0
	Intra-cranial lesion	1	2.86	1	5.56
	Burn	3	8.57	1	5.56
	None	28	80	11	61.11
	Others	3	8.57	4	22.22
	Total	35	100	18	100
Water supply	Bottled	8	22.86	4	22.22
	Tap	25	71.43	12	66.67
	Others	2	5.71	2	11.11
	Total	35	100	18	100

The young child is less likely to give an accurate description of symptoms, may be because of that there is a delay in the diagnosis of the disease so it appears more common in younger age group. This current study founded that of the 53 children 38 were males and 15 were females with a male to female ratio 2.5:1. This predominance of males agrees with that reported by Murphy et al, in Newcastle [12], which showed male to female ratio of 3.8:1. Chiang Bor-Luen, Taiwan [13], founded male to female ratio of 4.5:1, and in Goggin in Ireland [14], a sex ratio of 2:1, with the same results showed in Brendan Drumm et al in Toronto [7], with a ratio of 1.4:1.These higher results in males may be due to genetic elements.

Regarding family history, current study founded that only 28.3% have a positive family history and it is negative in 71.70% and this disagree with Murphy et al in Newcastle [12], that showed 62% at least one first or second degree relative had confirmed duodenal ulcer disease and in Goggin in Ireland [14], nine of 15 patients (60%) had a positive family history; but it goes with the results of Chiang in Taiwan [13] that founded a positive family history in 36% of 33 patients and close to the results of Drumm et al in Toronto [7], in which 26% of patients with primary duodenal

ulcer had a first degree relative with peptic ulcer disease. Genetic factors appear to play a role in the disease. A polygenic mode of inheritance has been proposed, studies in twins not only support this independence but provide strong evidence that the increased familial prevalence of peptic ulcer disease is due to genetic factors [15]. There is a study from Calcutta has shown that the (interleukin) IL-1B polymorphism is strongly associated with *H. pylori* related duodenal ulcer [16].

Table3. Distribution of the study group according to the clinical presentation and type of ulcer

Clinical features		Type of ulcer				*P* value
		Duodenal		Gastric		
		N	%	N	%	
Hematemesis	Yes	23	65.71	14	77.78	>0.05
	No	18	34.29	4	22.22	
Malena	Yes	18	51.43	10	55.56	>0.05
	No	17	48.57	8	44.44	
Epigastric pain	Yes	21	60.00	10	55.56	>0.05
	No	14	40.00	8	44.44	
Nausea	Yes	9	25.71	6	33.33	>0.05
	No	26	74.29	12	66.67	
Vomiting	Yes	10	28.57	9	50	>0.05
	No	25	71.43	9	50	
Feeding difficulty	Yea	8	22.86	5	27.78	>0.05
	No	27	77.14	13	72.22	
Crying episodes	Yes	4	11.43	6	33.33	0.054
	No	31	88.57	12	66.67	
Generalized abdominal pain	Yes	7	20	4	22.22	>0.05
	No	28	80	14	77.78	
Epigastric tenderness	Yes	15	42.86	8	44.44	>0.05
	No	20	57.14	10	55.56	
Bleeding per rectum	Yes	5	14.29	0	0	>0.05
	No	30	85.71	18	100	

Hematemesis occurred in 69.81% of cases, and malena was present in 52.83% of cases, that goes with the results of Drumm et al in Toronto [7], in which 10 of 17 children (58.8%) of secondary peptic ulcer had hematemesis and only one patient (5.2%) with primary peptic ulcer; but higher than Goggin in Ireland [14], that showed 6 out of 15 children (40%) had gastro-intestinal bleeding. It is important to realize that many ofthese children do not have the typical clinical pain syndrome of non-radiating epigastric pain, which begins several hours after eating, and is relieved by food, antacids, or vomiting. History of nocturnal pain in these children, however, is important. Apley's series of recurrent abdominal pain showed that only 7% of 118 children were woken at night by their pain [17].

Table4. Distribution of the study group according to the outcome at discharge and type of ulcer

Outcome at discharge	Type of ulcer			
	Duodenal		Gastric	
	N	%	N	%
Complete healing	23	65.71	12	66.67
Chronic ulcer	12	34.29	6	33.33
Total	35	100	18	100

This observation in the present study may be due, in part, to the inability of young children to verbalize the existence of pain. Episodes of vomiting had occurred in 35.85% that goes with Murphy et al [12], in which vomiting occurred in 39% of cases, while it is higher than the results of Goggin [14], in which 3 of 15 cases (20%) had vomiting. Vomiting is not infrequently a presenting complaint, but it usually occurs in association with abdominal pain and nausea. The exception is in children younger than 4 years of age, in as many as two thirds of whom it may be the only presenting symptom [15]. Epigastric tenderness was present in 43.4% of cases that is lower than percentage seen in Murphy et al [12], in which it was noted in 54% of cases. It was not usually a striking feature. Some degree of tenderness was noted, but its absence may relate to the state of activity of the disease at the time of examination found to have a duodenal ulcer subsequently suffer an episode of bleeding and with treatment the risk of this occurrence can be reduced. Bleeding per rectum founded in 9.43% of cases in the current study that is muchlower than Drumm et al, in Toronto [7], in which 14 of 17 children (82.3%) had lowergastro-intestinal bleeding.

The simultaneous decrease in the proportion presenting with overt gastrointestinal bleeding illustrates the fact that more of those being diagnosed in recent years have presented with abdominal pain rather than a complication of ulcer disease. Thirty-five of cases (66.03%) showed complete healing at time of discharge, 18 patients were already known to have achronic ulcer (33.96%) and there are no cases of death. The natural history of peptic ulcers changed again after the discovery of *H pylori*, when even in children, eradication of the infection was associated with a cure of the ulcer without long-term recurrence.

We conclude that duodenal ulcer is more common than gastric ulcer; males are more commonly affected than females; the most common presentation in the current study was hematemesis and abdominal pain; it is suspected that awareness of the commonly atypical presentation of this disorder, especially in young children might result in earlier diagnosis. It is important to advice long term follow up observation and maintenance therapy in children with chronic ulcer disease.

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Received 18th Sep. 2013: Accepted 16th Dec. 2013.

Iraqi JMS

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

Re-Evaluation the Frequency of Cutaneous Manifestations in Patients on Hemodialysis

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Abstract

Background Hemodialysis patients experience frequent and various cutaneous manifestations. Chronic renal failure (CRF) is a clinical state, which results in declining of kidney functions.

Objective To evaluate the frequency and nature of cutaneous lesions associated with CRF patients on hemodialysis.

Methods Fifty patients with chronic renal failure on hemodialysis in Al-Kadhimiya Teaching Hospital, Dialysis Center from the period of February 2012- May 2012 were conducted in this case series study.

Results All patients included in this study had at least one cutaneous manifestation related to chronic renal failure. The most frequent findings were pruritus (100%), pallor (100%), xerosis (100%), hyperpigmentation (74%), petechae (40%), ecchymosis (30%) and wrinkles (30%). Nail changes were half and half nail (76%), koilonychias (48%) and splinter hemorrhage (28%). Oral changes were xerostomia (88%) and ulceration (52%). Other manifestation like hair changes (42%) was seen.

Conclusion Chronic renal failure is associated with various cutaneous manifestations caused by hemodialysis or the disease itself. The most frequent findings; pruritus, pallor and xerosis. The early diagnosis of cutaneous changes may decrease the morbidity and eventually lead to the improvement of life quality in these patients.

Keywords Chronic renal failure, hemodialysis, cutaneous manifestations.

Introduction

Chronic renal failure (CRF) is a progressive loss of renal function over period of months or years through five stages, each stage is a progression through an abnormally low and deteriorating glomerular filtration rate [(1)]. End stage renal disease (ESRD) is a progressive and irreversible renal damage for more than three months duration [(1)].

Hemodialysis is one of the therapeutic modalities which can improve the quality of life in patients with ESRD [(1)]. In hemodialysis, blood is allowed to flow through special filters that remove wastes and extra fluids. Hemodialysis help to control blood pressure and keep the proper balance of chemicals like potassium and sodium in the body [(1)].

Cutaneous manifestations occurring in patients with chronic renal failure are those of full-blown end stage renal disease (ESRD) although cutaneous findings may appear in patients with moderate kidney disease. Persistent cutaneous complaints such as xerosis and intractable pruritus may allow searching for underlying renal dysfunctions [(2)].

Nearly all patients with ESRD have at least one cutaneous manifestation [(3)] these include:

Pruritus: It is a very frequent complaint affecting about 50-90% of ESRD patients [(3)], it tend to

become more severe with deteriorating renal function [3].

The mechanism of pruritus associated with ESRD related to xerosis, atrophy of sweat and sebaceous glands, secondary hyperpara-thyroidism, iron deficiency anemia and neuropathy [3]. Hemodialysis is useful in lowering the magnesium concentration.

- **Xerosis cutis:** It is very common in patients with ESRD and those on hemodialysis due to atrophy of sweat glands [4]. It increase the susceptibility to infections and this is aggravated by delayed wound healing of the skin [5].
- **Alteration in cutaneous pigmentation**: Macular hyperpigmentation of the palm, soles and mucous membrane [4].
- **Ecchymosis and petechae:** ESRD affect hemostatic process in the body by affecting platelets function and aggregation resulting in bleeding disorders; as ecchymosis, petechae and subsequent pallor, which are common in patients with ESRD and those on hemodialysis [1].
- **Skin infections:** ESRD is a state of immunosuppression that leads to various types of infections whether viral, bacterial or parasitic infections [1]. Infections with exotic agents as pseudomonas or even tuberculosis may occur [6,7]. Impaired immunity seen even before dialysis. The immune defect is mainly lymphopenia, decreased B-cell activity, and alteration of the T-cell subset and activity [7,8].
  - **Nail disorder:**
  - ❖ **Half and half nail:** is more common in patients on hemodialysis, it is characterized by proximal white discoloration and distal red/brownish color due to edema of the nail bed and capillary network, the nail plate is unaffected [4].
  - ❖ **Koilonychia:** It is more common in fingernail, but occasionally seen in toenail. It affects patients on hemodialysis as they are subjected for bleeding with resultant iron deficiency anemia [4].
  - ❖ **Splinter hemorrhage:** It is extravasation of blood from the longitudinally oriented blood vessels of the nailbed [4].
  - ❖ **Onycholysis**: Distal separation of the nail plate from the underlying nail bed.
  - ❖ **Muehrckes lines:** Narrow, white transverse bands occur in pairs due to hypoalbuminemia
- **Oral changes:** Ulceration of the mucous membrane, xerostomia, metallic taste or unpleasant mouth odor and loose teeth is common in ESRD patients and those on hemodialysis [4].
- **Premature aging of the skin:** As actinic elastosis which leads to extensive wrinkling at the neck (cutis rhomboidalis nuchae) and leads to telangiectasia [9].
- **Poor wound healing:** As a result of reduced cutaneous blood flow which proportionate with the duration of dialysis [10].
- **Raynaud's syndrome:** Because of increase susceptibility of to low temperature associated with ESRD and become more sever on dialysis [11,12].
- **Duputrens contacure:** It occurs due to cutaneous calciphylaxis [11,12].

The goal of our study is to Re-evaluate the frequency and nature of cutaneous lesions associated with chronic renal failure patients on hemodialysis.

Methods

A case series study was conducted in Al-Kadhimiya Teaching Hospital, Dialysis Center from the period of February 2012-May 2012. Fifty patients comprised 27 (54%) males and 23 (46%) females with chronic renal failure (CRF) were studied. Their age range from 25-70 with a mean±SD of (47.16±12.1) years, on regular hemodialysis were enrolled in the study. Each patient was subjected to hemodialysis for 3-4 hours in 2-3 sessions per week.

Especial questionnaire was performed including: name, age, sex, onset of skin manifestations, duration, and frequency of hemodialysis. All patients were examined thorough dermato-logical examination including skin, hair, nail and mucous membrane.

Results

The most frequent finding were pruritus 50 (100%), pallor 50 (100%), xerosis 50 (100%), hyperpigmentation 37 (74%), petechae 20 (40%), ecchymosis 15 (30%) and wrinkles 15 (30%) as seen in table 1.

Table 1. Number and percentage of patients with cutaneous manifestations on hemodialysis

Skin manifestations	Males		Females		Total	
	No.	%	No.	%	No.	%
Pruritus	27	54	23	23	50	100
Xerosis	27	54	23	23	50	100
Pallor	27	54	23	23	50	100
hyperpigmentation	20	54.1	17	17	37	100
Petechiae	13	65	7	7	20	100
Ecchymosis	9	60	6	6	15	100
Wrinkles	12	80	3	3	15	100

Nail changes were half and half nail 38 (76%), koilonychias 24 (48%) and splinter hemorrhage 14(28%). Other manifestations were hair changes 21 (42%). Oral changes were xerostomia 44 (88%) and ulceration 26 (52%) as noticed in table 2.

Table 2. Number and percentage of changes in skin appendages and oral changes in patients on hemodialysis

Changes		Males		Females		Total	
		No.	%	No.	%	No.	%
Nail changes	Half and half nail	25	65.8	13	34.2	34	100
	Koilonychias	15	62.5	9	37.5	24	100
	Splinter hemorrhage	10	71.4	4	28.6	14	100
Hair changes		8	38.1	13	61.9	21	100
Oral changes	Xerostomia	21	47.7	23	52.3	44	100
	ulceration	9	34.6	17	65.4	26	100

The onset of cutaneous manifestations in relation to hemodialysis were 27 (54%) patients after hemodialysis, 15(30%) patients during hemodialysis and 8 (16%) patients before hemodialysis (Table 3).

Table 3: Onset of symptoms in relation to hemodialysis

Onset of symptoms	Males		Females		Total	
	No.	%	No.	%	No.	%
After	12	44.4	15	55.6	27	100
With	6	40	9	60	15	100
Before	5	62.5	3	37.5	8	100

The response to hemodialysis in relieving cutaneous manifestations was 18(36%) patients responding to hemodialysis and 32(64%) patients with no response.

Discussion

Pruritus was the most prevalent cutaneous manifestations seen in 100% patients. Its frequency ranged from 19-90% in previous studies [13,14]. It is one of the most characteristic symptoms of CRF [15]. It does not necessarily subside with dialysis although it improves with kidney transplantation [14]. The exact etiology for pruritus is unknown, however it associated with the degree of renal insufficiency (urine output of < 500 mL) [16]. Hypervitaminosis A may be a cause as result for regular ingestion of fat soluble vitamins (Vit A) for the replacement of what is lost within dialysis making the patients in increased risk for accumulation and toxicity secondary to impaired excretion [17]. Xerosis is another possible cause for pruritus [5]. Neural theory is also suggested to be a possible cause for pruritus in CRF patients, as neuron-specific, enolase positive fibers may sprout throughout the epidermis in uremic patients as these nerves ending reaches the stratum basale [18]. Another possible cause is increased serum histamine levels due to allergic sensitization to various dialyzer membrane components and due to impaired renal excretion of histamine, so UVB radiation is effective in uremic patients by suppressing histamine-releasing factors in the sera of uremic patients, also in reducing vitamin A level in the epidermis. Increased serum levels of magnesium and albumin, iron deficiency anemia are considered other possible mechanisms for pruritus in chronic renal failure patients [19].

Xerosis was found in 100% patients in comparing other study were (46-90) % [20,21]. Atrophy of the sweat glands is a possible cause, although high dose diuretics regimens also implicated [21].

Pallor was seen in100% in comparing to 60% in other study done on CRF Indian patients [22]. Pallor is due to anemia mainly due to inadequate erythropoietin production, iron deficiency, folic acid or vitamin B12 deficiency and decreased erythrocyte survival [4].

Hyperpigmentation were seen in 74% in comparison to other studies as 63% [23]. Diffused hyperpigmentation in sun-exposed areas was noticed due to increase melanin in the basal layer and due failure of the kidneys to excrete beta-melanocyte stimulating hormone (B-MSH) [24].

Petechae was seen in 40% and ecchymosis was seen in 30% in comparing to 20% in other study [22]. The causes are could be a defect in the primary hemostasis like increased vascular fragility, abnormal platelets function and the use of heparin during [25].

Wrinkles were seen in 30% when compared to 16% [13]. The cause may be due to early actinic elastosis especially in patients undergo long term hemodialysis [26].

Half and half nails was found in 76% while found in 21% and 20% [22,27]. The cause still unclear and could be attributed to renal dysfunction or due to medication or to the procedure of hemodialysis [27]. Koilonychia found in 48% while it found in 17% and splinter hemorrhage was found in 28% in comparison to 7% in other studies [13].

Hair changes were found in 42% in form of diffuse thinning which is possibly due to the use of heparin or associated hypothyroidism in comparison to 10-30% in other studies [22].

Xerostomia was found in 88% of patients in comparing to 31% in other studies [22]. It could be due to mouth breathing and dehydration [22].

Ulceration of the oral mucous membrane was found in 52% in comparing to 29% in other studies [22]. The cause is due to possible candidal infections, which are due to associated xerostomia [28], and also due to cigarette smoking [29].

We conclude that cutaneous manifestations are frequent in patients with chronic renal failure. They cause high degree of morbidity and tend to be very refractory to treatment. It seems that long periods of pre-existing renal insufficiency, which cause typical skin changes of its own, aggravate the lesions in the dialyzed patients. Early diagnosis of cutaneous changes may decrease the morbidity and eventually lead to the improvement of life quality in these patients.

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Received 11th Dec. 2012: Accepted 31st Dec. 2013.

Iraqi JMS

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

Maternal Ketonuria and Results of Fetal Testing in the Impending Post-Term Pregnancy

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Abstract

Background The observation of ketonuria and its severity in post- term pregnancies can be considered as one of the most important biochemical markers, which can help the obstetrician to predict the adverse outcome of post-term pregnancy with some sort of precision.

Objective To assess the effect of maternal ketonuria on fetal wellbeing in pregnant woman with post-term pregnancy, estimate the frequency of amniotic fluid volume changes in different degrees of ketonuria and to estimate the frequency of non-stress test abnormalities according to the severity of ketonuria.

Methods Two hundred and fifty post-term pregnant women divided into pregnant woman with ketonuria of different severity (27) and pregnant woman without ketonuria (223). Ketone bodies were tested in urine. Fetal testing had been done for all of them in terms of non stress test and amniotic fluid volume assessment in term of amnioticfluid index.

Results Ketonuria was found in 10.8% of post-term pregnant woman and 62.9% of them had moderate to severe ketonuria. Oligohydramnios had been observed in 62.9% and 22.4% of pregnant woman with ketonuria and those without ketonuria, respectively. 4(80%) with severe ketonuria had oligohydramnios. Abnormal non-stress test was observed in 85.1% and 45.7% of those pregnant women with and those without ketonuria, respectively. There is a significant statisticalassociation between the presence of ketonuria and the result of fetal testing.

Conclusion Ketonuriawas significantly associated with oligohydramnios and abnormal results of non-stress test. Higher frequency of oligohydramnios and abnormal results of non-stress test among those with severe ketonuria.

Keywords Post-term, ketonuria, oligohydramnios, non-stress test

Introduction

Ketonuria is a medical condition in which the ketone bodies are present in excess in the urine as an indication that it is using an alternative source of energy, the production of Ketone bodies is a normal response to a shortage of glucose, meant to provide an alternate source of fuel from fatty acid [(1)]. Several previous studies have shown that ketones elicit alteration in the amniotic fluid volume of human beings [(2)]. By this effect on the amniotic fluid volume and composition as well as on the general wellbeing of the fetus, Ketonuria and its degree can be proposed as a biochemical marker that might augment the accuracy of the decision in addition to other parameters included in the classic and modified biophysical profile. Ketonuria during pregnancy is commonly a transient phenomenon and has a negligible effect on maternal and fetal outcome,

but in a prolonged pregnancy, the prolonged exposure to the ketone bodies and possible higher production rate of them, might result in more adverse outcome[3].

Post-term or prolonged pregnancy is our preferred expression for an extended pregnancy [4]. Post maturity is a neonatal diagnosis and should be used to describe the infant with recognizable clinical features associated with peeling, parchment-like skin, meconium staining of the skin, membranes and the umbilical cord. In addition, these infants may have overgrown nails, well-developed creases on the palms and soles, abundance of scalp hair, little vernix or lanugo hair, scaphoid abdomen and minimal subcutaneous fat [5].

Pathology of ketonuria is gradual deterioration in oxygen supply and nutrition can be tolerated for some while by reducing the metabolic demand and oxygen requirements and by increasing hematocrit and catecholamine. Chronic hypoxemia associated with placental insufficiency in post-term pregnancy may develop with noticeable changes in fetal heart rate, or other biophysical activities until they become sufficient to result in academia. A reduction in the metabolic rate accompanied reduced oxygen delivery and reduced carbohydrate and fat storage will precede the onset of anaerobic metabolism and increase chance of development of academia [6]. A reactive non stress test is define as 2 or more fetal heart rate acceleration at least 15 beat/minute above the baseline and lasting at least 15 seconds within 20 minutes period [7].

Amniotic fluid changes during pregnancy, at 22 weeks; the average amniotic fluid volume is 630 ml and this increase in amount to 770 ml at 28 weeks. Between 29 and 37 weeks, there is little change in volume; the average is 800 ml. beyond 39 weeks, the amniotic fluid volume decrease sharply in amount to 515 ml at 41 weeks. Once a pregnant woman became post-date, there is a 33% decline in amniotic fluid volume/week, consistent with clinical observation of an increased incidence of oligohydramnios in post-term gestation [5].

The objectives of this study was to assess the effect of maternal ketonuria on fetal wellbeing in pregnantwoman with post-term pregnancy, estimate the frequency of amniotic fluid volume changes in different degrees of ketonuria and to estimate the frequency of non-stress test abnormalities according to the severity of ketonuria.

Methods

This cross sectional study was carried out at Al-Kadhiymia Teaching Hospital in the Department of Obstetrics and Gynecology during the period from 1st of January 2012 to 31th of October 2012. Two hundred and fifty post-date pregnant women were recruited from outpatient clinics and hospital admissions. They weredivided into two groups:

1. Group A: 223 pregnant women without ketonuria.
2. Group B: 27 pregnant women with ketonuria of different severity.

An informed verbal consent was obtained from all women included inour study. A full medical and obstetrical history and examination were done for each candidate. The exclusion criteria in our study were: Any previous history of diabetes, hypertension, vascular disease, heart disease, collagen disease, renal disease, and smoking, Obesity (body mass index more than 30 kg/m^2), unsure date of last menstrual period and no early ultrasound report, history of drug use.

The characteristics of patients participated in our study were singleton uncomplicated pregnancy without gross congenital anomalies, gestational age more than 40 week, not in labor, intact membrane.

Maternal investigation for the presence or absence of ketone bodies in the urine, amniotic fluid index and non-stress test were performed on the same day. The biochemical estimation of ketonuria was done when 3 ml of freshly voided urine was collected in a clean dry container and then centrifuged for 2 minutes in macro centrifuge machine, Bayer reagent strip (KETOSTIX), which is specific for acetoacetic acid

detection in urine. The strip color was compared with the standard color block on the bottle label to determine the presence or absence of ketonuria and its degree if present. The results were either: light pink colors (no ketone bodies), pink (mild ketonuria), and one plus=15 mg/dl, dark pink (moderate ketonuria), 2 plus=40 mg/dl, violate (severe ketonuria), 3 and 4 plus=80-160 mg/dl.

Real time ultrasound was performed to measure the amniotic fluid index for each pregnant woman, an amniotic fluid index of less than 5 cm was regarded as oligohydramnios, thereafter, and non-stress test was done for each pregnant women. This was performed by cardio-tocography at the labor ward.

Statistical analysis for the association between maternal ketonuria and fetal test results was made. Data were collected and analyzed using computer facility programs, statistical package for social sciences (SPSS) version 16.0 for windows. The frequencies, percentages, means and standard deviation for variables were analyzed. Chi-square was used to detect the significant of relationship between various variables. Statistical significance was considered when P value less than 0.05.

Results

During the study period, a total of 250 post-term pregnant were included; 27 (10.8%) of them show different degree of ketonuria, 203 (89.2%) without ketonuria. Our study showed that out of the 27 pregnant women with positive ketonuria, 12 (44.4%) had moderate ketonuria, 10 (37.1%) had mild ketonuria, and 5 (18.5%) had severe ketonuria (Table1).

Table1. Patients' distribution according to the severity of ketonuria

Severity of ketonuria	No.	%
Mild	10	37.1
Moderate	12	44.4
Severe	5	18.5
Total	27	100

Statistical analysis comparing different demographic features of the pregnant women included in this study revealed no statistically significant difference between pregnant women with ketonuria and those without ketonuria in term of maternal age and parity (Table 2).

Table2. Maternal demographic parameters and their statistical significance.

Maternal demographic parameter	Ketonuria		*P* value
	present (n=27)	absent (n=223)	
Age(years)	27.7±6.4	27.4±6.7	>0.05
Primigravida	44.5%	34.5%	>0.05

Of pregnant women with ketonuria, 16 (59.3%) had passed the 41week of gestation. Thirteen out of 16 pregnant women with moderate to severe ketonuria had passed the 41week of gestation (81.2% of those with moderate to severe ketonuria) including 4 (80%) with severe ketonuria and 9 (75%) with moderate ketonuria (Table3).

Amniotic fluid index (AFI) in those pregnant women with ketonuria ranged between 1 and 8 (mean±SD= 4.8±1.8), while it was between1-19 (mean±SD = 9.4±4.8) in those with no ketonuria. This study indicated that 17 pregnant women with ketonuria (63% of the sample) had an AFI ofless than 5 i.e. oligohydramnios, on the other hand only 50 pregnant women with post-term pregnancy without ketonuria had oligo-hydramnios (22.4% of the sample). Furthermore, 80% of those with severe ketonuria (4 pregnant women) had oligohydramnios.

Statistical analysis revealed that there was a statistically significant difference in thevalue of AFI between those with and those without ketonuria, (P<0.001) (Table 4).

Regarding the results of non-stress test (NST), our study showed that non-stress test was abnormal in 23 pregnant women (85.1%) with ketonuria and post-term pregnancy, whether this abnormality was in terms of spontaneous

deceleration in the fetal heart rate in 10(37%) or in terms of non reactive NST 13 (48.1%). In contrast, 121 pregnant women (54.3%) without ketonuria had reactive non stress test versus 99(14.8%) of those without ketonuria had abnormal non stress test.

In addition, this study shows that all the pregnant women with severe ketonuria (5 pregnant women) had abnormal non stress test, either in the form of spontaneous deceleration in the fetal heart rate (4 pregnant women, 80% of those with severe ketonuria) or in the form of non-reactive fetal heart (1 pregnant woman, 20% of those with severe ketonuria). Statistical analysis using chi-square test indicated that the presence of ketonuria in pregnant women with post-term pregnancy is associated with higher risk of having abnormal results of non stress test (P<0.05) (Table 5).

Table 3. Patients' distribution according to their gestational age

Gestational age	ketonuria						Total	
	Mild		Moderate		Severe			
	No.	%	No.	%	No.	%	No.	%
40thweeks	7	70	3	25	1	20	11	40.7
41thweeks	3	30	9	75	4	80	16	59.3
Total	10	100	12	100	5	100	27	100

Table 4. Patients' distribution according to their amniotic fluid index

Amniotic fluid index(cm)	Ketonuria present						Ketonuria Absent	
	Mild		Moderate		Severe			
	No.	%	No.	%	No.	%	No.	%
<5	5	50	8	66.7	4	80	50	22.4
5-9.9	5	50	4	33.3	1	20	67	30.1
10-14.9	0	0	0	0	0	0	60	26.9
15-19.9	0	0	0	0	0	0	46	20.6
Total	10	100	12	100	5	100	223	100
Range	1-8						1-19	
Mean ±SD	4.8±1.8						9.4±4.8	
Statisticalanalysis	P value < 0.001							

Table 5. Patients' distribution according to the results of non-stress test

Results of Non-stress test	Ketonuria present						Ketonuria Absent	
	Mild		Moderate		Severe			
	No.	%	No.	%	No.	%	No.	%
Spontaneous deceleration of fetal heart rate	2	20	4	33.3	4	80	36	16.1
Non-reactive	5	50	7	58.4	1	20	66	29.6
Reactive	3	30	1	8.3	0	0	121	54.3
Total	10	100	12	100	5	100	223	100
Statisticalanalysis	P value < 0.05							

Discussion

There are general consensuses that perinatal mortality and morbidity are increased several folds when pregnancies are prolonged especially beyond the 42 week of gestation so its management is a subject of concern [8].

Assessment of amniotic fluid has become an integral component in the ante-partum assessment of pregnancies which are at risk of fetal death. The amniotic fluid index and the largest vertical pocket are semi-quantitative ultrasonogrphic techniques used to estimate amniotic fluid volume [9].

Post-term pregnancy is a universally accepted indication for antenatal fetal monitoring [10,11]. Option for evaluating fetal wellbeing include non-stress testing with amniotic fluid assessment, the biophysical profile or modified biophysical profile, the oxytocin challenge test, or a combination of these modalities. There is some observational evidence that some pregnancies at risk of adverse outcome can be identified, but less evidence that the prediction of adverse outcome confers prevention [12]. No single method has been shown to be superior [10,11,13].

Ketonuria is a commonly assessed as a urinary marker of maternal starvation and dehydration [3]. These findings stimulate many researches workers to hypotheses that ketonuria in the setting of post-term pregnancy can have a potential effect on the fetal wellbeing which can be evaluated by assessing any possible relation between the presence of Ketonuria and its severity and the results of fetal testing including mainly non stress test and amniotic fluid volume [2,3,14,15].

This study has shown that not only the presence of ketonuria but its severity had a potentially adverse effect on the results of non stress test and the amniotic fluid volume. All of them had an abnormal fetal non stress test in term of spontaneous deceleration in the fetal heart rate [16].

The results of this study in regard to the frequency of having minor levels of ketone bodies in the urine are similar to the results of a study done by Onyeije and Divon, enrolling 3601 patients with post-term pregnancy over a period of 4 years [3].

In regard to the effect of ketonuria on the amniotic fluid volume in term of alteration in the amniotic fluid index, this study had shown that ketonuria is associated with higher frequency of oligohydramnios with a significant statistical association between ketonuria and the presence of oligohydramions.

A study done recentlyby Rhee et al in South Korea (2005) showed that amniotic fluid assessment is a weaker predictor of poor perinatal outcomes than has been classically suggested [17].

On reviewing the results of this study regarding the relationship of various demographic features of the sample included in this study and the severity of ketonuria, one can conclude that the most important factors that potentially enhances the risk of having severe ketonuria is the gestational age. 80% of patients with severe ketonuria and 75% of patients with moderate ketonuria had passed the 41 week of gestation. This finding can explain the longer time of exposure to the phenomenon of accelerated starvation that can be regarded as the main mechanism used to describe the increased risk of ketonuria in post-term pregnancy.

The process of enhanced ketone production as a result of accelerated starvation becomes significant in the latter portion of pregnancy [18,19]. It becomes progressive and more potentially effective in enhancing ketone production from week to week in the later portion of pregnancy and of course it becomes more important in the setting of post-term pregnancy.

In final conclusion one could say that the most powerful factor that can affect the severity of maternal ketonuria is the gestational age, and that ketonuria and its severity had statistically significant association with oligohydramnios and abnormal results of non-stress test, in term of fetal heart rate deceleration or non reactive non stress test.

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Received 18th Sep. 2013: Accepted 16th Dec. 2013.

Iraqi JMS

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

Sudden Death due to Intracranial Colloid Cyst "A Case Report"

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Abstract

Intracranial colloid cysts are non-neoplastic epithelium-lined cysts of the central nervous system that almost always arise from the anterior third ventricle roof (immediately posterior to the foramen of Monro). These epithelium-lined, mucin-containg cysts can be found in asymptomatic patients; however, depending on their location, size, and degree of cerebrospinal fluid obstruction, patients may present with a variety of neurological symptoms. These symptoms can range from headaches to sudden death on rare occasions when there is acute hydrocephalus.

This study describes a colloid cyst located between the two lateral ventricles at the level of foramen of Monro obstructing the cerebrospinal fluid (CSF) flow leading subsequently to sudden death and discovered incidentally during autopsy. The study was done in the Medico-legal institute in Baghdad from 1st of November 2012 to 1st of May 2013.

Key words Sudden Death, Colloid Cyst, Autopsy, Intracranium.

Introduction

The dynamics of cerebrospinal fluid (CSF) production in the choroid plexus, its circulation throughout the ventricular system and subarachnoid space and then its reabsorption via the arachnoid granulations into the superior sagittal sinus is critical for homeostasis of the brain. Tumors can easily obstruct the circulation of the CSF and lead to abrupt enlargement and increase in the pressure of the ventricular system. This condition is known as acute hydrocephalus and it is potentially fatal. Example of this type of mechanism is the colloid cyst (which is not truly a tumor) [(1)].

Brain cysts are sacs filled with fluid and other organic debris. Almost all brain cysts form during fetal development; the colloid cysts form during embryonic development of the central nervous system [(2,3)]. In 1910, Sjovall hypothesized that colloid cysts were remnants of the paraphysis, an embryonic midline structure with diencephalic roof immediately rostral to the telencephalic border (old name is paraphyseal cysts) [(4)]. The origin of these cysts continues to be uncertain. Diencephalic ependymal, invagination of the neuroepithelium of the ventricle, or the respiratory epithelium of the endodermal origin are other etiologic possibilities so the colloid cysts are believed to derive from either primitive neuroepithelium of tela choroidea or from the endoderm [(5)]. One leading theory is that colloid cysts form when ectopic elements migrate into the velum interpositum during ventral system embryonic development [(2,5)].

Colloid cysts usually arise in the anterior portion of the third ventricle between the fornices. The cysts are attached the roof of the third ventricle and frequently to the choroid plexus. Usually, the cysts are immediately dorsal the foramen of Monro.These cysts have also been reported to frequently arise in the septum pellucidum, the

fourth ventricle, and the sella turcica[6]. In 1858, Wallmann first reported on colloid cyst and in 1921, Dandy accomplished the first successful resection of a colloid cyst [7].

Colloid cysts usually afflict adults (the youngest reported cases involve a 2-month-old infant) with uncertain gender differences; eight percent of the patients reported in the literatures are aged 30-60 years, approximately 0.1-1% of all primary intracranial brain tumors and 15% of all the intraventricular masses are colloid cysts and are the most common type of the neuroepithelial cysts, as well as the most common tumor in the third ventricle [2,4,5]. No known genetic relationship has been described, although familial occurrences of colloid cyst have been reported [5,7,8]. Typically, colloid cysts are clinical silent and are found incidentally when patients are imaged for other reasons and some types during autopsy, either computerized tomography scanning (CT) or magnetic resonance imaging (MRI) may help in diagnosing a colloid cyst, although MRI has fewer advantages. MRI typically demonstrates the location of the cyst, and the nature of intacystic contents [9,10]. These cysts can trigger headaches, fever and dizziness. The patient may also experience bouts of nausea and vomiting. Cysts rupture can be particularly dangerous; the contents spill into the brain can lead to meningitis. Cyst growth can cause hydrocephalus, a condition in which blockage prevents normal flow of fluid through the brain, causing the fluid to accumulate to dangerous level. In particularly severe cases, the afflicted person dies suddenly, without having experienced any prior symptoms [1-4].

Case report

On 25th of February, 2013; a natural sudden death case was referred by the police from Al-Mahmudiya to the medico-legal institute in Baghdad. The victim was 20 years old female with history of migrainous headaches since 2 years. She was treated with amitryptyline 25 mg once daily, 1 year before her death. Her medical history was negative apart from two previous cesarean sections. The night before her death; she complained of severe headache, which was relieved by a non-steroidal anti-inflammatory drug (oral Ibuprofen 200 mg) and then she went to bed after feeling well; during the night she had frequent projectile vomiting and the next morning she was found dead; face down in the bathroom. At autopsy the brain appeared swollen 1250 g in weight with flattening of the gyri throughout the convexities. The brain was symmetrical with no shifting of the midline structures. At the base; the unci of the temporal lobes were grooved and the hypothalamus appeared prominent but there was no definite herniation grossly.

On sagittal section the lateral ventricles were enlarged and containing a colloid cyst, 2.5 cm in diameter attached to the septum pellucidum at the level of foramen of Monro. The lateral ventricles were enlarged but the third and fourth ventricles were of normal size (Fig. 1.).

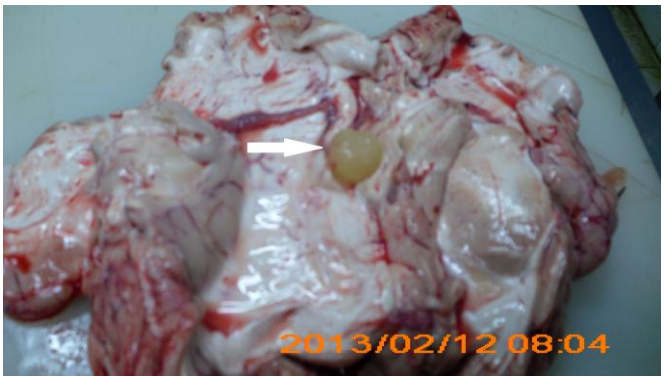

Fig. 1. **Colloid cyst of the ventricles at the level of foramen of Monro attached to the septum pellucidum.**

The cyst wall was elastic and yellowish in color, lined with mixed array of epithelial and goblet cells (Fig. 2) and full of proteinaceous mucinous fluid, slightly turbid-yellow and its consistency was thin gelatinous.

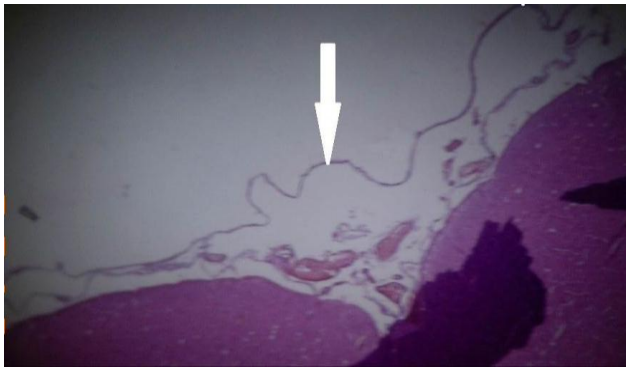

Fig. 2. Colloid cyst showing a cystic lesion lined by a thin fibrous capsule (X40 H. & E. stain).

It was concluded that her cause of death was due to acute hydrocephalus resulting from obstruction of CSF flow by the colloid cyst. No other pathological findings both grossly and microscopically were seen in her body and toxicological screening tests were negative.

Discussion

Undiagnosed brain tumors are rare cause of sudden natural deaths observed in medical examiners settings. Benign tumors are more likely than their malignant counter-parts to present with sudden death [11,12]. On rare occasions, a colloid cyst may obstruct the foramen of Monro completely and irreversibly, resulting in sudden loss of consciousness and, if patients are not treated, coma and subsequent death due to herniation. An alternative theory suggests that sudden death in patients with colloid cysts may be related to acute neurogenic cardiac dysfunction (secondary to acute hydrocephalus) and subsequent cardiac arrest rather than herniation [2]. The risk of sudden death remains difficult to predict, the risk of sudden death does not seem to correlate with tumor size, degree of ventricular dilatation, or duration of symptoms [13]. A study found that 8% of asymptomatic patients with a colloid cyst of the third ventricle eventually became asymptomatic [4], whereas a different study found 34% patients presented to a hospital with acute deterioration and in some cases sudden death [1]. Cyst size and extent of ventricular dilatation do not seem to predict for acute deterioration [6]. Pollock et al. found that ventriculomegaly is the most important variable associated with cyst-related symptoms, other variables include age and cyst size [2]. Eberhardt et al. described their 20 years of experience of previously undiagnosed brain tumors resulting in sudden death. In Eberhardt series of 11 neoplasms associated with sudden death, seven (63%) were gliomas, two (18%) were intraventricular colloid cysts, one (9.09%) was pituitary adenoma and one (9.09%) was schwannoma [12]. Following age-related regional frequency of brain tumors, lesions that involve the fourth ventricle are more common in children, whereas those of the third and lateral ventricles predominate in adults and this agrees with this study where the tumor was found in 20 years old female [1,4-7,14]. A study by Shemie et al. from Hospital for Sick Children in Toronto indicated that from 1990 through 1997, seven children died unexpectedly with acute obstructive hydrocephalus. All children had an intracranial tumor located at a critical site for CSF circulation, three with colloid cyst, two with asrocyrtoma, one with ependymomma and one with suspected lymphoma. This study was limited to children only where colloid cysts are more prevalent [14].

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Received 22nd Sep. 2013: Accepted 11th Dec. 2013

المجلد الحادي عشر، العدد الرابع، 1435 هـ، 2013م

المجلة العراقية للعلوم الطبية

عنوان المراسلات إلى المجلة العراقية للعلوم الطبية، صندوق بريد 14222 بغداد، العراق. تلفون وفاكس (964-1-5224368).

رقم الإيداع في دار الكتب والوثائق ببغداد 709 لسنة 2000