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STRESS RESPONSE FOLLOWING USE OF LARYNGEAL MASK AIRWAY AND TRACHEAL INTUBATION IN NORMOTENSIVE AND HYPERTENSIVE PATIENTS DURING INDUCTION OF GENERAL ANESTHESIA

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Abstract

Background: *The present study was accomplished to compare the use of laryngeal mask airway and tracheal tube in normotensive and hypertensive patients during anesthesia.* ***Methods :*** *80 patients subjected to elective surgical procedures under general anesthesia at Mansoura University Hospital were enrolled in this study. They were classified into normotensive and hypertensive group each is 40 patients. Each group is randomly classified into endotracheal tube group and laryngeal mask group each is 20. hamodynamics, blood glucose and blood cortisol level were recorded preoperative and 1,3,5 minutes after induction of anesthesia and at extubation. postoperative complications were recorded.*

Results:*hemodynamics and blood glucose and cortisol level increase after induction of anesthesia in all groups. There was significant increase in endotracheal group in hemodynamics, blood cortisol and glucose level compared to laryngeal mask group.*

Conclusion: *laryngeal mask is better to be used than endotracheal tube for airway management during induction of anesthesia especially in hypertensive patients.*

Key words : *laryngeal mask airway, tracheal intubation complications, hypertension.*

Introduction

Laryngoscopy and tracheal intubation after induction of anesthesia are frequently associated with transient hypertension,tachycardia and arrhythmias. Although these hemodynamic responses are probably of little consequence in healthy individuals, they may be more severe and more hazardous in hypertensive patients.[1] Laryngeal mask airway (LMA) insertion, originally described by Brain, has recently become widely used in airway management. [2] Insertion of the LMA after induction of anesthesia causes less hemodynamic change than tracheal intubation.[3,4] To our knowledge, especially in hypertensive patients, only few studies compare pressor responses and blood cortisol level following use of LMA. This study was performed to compare the pressor response for LMA insertion and tracheal intubation in normotensive and hypertensive patients.

Patients and Methods

The study was approved by the local ethical committee of department of anesthesia mansoura university hospital and informed consent was obtained from each patient. Forty normotensive patients (ASA I) and forty controlled hypertensive patients (ASA II) of either sex, aged between 20-50 years scheduled for elective surgical procedures under general anesthesia were the subject of the study. Exclusion criteria were a history of difficult intubation, respiratory, cardiac, CNS, cervical spine or esophageal disease. Patients were eligible to be normotensive when they had no history of hypertension and their admission blood pressure was <140/90 mmHg after hospitalization. Patients were eligible to be controlled hypertensive when they had a history of hypertension for which they were being treated and their admission blood pressure on was <150/100 mmHg after hospitalization.

All hypertensive patients received their antihypertensive medication approximately 3 hours before induction of anesthesia. Patients were premedicated with i.v midazolam 1-2 mg 15 minutes before surgery. An intravenous line was secured before induction of anesthesia for drugs injection

and an arterial line was used for blood sampling. Both normotensive and hypertensive Patients (40 patients each) were randomly assigned into either tracheal intubation group (ET group) or LMA insertion group(LMA group) 20 patients each.

After preoxygenation for 3 minutes with face mask , anesthesia was induced by using 1μg/kg fentanyl and 2.5mg /kg propofol followed by succhinylcholine 2mg/kg to facilitate tracheal intubation or LMA insertion. Tracheal tube or LMA were connected to anesthesia breathing circuit for IPPV. Anesthesia was maintained with 60% oxygen in air and sevoflurane 2%, muscle relaxation was achieved 0.08 mg/kg of vecronuim. Recording heart rate, non invasive blood pressure before insertion of tracheal tube or LMA and 1, 3 and 5 minutes after. Blood samples for basal blood glucose and basal cortisol level in blood before induction of general anesthesia were taken and then 3 minutes after tracheal intubation or laryngeal mask insertion. At the end of the surgery, residual neuromuscular block was reversed with (0.04mg/kg) neostigmine and (0.02mg/kg) atropine followed by removal of tracheal tube or LMA. Patient blood pressure and heart rate were recorded at time of extubation and 5 minutes after.Sore throat and hoarseness were graded on an established 4-point scale.

Statistical Analysis

The statistical analysis of data done by using excel program and SPSS program (statistical package for social science version 10). To test the normality of data distribution K-S (Kolmogorov-Smirnov) test was done. The description of the data done in form of mean (+/-) SD for quantitative data and Frequency and proportion for Qualitative data. The analysis of the data was done to test statistical significant difference between groups. ONE WAY ANOVA test to compare more than two groups, followed by Post Hoc test LSD (least significant difference) for inter groups comparisons. For quantitative date student t-test was used to compare between two groups. Paired sample t-test to compare one group at different time. Chi square test was

used for qualitative data. P is significant if ≤ 0.05.A sample size of 20 patients per group was required to achieve a power 85%.

Results

Normotensive and hypertensive groups were comparable in age, sex and duration of surgery. There were no differences noted among groups in age, duration of surgery. Male patients(42) more than female patients (38) (table 1). Normotensive patients: Baseline values of mean arterial blood pressure were comparable in groups .Mean blood pressure shows significant increase at 1,3,5 minutes after intubation and laryngeal mask insertion. But there was a significant decrease in mean arterial blood pressure in LMA group when compared with ET group ($p<0.05$). There was a significant increase in MAP at extubation and LMA removal and 5 min after, compared to the baseline but there were no significant difference among groups (table 2). No difference in the baseline values of blood cortisol. There was significant increase after intubation and LMA insertion compared to the basal values, the increase was significantly less in LMA than in ET group (table 3).

Hypertensive patients: Baseline values of mean arterial blood pressure were comparable in groups. Mean blood pressure shows significant increase at 1,3,5 minutes after intubation and laryngeal mask insertion when compared to the baseline values. But there was a significant decrease in MAP in LMA group compared to ET group ($p<0.05$). There was a significant increase in MAP at extubation and LMA removal, 5 min after, compared to the baseline but there were no significant difference among ET and LMA groups (table 4). No difference in the baseline values of blood cortisol level. There was significant increase after intubation or LMA insertion. The increase was significantly less in LMA than in ET group (table 5).

Table (1) : Patients characteristics of the studied groups.

	normotensive		hypertensive	
	ET	**LMA**	**ET**	**LMA**
Age (years)	***40.74±7***	***38.45±7***	**43.25±5**	**44.1±4**
Gender (male/female)	(9/11)	(12/8)	(8/12)	(13/7)
Duration of surgery	121.21±19.67	***108*±18.32**	**105.4±19.37**	110.9±18.15

Values are expressed as mean ± SD or number
ET =endotracheal tube.
LMA=laryngeal mask airway.

Table (2) : Perioperative mean arterial blood pressure (mmHg) of normotensive groups.

Groups	ET	LMA
basal	94.25±9.74	95.48±6.42
preintubation	96.72±6.85	96.25±5.83
1min	129.56±9.56*	106.23±6.98*#
3min	125.26±7.9*	105.35±4.26*#
5min	117.83±10.48*	100.67±6.58*#
extubation	122.16±11.74*	104.33±7.03*
5min after extubation	109.97±9.07*	98.81±5.41*

Values are expressed as mean ± SD.
Significant when ($p < 0.05$).
* Significant compared to the basal value in the same group.
\# Significant compared to ET group.

Table (3) : Perioperative blood cortisol level (mcg/dL). of normotensive patients.

Groups	ET	LMA
Before intubation	234.14±75.16	207.75±43.21
After intubation	453.74±85.15*	285.1±54.62*#

Values are expressed as mean ± SD.
Significant when ($p < 0.05$).
* Significant compared to the basal value in the same group.
\# Significant compared to ET group

Table (4) : Perioperative mean arterial blood pressure (mmHg) of hypertensive groups.

Groups	ET	LMA
basal	128.4±5.85	130.4±7.39
preintubation	130.9±4.82	133.45±6.67
1min	159.65±6.82*	146.55±6.74*#
3min	160.1±7.25*	147.6±6.68*#
5min	155.85±5.56*	143.9±7.15*#
extubation	160.85±7.39*	145.6±6.19*
5min after extubation	153.4±8.44*	140.7±7.69*

Values are in Mean ± SD
Significant when ($p < 0.05$)
* Significant compared to the basal value in the same group.
\# Significant compared to ET group.

Table (5) : Perioperative blood cortisol level (mcg/dl) of hypertensive groups.

Groups	ET	LMA
Before intubation	216±72.26	197.1±32.59
After intubation	494.05±68.66*	268.3±51.27*#

Values are in Mean ± SD.
Significant when ($p < 0.05$).
* Significant compared to the basal value in the same group.
\# Significant compared to ET group.

Discussion

Hypertensive patients are prone to much greater haemodynamic changes after tracheal intubation than normotensive patients.[5] An increase in blood pressure associated with laryngoscopy and tracheal intubation is dangerous and may cause left ventricular failure, myocardial ischaemia or cerebral haemorrhage.[6] Therefore, the prevention of hypertension following intubation of the trachea is of major importance in hypertensive patients.

Use of the LMA avoids the need for laryngoscopy and tracheal intubation, so that the marked hemodynamic response to these procedures should be prevented.[4]

Our results in normotensive patients confirmed that increases in HR, MAP and blood cortisol after tracheal intubation were greater than those after LMA insertion.

This study has also shown that insertion of the LMA reduces the increases in haemodynamic variables after laryngoscopy and tracheal intubation in hypertensive as well as in normotensive patients. Our results are in agreement with many authers[3,7,8] The results of this study contradict with others[9,10], they found no significant difference in heart rate

and mean arterial blood pressure in between tracheal intubation and LMA insertion.

The change in blood cortisol and blood glucose level is part of the stress response to induction of anesthesia. The changes in blood cortisol level lead to an associated increase in blood pressure and heart rate.[11] Use of LMA showed less pressor response during induction of general anesthesia ,this could be in turn due to the fact that it is relatively simple and atraumatic to insert and does not require stimulation of the airway with laryngeoscopy.[12] this ease of insertion evokes less pressor and sympathoadrenal response than endotracheal intubation without any associated increase in incidence of complications as shown from results of our study.

Conclusion

The LMA should be used instead of the tracheal tube for airway management during general anesthesia in fasting patients especially in situations where minimal changes in hemodynamics are desirable like patients with hypertension and ischemic heart disease.

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POST - MENOPAUSAL - OSTEOPOROSIS ROLE OF LOW LEVEL - LONG TERM CADMIUM EXPOSURE

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Abstract

The material for the present study included 28 post - menopausal osteoporotic women selected from Rheumatology and Rehabilitation out patient clinic Mansoura University Hospital with age range 45 - 68 years. Determination of bone Mineral density (BMD) was done for all patients using Dual - Energy - x- ray Absorptiometry (DEXA). Assessment of probability for osteoporotic bone fractures was determined using FRAX- fracture risk assessment score. 8 healthy volunteers with matched age and sex served as control group. Determination of serum calcium (Ca), serum Alkaline phophatase and 24 hours urinary Ca excretion were done for all patients and volunteers (control group). Serum and urinary cadmium (Cd) concentrations were determined using perkin - Elmer atomic absorption spectrophotometery. Serum and urinary Cd concentrations were found to be significantly increased among osteoporotic women (P = < 0.05) with concomitant significant decrease in (BMD) and significant increase in FRAX scores (P=< 0.05). these changes were particularly noticed among elderly osteoporotic women > 60 years.

Increase in 24 hours urinary Ca excretion associated with significant hypocolcaemia (P= <0.05) was observed among osteoporotic women particularly elderly patients with age > 60 years. However serum Alkaline phosphatase concentration, illustrated non-significant changes. Our results were discussed in light of previous literature and we concluded that low level long term Cd exposure is associated with increased risk for post menopausal osteoporosis. Enthusiastic efforts should be done to fight environmental pollution and Cd exposure must be limited as

much as possible from early years of life since Cd has cumulative toxic effects on bones.

Introduction

A number of risk factors for osteoporosis have been identified or suggested such as age, female gender, smoking, Alcohol, inactivity, low Ca intake and several medical disorders as well as drugs[1][2]. Cadmium was recently identified as a possible risk factor for osteoporosis[3][5][6] Skeletal damage may be the critical effect of low level long term exposure to Cd but the mechanism behind this is not clearly understood[4]. Cd is a heavy metal that is widely present in our environment as a pollutant[5] the environmental presence of Cd is attributed to industrial emissions and agriculture[6]. Populations world wide are exposed to Cd by inhalation of Tobacco smoke. Presence of Cd in the atmosphere due to industrial activities and the use of fertilizers containing Cd may lead to contamination of land and increase uptake of Cd by crops and vegetables cultivated for human consumption[7]. Dust is potentially an important route of exposure to Cd in areas with contaminated soil[8]. The aim of the present study was to investigate the possible role of low level long term Cd exposure as a risk factor for postmenopausal osteoporosis.

Material and methods

The material for the present study included 28 postmenopausal osteoporotic women. Selected from Rheumatology and Rehabilitation out - patient clinic Mansoura University Hospital with age range 45 - 68 years. Determination of bone mineral density (BMD) was done for all patients using Dual - Energy - x ray Absorptiomety (DEXA). Assessment of probability for osteoporotic bone fractures was determined using FRAX fracture risk assessment score [9] 8 healthy volunteers with matched age and sex served as control group. All patients and control group were subjected to the following study.

1) serum and urinary Cd concentrations were determined using Perkin Elmer atomic absorption spectrophotometry [10].

2) Determination of both serum Ca and serum alkaline phosphatase, were done according to the methods described by Tietz N W [11].

3) 24 hours urinary Ca excretion was determined according to the method described by Rochwell[12].

Results

1) Serum and urinary Cd concentrations were found to be significantly increased among osteoporotic woman (P=< 0.05) with concomitant significant decrease in (BMD) and significant increase in FRAX scores (P=0.05). these changes were particularly noticed among elderly patients with age > 60 years (Tables I , II).

2) 24 hours urinary Ca excretion was found to be significantly increased (P=<0.05) among osteoporotic women, particularly elderly patients (Tables III, IV).

3) Significant hypocalcaemia (P = < 0.05) was observed among osteoporotic women particularly in old patients with age above 60 years, however serum alkaline phosphatase illustrated non significant changes (P=>0.05) (tables IV, VI).

Table I: Serum and urinary Cd concentrations in osteoporotic patients versus healthy volunteers (control group).

	Serum Cd µg/dL M± SD	**Urinary Cd µg/dL M ± SD**
Osteoporotic women	0.16 ± 0.018	0.17 ± 0.01
Healthy volunteers	0.08 ± 0.011	0.045 ± 0.012
P value	< 0.05	< 0.05

Table II : Serum and urinary Cd concentrations , in relation to (DEXA) and FRAX scores in elderly osteoporotic women > 60 years versus middle aged patients.

	Serum Cd M ± SD	Urinary Cd M ± SD	DEXA T score hip M ± Sd	FRAX score facture risk % M ± SD
Elderly Osteoporotic patients > 60 years (20 patients)	0.188 µg / dL ± 0.01	0.19 µg / dL ± 0.02	-3.4 ± 0.12	13 ± 2.2
Middle aged patients < 50 years 8 patients	0.142 µg /dL ± 0.02	0.152 µg/dL ± 0.01	- 2.8 ± 0.13	5.7 ± 0.35
P value	< 0.05	< 0.05	< 0.05	< 0.05

Table III : 24 hours urinary Ca excretion in osteoporotic patients versus healthy volunteers (control group).

	Osteoporotic Patients	control group	P Value
24 hours urinary calcium excretion M± SD	**352** ±26 mg/24h	210 ± 15 mg/24 hours	< 0.05

Table IV : 24 hours urinary Ca excretion in elderly osteoporotic patients > 60 years versus Middle aged patients.

	Elderly osteoporotic Patients > 60 years	Middle aged patients < 50 years	P Value
24 hours urinary calcium excretion M± SD	398 ± 28 mg/dL	308 ± 14 mg/dL	< 0.05

Table V : Serum Ca and alkaline phosphatase concentrations in osteoporotic patients versus healthy volunteers (control group).

	Serum Alkaline phosphatase M ± SD	**Serum Ca M ± SD**
osteoporotic patients	103 U/L ± 6.1	7.8 mg/dL ± 0.33
healthy volunteers (control group)	93 U/L ± 7.4	9.8 mg/dL ± 0.31
P value	> 0.05	< 0.05

Table VI : Serum Ca and alkaline phosphatase concentration in elderly osteoporotic patients > 60 years versus middle aged patients <50 years.

	Serum Alkaline phosphatase M ± SD	**Serum Ca M ± SD**
Elderly osteoporotic patients > 60 years (20 patients)	102 ± 8.5 U / L	7.2 mg/dL ± 0.21
Middle aged patients < 50 years (8 patients)	91 ± 7.5 U / L	8.1 mg/dL ± 0.32
P value	> 0.05	< 0.05

Discussion

Populations world wide are exposed to Cd by low level intake, mainly through their contaminated water and food, inhalation of tobacco smoke and exposure to Cd contaminated air born particles (dust)[8] Cd is stored in bones and kidney from birth therefore its urinary excretions shows long term life time exposure[13]. Serum and urinary cd concentrations were found to be significantly increased among our patients with post. menopausal osteoporosis with concomitant significant decrease in (BMD) and increase in FRAX scores. Studies among populations from Belgium[14] Sweden[15] Japan[16] and china[17] showed similar association between osteoporosis and low level long term environmental Cd exposure. The generally accepted interpretation has been that Cd induces renal damage, decreasing Ca reabsorption in nephron thus resulting into hypercalciuria[18] and low bone mineral density (BMD) and hence increased fracture risk [14] particularly in post - menopausal women.

Significant hypercalciuria with increased 24 hours urinary ca excretion was found among our patients with post menopausal osteoporosis a finding which is in agreement with previous studies[14][15][16] Cd long term exposure causes tubular kidney damage which may progress to renal impairment, sever osteoporosis and osteomalacia. Also environmental Cd exposure promotes Skeletal demineralization leading to increased fracture risk[18].

Independently of the status of kidney function other studies support a direct osteotoxic effect of Cd on the basis of an increase in the urinary excretion of pyridinium cross links from bone collagen even at low non-nephrotoxic concentration of urinary Cd in post-menopausal women[19]. In a recent study[20] low level Cd exposure was found to activate osteoclastic functions and decrease osteoblastic viability and activity. Serum alkaline phosphatase a marker of osteoblastic activity illustrated non significant changes in our patients with osteoporosis in spite of presence of significant hypocalcaemia, decreased BMD

and increased FRAX scores a finding which may suggest that low level of Cd exposure may have direct osteotoxicity and adverse effects on osteoblastic activity and viability.

In the present study Cd toxicity, hypercalciuria, osteotoxicity, decreased (BMD) and increased fracture risk was particularly noticed among postmenopausal women in older age group above 60 years. Cd is a cumulative toxic element the main organ for long term Cd accumulation is the kidney and bones[21] the half life period for Cd is approximately 10 years, Cd Nephrotoxicity and osteotoxicity risks increase with age.

Our result were discussed in light of previous literature and we concluded that low level long term Cd exposure is associated with increased risk for post- menopausal osteoporosis. Enthusiastic effort should be done to fight environmental pollution and Cd exposure must be limited as mush as possible from early years of life since Cd has cumulative toxic effects on bones.

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CONSERVATIVE LAPAROSCOPIC ELECTROCOAGULATION ADENOMYOLYSIS (CLEA): AN INNOVATION FOR THE MANAGEMENT OF ADENOMYOSIS

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Abstract

Objective: To assess the efficacy of a laparoscopic novel technique for management of women with adenomyosis. Patients and methods: From June 2008 to June 2011 in Mansoura university teaching hospital, 30 perimenopausal women (between 40-50 years old) with chronic pelvic pain and/or menorrhagia with ultrasonic features suggestive of adenomyosis by transvaginal ultrasound and/or MRI were subjected to unipolar adenomyolysis. Women were assessed before the intervention and after 12 months as regard the uterine volume measured by TVS as well as the magnitude of pain using Visual Analogue Scale (VAS). The overall level 12 months of the procedure was assessed by questionnaires. Results: There were a significant improvement in every scale of the SF-36 ($p < 0.0001$) , a significant reduction in the mean volume of the uterus after 12 months of the procedure (435.3- 247.5 cm^3) initially and after 12 months, respectively, $p <0,0001$). There was significant reduction in the mean scoring system of pain (59,41) initially and after 12 months, respectively ($p <0,0001$). seventeen cases (56%) expressed their moderetly satisfied with the procedure, eight cases (27%) were highly satisfied while five cases (17%) were not satisfied with the procedure. Two patients got pregnant after the procedure. One of them had first trimester miscarriage. The other one was had a full term pregnancy and was delivered cesarean section. Conclusion: Laparoscopic Electrocoagulation Adenomyolysis (CLEA) may be an effective and safer

minimal invasive procedure for management of symptomatic perimenopausal women with adenomyosis. More studies are warranted.

Keywords: *adenomyosis, pain, laparascopy, adenomyolysis.*

Introduction

Adenomyosis is a benign gynecologic disease in which the endometrial stroma invades the uterine myometrium, usually on the posterior wall of the uterus. Adenomyosis is divided into diffuse and localized forms according to the extent of the lesion. Localized adenomyosis is also known as adenomyoma[1].

The incidence rate of the disease varies between 5 and 70%. Generally it occurs in women aged between 40 and 50 years with prevalence rate of 70-80%. Adenomyosis was found in 23% of uterus which has been removed due to fibroma uteri[2].

The etiology of this disease has not been clearly elucidated. However, several pathophysiological mechanisms have been proposed, such as damage of endometrial-myometrial border due to trauma and high estrogen biosynthesis associated with increased activities of aromatase enzyme[3].

The clinical manifestations include dysmenorrhea, chronic pelvic pain, and menorrhagia. It is usually combined with pelvic endometriosis, endometrial cysts of the ovary, uterine myomas or other estrogen-dependent diseases[4].

Chronic pelvic pain (CPP) can be defined as a nonmalignant pain perceived in structures related to the pelvis; constant or recurring over a period of 6 months. In some cases it might be associated with negative cognitive, behavioral and social consequences[5].

The diagnosis of adenomyosis was based on clinical symptoms. In recent years, the development of imaging techniques has made diagnosis more accurate. It has been reported that the sensitivity of diagnosis by vaginal ultrasound is 80% to 86%and its specificity is 74% to 86%; the sensitivity of magnetic resonance imaging is 80% to 86% and its specificity is 74% to 86% [6].

Treatment with different strategies has been tried, any conservative treatment is a first choice when preservation of fertility is desired. Unfortunately, treatment options based on such symptoms have been variably effective, with the result that it is still difficult to manage symptomatic patients[7]. For refractory patients who prefer conservative measures and also decline hysterectomy, the treatment options are ill-defined Among the conservative treatments, medical therapy may be the least invasive and most acceptable strategy, and includes the use of prostaglandin inhibitors, oral contraceptive pills, progestogens, danazol, gestrinone, and gonadotropin releasing hormone (GnRH) agonists. Unfortunately, the effect of these medical treatments is often transient; and the symptoms (especially pain) of uterine adenomyoma related diseases nearly always reappear after discontinuing medication [8].

The surgical approach for preserving the uterus can be considered when dysmenorrhea does not respond to drug treatment, the patient can not tolerate the side effects of longterm drug treatment, or the patient wants to have a definite diagnosis. The standard conservative surgical approach has been excision of the myometrial adenomyoma through exploratory laparotomy, but the results have been varied. The development of advanced techniques has led to other, less invasive conservative surgical approaches, including endomyometrial ablation, laparoscopic myometrial electrocoagulation, and laparoscopic surgery. In addition, uterine artery embolization has also been available in the management of women with adeno¬myoma or adenomyosis[9].

All conservative surgical treatments have been proven effective in up to 50% of patients; however, the follow-up assessment periods have been of short duration[10].

The conventional and definitive management of symptomatic adenomyosis is hysterectomy. It is estimated that in the United States approximately 650,000 hysterectomies were performed each year[11].

Phillips et al., 1996 study de-

signed as prospective observational study (laparoscopic bipolar coagulation for conservative treatment of adenomyomata) Preoperative GnRH analogue, In 10 patients(limited number) performed on adenomyomata, and concluded that further evaluation of this technique is necessary to determine its definitive role[12].

Wood's study (1998) showed that conservative surgeries, such as endometrial ablation, myometrial electrocoagulation, or laparoscopic excision were effective in >50% of patients, although the follow-up was limited and it is difficult to ensure recovery after excision or electrocoagulation[10].

Laparoscopic resection versus myolysis in the management of symptomatic uterine adenomyosis (Wachyu Hadisaputra, T. Dewi Anggraeni 2006) study concluded that, no significant differences were found in median reduction of menorrhagia and dysmenorrhea scores between the resection and myolysis groups. The findings of this study had not given enough evidence to provide guidance for managing symptomatic adenomyosis conservatively in the future because of the limitations in data, sample, and duration of the study. It is necessary to conduct further studies with an adequate number of subjects as well as objective data and instruments. In addition, long-term observation is still essential in evaluating the effectiveness, side effects, and recurrence as well as the effects of the intervention on pregnancy and delivery. If persistent symptoms were found in women with enough parity, the most appropriate therapy for adenomyosis would be a total hysterectomy[2].

Methods

This prospective observational study was assigned to know the effectiveness of unipolar adenomyolysis by laparoscopy in eliminating subjective symptoms, such as pelvic pain and menorrhagia as well as objective signs such as reduced size of adenomyosis. Full ethical approval was obtained from the local ethics committee to the study protocol.

Protocol :

From June 2008 to June 2011, in obstetric and gynecologic de-

partment in Mansoura University Hospital Egypt, 30 patients with preoperative diagnosis of adenomyosis had done CLEA and the method of contraception postoperative was condom and agreed to take part to the study protocol, inclusion criteria were as follows: Premenopausal women (40-50 years), they complete their families, they suffer from chronic pelvic pain and / or menorrahgia, no association with other pelvic pathology, they refuse hysterectomy as a treatment option.

All women underwent gynaecological examination, pelvic transvaginal and abdominal ultrasonography and MRI in order to evaluate the presence of adenomyosis

Surgical techniques :

We used unipolar needle for myolysis. The anterior surface of adenomyosis was visualized to protect the bladder and The unipolar needle was put directly opposite to adenomyosis through the supra pubic Secondary puncture and the depth of penetration about 10-15mm depending on the thickness of the adenomyotic myometrium determined preoperatively by ultasonography and MRI. After the depth for safe penetration had been determined, the unipolar needle was applied to adenomyosis using one way current at (80-100 watts) continuous power and then moved slowly to reach maximum coagulation, needle punctures were made at 1-2 cm intervals and the punctures about 10.

The posterior surface of adenomyosis was visualized to protect the colon, and uterine blood vessels. The unipolar needle was put directly opposite to adenomyosis through the subumbilical primary puncture and the the depth of penetration about 10-15mm depending on the thickness of the adenomyotic myometrium determined preoperatively by ultasonography and MRI. After the depth for safe penetration had been determined, the unipolar needle was applied to adenomyosis using one way current at (80-100 watts) continuous power and then moved slowly to reach maximum coagulation, needle punctures were made at 1-2 cm intervals and the punctures about ten, Excessive blood was suctioned

and the tissue was cleaned up thoroughly.

Finally, the laparoscopy is withdrawn and gas is allowed to escape from the cannula which is then removed and closure of the skin at sites of punctures was done by interrupted mattress sutures. After the surgical procedure, we observe the patients for 24 hours. If their conditions were stable, they were discharged in the next day. We gave antibiotics and NSAID for 3 to 5 days .

Follow up:

After surgical treatment patients were recommended to use condom as contraceptive device . All patients were asked to undergo a follow-up visit 3, 6,12 months after surgery.

During the follow-up visit, patients underwent physical examination and trans-vaginal ultrasonography and or MRI to evaluate symptoms and uterine volume. Women were asked to complete the SF-36 Questionnaire and to rank their symptom intensity using the same numerically rated VAS used preoperatively.

Results

The 30 patients assessed for eligibility, underwent conservative laparoscopic electrocoagulation adenomyolyisis (CLEA), Significant complications did not occur. Subjective symptoms could be evaluated in all patients.

SF 36 Scores :

After (CLEA), at 12-months follow up, a significant improvement was observed in the SF-36 total score, in the SF-36 component summaries and in every scale of the SF-36 ($p < 0.0001$) (Table 1).

Pain scores (VAS) :

VAS were significantly improved after 12-months from surgical treatment ($p<0.0001$). Preoperatively 83% chronic pelvic pain (mean VAS score of 59). Postoperatively, at 12-months follow up, (meanVAS score of 41) (table 2).

Uterine volume :

On pelvic examination and through ultrasound exam, there were significant reduction was found in mean adenomyosis volume (43%) at 12 - months

follow-up (table 3).

Improvement :

Symptomatic improvement after CLEA according to the complaint (Table 4).

Vascularity :

Vascularity by Doppler after CLEA (Table 5).

Success :

Technical success rate after CLEA (Table 6).

Table (1) :

Scale	Pre	12	P value
Physical functioning	13 ± 1.6	39 ± 14	.000
Role limitations due to physical health	27 ± 16	48± 24	
Role limitations due to emotional problems	36.3±21.8	36.5±22.1	
Energy/ Fatigue	39± 4.9	54± 13	
Emotional well being	58± 6.7	69± 12	
Social functioning	28± 5.6	52± 16.5	
Pain	30± 3.7	62± 18.8	
General health	31± 5.3	59± 15.9	

Table 2 :

VAS	N	Mean	Std.Deviation
Preoperative	30	59.0000	6.6176
3 months follow up	30	50.6667	9.8027
6 months follow up	30	41.0000	14.7040
12 months follow up	30	41.0333	14.7028

Table 3 :

Item	Preoperative (mean) (cm^3)	Postoperative (mean) (cm^3)			Average reduction (%)	P VALUE
		3 months	6 months	One year		
Uterine size	435.3	262.5	247.4	247.5	43%	P < 0.001

Table 4 :

	CPP		Menorrhagia		improved	
	No	%	No	%	No	%
Improved:						
Significant	8	26.66	6	33.33		33.33
Slight	17	56.66	7	38.88		50.00
Total	25	83.33	13	72.22		83.33
Not improved	5	16.66	5	27.77		16.66
P						.000

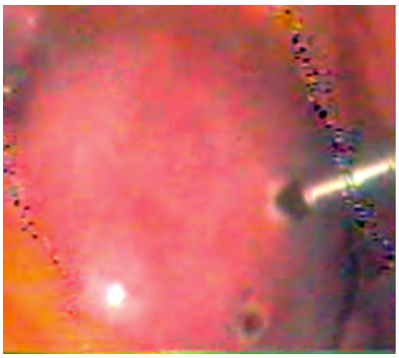
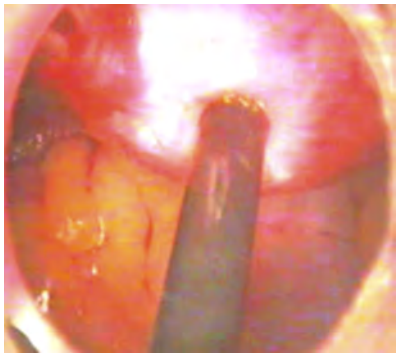
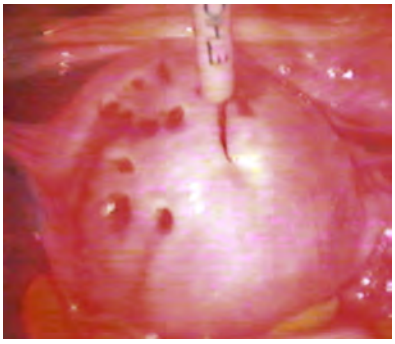
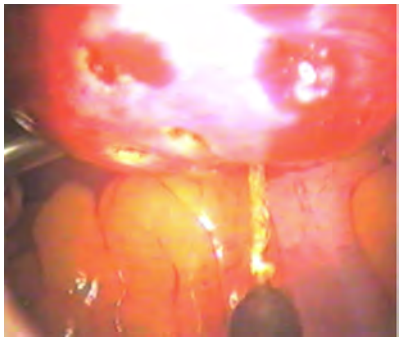
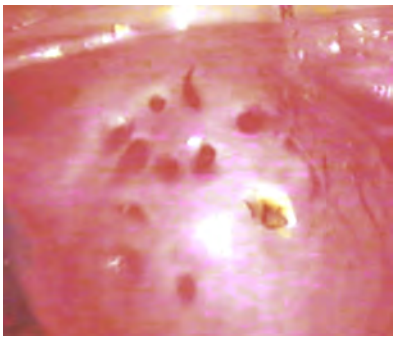
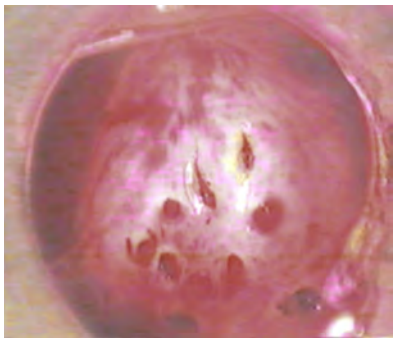
Table 5 :

Vascularity by Doppler	No	%
Decreased	25	83.33
Unchanged	5	16.66

Table 6 :

Result	No	%
Succeeded	25	83.33
Failed	5	16.66
Total	30	100

Anterior wall **Posterior wall**

Discussion

In our study we design a prospective observational study (on 30 women aged between 40-50) years old suffering from chronic pelvic pain and or menorrhagia diagnosed adenomyosis by TVS and MRI , the thirty patients had done CLEA and the method of contraception postoperative was condom.

The adenomyosis can be detected by MRI, TVS, inspection of the uterus at laparoscopy, electrocoagulation has the capability of shrinking adenomyosis by causing necrosis., the technique has been applied to localized or extensive disease, the ectrocoagulation may be less accurate as electrical conduction in the abnormal tissue may be incomplete and this cannot be checked at the time of surgery and It may also reduce the strength of the myometrium by replacing abnormal myometrium with scar tissue and the width of the scar may be extensive to be sufficient to control symptoms and the risk of uterine rupture following extensive electrocoagulation is demonstrated by the following experience.

Electrocoagulation is best suited to women over 40 years of age, who do not wish to conceive, and who wish to avoid more extensive surgery such as excision or hysterectomy. We avoid using of vasoconstricting agents such as adrenaline and vaso-pressin as excessive bleeding has not been experienced and the blanching of the myometrium after vasoconstriction makes it difficult to determine the devascularizing effect of electro-coagulation instead we use PGE1 analog preoperative.

Electrocoagulation of the adenomyosis carried out with unipolar needles, using (80-100 W) coagulation current, as bipolar needles concentrate current between the two needles and their effectiveness is diminished by the tendency of the two needles to move close together as they penetrate the myometrium. Additionally, the area of coagulation may spread outwards from each needle and there is a possibility of surface necrosis in which a future adhesion may be formed, needle punctures are made at 1-2 cm intervals, depending on the spread of the coagulative effect. The

depth of needle puncture may vary, depending on the thickness of the adenomyotic myometrium determined preoperatively by ultrasound or MRI. This varies from 1 to 1.5 cm., so laparoscopic unipolar myometrial electrocoagulation may be useful as penetration of coagulation may be achieved over the full depth of the myometrium.

No complications have been observed including post-operative infection, bleeding or subsequent adhesion formation. The result of the surgery was assessed by symptom relief and MRI or TVS. Loss of features of adenomyosis including reduction of myometrial thickness, reduced vascularity and normal myometrial appearance have all been observed.

Contraception should be offered to all women having myometrial electrocoagulation because of the possible future risk of uterine rupture in pregnancy, One woman had ruptured uterus in the twelfth week of pregnancy in Phillips et al. (1996) study and One woman had ruptured uterus in the seven month of pregnancy in Wachyu Hadisaputra, T. Dewi Anggraeni 2006) study.

SF 36 Scores After laparoscopic surgery, at one year follow up, a significant improvement was observed in the SF- 36 total score, in the SF-36 component summaries and in every scale of the SF-36 ($p<0.0001$) (Table 1).

In our study SF-36 Scores after CLEA at one year follow up ,a significant improvement was conducted, The SF-36 is a multipurpose health survey with 36 questions. It yields an eight-scale profile of functional health and well-being scores, as well as psychometrically based physical and mental health summary measures (standardized).[13]

From table (1) the mean physical functioning preoperative and 12 months postoperative was (13.3±1.6, 39.03±14.7) respectively, the mean Role Limitations due to physical functioning preoperative and 12 postoperative was (27.5±16.5, 48.36±24.5) respectively, the mean Role Limitations due to emotional problems preoperative and 12 postoperative was

(36.30±21.8, 36.49±22.05) respectively, the mean Energy/ Fatigue preoperative and 12 postoperative was (39.66±4.9, 54.86±13.03) respectively, the mean Emotional well being preoperative and 12 postoperative was (58.4±6.7, 69.50±12.09) respectively, the mean Social functioning preoperative and 12 postoperative was (28.33±5.6, 52.53±16.54) respectively, the mean pain preoperative and 12 postoperative was (30.83±3.7, 62.68±18.78) respectively, the mean General health preoperative and 12 postoperative was (31.83±5.3, 59.70±15.92) respectively and there were significant improvement on patients from preoperative to postoperative 12 months followe up ($p < 0.0001$).

This study reported that, 83.33% of patients improved after laparoscopic surgery (CLEA) while 16.66% of patients at their 12 months follow up appointment reported that their symptoms did not improve. 33.3% of women reported significant improvement, 50.00% reported slight improvement,16.66% not improved and NO reported cases had complete improvement .

From table (2) the mean VAS as assessment of Pain preoperative and 3,6,12 postoperative was (59.00±6.6, 50.66±9.8, 41.00±14.70, 41.03±14.70) respectively and there were significant improvement on patients from preoperative to postoperative 12 months followe up ($p<0.0001$).

Improvement (table 4) of chronic pelvic pain and bleeding problem had been reported in (25) patients 83.33%, NO improvement in (5) patients 16.66% one case missed in follow up and another four cases had done hysterectomies.

Chronic pelvic pain cases had been improved in 25 cases (83.33%) and no improvement had been found in 5 cases (16.66%). Of improved patients 8 cases (26.66%) showed significant improvement, 17 cases (56.66%) revealed slight improvement, No cases reported complete improve.

Menorrhagia had been improved in 13 cases (72.22%) and no improvement had been present in 5 cases (27.77%). Of improved patients, 6 patients (33.33%)

showed significant improvement, 7 cases (38.88%) revealed slight improvement, and No cases reported complete improvement.

From patients (table 4), 25 patients(83.33%) had been improved and 5 patients (16.66%) not improved. Of improved patients, 8 patients (26.66%) showed significant improvement, 17 patients (56.66%) revealed slight improvement NO complete improvement.

Follow up US (Table 3) at preoperative, 3 months, 6 months and 12 months disclosed significant reduction in uterine volume after CLEA. Average mean preoperative uterine volume (435.27 cm^3 ± 61.55). Average mean postoperative uterine volume were (262.49 cm^3±79.94, 247.44 cm^3± 84.04, 247.47 cm^3 ± 84.04 respectively (average reduction 43%) there were significant reduction in uterine volumes on patients from preoperative to postoperative 12 months followe up ($p < 0.0001$).

These findings NOT agree with results of study done by (Wachyu Hadisaputra, T. Dewi Anggraeni 2006)in which there were increase in postoperative uterine size.

Volume reduction analysis by patient revealed significant reduction in 8 cases (26.66%) and slight reduction in 17 cases (56.66%), No reduction in 5 cases (16.66%) on patients from reoperative to postoperative 12months follow up.

Uterine vascularity (Table 5) as studied by color Doppler after CLEA showed reduction in 25 cases (83.33%).

All above data (table 6) showed that; Thirty patients underwent laparoscopy for CLEA and completed follow up inquiries 12- months, 25(83.33%) technically succeeded, and 5 patients(16.66%), technically failed (four cases need hysterectomy, last case missed follow up).

Two patients was pregnant, inevitable abortion was found in 1 patient at the age of 8 weeks of pregnancy, the other one was complete her pregnancy up to full term delivery at 38wk gestation by CS.

Outcomes pregnancy:

The replacement of myometrium by tissue scars in significant amount will create problems, such as reduced capacity of myometri-

um and uterus during pregnancy such that it may trigger abortion or premature birth;and the formation of scar tissue which can contain undetectable adenomyosis focus that may reduce the strength of stretch. The capacity of uterus expansion during pregnancy is more dependent on the increased plasticity, rather than elasticity. The expansion capacity of the uterine myometrium and its supporting tissue makes this maneuver is possible only to be performed in a certain patients(inclusion criteria).

The possibility of uterine rupture is associated with the formation of broad scar tissue after coagulation. It is advisable that myolysis is followed by sterilization in women with enough children, in view of the possible uterine rupture.

However, adenomyosis itself can reduce the capacity of uterus by replacing normal myometrium and supporting tissue which can interfere the arrangements of muscular fibers and three-dimensional structure of collagen. In this way, the scar tissue which was formed after intervention constituted a separate factor.

Thus, myolysis is more appropriate for women aged above 40 years with enough parities who do not want any pregnancy anymore but wish to maintain their uterus or reject broad operation or hysterectomy.

In this study two patients became pregnant, with one patient experienced inevitable abortion at 8 weeks of pregnancy,full term delivery was found in the another one .Of two patients in this group who became pregnant, one was 42 years old; she experienced reduction in adenomyosis volume and chroni pelvic pain and was pregnant at 10 months after the intervention and had her pregnancy terminated by cesarean section at 38 weeks of gestation Her baby had birth weight of 3,000 grams. The patient experienced neither any symptom nor complication during pregnancy. The other patient was 43 years old. She had a reduced volume of adenomyosis and was pregnant in 12 months after intervention, she was pregnant for 8 weeks. Unfortunately, she had inevitable abortion.

Hospital stay revealed short duration: patients were admitted the

day before the procedure and all patients discharged the same day or next day after the procedure with average hospital stay 1 day.

Conclusions

An optimal method for CLEA is not yet defined; however, the result CLEA may be related to the level of experience of the physician performing the procedure.

Recommendations :

The findings of this study had given enough evidence to provide guidance for managing symptomatic adenomyosis conservatively in the future. It is necessary to conduct the procedure. In addition, long-term observation is still essential in evaluating the effectiveness, side effects, and recurrence as well as the effects of the intervention on pregnancy and delivery. It is advisable not to perform myolysis in women who still want pregnancy. If persistent symptoms were found in women with enough parity, the most appropriate therapy for adenomyosis would be a total hysterectomy.

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CONSERVATIVE LAPAROSCOPIC ELECTROCOAGULATION ADENOMYOLYSIS (CLEA): AN INNOVATION FOR THE MANAGEMENT OF ADENOMYOSIS

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EFFECTIVENESS OF EXERCISE PROGRAMS IN FEMALE PATIENTS WITH FIBROMYALGIA

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Abstract

Aim of the work: *The aim of this study was to examine the effectiveness of exercise programs on female patients with fibromyalgia (FM) and to determine the effect of exercise on pain, fatigue, number of tender points, health-related quality of life (HRQOL) and fibromyalgia impact questionaire (FIQ).*

Subjects and Methods: *Forty female patients with FM received 20 weeks of treatment aerobic, followed by strengthening exercise program assessed before and after the study period using wide spread pain, fatigue, sleep disturbance, duration of morning stiffness, number of tender points, quadriceps strength, HRQOL and FIQ were enrolled in this study.*

Results: *Statistically significant improvement of wide spread pain ($t=3.99$, $p<0.05$), fatigue ($t=3.91$, $p<0.05$), sleep disturbance ($t=0.2.22$, $p<0.05$), duration of morning stiffness ($t=4.21$, $p< 0.05$), number of tender points ($t=4.82$, $p< 0.05$), HRQOL ($t=3.66$, $P< 0.05$) and FIQ ($t=3.71$, $p< 0.05$) in FM patients after the study period.*

Conclusions: *This study supports the beneficial role of exercise program rehabilitation in the management of female patients with FM. Exercise program rehabilitation led to significant improvement in pain, fatigue, number of tender points, HRQOL and FIQ, so it is recommended for every FM patient.*

Keywords: *Fibromyalgia; Pain; Exercise; Fatigue; Health-related quality of life; Fibromyalgia impact questionaire*

Introduction

Fibromyalgia FM) is a chronic widespread (both sides, above and below waist line, and axial skeletal) pain, fatigue, mood disturbances, sleep disturbances, tenderness on palpation[1], functional disability, and psychological distress[2]. Comorbidities with other functional somatic syndromes and mental disorders are common[3], it and affects women about 8 times more than men[4]. In young and middle-aged women, fibromyalgia is the most common cause of generalized musculoskeletal pain [5].

Athough the precise pathogenesis of FM remains unknown, peripheral and central hyper-excitability of the spinal cord or brain stem and altered pain perception and somatization have been hypothesized and demonstrated in some patients[6].

Treatment of FM is usually symptomatic due to lack of understanding of its etiology and pathophysiology, due to the lack of effective treatment options, treating patients with FM poses a great challenge to clinicians[7]. There is no cure for FMS and treatment usually requires both pharmacological and non-pharmacological approaches[8]. Current treatment recommendations for fibromyalgia strongly support a multifaceted regimen, including patient education, cognitive behavioral therapy, specific pharmaco-therapy, restorative sleep, and exercise; exercise may include low-impact aerobic exercise, strengthening and flexibility[5].

Although it is known that better clinical findings and disappeared symptoms in patients with FM were obtained from aerobic exercises prescribed for treatment, exact mechanism remains unclear[9]. Exercise programs have been reported to be helpful in FM patients in several studies, however, standardization of the type, intensity and duration of exercise has not yet been well established[10].

The aim of this study was to examine the effectiveness of exercise programs on female patients with fibromyalgia (FM) and to determine the effect of exercise on pain,

fatigue, number of tender points, health-related quality of life (HRQOL) and fibromyalgia impact questionaire (FIQ).

Subjects and Methods

The present study included forty female patients with fibromyalgia (FM) diagnosed according to the American College of Rheumatology (ACR) Criteria [1], who were attending the outpatients clinics of the Rheumatology and Rehabilitation Department of Benha University Hospitals, the mean age ranged was 29.67±5.56 years and the mean disease duration was 3.74 ± 2.14 years. Of the 40 patients started the study only 32 completed it, 8 were withdrawn from the study because of non medical causes.

Exclusion Criteria: Individuals with major diseases which may prevent exercise (cardiac failure or late phase renal or pulmonary diseases), high-grade mechanical or degenerative disorders, inflammatory rheumatological diseases history or orthopedic or neurological disorder of walking were excluded from the study.

Informed written consents were obtained from all participants and the study was approved by the local Ethical Committee.

All Patients Were Subjected to the Following:

Measurement of weight and height, as well as body mass index (BMI) which was estimated with the following formula: Weight / Height^2 (Kg/m^2) [10].

- Evaluation at both the beginning and the end of the study (i.e. after the twenty weeks of the training program).

A- Clinical Assessment:

- Full history taking with special attention to the disease duration, history of widespread pain, fatigue and sleep disturbance (using visual analogue scale ranged from 0-10 where 0 = no, 10 - severe symptoms), together with the presence of morning stiffness and other associated symptoms e.g. irritable bowel disease.

- General examination with special attention to the pulse, blood pressure, lymph nodes and thyroid gland. Cardiac,

neurological and joint examination to exclude secondary causes of fibromyalgia syndrome.

B- Assessment of Physical Function:

1- Number of tender points:

Tender points were assessed according to the American College of Rheumatology Criteria of Fibromalgia [1]. Tender points were recorded as the number of positive tender points (range 0-18),

2- Muscle strength measurement:

Muscle strength was assessed in both knee extensors using a 1-repetition maximum (1-RM) technique[11]. The test requires the patient to perform repeated single repetitions of a particular anatomic motion, separated by periods of rest. Resistances are systemically added until the patients' maximum voluntary muscle force cannot move the resistance through the full range of motion[12]. The greatest weight a patient can move through a full range of motion is recorded as the (1-repetition maximum). Tests were performed on (Model GT. 30 musculator chair-Tokyo OGGI Ken Ltd, Japan). Patients were seated with hips flexed at 90°, and pelvis secured, fixing belt was placed over the trunk and another one over the thigh. The lever of the musculator was attached to the lower leg just above the ankle Joint.

3- Health-Related Quality Of Life[13]:

General HRQOL was measured with the Medical Outcome Study 36-item Short Form Survey (SF-36). The SF-36 consists of 36 items representing 8 subscales that cover the domains of physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health. Of the 8 subscales, 4 scales (physical functioning, role-physical, bodily pain, and general health) principally relate to physical health, and the other 4 scales (vitality, social functioning, role emotional, and mental health) relate to mental health. The 8 subscale scores range from 0 to 100, with lower scores indicating poorer levels of function and higher scores better levels of function.

4- Fibrmnyalgia Impact Questionnaire (F1Q):

The FIQ is a self-administered questionnaire developed and validated for its use in patients with FMS[14]. It measures physical function, work status, and overall well-being; it also contains 6 visual analog scales (VAS) for pain, fatigue, morning tiredness, stiffness, anxiety and depression. The range of the total score is between 0 and 80 where a higher score indicates a negative impact[15]. The F1Q has shown to be the most responsive measure to perceive clinical improvement in FMS patients[16].

Program of exercise training :

After primary assessment measures, patients were enrolled into a program of exercise training that consisted of 2 phases of exercise. The first phase (8 weeks) comprised of graded aerobic exercise, while the second phase (12 weeks) involved a program of progressive strengthening training. Each session was 60 minutes in duration. The frequency of the training sessions was three sessions per week.

Graded Aerobic Exercise[17]:

This program lasted 8 consecutive weeks, mostly walking on treadmills, cycling on exercise bicycles and walking on a track. Each patient was encouraged to increase the amount of exercise steadily as tolerated. The aerobic component was designed to generate heart rate equivalent to 60-80% of age adjusted maximum heart rate.

Strength Training Exercise[18]:

Strength training exercise included static contractions for pelvic and lumbar spine stabilization, and dynamic movement of large muscles and multi-joint action hip flexion / extension, knee flexion / extension, ankle planter / dorsiflexion, shoulder flexion, extension, abduction and adduction, elbow flexion/extension and trunk flexion and rotation. Strength training exercises were performed using a combination of machines (as quadriceps drill), hand weights and body weight. Patients began with resistance levels they could do easily, and progressed in 8-10-12-14 repetition.

Laboratory tests : Complete blood count, erythrocyte sedi-

mentation rate, rheumatoid factor, random blood sugar and thyroid function tests to exclude secondary-causes of fibromyalgia.

Statistical analysis:

The collected data were presented and analyzed using SPSS version 17 soft ware. Suitable statistical techniques were calculated as mean and standard deviation. Student "t" test, was used as a test of significance. P< 0.05 was considered significant.

Results

The results of the present study are summarized, statistically analyzed and presented in 4 tables:

Baseline characteristics of FM patients is shown in (table 1).

There was statistically significant improvement of wide spread pain (t=3.99, p<0.05), fatigue (t=3.91, p<0.05) and sleep disturbance (t=0.2.22, p<0.05) in FM patients after the study period (table 2).

There was statistically significant decrease in duration of morning stiffness (t=4.21, p< 0.05) and number of tender points (t=4.82, p< 0.05) and increase of quadriceps strength (t=3.55,p< 0.05) in FM patients after 20 weeks period of exercise (table 3).

As regards HRQOL and FIQ in FM patients there were statistical significant difference before and after the study period (t=3.66, P< 0.05) and (t=3.71, p< 0.05) respectively (Table 4).

Table (1): Baseline characteristics of FM patients (no=32).

Variable	Mean	±SD
Age (years)	29.67	5.56
Disease duration (yrs)	3.74	2.14
BMI (Kg/m2)	28.43	7.35
Morning stiffness (min)	15.67	6.7
Wide spread pain (VAS)	6.59	3.51
Fatigue (VAS)	7.89	4.11
Sleep disturbance (VAS)	4.9	3.54
No of tender points	15.8	4.7
Quadriceps strength (QdS) (kg)	24.27	4.76
health-related quality of life (SF-36)	45.87	10.76
Fibromyalgia Impact Questionaire (FIQ)	52.76	12.52

Table (2): Comparison of wide spread pain, fatigue and sleep disturbance in FM patients before and after the study (no=32).

	Wide spread pain (VAS)		Fatigue (VAS)		Sleep disturbance (VAS)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Mean	6.59	3.73	7.89	4.53	4.9	3.23
±SD	3.51	2.01	4.11	2.59	3.54	2.36
t-test	3.99		3.91		2.22	
p-value	P< 0.05		P< 0.05		P< 0.05	

P< 0.05 significant
Pre=at beginning of the study Post=after 20 weeks of study.

Table (3): Comparison of morning stiffness, number of tender points and quadriceps strength in FM patients before and after the study (no=32).

	Morning stiffness (min)		No of tender points		Quadriceps strength (QdS) (kg)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Mean	15.67	9.62	15.8	10.93	24.27	27.73
±SD	6.7	4.59	4.7	3.26	4.76	2.76
t-test	4.21		4.82		3.55	
p-value	P< 0.05		P< 0.05		P< 0.05	

P< 0.05 significant
Pre=at beginning of the study Post=after 20 weeks of study.

Table (4): Comparison of health-related quality of life (SF-36) and fibromyalgia impact questionaire (FIQ) in FM patients before and after the study (no=32).

	Health-related quality of life (SF-36)		Fibromyalgia Impact Questionaire (FIQ)	
	Pre	**Post**	**Pre**	**Post**
Mean	45.87	54.29	52.76	42.61
±SD	10.76	7.34	12.52	8.28
t-test	3.66		3.71	
p-value	P< 0.05		P< 0.05	

P< 0.05 significant
Pre=at beginning of the study Post=after 20 weeks of study.

Discussion

Fibromyalgia is a complex and heterogeneous condition in which there is abnormal pain that results in a wide range of symptoms [19-20].

The aim of this study was to examine the effectiveness of exercise programs on female patients with FM and to determine the effect of exercise on pain, fatigue, number of tender points, HRQOL and FIQ.

In healthy individuals changes in pain sensitivity occur after exercise is indicative of analgesic response (e.g., increases in pain threshold and tolerance and lower pain rating). This phenomenon has been termed exercise induced analgesia (EIA) and has been demonstrated after exercise for several different experimental pain stimuli [21].

The most commonly tested hypothesis for analgesia after exercise is that EIA is produced by exercise-induced release of endogenous opioids at peripheral, spinal, and/or central sites capable of pain modulation[22]. Opioid antagonists have been found to attenuate the analgesic response after exercise of mild severity but have not had a consistent effect on analgesia after more severe exercise[23]. The specific neurochemistry of non-opioid analgesia is not fully understood, but several neurotransmitters, such as serotonin and norepinephrine, have been implicated, in addition, involvement of N-methyl-D-aspartic acid subtype of excitatory amino acid receptors have received some attention[24].

In this study, 32 female patients with FM received 20 weeks of treatment aerobic, followed by strengthening exercise program assessed before and after the study period using wide spread pain, fatigue, sleep disturbance, duration of morning stiffness, number of tender points, quadriceps strength, HRQOL and FIQ.

Our results showed statistically significant decrease of wide spread pain and number of tender points in FM female patients after the study period. This result was in agreement with previous study of Jones and Liptan 2009, who

found that aerobic exercises appears to be most beneficial for improving the physical fitness and self-efficacy of FM patients, as well as decreasing their tender point pain threshold. In the same context, Hauser et al 2010 found the same result and reported that subjects that continue exercising tend to do better in long term with less pain and better maintenance of function. Carbonell-Baeza et al 2011[25], in three month exercise program study three times/week revealed that exercise had a positive effect on pain threshold in several tender points in women with FM.

Significant improvement of fatigue in female FM patients after the study period was observed in this study. This result was in accordance with those of Häuser et al 2010[27], who found that exercise reduces fatigue and improves physical fitness and that there is no evidence of a superiority of water-based over land-based exercise, they reported that frequency of 2 to 3 times per week for at least 4 to 6 weeks is necessary for a reduction of symptoms in FM patients.

Fibromyalgia has no cure, and clinical management is difficult. Pharmacological and non pharmacological interventions, is optimal [19]. Medications for managing FM symptoms tend to have modest and inconsistent benefits on pain, other symptoms, and quality of life [27]. Non pharmacological treatments and complementary and alternative therapies-massage therapy, psychological therapies, education, cognitive-behavioral therapy, relaxation training, and social supportmay be helpful [19].

Patients with fibromyalgia have lower functional capacity for daily activities[28] and health-related quality of life than healthy age- and sex-matched people[29].

A statistically significant improvement of HRQOL was revealed in FMS female patients at the end of the study. This result was in agreement with previous studies of Busch et al. 2011, Thomas and Blotman 2010[30-31] who found that exercise in patients with fibromyalgia have found beneficial effects on physical fitness, quality of life, in subjects participating in

exercise training compared with control patients. Jones and Liptan 2009 [32], reported that exercise has been shown to improve patients' symptoms and quality of life in FM patients.

In the present study, there were significant decreases in FIQ score in female patients with FM. Kingsley et al. 2010 [33], found the same result in FM patients after resistance exercise training.

Most studies recommend aerobic and strengthening exercise for FM patients[34]. Recently, researchers have begun to explore the effects of a wide range of exercise techniques that extend beyond more conventional exercise training modes (e.g., low-impact aerobic training and strength training) [30]. Most authors recommend a gradual progression from low intensity exercise [31] using the "start low and go slow" approach [32] with the goal of achieving at least moderate intensity [35].

Conclusions

This study supports the beneficial role of exercise program rehabilitation in the management of female patients with FM. Exercise program rehabilitation led to significant improvement in pain, fatigue, number of tender points, HRQOL and FIQ, so it is recommended for every FM patient.

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EFFECTIVENESS OF EXERCISE PROGRAMS IN FEMALE PATIENTS WITH FIBROMYALGIA

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CORRELATION OF SURVIVIN AND KI-67 EXPRESSION WITH CLINICOPATHOLOGICAL FEATURES OF GASTRIC CARCINOMA

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Abstract

Introduction: *Gastric cancer is ranked as the world's second leading cause of mortality due to cancer next to lung cancer. It continues to be a major health problem because of the slow decrease in incidence in East and high mortality rate of diagnosed gastric carcinoma in West. Inhibition of survivin expression and function resulted in spontaneous apoptosis and mitotic catastrophe. On the other hand, proliferation is a key feature of progression of any tumor.*

Patients and Methods: *We studied the expression of survivin and ki-67 in gastric carcinoma and investigated its relation to clinical and pathological parameters. Archival material from tumor tissue of 36 patients with gastric carcinoma who underwent radical surgery from Jan 2006 to Jan 2008 at the Gastroentrology centre, Mansoura University, Egypt were collected. Paraffin sections of all samples were submitted for immunohistochemistry using survivin and ki-67.* ***Results:*** *In 36 cancer tissue specimens, survivin was expressed in 16 cases (44.4%) while the high mitotic count ki-67 was found in 17 cases (47.3%). Positive expression of survivin was significantly associated with larger tumor size, and higher clinical stage. Positive expression of Ki67 is significantly associated with Lauren pathological classification, higher clinical stage.* ***Conclusion:*** *Survivin expression in gastric carcinoma is significantly related to ki-67 expression; both are related to the higher stage of the disease.*

In conclusion, *Immunohistochemical staining of the survivin and ki-67 is a very useful tool in differentiating the higher stage of the disease. Both could be a potential predictive factor for recurrence or metastasis.*

Key words: *survivin; Ki67; gastric carcinoma; clincopathological data.*

Introduction

Gastric cancer represents the fourth most common cancer and second leading cause of cancer-related death worldwide with highest incidences in Eastern Asia, Eastern Europe and South America [(1)].

Apoptosis, also called programmed cell death, plays an important role in the development and homeostasis of tissues. Deregulation of apoptosis is involved in carcinogenesis by abnormally prolonged cell survival, facilitating the accumulation of transforming mutations and promoting resistance to immunosurveillance[(2)]. Survivin is expressed in most human neoplasms, but is absent in normal, differentiated tissues. Survivin is a bifunctional inhibitor of apoptosis proteins that has been implicated in protection from apoptosis and regulation of mitosis so it may be an important factor in carcinogenesis[(3-4)].

Cell proliferation characteristics may reflect the aggressiveness of gastric tumors and their eventual prognosis. The monoclonal antibody Ki-67 detects a nuclear antigen which is exclusively expressed in proliferating cells (GI,S and G2 phases and mitosis) but not in Go. Hence the Ki-67 antibody permits the immunohistochemical detection of cycling cells and its expression provides a direct measure of the growth fraction of the tissue [(5)].

In this study, we aimed to investigate the expression of survivin and ki67 in human gastric cancer specimens using immunohistochemistry and study its relation with different clinicopathological data.

Material and Methods

Study design : This retrospective study was performed on archival material of 36 cases with primary diagnosis of gastric carcinoma in the Gastroentrology

center, Mansoura University, Egypt in the period from Jan 2006 to Jan 2008. The medical records of patients were reviewed. None of the patients received any neoadjuvant chemotherapy or radiotherapy.

Patients: All patients had a baseline history and physical examination records. Preoperative, intraoperative and postoperative data were collected. Preoperative variables included patient demographics (age, sex, and medical history), patients' symptoms (abdominal pain, vomiting, loss of weight, heart burn, haematemesis and melena), physical signs (abdominal mass, and body weight), laboratory tests, preoperative image studies.

Methods: The paraffin blocks were retrieved and 4 µm thickness sections were prepared for routine H&E. Other sections were prepared on coated slides for immunohistochemistry. Examination of the slides was done for histopathologic diagnosis and TNM staging.

The age of the studied cases are divided into two categories above 60 years (13 cases) and below 60 years (23 cases); regarding gender; the females are 15 cases while the males are 21 cases

Immunohistochemistry: Immunohistochemical staining for survivin was performed for all cases of the study with rabbit polyclonal antibodies (Thermo Scientific Clone RB-9245-R7). It is performed by using ImmunoPure Ultra-Sensitive ABC Peroxidase (Thermo Scientific Cat. # 32052), using (DAB) as chromogen. All specimens are incubated with primary antibody overnight. No enzyme digestion is required as indicated by the procedures. The polyclonal antibody is prediluted. Positive control is pancreatic tissue. Antigen retrival occurs by using EDTA buffer solution ph 8.0 and heating in the microwave for 30 minutes. The cytoplasmic stain is considerd positive as indicated by the procedures,

Monoclonal rabbit Anti-Human ki-67 antigen Clone MIB-1 (Thermo Scientific Cat # MA1-90584). It is diluted to 1:100. Antigen retrieval occurs by using citrate buffer and heating in the microwave

at full power for 30 minutes. Positive control is tumor tissue of colorectal adenocarcinoma proved in previous study to be ki-67 positive

Immunohistochemical scoring: survivin staining intensity was classified as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The percentage of positive cells examined in 200 cells were divided into 0 (<5%), 1 (5%-25%), 2 (26%-50%), 3 (51%-75%) and 4 (> 75%). According to the multiplication product of the intensity and the percentage of positive cells, the IHC results was classified as 0, negative (-); 1-4, weakly positive (+); 5-8, moderately positive (++) and 9-12, strongly positive (+++)[6].

Ki-67 labelling index (LI) was done manually. Ten different fields at a magnification of 400 X were examined at first in those tumor areas with positive stained nuclei. Five random areas within those different fields were chosen and counted under 200-fold magnification. The ki-67 labeling index was expressed as a percentage of tumor cells with positively stained nuclei divided by the total number of tumor cell nuclei counted. The average percentage of positivity was recorded as the ki-67 LI for each case. The cut off value between low and high mitotic count is 45%[7-8].

Statistical analysis: Statistical analysis of the data in this study was performed using SPSS software, version 10. For continuous variables, descriptive statistics were calculated and were reported as mean ± standard deviation (SD). Categorical variables were described using frequency distributions. Independent sample t-test was used to detect differences in the means of continuous variables and Chi-square test was used in cases with low expected frequencies. P values < 0.05 were considered to be significant.

Results

Thirty six patients with gastric carcinoma who underwent radical gastrectomy were included in the study. They were admitted to the Gastro-entrology centre, Mansoura University between January 2006 and January 2008. The mean age of patients was 52.58 ± 10.82 years. The studied cases

were divided into two age-related categories; above 60 years (13 cases) and below 60 years (23 cases). Regarding gender cases included 21 males and 15 females.

The age and the gender showed no statistically significant difference regarding either survivin or ki-67. The clinicopathological data of all patients in relation to survivin expression were summarized in Table (1); Figure (1) & (2).

There is statistically significant relationship between the size of the tumor and survivin expression; the larger the size; the more expression of survivin. In patients with gastric mass less than 5 cm; the negative cases were 12 while the positive was 2 on other hand in patients with gastric mass more than 5 cm; 8 cases were negative and 14 cases were positive (P=0.024).

According to Lauren pathological classification; 19 cases were intestinal type (11cases had negative expression of survivin while 8 cases showed positive expression of survivin), 13 cases were diffuse type (among which 6 cases showed negative expression of survivin and 7 cases were positive) and 4 cases were mixed (one case was negative and three cases were positive) (P=0.07).

There was insignificant relationship between survivin expersssion and Helicobacter pylori status, lymph node status or neural infiltration (P=0.14, P=0.56, and P=0.08 respectively). Regarding T (tumor depth) and TNM staging both are statistically significant; the higher the T tumor depth; the higher TNM staging, the more expression of survivin protein (P=0.001).

Association between ki-67 and some pathological data of the studied cases were summarized in Table (2); figure (3) the parameters mentioned in the table were size; Lauren pathological classification; lymph node metastasis; T (tumor depth); neural infiltration and TNM staging. Ki-67 showed statistically significant relationship with Lauren pathological classification (P=0.012). In addition, ki-67 showed a statistically significant relation with TNM stag-

ing; the higher the stage was associated with more expression of ki-67 (P=0.048).

The relation between ki-67 and survivin was shown in table (3). Higher survivin expression was associated with higher ki-67 milotic count. The number of cases which had high count for ki-67 were 17 cases (47.3%) while the low count cases were 19 cases (52.7%). On the other hand the number of survivin positive cases were 16 cases (44.4%) while the negative and weak cases were about 20 cases (56,6%). Marked staining of survivin co-existing with high count of ki-67 was found in 6 cases.

Table (1) : Survivin and the clinicopathological data of the studied cases.

Variable	No.	%	Survivin			P-value
			Weak Or Negative	moderate	marked	
Size						
Less than 5cm	14	33.3	12	2	0	0.024
more than 5cm	22	66.6	8	7	7	
Lauren types						
Intestinal type carcinoma	19	52.7	11	4	4	0.078
Diffused type carcinoma	13	36.1	7	4	2	
Mixed	4	11.1	2	1	1	
Helicobacter pylori infection						
Positive	19	52.7	13	5	1	0.144
Negative	17	47.2	8	3	6	
Lymph node metastasis						
(−)ve	15	41,6	16	7	6	0.567
(+)ve	21	58.3	4	2	1	
Neural infiltration						
(−)ve	32	88.8	19	6	7	0.080
(+)ve	4	1.1	1	3	0	
T Stage						
T1	1	2.7	1	0	0	
T2	21	58.3	15	4	2	0.027
T3	9	25	4	4	1	
T4	5	13.8	0	1	4	
TNM stage						
I+II	21	58.3	17	4	0	0.001
III+ IV	15	41.6	3	5	7	
*P value is significant at 0.05 levels (P≤ 0.05).						

Table (2): Association between ki-67 and some pathological data of the studied cases.

Variable	No.	%	KI67		P-value
			Low count Less than 45%	High count more than 45%	
Size					
Less than 5cm	14	38.9	11	3	0.138
more than 5cm	22	61.1	8	14	
Lauren types					
Intestinal type carcinoma	19	52.7	8	11	0.012
Diffused type carcinoma	13	36.1	7	6	
Mixed	4	11.1	3	1	
Helicobacter pylori infection					
Positive	19	52.7	7	10	0.187
Negative	17	47.2	12	7	
Lymph node metastasis					
(−)ve	15	41,6	10	5	0.158
(+)ve	21	58.3	9	12	
Neural infiltration					
(−)ve	32	88.8	18	14	0.238
(+)ve	4	1.1	1	3	
T Stage					
T1	1	2.7	1	0	
T2	21	58.3	12	9	0.356
T3	9	25	5	4	
T4	5	13.8	1	4	
TNM stage					
I+II	21	58.3	14	7	0.048
III+ IV	15	41.6	5	10	
*P value is significant at 0.05 levels (P≤ 0.05).					

Table (3) : The relation between Ki67 and survivin expression.

		No.	%	Survivin			P value
				Weak Or Negative	Moderate	Positive	
Ki67	Low count Less than 45%	19	52.7	16	2	1	0.002
	High count more than 45%	17	47.3	4	7	6	
*P value is significant at 0.05 levels (P≤ 0.05).							

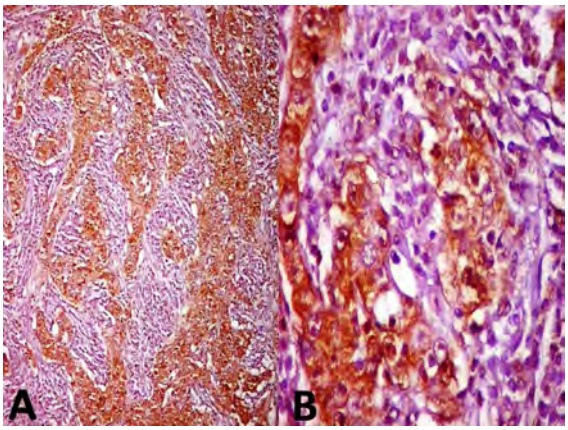

Fig. 1 : Poorly differentiated adenocarcinoma (diffuse type) with marked cytoplasmic survivin expression of tumor cells (A) low power (Immunoperoxidase DAB X 200) (B) high power (Immunoperoxidase DAB X 400).

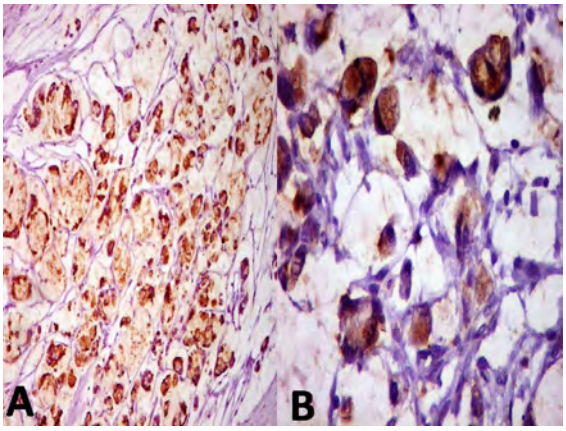

Fig. 2 : Mucinous adenocarcinoma with scattered signet ring cells (intestinal type) with moderate cytoplasmic survivin expression of tumor cells (A) low power; the remnants of the acini appear with pools of extracellular mucin (Immunoperoxidase DAB X 200) (B) high power; some signet ring cells stained with survivin (Immunoperoxidase DAB X400).

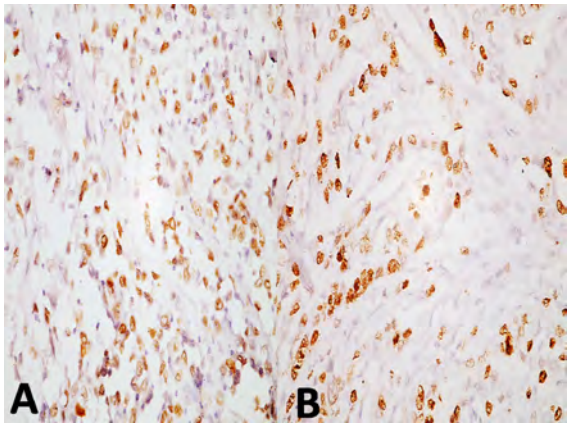

Fig. 3 : High count Ki-67 in (A) and (B) the percentage of tumor cells with positively stained nuclei divided by the total number of tumor cells nuclei counted more than 45% (Immunoperoxidase DAB X400).

Discussion

Gastric carcinoma had been regarded as a top killer among cancers of the gastrointestinal tract[9]. Despite its decreasing in incidence, cancer stomach is still one of the most common malignant tumors worldwide. However, with aggressive treatment, prognosis of patients remains poor. It is linked not only to the high disease stage, but also to the aggressive biologic behavior, which is characterized by high potential for metastasis as well as resistance to anticancer therapy[10].

Our current study found that no statistical relationship between age, gender and survivin expression. This comes in agreement with Da et al. and Zhu et al. who found no relation between both variables and survivin[3'6].

In the present study; there is significant positive correlation between survivin expression and Lauren pathological classification as well as TNM staging system while other clincopathological crieteria were insignificant including Helicobacter pylori, lymphatic, neural, and T (tumor depth) Many studies study this relationship; Zhu et al., (2003) found that the expression of survivin had no relation with the parameters of, tumor depth, tumor size, and stage of the disease, but was significantly related to histological type. Intestinal type also was significantly higher of survivin expression than that in cases of diffuse type[3]; another study was done by Da et al., (2009) found that there is significant positive correlation between survivin expression and lauren pathological classification as well as lymph node metastasis[6]; Song et al., (2009) found that the only statistically significant factor is the size[10]. Krieg et al., (2002) found that stage and grade are significantly related to survivin while other parameters are not[11]. Bertazza et al., (2009) documented that survivin is related to the TNM staging system of patients with gastric carcinoma[12]. Survivin mRNA is a poor prognostic parameter and that non-antiapoptotic survivin-2B was correlated to tumor stage, histological type and depth of tumor invasion with poor outcome in gastric carcinoma[7'10].

Helicobacter pylori causes gastritis and peptic ulceration and it is an important risk factor for gastric adenocarcinoma[13]. Yu et al., found that expression of survivin in gastric cancer was associated with reduced apoptosis so these findings indicate that inhibition of apoptosis regulated by survivin is important in the pathogenesis of gastric cancer[14]. Morever; Zhenning et al., (2004) in their study found that survivin expression even in the peritoneal cavity significantly correlated with TNM stage, depth of cancer invasion, and lymph node metastasis[9].

Stable inhibition of survivin expression and function resulted in spontaneous apoptosis and mitotic catastrophe, enhanced sensitivity to cytotoxic drugs, and suppression of de novo tumor formation with reduced development of angiogenesis in gastric cancer these data suggest that targeting the survivin pathway alone or with cytotoxic drugs may be useful in the treatment of gastric cancer[15-16]. Some other data indicate that high expression of survivin is correlated with poor prognosis and chemotherapy resistance[3] Survivin protein is an important predictive and prognostic parameter of poor outcome in gastric carcinoma[10-11]. Moreover, up-regulation of survivin is found in gastric cancer cell lines after treatment with cytotoxic drugs, indicating that survivin contributes to chemoresistance in gastric cancer[17].

In the current work there is significant positive correlation between Ki67 expression and Lauren pathological classification as well as TNM staging system. The other clincopathological criteria were insignificant including Helicobacter, lymphatic, neural, and T (tumor depth). Some studies study this relationship; Zheng et al., documented ki-67 immunostaining done in this study indicated that the intestinal-type carcinomas had high levels of proliferation and apoptosis compared with diffuse-type carcinoma[18]; Rezaii et al., mentioned that Ki67 expression in relation to Helicobacter pylori seropositivity are associated with gastric cancer[19] Ki-67 immunohistochemically to in preoperative biopsy specimens obtained from extremely well-

differentiated gastric adenocarcinoma will be very helpful for pathologic diagnosis[20]. On the other hand; Lazar et al mentioned that there is a close correlation between the degree of tumor differentiation and the Ki-67 score. The results do not reveal any correlation between the Lauren's pathological classification of gastric carcinomas, the lymphovascular invasion, the depth of tumor invasion; the TNM stage and the Ki-67 score[21]. Czyzewska et al., documented a statistically significant correlation was found of Ki-67 in tumor tissue and metastatic lymph nodes with depth of wall invasion and local lymph node involvement[22]. Ki-67 was independent prognostic factor [23].

In the current work; the survivin expression occurs in 16 cases (44%) while the high mitotic count of the ki-67 occurs in 17 cases (47.3%). Zhu et al., (2003) found that survivin was expressed in 27 of 56 (48.2 %) cases of human gastric carcinoma tissues (3). In addition; there is significant positive correlation between survivin and Ki67 the high count of ki-67 were associated with more survivin expression. Meng et al., (2004) found no relation between them [7].

In conclusion, immunohistochemical staining of the survivin and ki-67 is a very useful tool in differentiating the higher stage of the disease. Both could be a potential predictive factor for recurrence or metastasis. Suvivin may be a potential target for specific therapeutic interventions in the future.

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CORRELATION OF SURVIVIN AND KI-67 EXPRESSION WITH CLINICOPATHOLOGICAL FEATURES OF GASTRIC CARCINOMA

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CLINICAL ASSESSMENT OF MILD COGNITIVE IMPAIRMENT

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Abstract

Objective : *This study is carried out to evaluate mild cognitive impairment (MCI) clinically and neuropsychologically, furthermore we followed those patients althrough the period of 2 years (from March, 2009 to Februrary, 2012) to evaluate whether or not these patients will convert to dementia.*

Methods : *The participants were 50 patients with MCI in addition to 20 normal control subjects .We performed a group of neuropsychological test battery including measures of global cognitive abilities, memory, orientation, attention, executive functions, language, and visuospatial abilities. The Functional Activities Questionnaire (FAQ) was used for instrumental activities of daily living (IADLs).* ***Results :*** *There were no statistically significant differences between MCI patients as a whole and control as well as between convertor and stable MCI patients as regards age, sex, education, vascular risk factors "Diabetes, Hypertension, Heart disease, Dyslipidaemia" and neuropsychiatric symptoms" depression, anxiety" ($P>0.05$). There were statistically significant differences between MCI patients as a whole and control as regard all the neuropsychological tests ($p<0.05$) except intelligent quotient(IQ). Regarding convertor and stable MCI, there were statistically significant differences between convertor and stable MCI patients as regard all the neuropsychological tests except minimental state examination (MMSE),working memory and associate learning ($p<0.05$).*

Conclusions : *Neuropsychology has contributed importantly to the characterization of MCI patients and its differentiation from cognitive*

changes associated with normal aging.

Keywords : *Mild cognitive impairment ; Alzheimer's disease; Neuropsychological characteristics.*

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative illness associated with aging, accounting for 60-70% of age-related dementia cases. In 2000, approximately 25 million people over the age of 60 were diagnosed with dementia worldwide, and the number afflicted is expected to reach over 80 million by 2040 [1]. Earlier diagnosis of AD is widely considered to be an important goal for researchers. Characterization of the earliest known clinical signs has led to the development of the classification of MCI which is thought to be a transitional stage between normal aging and the development of AD [2].

MCI is frequently prodromal to AD , to the extent that MCI has been referred to as early-stage AD , and clinical manifestation of AD has been recently subdivided into the stage of MCI and the subsequent stage of dementia [3]. The patient's history typically raises the suspicion of a decline in cognition, usually memory, and neuropsychological testing may be necessary to corroborate the decline. Neuropsychological testing may be helpful to distinguish MCI cases from normal aging, but testing is not routinely needed to make the clinical diagnosis. A brief mental status examination in the physician's office, such as the Mini-Mental State Examination (MMSE), is often insensitive to MCI [4].

The gold standard for the diagnosis of MCI is a clinical history with corroboration from an informant. There are no definitive diagnostic tests for MCI, and neuropsychological tests and laboratory or brain imaging findings should be interpreted in the light of the clinician's history and assessment [5].

Subjects and Methods

Subjects :

A prospective study was conducted on patients with MCI admitted to neurology department of

Mansoura University Hospital in the period of March, 2009 to Februrary, 2012. The study included 2 groups.

1- MCI group :

Fifty patients with MCI selected according to the following inclusion criteria based on the modified Petersen criteria (2) : 1- Subjective cognitive complaint. 2- Essentially intact activities of daily living. 3- Objective cognitive impairment. 4- Not demented. Subjects were considered cognitively impaired if their performance on any domain was at least 1.5 SD below published age- and education-adjusted normative means[6,7]. A cutoff of 1.5 SD was used so as to not over diagnose MCI.

The single domain amnestic subtype of MCI (aMCI-S) was diagnosed if scores on memory measures were lower than 1.5 SDs below the normative mean & performance on measures of the other cognitive domains was greater than or equal to 1.5 SDs below the demographically corrected mean. The multiple cognitive domain amnestic subtype (aMCI-M) was defined as impaired memory with impairment 1.5 SDs below norms in 1 or more tests in another cognitive domain.

Exclusion criteria included: (a) Cerebrovascular disease, hydrocephalus, intra-cranial mass, traumatic brain injury ,Parkinson disease, Huntington disease, seizure disorders. (b) Major depressive disorder, bipolar disorder, schizophrenia, substance use disorder, or mental retardation. (c) Abnormalities in serum Vitamin B12, or thyroid hormones' levels. (e) Significant medical problems (e.g. poorly controlled diabetes or hypertension; clinically significant hepatic, renal, cardiac or pulmonary disorders).

2- Control group :

Twenty normal control subjects were selected to match the patient's age, sex and education level. The controls showed no impairment in the cognitive tests, and had no history of neurological or psychiatric disease.

Routine laboratory investigations :

Basic laboratory tests were

performed to rule out the presence of diseases that can produce cognitive impairment other than neurodegenerative. These tests included; complete blood picture (to role out anemia), random blood sugar, serum creatinine, liver function tests, vitamin B12 level and hormonal assay (T3, T4, TSH to rule out hypothyroidism).

Neuropsychological Assessment :

The neuropsychological test battery included measures of global cognitive abilities, memory, orientation, attention, executive functions, language, and visuospatial ability. Global cognitive abilities were tested using the mini-mental state examination (MMSE)[8] and the full-scale intelligence quotient (IQ) from the Wechsler Adult Intelligence Scale (WAIS)[9]. Working memory was tested with digit span subtest of Wechsler Memory Scale (WMS)[10,11]. Episodic memory was tested using Logical memory (LM), Associate learning and Visual reproduction subtests of WMS. Semantic memory was tested using similarities subtest of WAIS. Orientation was tested using orientation subtest of MMSE[8]. Attention was tested using trail making test -A (TMT-A). The subject has to connect consecutively numbered circles from 1 to 25 in a sequence[10]. Executive functions were tested using-Trail making test-B (TMT-B) and Mental control subtest of WMS. Language was tested using comprehension subtest of WAIS. Visuospatial abilities were tested using Picture completion and Block design subtests of WAIS. The Functional Activities Questionnaire (FAQ) was used for instrumental activities of daily living (IADLs). This instrument was administered to rate each subject's performance on 10 separate categories of IADLs: (1) writing checks, paying bills, keeping financial records; (2) assembling tax or business records; (3) shopping alone; (4) playing a game of skill; (5) making coffee or tea; (6) preparing a balanced meal; (7) keeping track of current events; (8) attending to and understanding a television program, book, or magazine; (9) remembering appointments, family occasions, medications; and (10) traveling out of the neighborhood. Higher scores in

each category denote increasing impairment: 0 = normal; 1 = has difficulty, but does by self; 2 = requires assistance, or 3 = dependent [12].

Statistical Procedures

Frequency, mean and standard deviation were used to describe data. Chi-square test was used to test for significant association between conversion and other categorical parameters. As most of the data showed obvious deviation from normal distribution curve, non-parametric Mann-Whitney μ test was used to test for significance of difference between groups in quantitative variables.. P value was considered significant if less than 0.05. These tests were run on an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS) for windows 17 (SPSS Inc., Chicago, IL, USA).

Results

All of our MCI patients fulfilled the criteria of amnestic MCI, with 12 (24%) presented with memory impairment alone and 38 (76%) presented with memory impairments plus impairment in one or more of other cognitive domains (memory, orientation, attention, executive functions, language and visuospatial abilities. During the 2 years follow- up period, 11(22%) of patients has converted to Probable AD (convertor) and 39(78%) remained as MCI (stable).

A-Demographic and Neuropsychological characteristics of MCI patients as awhole:

Demographic characteristics :

There were no statistically significant differences between MCI patients and control as regards age, sex, education, vascular risk factors "DM, HTN, heart disease, dyslipidaemia" and neuropsychiatric symptoms"depression, anxiety" ($p>0.05$) "Table 1".

Neuropsychological characteristics :

1- Neuropsychological performance of MCI patients as a whole at baseline:

All Neuropsychological performance of control and MCI patients were statistically significant ($P<0.05$) except IQ scores which showed statistically insignificant difference ($P>0.05$) "Table 2".

2- Neuropsychological performance of MCI patients as a whole after 1 year :

The mean scores of all the neuropsychological tests except IQ showed statistically significant differences between MCI patients and control (P<0.05)"Table 3".

3- Neuropsychological performance of MCI patients as a whole after 2 years :

The mean scores of all the neuropsychological tests except IQ showed statistically significant differences between MCI patients and control (p<0.05) "Table 4".

B- Demographic and Neuropsychological characteristics of convertor and stable MCI patients:

Demographic characteristics :

There were no statistically significant differences between convertor and stable MCI patients as regards age, sex, education, vascular risk factors "DM, HTN, heart disease, dyslipidaemia"and neuropsychiatric symptoms "depression, anxiety" (p>0.05). (Table 5).

Neuropsychological characteristics :

1- Neuropsychological performance of convertor and stable MCI patients at baseline:

All Neuropsychological performance of convertor and stable MCI were statistically significant (P<0.05) except MMSE, digit span, associate learning and mental control scores which showed statistically insignificant difference (P>0.05) "Table 6".

2- Neuropsychological performance of convertor and stable MCI patients after 1 year:

The mean scores of all the neuropsychological tests except the digit span showed statistically significant differences between convertor and stable MCI patients (p<0.05)"Table 7".

3- Neuropsychological performance of convertor and stable MCI patients after 2 years:

The mean scores of all the neuropsychological tests showed statistically significant differences between convertor MCI and stable MCI patients (p<0.05) “Table 8".

Table (1): The demographic characteristics of MCI patients as a whole.

	Control	MCI	P value
Age (mean ±SD)	56.75±7.09	60.64±9.69	0.167
Sex: No(%) F	8(40%)	26(52%)	0.364
Education (mean ±SD/year)	3.55±4.32	4.84±5.5	0.443
Vascular risk factors			
DM: No (%)	6(30%)	9(18%)	0.269
HTN: No (%)	3(15%)	12(24%)	0.407
Heart disease: No (%)	2(10%)	2(4%)	0.329
Dyslipidaemia: No (%)	2(10%)	9(18%)	0.404
Neuropsychiatric symptoms			
Depression: No (%)	0(0%)	7(14%)	0.078
Anxiety: No (%)	0(0%)	5(10%)	0.142

(DM : Diabetes , HTN : Hypertension)

Table (2) : Baseline Neuropsychological performance of MCI patients as a whole.

Domain , cognitive measure	**Control Vs MCI patients**		
	Control	**MCI**	**P value**
FAQ	0.15±0.37	2.62±2.66	<0.001
Global cognitive abilities :-MMSE	29.25±.85	25.04±3.86	<0.001
-IQ	101.4±7.37	95.86±21.64	0.507
Memory			
Working memory (Digit span)	9.4±1.47	8.44±1.93	0.027
Episodic memory: -L.M	15.05±3.63	9.17±4.28	<0.001
-Associate learning	5.3±1.22	3.51±0.94	<0.001
-Visual reproduction	11.05±1.10	6.32±3.48	<0.001
Semantic memory (Similarities)	14.35±2.78	11.3±4.01	0.002
Orientation (MMSE)	9.65±0.49	7.78±2.15	<0.001
Attention (TMT-A)	87.3±21.39	143.04±53.3	<0.001
Executive function : -TMT-B	173.4±46.58	253.2±77.77	<0.001
-Mental control	5.9±1.89	3.98±1.91	0.001
Language(comprehension**)**	15±1.21	11.9±3.47	<0.001
Visuospatial abilities:			
- Picture completion	14.95±0.83	9.54±3.46	<0.001
- Block design	15.1±0.91	10.8±2.97	<0.001

Table (3): Neuropsychological performance of MCI patients as a whole after 1 year.

Domain , cognitive measure	Control Vs MCI patients		
	Control	MCI	P value
FAQ	.15±.37	3.96±3.9	<0.001
Global cognitive abilities			
-MMSE	29.25±.85	24.38±4.16	<0.001
-IQ	101.4±7.37	92.9±23.15	0.301
Memory			
Working memory (Digit span)	9.4±1.47	8±2.08	0.003
Episodic memory:-L.M	15.05±3.36	8.85±4.47	<0.001
-Associate learning	5.3±1.22	3.19±1.1	<0.001
-Visual reproduction	11.05±1.1	5.96±3.51	<0.001
Semantic memory(Similarities)	14.35±2.78	10.72±3.92	0.001
Orientation(MMSE)	9.65±49	7.52±2.33	<0.001
Attention(TMT-A)	87.3±21.39	156.24±59.99	<0.001
Executive function: -TMT-B	173.4±46.58	264.1±80.17	<0.001
-Mental control	5.9±1.89	3.66±2.02	<0.001
Language - Comprehension	15±1.21	11.4±3.87	<0.001
Visuo-spatial abilities:			
-Picture completion	14.95±.83	9.02±3.7	<0.001
- Block design	15.1±.91	10.2±3.33	<0.001

(Results are presented in mean± SD. FAQ : Functional activities questionnaire ;
MMSE :Mini-mental state examination ; IQ :Intelligent quotient ;LM: Logical memory;
TMT-A:Trail making test A; TMT-B:Trail making test B)

Table (4): Neuropsychological performance of MCI patients as a whole after 2 years.

Domain , cognitive measure	Control Vs MCI patients		
	Control	MCI	P value
FAQ	0.15±.37	5.62±5.24	<0.001
Global cognitive abilities			
-MMSE	29.25±.85	22.84±5.25	<0.001
-IQ	101.4±7.37	89.28±25.16	.118
Memory			
Working memory(Digit span)	9.4±1.47	7.42±2.2	<0.001
Episodic memory: -L.M	15.05±3.36	8.28±4.44	<0.001
-Associate learning	5.3±1.22	2.89±1.31	<0.001
-Visual reproduction	11.05±1.1	5.48±3.52	<0.001
Semantic memory:-Similarities	14.35±2.78	10.0±4.26	<0.001
Orientation (MMSE)	9.65±49	7.04±2.55	<0.001
Attention (TMT-A)	87.3±21.39	167.54±63.37	<0.001
Executive function:-TMT-B	173.4±46.58	275.3±82.56	<0.001
-Mental control	5.9±1.89	3.14±2.08	<0.001
Language:-Comprehension	15±1.21	10.84±4.48	<0.001
Visuo-spatial abilities: -Picture completion	14.95±.83	8.42±3.87	<0.001
- Block deign	15.1±.91	9.7±3.51	<0.001

(Results are presented in mean± SD. FAQ : Functional activities questionnaire;
MMSE : Mini-mental state examination ;IQ :Intelligent quotient ;LM: Logical memory ;
TMT-A:Trail making test A; TMT-B:Trail making test B)

Table (5): The demographic characteristics of convertor and stable MCI.

	MCI patients		
	Convertor	**Stable**	**P value**
Age (mean ±SD)	63.00±7.18	59.97±10.26	0.43
Sex: No(%)F	3(27%)	23(59%)	0.118
Education (mean ±SD/year)	2.18±2.40	5.59±5.9	0.587
Vascular risk factors			
DM: No (%)	3(27%)	6(15%)	0.379
HTN: No (%)	4(36.4%)	8(20.5%)	0.374
Heart disease:No (%)	1(9.1%)	1(2.6%)	0.442
Dyslipidaemia: No (%)	1(9.1%)	8(20.5%)	0.464
Neuropsychiatric symptoms			
Depression: No (%)	2(18.2%)	5(12.8%)	0.184
Anxiety:No (%)	0(0.0%)	5(12.8%)	0.118

(DM : Diabetes , HTN: Hypertension)

Table (6) : Baseline Neuropsychological performance of convertor and stable MCI patients:

Domain , cognitive measure	**MCI patients**		
	Convertor	**Stable**	**P value**
FAQ	5.09±2.21	1.92±2.37	<0.001
Global cognitive abilities: -MMSE	24.0±3.26	25.33±4.01	0.215
-IQ	77.09±8.77	101.15±21.28	<0.001
Memory			
Working memory : - Digit span	8.36±1.56	8.46±2.05	0.765
Episodic memory :			
- L.M	6.36±2.29	9.96±4.39	0.007
- Associate learning	3.14±.71	3.62±0.98	0.171
- Visual reproduction	5.0±1.48	6.69±3.79	0.04
Semantic memory :(Similarities)	7.64±2.20	12.33±3.8	<0.001
Orientation: (MMSE)	5.91±1.76	8.31±1.96	0.001
Attention: (TMT-A)	199.09±34.41	127.23±46.75	<0.001
Executive function:-TMT-B	341.55±50.95	228.28±64.95	<0.001
-Mental control	3.36±.92	4.15±2.08	0.102
Language:(comprehension)	8.64±3.67	12.82±2.83	0.003
Visuospatial abilites:			
- picture completion	6.82±1.72	10.31±3.45	0.003
- Block design	7.55±2.16	11.72±2.49	<0.001

(Results are presented in mean± SD .FAQ :Functional activities questionnaire ;
MMSE :Mini-mental state examination ;IQ :Intelligent quotient ; LM: Logical memory ;
TMT-A:Trail making test A; TMT-B:Trail making test B)

Table (7): Neuropsychological performance of convertor and stable MCI patients after 1 year:

Domain , cognitive measure	MCI patients		
	Convertor	Stable	P value
FAQ	8.91±3.18	2.56±2.8	<0.001
Global cognitive abilities			
- MMSE	21.18±2.93	25.28±4.04	0.001
- IQ	67.82±10.28	99.97±20.74	<0.001
Memory			
Working memory :(Digit span)	7.18±1.40	8.23±2.19	0.136
Episodic memory:-L.M	4.82±1.89	9.99±4.33	<0.001
- Associate learning	2.27±.72	3.45±1.05	0.001
-Visual reproduction	3.82±1.08	6.56±3.73	0.004
Semantic memory: (Similarities)	6.36±1.69	11.95±3.47	<0.001
Orientation: (MMSE)	5.09±1.97	8.21±1.95	<0.001
Attention: (TMT-A)	229.27±37.15	135.64±47.94	<0.001
Executive function:-TMT-B	361.0±48.0	236.77±64.79	<0.001
- Mental control	2.45±1.29	4.0±2.06	0.015
Language:(Comprehension)	6.64±3.17	12.74±2.87	<0.001
Visuo-spatial abilities:			
- Picture completion	5.09±1.22	10.13±3.4	<0.001
- Block design	5.91±1.81	11.41±2.57	<0.001

(Results are presented in mean± SD FAQ : Functional activities questionnaire ;
MMSE :Mini-mental state examination ; IQ :Intelligent quotient ;
LM: Logical memory ;TMT-A:Trail making test A; TMT-B:Trail making test B)

Table (8): Neuropsychological performance of convertor and stable MCI patients after 2 years.

Domain , cognitive measure	MCI patients		
	Convertor	Stable	P value
FAQ	13.27±3.93	3.46±3.09	<0.001
Global cognitive abilities			
- MMSE	17.36±3.44	24.38±4.61	<0.001
- IQ	59.09±14.35	97.79±20.58	<0.001
Memory			
Working memory: (Digit span)	5.55±1.69	7.95±2.05	0.001
Episodic memory:-L.M	3.82±2.04	9.54±4.12	<0.001
-Associate learning	1.27±75	3.35±1.04	<0.001
-Visual reproduction	2.73±1.27	6.26±3.57	0.001
Semantic memory :(Similarities)	4.73±1.85	11.49±3.49	<0.001
Orientation: (MMSE)	3.91±2.02	7.92±1.91	<0.001
Attention: (TMT-A)	250.64±37.9	144.1±47.31	<0.001
Executive function: -TMT-B	381.18±41.32	245.44±64.64	<0.001
-Mental control	1.18±1.33	3.69±1.92	<0.001
Language: (Comprehension)	4.91±3.21	12.51±3.19	<0.001
Visuo-spatial abilities:			
-Picture completion	3.91±1.14	9.69±3.38	<0.001
- Block deign	4.82±1.72	11.08±2.51	<0.001

(Results are presented in mean± SD .FAQ :Functional activities questionnaire ;MMSE :Mini-mental state examination ;IQ :Intelligent quotient ;LM: Logical memory ;TMT-A:Trail making test A; TMT-B:Trail making test B)

Discussion

Earlier diagnosis of AD is widely considered to be an important goal for researchers. Characterization of the earliest known clinical signs has led to the development of the classification of MCI which is thought to be a transitional stage between normal aging and the development of AD [2].

Our study included 50 patients, all of them fulfilled the criteria of amnestic MCI, with 12 (24%) presented with memory impairment alone (aMCI-S), and 38 (76%) presented with memory impairments plus impairment in one or more of other cognitive domains (aMCI-M). This low incidence of isolated memory impairment is consistent with other reports in the literature. Lopez et al.[13] for example,found that about 28% had isolated memory impairment, whereas the remaining 72% had deterioration in at least 1 non-memory cognitive domain.

During the 2 years follow- up period, 11(22%) of patients has converted to probable AD (convertor) and 39 (78%) remained as MCI (stable). These results are consistent with reports from other groups indicating an annual conversion rate to dementia ranging from 5% - 15% [14].

As regards to age in our work, the mean age of MCI patients was 60.64±9.69 years. The mean age of control was 56.75±7.09. There was no statistically significant difference between patients and control as regards to age (P = 0.167). The mean age of convertor MCI patients was 63.00 ± 7.18 years. The mean age of stable MCI patients was 59.97±10.26. There was no statistically significant difference between convertor and stable MCI as regards to age (P=0.430). Although statistically insignificant, the MCI patients were older than control and convertor MCI were older than stable. Our results are in agreement with several studies which showed increased incidence rates between the ages of 65 and 85 years, doubling every 5 years in the AD incidence[15].

In Our study, the MCI patients included 26 female (52%) and 24 males (48%). The control group included 8 females (40%) and 12

males (60 %). There was no statistically significant difference between the sex of MCI patients and control (P=0.364). There was also no statistically significant difference between the sex of convertor and stable MCI patients (P=0.118). Reports of the association between gender and AD have been controversial .Petersen et al.(16) had studied 329 subjects with MCI; the prevalence of MCI was higher in men compared with women. By contrast , Genin et al. (17) found that the estimated lifetime risk of AD is 10–11% in males and 14–17% in females.

In our study, There was no statistically significant difference between patients and control as regards to education (P=0.443). There was also no statistically significant difference between convertor and stable MCI as regards to education (P=0.587). In agreement with our findings, Manly et al.[18] could not found significant association between the level of education and the incidence of MCI. In contrast to our findings, lower educational attainment is found to increase the risk of cognitive impairment and dementia[19].

Regarding hypertension in our study, there was no statistically significant difference between patients and control (P=0.407). There was also no statistically significant difference between convertor and stable MCI (P=0.374). In agreement with our findings Solfrizzi et al.,[19] could not found significant association between the presence of hypertension and the incidence of MCI. However, hypertension could be identified by Tervo et al.[20] as a risk factor for MCI .

As regard dyslipidaemia in our study, There was no statistically significant difference between patients and control and between convertor and stable MCI (P>0.05). In agreement with our findings, studies of older subjects showed no association between hypercholesterolemia and cognitive decline[21]. In contrast to our findings, Large population-based studies have revealed that hyperlipidemia is associated with the risk of occurrence of MCI[22].

In our work, heart disease showed no statistically significant difference between patients and

control (P=0.329) and between convertor and stable MCI (P=0.442). In agreement with our findings, Manly et al.[18] could not found significant association between the presence of heart disease and the incidence of MCI. In contrast, Tervo et al.[20] have found cardiovascular diseases to be significant risk factor for the development of MCI.

Regarding diabetes in our study, there was no statistically significant difference between patients and control (P=0.269) and between convertor and stable MCI (P=0.379). Our findings are in agreement with Curb and colleagues [23] who did not found the incidence of diabets to be higher in MCI patients. In contrast to our findings, other studies reported that the presence of diabetes to be associated with an increased risk for MCI [24,25].

Regarding depression in our study, there was no statistically significant difference between patients and control (P=0.078). Anxiety also showed no statistically significant difference between patients and control (P=0.142). There was no statistically significant difference between convertor and stable MCI as regards depression or anxiety (p>0.05). In agreement with our findings, Some studies have been unable to link MCI with depression[26,27] but other longitudinal and case-control studies have found an increase in the risk of MCI in individuals with a history of depression [28,29].

Regarding Activity of daily livings (ADL) in our study, there was statistically significant difference between patients and control as regards initial initial FAQ (P<0.001). There was also statistically significant difference between convertor and stable MCI patients as regards to initial FAQ (P<0.001). After 1 and 2 years of follow-up, there were statistically significant difference between patients and control as well as between convertor and stable MCI as regards to FAQ (P<0.001). This is in agreement with with other studies[30]. MCI patients who progressed to AD 1 year later presented as more impaired on financial capacity at baseline and had greater decline

than non-progressors [31].

Regarding MMSE in our study, the mean initial MMSE in MCI patients was 25.04±3.86. The mean initial MMSE in control group was 29.25±0.85. There was statistically significant difference between patients and control as regards initial MMSE (P<0.001). After 1 and 2 years of follow up the differences between control and MCI patients as well as between convertor and stable MCI as regards MMSE were statistically significant (P<0.05). This is in agreement with Libon et al.[32] who compared between MCI patients and control and found statistically significant differences (the mean MMSE of MCI patients was 25.83±3.22 and the mean MMSE of control was 28.64±1.22). In contrast to our findings, Petersen et al. [33] compared controls, MCI and mild AD patients by a neuropsychological assessment comprising the MMSE, WAIS, WMS. The re¬sults showed similar performance on measures of general cognition between controls and MCI.

Regarding IQ in our work, there was no statistically significant difference between patients and control as regards initial IQ (P>0.05). There was statistically significant difference between convertor and stable MCI patients as regards initial IQ (P<0.001).After 1 and 2 years, the mean IQ showed statistically significant differences between convertor and stable MCI (P<0.001) .This is in agreement with Petersen et al.[33] who found that on measures of general cognitive function such as full scale IQ, the individual with MCI performs more like the normal elderly subject.

Regarding logical memory (LM) in our work, the mean initial LM in MCI patients was 9.17±4.28. The mean initial LM in control group was 15.05±3.63. There was statistically significant difference between patients and control as regards initial LM (P<0.001). After 1 and 2 years of follow up, the mean LM was also statistically significant between MCI patients and control. There were statistically significant differences between convertor and stable MCI patients as regards initial LM and After 1 and 2 years of follow up (P<0.05).

In agreement with our findings, several other studies showed statistically significant differences between MCI patients and control in scores of LM [34,35]. Recent studies associate the presence of impairment in LM with a higher rate of conversion to AD as compared with other episodic memory tests. Furthermore, the LM test was recently proposed as a screening tool for MCI in the Alzheimer 's disease Neuroimaging Initiative (ADNI) study [36].

Regarding associate learning in our study, there was statistically significant difference between patients and control as regards initial associate learning and After 1 and 2 years of follow-up (P<0.001). There was no statistically significant difference between convertor and stable MCI patients as regards to initial associate learning (P>0.05). After 1 and 2 years of follow-up, the associate learning showed statistically significant differences between convertor and stable MCI patients (P<0.001). Regarding visual reproduction in our work, there was statistically significant difference between patients and control as regards initial visual reproduction and After 1 and 2 years of follow-up (P<0.001). There was statistically significant difference between convertor and stable MCI patients as regards initial visual reproduction and After 1 and 2 years of follow-up (P<0.05).

In agreement with our findings, a number of prospective longitudinal studies of cognitive function in non-demented older adults have shown that a subtle decline in episodic memory often occurs before the emergence of the obvious cognitive and behavioral changes required for a clinical diagnosis of AD[37,15]. Chen et al.[38] and Lange et al.[39] showed a significant and steady decline in episodic memory beginning 3 years before the dementia diagnosis in individuals who were either initially asymptomatic or met criteria for MCI at enrollment in these longitudinal studies. The earliest neurofibrillary changes that are part of the pathology of AD usually occur in medial temporal lobe structures (e.g., hippocampus, entorhinal cortex), interrupting the neural network critical for episodic memory function. Thus, it is not

surprising that a deficit in episodic memory is the clinical hallmark of AD pathology [40].

Regarding semantic memory in our study, testing semantic memory with similarities subtest of WAIS showed statistically significant difference between patients and control as regards initial similarities score and after 1 and 2 years of follow-up (P<0.05). There was also statistically significant difference between convertor and stable MCI patients as regards initial similarities score and after 1 and 2 years of follow-up (P<0.001). Although, as in AD, episodic memory impairment is considered to be the main clinical feature of aMCI, recent studies , in agreement with our findings, have demonstrated the presence of semantic memory impairment as well[41,42]. Vogel et al. [43], found semantic memory to be impaired in MCI patients who later on converted to AD.

In studying working memory by the digit span subtest of WMS, we found statistically significant difference between patients and control as regards initial digit span and after 1and 2 years of follow-up (P<0.05). There was statistically insignificant difference between convertor and stable MCI patients as regards initial digit span and after 1 year of follow-up(P>0.05) but the differences were statistically significant after 2 years of follow-up (p<0.05).

In agreement with our work, several studies showed evidence of impaired working memory in MCI patients[44,45,46]. Belleville et al.[47] evaluated working memory in older persons MCI and found that performance of individuals with MCI was impaired compared to controls. In addition, their results showed that impairment in working memory predicted future progression from MCI to AD.

In studying attention with TMT-A, we found statistically significant difference between patients and control as regards initial TMT-A score and after 1 and 2 years of follow-up (P<0.001). There was also statistically significant difference between convertor and stable MCI patients as regards initial TMT-A score and after 1 and 2 years of follow-up (P<0.001). In

agreement with our findings, other investigators have also reported clinically evident impairment in attention in MCI[48.49]. Bozoki and colleagues [50] reported that 29% of aMCI patients who converted to AD over 2 years had impaired attention at initial presentation.

Our work showed that the executive functions (measured with TMT-B and mental control tests) were statistically significant in MCI patients when compared to control at initial testing, after 1 year and after 2 years of evaluation ($P < 0.05$). The convertor and stable MCI patients showed statistically significant differences as regards initial TMT-B scoring but the difference in mental control scoring was statistically insignificant ($p > 0.05$). After 1 and 2 year of evaluation, the differences were statistically significant ($P < 0.05$). Deficits in executive functions occur early in the course of AD and are often evident in the MCI stage[38]. Executive function deficits also predict subsequent progression to AD dementia. These deficits in executive functioning have been hypothesized to reflect AD pathology, especially neurofibrillary tangle burden, in prefrontal cortex[40]. In contrast to our findings, Farias et al.[51] indicated that executive dysfunction was not associated with an increased risk of progression of MCI patients to dementia.

Regarding language testing in our study, we tested language using the comprehension subtest of WAIS. There were statistically significant differences between patients and control as regards initial comprehension score, after 1 and 2 years of follow-up ($P < 0.001$). There were also statistically significant difference between convertor and stable MCI patients as regards initial comprehension score, after 1 and 2 years of follow-up ($P < 0.05$). In agreement with our finding, several other studies showed deficits in language in patients with MCI[52,53]. In contrast to our findings, Tabert et al. [54] did not find statistically significant difference between convertor and stable MCI patients as regard language ($p = 0.12$) .

In studying visuospatial abilities using picture completion and

block design subtests of WAIS , we found statistically significant differences between MCI patients and control at initial presentation, after 1 and 2 years ($P<0.001$). We also found statistically significant differences between convertor and stable MCI patients at initial presentation, after 1 and 2 years ($P<0.05$).

In agreement with our findings, other studies showed deficits in visuospatial abilities in MCI patients[55,56]. In contrast to our findings, Lonie et al.[57] did not find statistically significant differences between MCI patients and control as regard visuospatial abilities ($p=0.72$).

Conclussion

Neuropsychology has contributed importantly to the characterization of MCI patients and its differentiation from cognitive changes associated with normal aging .Differentiating patients of MCI who will convert and patients who will remain stable can be determined using neuropsychological tests and this may have potential implications on early treatment of AD.

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CLINICAL ASSESSMENT OF MILD COGNITIVE IMPAIRMENT

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PIOGLITAZONE AND FLUOXETINE ATTENUATE PATHOLOGICAL NEURAL CHANGES AND NEUROPATHIC PAIN INDUCED EXPERIMENTALLY IN DIABETIC RATS

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Abstract

Diabetic neuropathic pain, an important microvascular complication in diabetes mellitus, is recognized as one of the most difficult types of pain to treat due to underlying neural pathological changes such as inflammation and fibrosis. It is not possible to nominate a single drug as a first-line treatment for diabetic peripheral neuropathic pain. The aim of this work is to study the neural pathological changes and tissue levels of proinflammatory cytokines, TNF-α and IL-6 that may underlie the different types of neuropathic pain (tactile allodynia and thermal hyperalgesia) and to explore the effects of pioglitazone and/or fluoxetine on these changes. Sixty adult male white albino rats were assigned into two main groups, diabetic group with induction of diabetes by single intra-peritoneal injection of streptozotocin with high cholesterol diet and non-diabetic group fed on normal diet. Each main group was divided into subgroups (n=6 rats each) as follows: [I] control, with no treatment; [II] subjected to peripheral sciatic nerve ligation (PSL) only with no treatment; [III] PSL with pioglitazone treatment; [IV] PSL with fluoxetine treatment and [V] PSL with pioglitazone and fluoxetine combined treatment. All subgroups were tested before, at day 7 and day14 after PSL for tactile allodynia and thermal hyperalgesia followed by measurement of nerve tissue levels of TNF-α and IL-6, quantification of collagen deposition and macrophages counting. We found that PSL significantly increased inflammatory cell infiltration mainly macrophages and collagen deposition with significant increase of nerve tissue levels of TNF-α and IL-6 in both groups. These changes were associated with significant increase of tactile al-

lodynia and thermal hyperalgesia. Administration of pioglitazone and/or fluoxetine significantly decreased both macrophages infiltration and collagen deposition and nerve tissue levels of TNF-α and IL-6. These effects were associated with significant attenuation of tactile allodynia and thermal hyperalgesia produced by PSL in both diabetic and non-diabetic groups but fluoxetine alone had weaker effect in diabetic group. These results suggested that macrophages infiltration and collagen deposition with associated elevation of tissue proinflammatory cytokines could be a cause of neuropathic pain and administration of pioglitazone and fluoxetine can attenuate neuropathic pain by abolishing these changes.

Introduction

Neuropathic pain is characterized by pain in the absence of a stimulus and by reducing nociceptive thresholds so that, normally innocuous stimuli produce pain[1]. Diabetic neuropathic pain, an important microvascular complication in diabetes mellitus, is recognized as one of the most difficult types of pain to treat. The management of peripheral diabetic neuropathy consists of excluding other causes of painful peripheral neuropathy, maximizing diabetic control and using medications to alleviate pain[2]. It is not possible to nominate a single drug as the first-line treatment for diabetic peripheral neuropathic pain. Numerous studies using animal models have proposed candidates for therapeutic targets to reduce neuropathic pain. However currently, there are no good pharmacotherapies for neuropathic pain[3]. Inflammatory injury and subsequent fibrosis with the proinflammatory cytokines that contribute to neuropathic pain as TNF-α and IL-6 are expected mediators underlying tactile allodynia and thermal hyperalgesia induced by peripheral nerve injury[4]. Proinflammatory cytokines and the mRNA of TNF-α and IL-1α increased in the brain with pain-associated behavior in the rat models of neuropathic pain[5]. Peroxisome proliferator-activator receptor (PPAR) is a ligand activated transcription factor belonging to a nuclear hormone receptor superfamily, containing three isoforms (α, β/δ, and γ) [6]. PPARγ agonist, pioglitazone, plays a

critical physiological role as a primary lipid sensor and regulator of lipid metabolism. Thus, its ligands are clinically used for treatment of some diseases, including type 2 diabetes[7]. Some studies evaluated that; pioglitazone attenuates the development and maintenance of allodynia and hyperalgesia in mice with neuropathic pain due to peripheral nerve injury [8]. Fluoxetine, one of antidepressant drugs and a selective serotonin reuptake inhibitor, is reported to be used as co-analgesics in clinical management of migraine and neuropathic pain[9]. There is increasing recognition that norepinephrine (NE) and serotonin (5-HT) reuptake inhibitors (NRIs and SRIs) are efficacious in treating some types of pain. Studies have not systematically evaluated the relative activity at the NE and/or 5-HT transporter required for maximal efficacy in rodent pain models [10]. Thus, the aims of the present work were to study a part of the possible underlying pathological neuroimmune mechanism of neuropathic pain and explore the effects of pioglitazone or/and fluoxetine on tactile allodynia and thermal hyperalgesia induced experimentally in non-diabetic and diabetic rats.

Materials and Methods

1- Drugs and chemicals:

- Streptozotocin (STZ) powder creamy white: (Sigma Chemicals Co., U.S.A).
- Urethane (Ethyl Carbamate); (Prolabo, Paris) white crystals.
- Fluoxetine powder (Misr Co., Egypt)
- Pioglitazone powder (Unipharma, Egypt)
- Carboxy-methyle cellulose (powder) (El Nasr Pharmaceutical Chemicals Co.)
- Ray Bio® Mouse IL-6: Ray Biotech.U.S.A.
- MEDGENIX TNF-α EASIA Kit.

2- Animals:

Adult male albino rats (n=60), weighting 150-200g. They were brought from (Experimental Animal Breeding Farm, Helwan - Cairo). All animals were housed in controlled laboratory condition at 20-25C in a 12h light/dark cycle and had free access to standard laboratory chow (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water. They have acclimatized for one week and were caged (6/cage) in fully ventilated room (at

room temperature) in pharmacology department, Benha Faculty of Medicine. All experimental protocols were approved by the committee of Benha University.

3- Study Design:

After acclimatization for 1 week, the rats were assigned into 2 main groups, diabetic and non-diabetic groups. Diabetes was induced in the diabetic group by a single intra-peritoneal injection of streptozotocin (STZ 60 mg/kg) dissolved in cold 0.1 mole citrate buffer (pH 4.5) after fed the rats with high cholesterol diet for 2 weeks as a model for type 2 diabetes[11] 72 hours after STZ injection, diabetes was confirmed in rats by showing blood glucose levels increased to > 300 mg/100 ml[12]. The blood glucose concentration was measured using a glucometer from blood samples obtained by tail prick. The non-diabetic group was fed on normal diet.

Each main group was subdivided into five experimental subgroups, 6 rats each and treated for 14 days as follows:

Subgroup (I): Control group: did not receive any drugs but the sciatic nerve of their right lateral hind limb was exposed, and not subjected to ligation.

Subgroup (II): Non-treated peripheral sciatic nerve ligation (PSL) group: subjected to peripheral sciatic nerve ligation according to the modified method of Seltzer Z et al., 2004[13].

Subgroup (III): Pioglitazone-treated group: Subjected to PSL and received pioglitazone (25mg/kg/day) by gavage according to Maeda et al., 2008 [14].

Subgroup (IV): Fluoxetine-treated group: Subjected to PSL and received fluoxetine (30mg/kg/day) by subcutaneous injection [15].

Subgroup (V): Pioglitazone + fluoxetine-treated group: Subjected to PSL and received combined pioglitazone (25mg/kg/day) and fluoxetine (30mg/kg/day).

4- Surgical procedure (peripheral sciatic nerve ligation):

The procedure used for induction of neuropathic pain was the model adopted by Seltzer Z et al.,

2004 [13]. Rats were anaesthetized with urethane (1.5-1.75 g/kg I.P). The left sciatic nerve was exposed at the upper-thigh level, and the dorsal third to half of the sciatic nerve was tightly ligated. The wound was then sutured and the rats were allowed to recover in their home cage.

5- Behavioral tests :

All the behavioral data were recorded before surgery and at day 7 and 14 for all subgroups. The paw pressure threshold in response to normal innocuous mechanical stimuli was measured by using an analgesimeter[16]. Rats were situated on the platform under the situation point, so that this is at 5mm distance. Once the rats are prepared we activate the start pedal. The values on the display will increase in a progressive way and once the selected algesic response has been reached (shaking of the stimulated mice, vocalization etc.), we free the start pedal. The force (g) being exerted at the moment of freeing the start pedal is considered the end-point of the test. Once the pedal is released, the motor will turn counter-wise, at the same time the value shown at the digital display is transmitted through the RS232 serial connector.

On the other hand, thermal sensitivity was determined by measuring hind-paw withdrawal latencies to a radiant heat stimulus according to Hargreaves wt al., 1988[17] at the same time points.

6- Histopathological examination:

After the behavioral testing, 14 days post-surgery, all rats were deeply anesthetized and the sciatic nerve was dissected, fixed with 10% formaldehyde; embedded in paraffin, cut at 5 um, stained with hematoxylin and eosin (H&E) to determine the pathological changes of the dissected nerves and with Masson trichrome for quantification of collagen. All sections were examined with slides unlabeled with the treatment protocol.

a- Quantification of Collagen Deposition:

Quantitative analysis of sciatic nerve collagen fibers deposition in masson trichrome-stained sections was performed by morphometric analysis according to

James et al., 1990[18]. Briefly, A total of 10 fields were randomly chosen per mouse and images were taken with a digital camera mounted on a CX41 Olympus optical microscope. Collagenous areas stained with masson trichrome were extracted and analyzed using the NIH Image software. The extent of collagen deposition was xpressed as the percentage of the stained area relative to the total area. The percentages obtained from the 10 fields were expressed as the mean ± standard deviation.

b- Immunohistochemical staining of macrophages:

Immunostaining of tissue sections was performed as follows, after treatment with pepsin for 10 minutes or heating in an autoclave for antigen retrieval, sections were incubated with rat anti-Mac-3 monoclonal antibody (Pharmingen, San Diego, CA; working dilution 1:10) for identification of macrophages. Dendritic cells of splenic white pulp ase used as control. Three independent fields of nerve tissue from each rat were examined and Mac-3-positive cells were counted [19].

7- Estimation of serum TNF-α and IL-6:

After the behavioral testing, the dissected nerves (1 cm in length, including the ligation region) were used to determine the change in expression of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in the sciatic nerve

a- Estimation of TNF-α by a sandwich ELISA with (MEDGENIX TNF-α EASIA Kit):

TNF-α was determined using a commercially available kit according to the manufacture instructions.

b- Estimation of IL-6 by ELISA technique using (Ray Bio® Mouse IL-6):

By following the manufacture instruction according the protocol of Howord et al., 1992[20].

Statistical analysis:

Data are shown as mean ±SD. The results were analyzed by one way analysis of variant (ANOVA) followed by t-test or turkey test with $p<0.05$ selected as the criterion for statistical significance. The numerical data were analyzed and compared using Student's t-test or Mann-Whitney's U test.

Results

1- Behavior test:

Peripheral sciatic nerve ligation (PSL) induced significant tactile allodynia ligation which calculated as the rats showing hind paw withdrawal in response to lower weight (grams) pressure applied to its hind paw. Administration of pioglitazone (25mg/kg, orally) daily and combination of pioglitazone and fluoxetine once daily alleviated the tactile allodynia in non-diabetic and diabetic groups. Also, administration of fluoxetine (30mg/kg, s.c) once daily significantly attenuated tactile allodynia in non-diabetic rats but, could not successfully improve tactile allodynia in diabetic rats.

Regarding the thermal hyperalgesia, it was observed that PSL - induced thermal hyperalgesia groups had significantly shorter withdrawal latencies, and this was reversed by pioglitazone administration and combination of pioglitazone and fluoxetine in non-diabetic and diabetic rats. On the other hand, administration of fluoxetine (30mg/kg, s.c) improved thermal hyperalgesia elicited by peripheral nerve injury in comparison to the control group in non-diabetic rats. But administration of fluoxetine (30mg/kg, s.c) produced no significant improvement in thermal hyperalgesia in diabetic rats (table 1).

2- Histopathological examination

a- Inflammatory infiltration and nerve fibrosis:

In both diabetic (Figure 1A,) and non-diabetic (Figure 1B), sciatic nerve ligation produced inflammatory cell infiltration with significant increase of collagen fibers deposition in the injured sciatic nerve compared to control group. Administration of pioglitazone and / or fluoxetine showed less inflammatory cell infiltration with significant percentage reduction of collagen deposition in both diabetic and groups non-diabetic group. But fluoxetine alone show lesser improvement in diabetic group ($P<0.006$vs. 0.0006 in non-diabetic group (Table 2).

a- Macrophages infiltration:

Immunohistological analysis

with Mac-3 demonstrated significantly increased number of macrophages accumulated in the nerve tissue after PSL in both diabetic and non-diabetic groups, whereas only few macrophages were observed in control rats. In pioglitazone / fluoxetine - treated rats, the number of macrophages was significantly reduced in both groups compared with PSL groups (Figure 2) and (Table 3).

3- Production of inflammatory cytokines:

TNF-α and IL-6 were at a low level in the control group. Sciatic nerve ligation was associated with marked increase in the expression of both cytokines TNF-α and IL-6 in all examined nerve tissue. Pioglitazone and fluoxetine, suppressed significantly the elevation of both cytokines in all examined tissues (table 4).

Table (1): Peripheral sciatic nerve ligation produced significant increase of tactile allodynia and thermal hyperalgesia which is significantly reduced after administration of pioglitazone/ fluoxetine in both diabetic and non-diabetic groups

Subgroups	Threshold (g)		Latency (S)	
	Diabetic groups	Non-diabetic groups	Diabetic groups	Non-diabetic groups
control subgroup	310.2±16	340.3±14	5.9±2.2	6.8±1.6
Sciatic nerve ligation subgroup (PSL)	90.8±8 *P1<0.05*	152.8±8 *P1<0.05*	3.1±0.12 *P1<0.05*	3.2±0.14 *P1<0.05*
PSL + Pioglitazone-treated subgroup	270.4±10 *P2<0.05*	260.4± 18 *P2<0.05*	4.7±0.1 *P2<0.05*	4.9± 0.1 *P2<0.05*
PSL + Fluoxetine-treated subgroup	160.8±13 *P3>0.05*	280.6±20 *P3<0.05*	3.8±0.19 *P3>0.05*	3.8±0.4 *P3<0.05*
PSL + combined treatment with Pioglitazone/Fluoxetine	290.6±14 *P4<0.05*	305.1±23 *P4<0.05*	4.8±0.13 *P4<0.05*	5.6±0.5 *P4<0.05*

P1: compares values in PSL subgroups with control values
P2: compares values in pioglitazone subgroup with PSL subgroup
P3: compares values in fluoxetine subgroup with PSL subgroup
P4: compares values in pioglitazone + fluoxetine with PSL subgroup

Table (2): Peripheral sciatic nerve ligation produced significant increase of collagen fibers deposition which is significantly reduced after administration of pioglitazone/fluoxetine in both diabetic and non-diabetic groups.

Subgroups	Diabetic		Non-diabetic	
	% Fibrosis	P-value	% Fibrosis	P-value
control subgroup	3.9±0.16		3.1±0.34	
Sciatic nerve ligation subgroup (PSL)	9.53±0.32	*P1<0.0001*	8.23±0.56	*P1<0.0001*
PSL + Pioglitazone-treated subgroup	7.12±0.91	*P2<0.0008*	6.65±0.76	*P2<0.0032*
PSL + Fluoxetine-treated subgroup	7.83±0.93	*P3<0.006*	5.64±1.0	*P3<0.0006*
PSL + combined treatment with Pioglitazone/Fluoxetine	5.021±0.63	*P4<0.0001*	4.16±0.56	*P4<0.0001*

P1: Significant inP1: reased of collagen deposition compared to control subgroup
P2: compares % fibrosis reduction in pioglitazone subgroup with PSL subgroup
P3: compares % fibrosis reduction in fluoxetine subgroup with PSL subgroup
P4: compares % fibrosis reduction in pioglitazone + fluoxetine with PSL subgroup

Table (3): Peripheral sciatic nerve ligation produced significant increase of mac-3 positive cell count in nerve tissue which is significantly reduced after administration of pioglitazone/ fluoxetine in both diabetic and non-diabetic groups

Subgroups	Diabetic		Non-Diabetic	
	Mac-3 positive cells	P-value	Mac-3 positive cells	P-value
control subgroup	3.2±0.9		2.9±0.6	
Sciatic nerve ligation subgroup (PSL)	14.3±3.4	*P1<0.0001*	11.6±2.5	*P1<0.0001*
PSL + Pioglitazone-treated subgroup	8.1±3.2	*P2<0.027*	6.9±2.1	*P2<0.008*
PSL + Fluoxetine-treated subgroup	9.3±3.7	*P3<0.046*	7.2±2.3	*P3<0.014*
PSL + combined treatment with Pioglitazone/Fluoxetine	4.5±2.9	*P4<0.0006*	3.1±2.2	*P4<0.0002*

P1: Significant increased macrophage count compared to control subgroup
P2: compares macrophage count reduction in pioglitazone subgroup with PSL subgroup
P3: compares macrophage count reduction in fluoxetine subgroup with PSL subgroup
P4: compares macrophage count reduction in pioglitazone + fluoxetine with PSL subgroup

Table (4): Peripheral sciatic nerve ligation produced significant increase of nerve tissue levels of TNF-α and IL-6 which is significantly reduced after administration of pioglitazone/ fluoxetine in diabetic and non-diabetic groups

Subgroups	Diabetic		Non-diabetic	
	TNF-α	IL-6	TNF-α	IL-6
control subgroup	0.03±0.001	0.12±0.01	0.25±0.01	0.08±0.001
Sciatic nerve ligation subgroup (PSL)	0.82±0.011 *P1<0.05*	0.29±0.012 *P1<0.05*	0.72±0.013 *P1<0.05*	0.25±0.011 *P1<0.05*
PSL + Pioglitazone-treated subgroup	0.35±0.03 *P2<0.05*	0.22±0.011 *P2<0.05*	0.45±0.03 *P2<0.05*	0.16±0.013 *P2<0.05*
PSL + Fluoxetine-treated subgroup	0.51±0.014 *P3<0.05*	0.21±0.012 *P3<0.05*	0.46±0.02 *P3<0.05*	0.18±0.002 *P3<0.0*
PSL + combined treatment with Pioglitazone/Fluoxetine	0.46±0.03 *P4<0.05*	0.17±0.005 *P4<0.05*	0.42±0.004 *P4<0.05*	0.13±0.005 *P4<0.05*

P1: Significant difference from control subgroup
P2: compares values in pioglitazone subgroup with PSL subgroup
P3: compares values in fluoxetine subgroup with PSL subgroup
P4: compares values in pioglitazone + fluoxetine with PSL subgroup

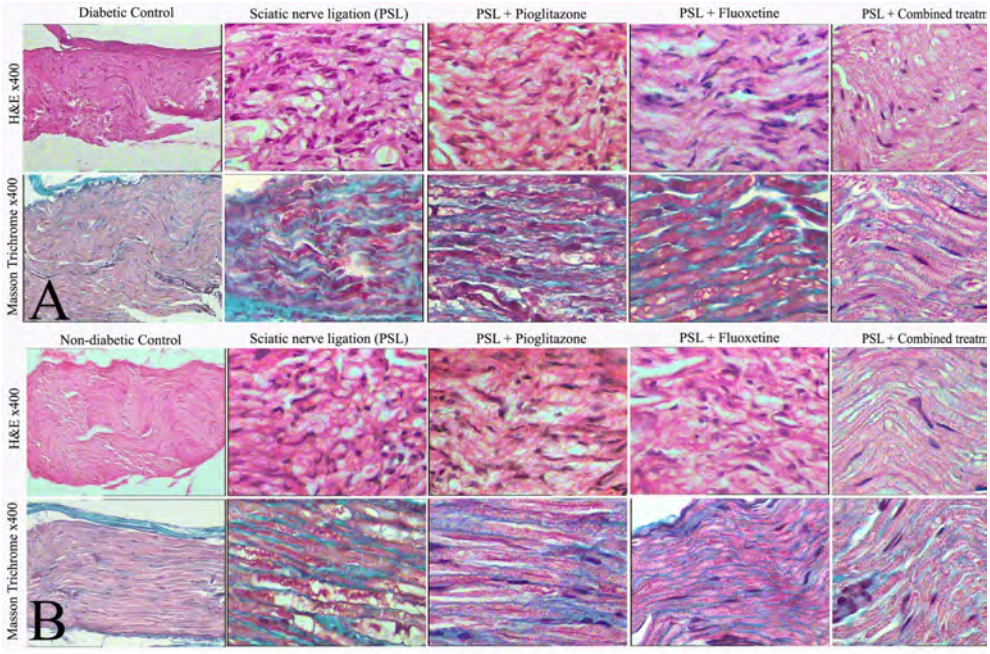

Figure (1): Histopathological changes in rats with peripheral sciatic nerve ligation (PSL): A) diabetic groups B) Non-diabetic groups. Sciatic nerve ligation produced inflammatory cell infiltration with marked increase in the collagen fibers deposition in the injured sciatic nerve in comparison to normal control group. Administration of pioglitazone and/or fluoxetine decreased the inflammatory cells and reduced the amount of collagen in both groups.

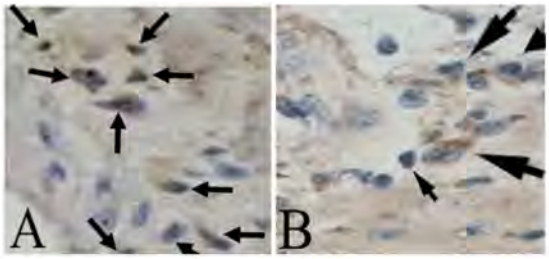

Figure (2): Effects of peripheral sciatic nerve ligation on macrophages infiltration before A) and after B) treatment with pioglitazone/fluoxetine. Sciatic nerve ligation produced increased count of mac-3 positive cells which is significantly reduced after administration of pioglitazone/fluoxetine in both diabetic and non-diabetic groups.

Discussion

Diabetic neuropathy is one of the most frequent complications of diabetes mellitus.

However, the mechanisms underlying these disorders are not yet well defined and it has been reported that currently available analgesics have hardly ameliorating effect on painful diabetic neuropathy. The purpose of the present study was to explore the possible underlying pathogenetic mechanisms that lead to the development of tactile allodynia and thermal hyperalgesia that occur in non-diabetic and diabetic rats subjected to partial sciatic nerve ligation and the possible antiallodynic and antinociceptive effects of pioglitazone and fluoxetine on established neuropathic pain in non-diabetic and diabetic rats.

Results of the present work revealed that some proinflammatory cytokines contribute to induction of neuropathic pain. TNF-α and IL-6 are accepted mediators underlying tactile allodynia and thermal hyperalgesia induced by peripheral nerve injury[4]. Sciatic nerve injury increases the levels of both cytokines in the sciatic nerve, dorsal root ganglion, and spinal cord in agreement with other studies[21,22,23,24]. Transcription of both cytokines is regulated by transcription factor binding sites within the promoter. It has been reported that promoter activities of both TNF-α and IL-6 are driven by binding of NF-κB, AP-1, and STAT, primary proinflammatory transcription factors[25]. We

therefore, tested whether pioglitazone and fluoxetine blocked partial sciatic nerve ligation induced up-regulation of both cytokines.

Histopathological examination of sciatic nerve showed significant increase of inflammatory cell infiltration mainly macrophages as a source of inflammatory and fibrogenic cytokines that explained the significantly increased amounts of proinflammatory cytokines, collagen deposition with subsequent aggravated neuropathic pain after partial sciatic nerve ligation. In our work, administration of pioglitzone and fluoxetine attenuated the histopathological changes in the sciatic nerve sections of the treated diabetic and non diabetic rats. These results are in line with Takahashi et al., 2011[26] who reported that, rosiglitazone treatment in the early phase of neuropathic pain significantly alleviated the development of tactile allodynia by regulating macrophage infiltration and production of proinflammatory molecules at the inflamed site. Administration of pioglitazone and fluoxetine after partial sciatic nerve ligation reduced increases in the expression level of both cytokines in all examined tissues. These results strongly suggest that pioglitazone and fluoxetine alleviates tactile allodynia and thermal hyperalgesia, at least in part, through inhibiting the upregulated proinflammatory cytokines. These results are in congruent with those of Maeda et al., 2008[12] who reported that pioglitazone alleviates allodynia and thermal hyperalgesia in mice with neuropathic pain due to peripheral nerve injury through attenuation of proinflammatory cytokine upregulation by perioxsone proliferative activated receptor gamma stimulation. Also, Papans et al., 2011[27] reported that pioglitazone has been shown to improve experimental diabetic neuropathy and alleviate neuropathic pain. Wiggin et al., 2008[28] observed that rosiglitazone reduced oxidative stress and prevented the development of thermal hypoalgesia in streptozotocin-induced diabetic mice. In addition, Churi et al., 2008[29] found that PPAR∝ ligand-induced activation of spinal PPAR-gamma rapidly reverses nerve injury-induced mechanical allodynia. On contrast to these finding, Shibata et al., 2000[30]

reported that troglitazone was less effective in controlling neuropathy in the Zucker diabetic fatty (ZDF) rats. This difference in our results may be due to uses of different doses and different species of animals.

In the present study, fluoxetine significantly attenuated tactile allodynia and thermal hyperplasia. These effects are in line with those reported by Kesim et al., 2006[31] that fluoxetine showed significant antinociceptive effect in non diabetic mice, but they could not successfully show this effect in diabetic mice. In disagreement with these findings, Sounvoravong et al., 2007[32] who stated that fluoxetine itself lacks antinociceptive properties in diabetic and sciatic nerve ligation mice, as model of neuropathic pain[13] added that fluoxetine (3-30mg/kg, s.c) did not inhibit either hyperalgesia or allodynia in formalin model and the L5/L6 spinal nerve ligation model of neuropathic pain.

In conclusion, neuropathic pain induced experimentally by nerve injury in diabetic and non-diabetic neuropathic pain may be in part due to macrophages infiltration with over expression of proinflammatory cytokines and subsequent fibrosis and administration of pioglitazone in combination with fluoxetine may be an effective combination in attenuating

Neuropathic pain through downregulation of these pathological changes.

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PIOGLITAZONE AND FLUOXETINE ATTENUATE PATHOLOGICAL NEURAL CHANGES AND NEUROPATHIC PAIN INDUCED EXPERIMENTALLY IN DIABETIC RATS

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COMPARATIVE STUDY OF HIGH-FLEX VERSUS CONVENTIONAL TOTAL KNEE ARTHROPLASTY

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Abstract

Recently, use of high-flex design was introduced in total knee prostheses. The purpose of this study was to prospectively compare the results of conventional and high-flex total knee prostheses. Differences in age, gender, diagnosis, preoperative range of motion of the knee, and Knee Society Score between the 2 groups were not statistically significant. At 24 month follow-up, average range of motion was 118.18 degrees for conventional, and 119.2° for high-flexion total knee prostheses.

Introduction

The goal of total knee replacement surgery is to provide the best possible outcome for the patient. There are many factors that influence the outcome of total knee replacement. There are also many measures of outcome[1].

Range of motion has been an important measure of outcome and is an important part of most knee scoring systems. The factors that one has some control over include surgical technique and implant design. Those that cannot be controlled include the patient's condition at the time of surgery, such as weight, history of previous surgery, multiple joint arthritis, pre¬operative knee score including preoperative motion, and complications [1].

Following total knee arthroplasty, most patients can rarely flex the knees beyond 120 degrees, and most clinical studies have a final range of motion that average between 100 and 110 degrees of flexion after conventional total knee arthroplasty [1,2].

The high flex total knee system was introduced to enhance knee flexion after total knee arthroplasty. The high flex prosthesis is specifically designed to aim for 150 degrees of flexion. The prominent differences seen from conventional prosthesis is by removing additional 2 millimeters of bone from the posterior femoral condyle to increase the articulation curvature during high flexion and making an anterior cut of the tibial insert to avoid patellar tendon impingement during high flexion [2,3,4].

Patient and Methods

From January 2007 to October 2012, 88 primary total knee arthroplasties were performed in 67 patients by a single surgical team. 44 knees had conventional total knee prostheses, whereas the remaining 44 knees had high-flexion total knee prostheses. There were no demographic differences between the two groups (table 1). The surgical procedure was the same in the two groups. All procedures were performed through a midline skin incision measuring 10 to 15 cm in length. The rehabilitation program also was the same in the two groups. Immediately after operation, patients were encouraged to begin quadriceps-strengthening exercise. Two days after operation, the drain was removed and patients were allowed to walk.

All patients were evaluated preoperatively, at six weeks, 3 months, 6 months, 1 year postoperative, and then every 1 year according to guidelines of the Clinical Knee Society Rating System[5]. Both high-flex and conventional groups were matched and did not differ significantly as regard to all demographic data, preoperative pain, range of motion, stability of the knee, flexion contracture, alignment, knee score and function score.

Results

Two years Postoperative range of motion and Knee Society Score are summarized in Table 2. The differences between groups in range of motion and Knee Society Scores (knee score and function score) were not statistically significant (P = 0.826, P=0.606 and P = 0.558, respectively).

In the high-flex group, the mean preoperative range of motion was 98.18°, increasing to 119.2° two years after surgery. In the conventional group, the mean preoperative range of motion was 98.86°, increasing to 118.18° two years after surgery. The mean preoperative knee score was 30.59 in the high-flex group and 32.39 in the conventional group. Two years after surgery, the mean knee score was 88.66 in the high-flex group and 87.57 in the conventional group. At 2 years follow up, 23 cases can flex the knee more than 125 degrees. In comparison with patients have postoperative flexion <125°, there was no statistically significant difference in the preoperative demographic data, pain score, stability score, knee score and function score. The only significant difference was the preoperative range of motion. Patients with postoperative knee flexion more than 125° had higher preoperative range of motion (mean 111.07±11.59°) than patients with postoperative range of motion <125° (mean 97.08±11.09°).

Table 1 : Patients demographic data.

	High-flexion group (44 cases)	***Conventional group(44 cases)***	***P value***
Age (years)	58.27 (SD± 4.98)	57.89 (SD±6.58)	0.75
Sex (male/female %)	13.6/86.4	18.2/81.8	0.38
Body mass index(kg/m2)	29.66 (SD 3.60)	29.43 (SD 4.03)	0.78

Table 2 : 2 years range of motion and Knee Society Score.

Postoperative Parameters	***High-flexion group (44 cases)***	***Conventional group(44 cases)***	***P value***
Range of motion(degrees)	119.2±13.1	118.18±12.3	0.826
Knee Society Score			
knee score	88.66 ± 10.48	87.57 ±9.25	0.606
function score	75.80 ±20.23	73.75± 11.06	0.558

Discussion

Although several reports on clinical results with high-flex prostheses have been published, the advantage of provision of a better range of motion of the knee with a high-flexion prosthesis has remained unclear. Some authors have reported that use of a high-flexion design increased postoperative range of motion compared with the conventional design[3,6,7]. However, Kim et al reported results for simultaneous bilateral total knee arthroplasty in 50 patients who received a high-flex design in one knee and a conventional design in the contralateral knee, and concluded that there was no significant difference between the knees with regard to range of motion.

In the presented study, we compared the clinical outcome of patients after high-flexion or conventional total knee arthroplasty. At the 2 years follow-up, there were no differences between the two groups, especially the range of motion (119.2° vs. 118.18°). Ranawat and Ritter described that the preoperative range of motion is the most important factor for the postoperative knee flexion unaffected by the implant design [9]. This statement is reflected by the results of the presented study. At the 2 years follow-up, there were no differences in range of motion in this study, unaffected by the implant design.

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COMPARATIVE STUDY OF HIGH-FLEX VERSUS CONVENTIONAL TOTAL KNEE ARTHROPLASTY

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GASTRO-PROTECTION AGAINST ASPIRIN INDUCED GASTRIC ULCER IN RATS

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Abstract

Gastric ulceration was induced in rats by oral administration of non-steroidal anti-inflammatory drug (NSAID). The aim of this study was to evaluate the protective effect of L-arginine (NO precursor) and ginger powder on aspirin induced gastric ulcer. It was carried out on 24 adult albino rats divided into 4 groups; Control group, ulcer control group, L-arginine group (200mg/kg/orally) and ginger treated group (200 mg/kg/orally). After 3 days of treatment, single oral dose of aspirin (250 mg/kg/orally) was administrated to induce gastric ulcer except for control group. Within 6 hours, animals were anesthetized; gastric secretion was collected to determine its volume, acidity and nitrate. Gastric lesions and ulceration were expressed as ulcer index. Statistical analysis was done using SPSS with P<0.05 is significant. This study has proved the noxious effect of aspirin on gastric mucosa. While, Administration of L - arginine and ginger 3 days before aspirin detect a gastroprotective effect which is more in L-arginine treated than ginger treated animals.

Keywords : *NSAIDS, L-arginine, ginger and gastric ulcer.*

Introduction

Gastric ulcer results from an imbalance between factors that damage gastric mucosa and normal mucosal defense and repair mechanisms. The most commonly ulcer predisposing factors are: H-pylori infection increased gastric acid secretion, stress, alcohol, tobacco, and non-steroidal anti-inflammatory drugs (NSAIDs)[1].

The prevention of gastric ulcer pathogenesis orits recurrence is the main desired goal for manyexperimental studies in the present era[2]. Aspirin is a potent NSAID that is used for the treatment of rheumatoid arthritis and prevention of cardiovascular thrombotic diseases[3]. Its gastrointestinal injury is a serious adverse effect, especially it is widely used, and so

effective strategies to protect the gastrointestinal mucosa are required[4]. This widespread use makes NSAID-associated peptic ulcer disease a significant public health concern particularly it is associated with high annual treatment costs [5].

Previous studies investigating the mechanisms for the development of NSAIDs-induced gastric mucosal ulceration were achieved. NSAIDs may cause gastric lesions by inhibiting cyclooxygenase (COX) and reducing prostaglandin (PG) production, especially PGE and PGI2, but the exact pathogenic mechanism remains unclear [6]. It is unlikely that PGs deficiency alone is sufficient to initiate the process that ultimately results in gastric ulceration. Direct cytotoxicity of NSAIDs may be involved in NSAID induced gastric lesions[7]. Different mechanisms of ulcer protection have been explained. The aim of the present study is to investigate the mechanisms of gastro- protection and detect which is more effective.

L-Arginine is an amino acid that plays several pivotal roles in cellular physiology. Like any amino acid, it is involved with protein synthesis, but it is also involved with cell signaling through the production of nitric oxide (NO) [8]. NO plays an important role in the regulation of various cellular functions through gastrointestinal tract. It has been shown that different concentrations of NO have completely opposite effects in the same tissue. In general, the mucosal and endothelial nitric oxide synthase (NOS) isoforms produce low amounts of NO. However, the high quantity of NO produced by inducible (iNOS) that damage the epithelium[9]. Additionally, NO exerts a beneficial influence on gastric mucosa by enhancing the mucosal defensive ability and preventing pathogenic events resulting from suppression of PG synthesis as a reduction in gastric microcirculation and leukocyte endothelial adherence. NO maintain blood flow around the ulcer and angiogenic process that help ulcer healing [10,11].

Ginger has been used as a spice and an ingredient of Chinese traditional stomach medicine for thousands of years. In traditional

medicine, ginger has been used to treat many inflammatory conditions and associated pain (12).The major pungent constituents of ginger have many interesting pharmacological effects, such as antioxidant, antitumor promoting and anti-inflammatory effects (13). However, the mechanism underlying the protective effects of ginger against gastric damage is unclear. So, the antiulcer effects of ginger in aspirin-induced gastric ulcer model rats were investigated in the present study.

Aim of the work

The aim of this study was to evaluate and compare the protective effect of L-arginine (NO precursor) and ginger powder on aspirin induced gastric ulcer.

Material and Method

This study was done using (24) adult male albino rats weighting from 140-160 mg. They were acclimated to the surrounding environment for 2 weeks before the beginning of the experiment. The animals were randomly classified into 4 groups.

1- Group 1 (control group): The animals received 3 mL of 1% carboxy methylcellulose in water as a vehicle orally for 3 days.
2- Group 2 (Ulcer control group): It was received the vehicle for the same period of time.
3- Group 3 (L- Arginine group): received L- arginine as oral dose of 200 mg\kg body weight for 3 days(14).
4- Group 4 (Ginger- group): It was administrated ginger in a dose of 200 mg/kg orally as a form of powder suspended in 3 mL of 1% carboxy methylcellulose in water for 3 days(3).

Induction of gastric ulcer:

By the end of the period specified, the rats were deprived of food but not water for 12 h. Groups 2, 3 & 4were orally administrated a single dose of aspirin (250 mg/kg body weight) suspended in 3 mL of 1% carboxy-methyl-cellulose in water (15).

Within 6 hours after aspirin administration(16), the animals were anesthetized using diethyl ether. Abdominal incision was performed; the pyloric portion of the

stomach was gently mobilized andcarefully ligated with around thepyloric sphincter. The stomach was cut open along the greater curvature,and the contents were collected in tubes and centrifuged for 10 min. to remove anysolid debris. The resultantsupernatant was used for the estimation of gastric juice output volume and acidity. The stomachsrinsedwith saline, Gastric tissues were pinned out flat on a board. The inner surface was photographedto allow the measurement of the area coveredby hemorrhagic ulceration. Next, the gastric mucosal tissues were removed and stored at -80˚C for estimation of reduced glutathione level and nitric oxide [3].

The mucosa of the stomach was scrapped, homogenized in cold potassium phosphate buffer and centrifuged for10 minutes at 4C; the supernatant was then kept at -80ºC for measurement reduced glutathione and nitrate level[33].

Gastric ulcer index

Macroscopically, with the help of hand lens (X10), the changes in gastric mucosa was detected and expressed as ulcer score according to this table Ulcer score for each animal is expressed as Ulcer Index by multiplying ulcer score X 100[15].

	Stomach color	Ulcer score
1	Normal color	0
2	Red color	0.5
3	Red spots	1
4	Hemorrhagic streaks	1.5
5	3 - 5 ulcers	2
6	More than 5 ulcers	3

The percentage of gastric lesions =

As mucosal lesions were different in rats of the same group, estimation of % ulceration had done[17].

$$\frac{(\text{Number of lesion rat} - \text{number of non-lesion rat}) \times 100}{\text{Number of rat in group}}$$

Gastric mucosa was normal in control group, while in ulcer control group, multiple lesions and hemorrhagic streaks were determined in all animals with 100% lesion percentage. In L-Arginine treated group, gastric mucosa had small red spots detected in only 2 animals and the remaining were

free and 50% ulceration percentage. Concerning ginger-treated group, red spots were detected in 4 rats and gastric mucosa was red colour with ulceration percentage is 33%.

The percentage ulcer protection

It was calculated using the formula:

Percentage ulcer protection = Ut / Uc X 100

Ut = Ulcer index of treated group

Uc = Ulcer index of the control group.

As regard, L-arginine group when compared with ulcer control group, percentage of protection is 40.6%. Meanwhile, ginger treated group protection percentage is 27.8%[15].

Statistical analysis

Statistical analysis was evaluated using independent t test (SPSS version 19 program, P values ≤0.05 were considered significant.

Results

Table (2): The results are expressed as mean±SD with * indicate significant change when compared with control group, while # indicates significant change when compared with ulcer control group.

	Control group	Ulcer control group	L-arginine-treated group	Ginger- treated group
Volume of gastric secretion	2.65±0.659	4.5±0.63 *	5.6717±0.543 *#	4.9333±0.35 *
Acidity	39.62±4.72	43.31±3.41 *	59.75±1.726 *#	47.533±2.477 *#
Ulcer index	00±0.00	533.3±160.2	216.66±51.63	148.33±42.62 *#
GSH	2.8±0.49	1.3±0.2 *	2.90±0.442 #	4.85±0.758 *#
NO	25.75±3.97	16.4±2.29 *	24.066±1.09 #	14.78±0.66 *

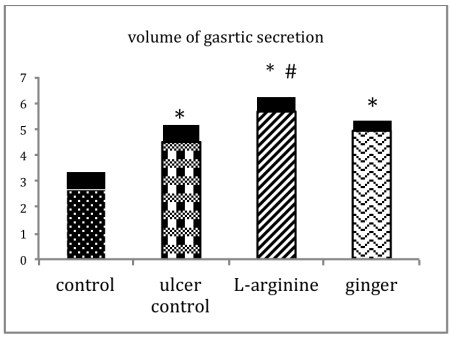


Fig. (1): Show changes in volume of gastric secretion expressed as mean ±SD. There is significant increase in gastric secretion volume in all treated groups when compared with control group (p<0.001). Also, L-arginine group is significantly more than ulcer control group (p<0.006).

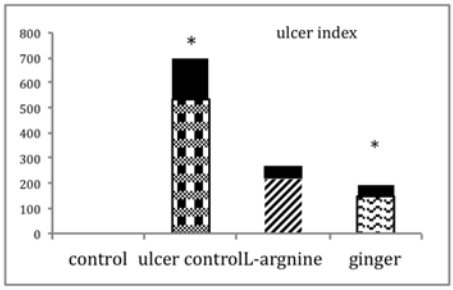


Fig (3): This figure clarifies ulcer index changes. Ulcer control group is significantly more than control group (P<0.001) while both l-arginine and ginger treated groups are less significantly (P<0.001). When compare with ulcer control group, ulcer index is less in l-arginine and ginger treated groups (P<0.001)

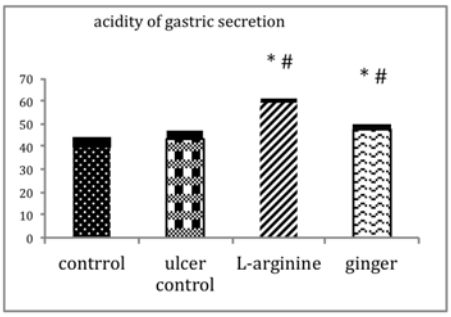


Fig. (2): This figure shows the changes in acidity of gastric secretion expressed as mean ±SD. Gastric acidity is significantly increased in L- arginine and ginger treated groups than control group (p<0.005). As regard ulcer control group, L- arginine treated group is significantly more (P< 0.0001) but ginger treated group is significantly less (p<0.03).

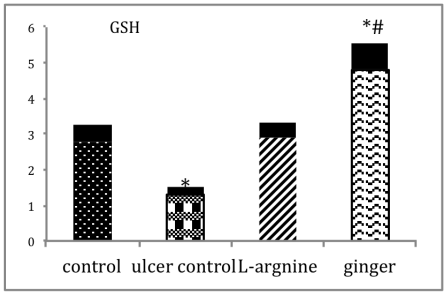
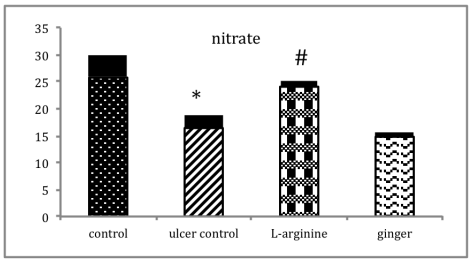
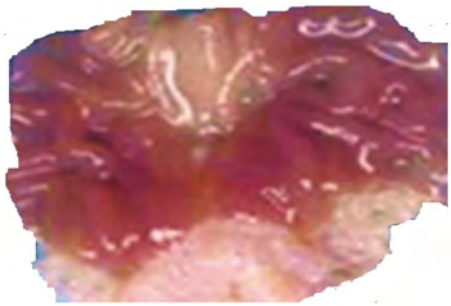
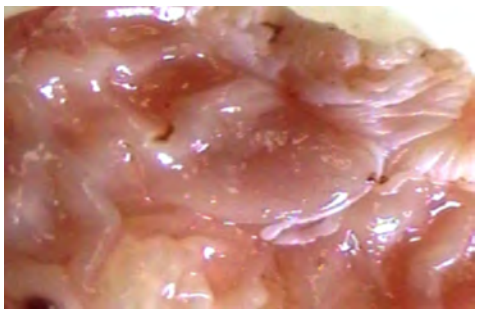


Fig. (4): This figure illustrates the changes in GSH level. As regard control group, ulcer control group is significantly less (P<0.001) while, ginger treated group is increased significantly (P<0.001). When compare with ulcer control group, l-arginine and ginger treated groups are significantly increased (P<0.001) for both.

Fig. (5): This figure shows the changes in nitrate level as both ulcer and ginger treated group are significantly less than control group (P<0.001) for both. As regard l-arginine treated group it is more significantly than ulcer control group

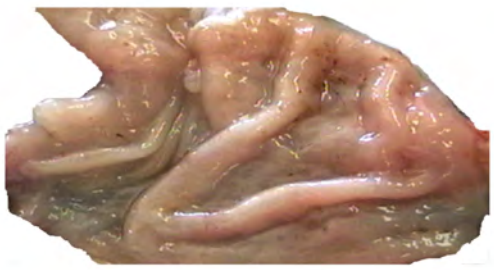


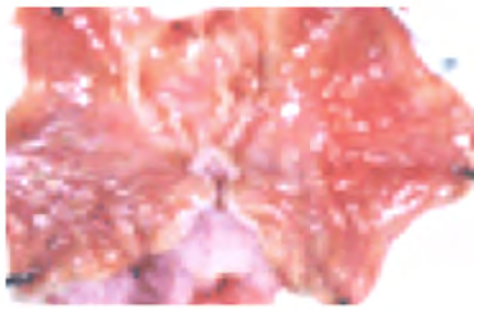




ɜa . Normal mucosa in control group

b- Mucosa in aspirin treated group





c- Mucosa in l-arginine treated group

d- Mucosa in ginger treated group

Discussion

As gastrointestinal complications of aspirin are widely recorded specially its effect on gastric mucosa. Macroscopic examination of gastric mucosa of aspirin treated group demonstrated a significant increase in ulcer index than control group with percentage of ulceration was 100%. Additionally, volume of gastric secretion also increased significantly. Manyprevious studies have investigated the mechanisms forthe development of NSAIDs-induced gastric mucosallesions[6].

Endogenous PGs play a role in gastric secretion, mucous- bicarbonate production [18] and mucosal blood flow [19]. It is considered that deficiency of PG plays a key role in NSAID induced gastrointestinal side effects. In the GI mucosa, the principle metabolic products of COX enzymes are the gastroprotective prostaglandins. So, in the presence of a COX inhibitor, such as aspirin or other NSAIDs variable degrees of GI mucosal injuries occur[20]. There is a cross talk between the products of NO synthatase and COX enzymes. First, NOS has been shown to interact with COX pathways. Second, it has also been reported that COX products interact with NOS pathway [21]. That can explain the demonstrated decrease in the level of nitric oxide in aspirin treated group in this study. Frankly, not only NO level is significantly decreased but also GSH. This is explained by David A. Hildeman et al., 2003[22] who has detected that aspirin administration lead to leuckocyte infiltration in gastric tissue that consider a major source of superoxide radicles that react with cellular lipids forming lipid peroxides.

The results of this study clearly detect the gastroprotective role of 3 days of amino acid L- arginine. There is significant decrease in ulcer index than aspirin treated group. That is explained by YoshijiOhta and Keiji Nishida, 2002 [23] as exogenously administered L-arginine exerts a protective effect in rats at least through preservation of gastric mucus synthesis. Also, there is significant increase in secretion of NO produced from the administered L-arginine via constitutive NOS (cNOS) in gastric mucosal tissue. Also, L-arginine

could cause nitric oxide-mediated inhibition of leukocyte infiltration of gastric mucosa. The protective effect of L-arginine (NO precursor) can be explained by restoration/ and or increase in NO production by quenching the release of superoxide anion from the endothelium, thus reducing oxidative stress[24]. Antioxidant role of L-arginine is demonstrated as GSH level is significantly increased than aspirin treated group which come in agreement with Adel A Anglo et al., 2010[16]. As L-arginine act as a scavenger for superoxide anion thorough its alpha amino group[25]. Furthermore, L-arginine decreases plasma xanthine oxidase activity and inhibits lipid peroxidation[26].

The group treated with ginger 3 days before aspirin administration showed significant increase in gastric secretion volume than control group. As regard gastric ulcer index it is significantly decreased than aspirin treated group which indicate gastroprotective role of ginger on gastric mucosa. The gastroprotective mechanisms of ginger as it reduce inducible nitric oxide synthatase (iNOS) which lead to secretion of high quantity of NO [9]. The excessive release of NO from gastric epithelial cells has been reported to exert detrimental effects. Aspirin administration increases iNOS that consider a mechanism of gastric injury [27,28]. Additionally, the inflammation induced in the gastric mucosa by aspirin is accompanied by increased TNF- production[29,17] which augments neutrophil-derived superoxide generation[30] and stimulates IL-1 production, leading to neutrophil accumulation[31,32]. Co-administration of ginger inhibit the increases in TNF- and IL-1 which ulcer formation progressing.

Conclusion

This study support noxious effect of aspirin on gastric mucosa. Administration of L - arginine and ginger for 3 days before aspirin detect a gastroprotective effect which is more in L-arginine treated animals than ginger treated animals.

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GENE PROMOTER 469 E/K POLYMORPHISM OF THE INTERCELLULAR ADHESION MOLECULE-1 GENE ASSOCIATION WITH MYOCARDIAL INFARCTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Abstract

Background: *Type 2 diabetes mellitus (T2DM) is one of the major risk factors for the development of coronary artery disease and subsequent myocardial infarction (MI). ICAM-1 has a vital responsibility in the adhesion of circulating leucocytes to the blood vessel wall and subsequent transendothelial migration to the vascular intima, one of the earliest stepladders in atherogenesis.* ***The object*** *of this study was to investigate the possible role of the K469E polymorphism of the ICAM-1 in genetic susceptibility to MI amongst the patients with type 2 diabetes.* ***Subjects & Methods:*** *The study included 70 patients divided into two groups: Group I included 35 patients suffering from T2DM with MI. Group II included 35 patients with T2DM with no history of coronary artery disease (CAD). In addition, 30 apparently healthy age and gender matched subjects were involved as a control group. For all subjects the followings were done: History taking & clinical examination, ECG, lipid profile, HbA1c, sICAM-1 by ELISA. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to determine the distribution of allele and genotype frequencies of the 469 E/K polymorphism of the intercellular adhesion molecule-1.* ***Results:*** *Diabetic patients with MI showed a higher statistical signifi-*

cant difference regarding HbA1c compared with diabetic patients without CAD. While, no statistical significant differences were found regarding total cholesterol, LDL-c levels, HDL-c or triglycerides levels between groups I & II. Regarding sICAM1 level, there were high statistical significant differences between diabetic patients with MI (468±40.4 ng/ml) and both diabetic patients without CAD (266.4±44.1 ng/ml) & the healthy controls (150.6±29.7ng/ml p<0.001 for both). As well as, a higher statistical significant difference was found between diabetic patients without CAD and the healthy control (p<0.001) regarding sICAM1. T2DM group with MI had a statistical significant higher distribution of the EE genotype and E allele frequency (42.9%, 64%) than those of T2DM without CAD group (20%, 41.7%) & the healthy controls (16.7%,41.2%). Meanwhile, there was no statistical significance between T2DM without CAD group & the healthy control group regarding EE genotype and E allele frequency. Also, T2DM with MI estratified by ICAM-1 E469K gene polymorphism showed no statistical significant differences regarding all studied parameters (P<0.05 for all). ***Conclusion:*** *the EE genotype was associated with MI in the present set of diabetic patients with increasing the level of circulating sICAM-1, but this increase did not sway by the existing polymorphism. Hence, the K469E polymorphism of theICAM-1 gene may be employed as a genetic indicator for MI in patients with T2DM.*

Introduction

Type 2 diabetes mellitus (T2DM) is one of the major risk factors for the development of CAD and subsequent (myocardial infarction) MI[1] Various inflammatory mediators such as tumor necrosis factor-α increase the expression of cell adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1 and E-selectin on endothelial cells[2]. Thus, upon inflammatory stimulation, the endothelial barrier function is rapidly lost

and preformed P-selectin is translocated to the luminal surface of endothelial cells, followed by expression and release of E-selectin, ICAM-1, and VCAM-1 substances that regulate the attachment and trans-endothelial migration of leukocytes. Both macrophages and endothelial cells produce ICAM-1 in response to infammatory cytokines[3]. ICAM-1 is a transmembrane glycoprotein of 505 amino acids, with a molecular mass of 80-114 kDa (depending on the degree of glycosylation), and contains five immunoglobulin (Ig)-like domains[4]. ICAM-1 has a vital responsibility in the adhesion of circulating leucocytes to the blood vessel wall and subsequent transendothelial migration to the vascular intima, one of the earliest stepladders in atherogenesis[5]. It provokes adhesive interactions by binding to two surface membrane β2 integrin molecules present on leucocytes, namely leucocyte function associated-antigen-1 and macrophage antigen-1[6]. The ICAM-1 gene is located on chromosome 19p13 and consists of seven exons[7]. Several single-nucleotide polymorphisms of the human ICAM-1 gene have been reported, and functional analyses regarding the association between each polymorphism and certain diseases have been performed[8]. Vora et al.[9] searched for polymorphisms in the exons of ICAM-1 and described single nucleotide polymorphism K469E, is an A→G substitution at position 1548 in codon 469 in exon 6, causing a lysine (AAG) to glutamine (GAG) change in Ig-like domain 5.1 E469K is known to be common in all populations and has been analyzed for its association with several inflammatory diseases. The object of this study was to investigate the possible role of the K469E polymorphism of the ICAM-1 in genetic susceptibility to MI amongst the patients with T2DM.

Subjects and Methods

The present study was carried out at Internatl medicine department in collaboration with cardiology, clinical pathology & medical

biochemistry departments, faculty of medicine, Benha & Menoufiya university hospitals in the period between November 2011 & September 2012. The study included 70 patients (47 males, 23 females), their age ranged between 38-62 years divided into two groups: Group I included 35 patients (24 males, 11 females) with T2DM patients with MI (diagnosis was based on the characteristic ECG findings and cardiac specific enzymes, using diagnostic criteria of the American College of Cardiologists), their ages ranged between 42-62 years. Group II included 35 patients with T2DM with no history of coronary artery disease (23 males, 12 females) lasting more than 10 years, their ages ranged between 38-62 years. In addition, 30 apparently healthy age and gender matched subjects were involved as a control group (21 males, 9 females), their ages ranged between 42- 62 years. Patients with a history of other metabolic, autoimmune and malignancy diseases or with a family history of such illness were excluded from the study. For all the subjects the followings were done: History taking and clinical examination, ECG, lipid profile (total cholesterol, triglyceride, LDL-c& HDL-c) & The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method was used to determine the distribution of allele and genotype frequencies of the 469 E/K polymorphism of the intercellular adhesion molecule-1. Written informed-consents were provided by all participants.

- **Sampling:** under complete aseptic conditions, 6 ml of venous blood were collected after 12 hour fasting & divided as follows: 2 ml of blood collected in citrate (to prevent clotting and DNA degradation) for DNA extraction and kept immediately at -20 C°. 3 ml were collected, left to clot; serum was divided into two aliquots, one kept immediately at -20 °C for further determination of sICAM and the other used for immediate assay of lipid profile. 1ml of blood collected in EDTA for determination HbA1c.

- Laboratory Methods:

- Total cholesterol, triglyceride, and HDL-c concentrations were determined by using an enzymatic colorimetric assay on Synchron Cx9. LDL-c concentration was calculated according to the Friedewald formula[10]. HbA1c was determined by column chromatography.

- sICAM-1 was measured by ELISA an anti-ICAM-1 monoclonal antibody is adsorbed onto microwells. Soluble ICAM-1 present in a sample or standard then binds to antibodies adsorbed to the microwells. A second, HRP-conjugated monoclonal anti-ICAM-1 antibody is added and binds to ICAM-1 captured by the first antibody. Unbound enzyme-conjugated anti-ICAM-1 is removed with a wash step and HRP substrate solution is added to the wells. An amount of colored product is formed, proportional to the amount of soluble ICAM-1 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from ICAM-1 standard dilutions and the ICAM-1 sample concentration is determined.

- DNA analysis: PCR-RFLP method was used to determine the distribution of allele and genotype frequencies of the 469 E/K polymorphism of the intercellular adhesion molecule-1. The DNA was extracted using commercially available Spin-column technique kit for DNA extraction from human whole blood (QIAamp®DNA Blood Mini Kit). The DNA concentration was determined by measuring the OD at 260 nm. The PCR was performed on 300 ng DNA in a total volume of 25 µl of the reaction mixture containing 2.5 µl of 10X PCR buffer, 0.75 µl of 10 µMdNTPs, 0.75 µl of 50 µM MgCl2, 1 µl of 20 ρM primers and 2 U of Taq DNA polymerase, forward and reverse primers: forward primers: 5'-GGTGAGATTGCATTAAGGTC-3' and the reverse primers 5'-GGAACCCATTGCCCGAGC3. PCR was carried out with an initial denaturation at 95 °C for 7 min and

35 cycles of 95 °C for 35 s, 57 °C for 45 s, and 72 °C for 45 s with a final extension of 5 min at 72 °C. The PCR fragments (223 bp) were digested using the restriction enzyme FastDigest® BstUI. The digested samples were separated by electrophoresis on 3% agarose gel stained with ethidium bromide and visualized on a UV trans-illuminator. This resulted in three genotypes identified: (i) EE was divided into two fragments (87 and 136 bp) (ii) KK not divided (223bp) (iii) heterozygous EK divided into three fragments (223, 87 and 136 bp) (fig. 1).

Statistical Analysis

The statistical analysis was undertaken using SPSS software (version 17; SPSS Inc., Chicago, IL, USA). Descriptive statistics in the form of mean and standard deviation for parametric data were used. Chi-square test ($\chi 2$) was used for two qualitative variables. ANOVA test for comparison between three or more groups having quantitative variables normally distributed followed by LSD (least significant difference). Kruskal-Wallis test for comparison between three or more groups not normally distributed having quantitative variables. Odd ratios (ORs) and 95% confidence intervals (CI) were calculated by logistic regression analysis. The significance level was set at 0.05 or less[11].

Results

The results were presented in the following figures and tables:

Table (1): Sociodemographic criteria & clinical parameters in the studied groups.

parameter	Studied groups			Test of significance	P value
	Group I (DM with MI) N = 35	Group II (DM without CAD) N = 35	Group III (control group) N = 30		
Age (years) X ± SD	51.71± 5.53	50.42± 7.32	50.90±5.86	f- test 0.37	0.691
Gender Male /Female (%)	25/10(71.4/28.6%)	22/13 (62.9/37.1%)	20/10 (66.7/33.3%)	χ^2 58	0.747
Hypertension Positive /Negative (%)	31/4 (88.6/11.4%)	24/11(68.6/31.4%)	0/30 (0.0/100%)	χ^2 50.8* 37.1** 2.36 ***	<0.001* <0.001** 0.124***
Smoking Positive/Negative (%)	24/11(68.6/31.4%)	22/13 (62.9/37.1%)	0/30 (0.0/100%)	χ^2 32.6* 28.5 ** 0.25 ***	<0.001 * <0.001** 0.0.61 ***

* Comparison between group I and group III ** comparison between group II and group III
*** Comparison between group I and group II
P: Probability of error P < 0.05 significant P> 0.05 non significant P< 0.001 highly significant
F test=analysis of variance (ANOVA) $\chi 2$=chi square

Table (2): Laboratory parameters in the studied groups.

parameter	Group I (DM with MI) N = 35	Group II (DM without CAD) N = 35	Group III (control group) N = 30	f- test	P value	Post Hoc test (LSD)
HbA1c (%) X ± SD	10.94± 1.49	9.96 ± 1.11	6.48 ± 0.42	139.4	<0.001	<0.001* <0.001** <0.001***
Triglycerides (mg/dl) X ± SD	212.5±23.3	208.85± 17.88	93.10±17.37	369.19	<0.001 HS	<0.001* <0.001** 0.442***
Cholesterol (mg/dl) X ± SD	200.31± 32.6	194.25 ± 25.66	178.93±17.33	5.56	0.005 HS	0.002* 0.021** 0.338***
HDL-c (mg/dl) X ± SD	33.97 ± 5.3	35.11 ± 4.03	41.76 ± 2.64	31.43	<0.001 HS	<0.001* <0.001** 0.260***
LDL-c (mg/dl) X ± SD	150.62 ±26.1	158.48 ± 20.53	114.23±11.53	41.28	<0.001 HS	<0.001* <0.001** 0.115**
sICAM1(ng/ml) X ± SD	468.11 ±40.42	266.48 ±44.15	150.63± 29.7	558.2	<0.001 HS	<0.001* <0.001** <0.001***

* Comparison between group I and group III ** comparison between group II and group III
*** Comparison between group I and group II P: Probability of error P < 0.05 significant
P> 0.05 non significant P< 0.001 highly significant F test=analysis of variance (ANOVA)
LSD least significant difference.

Table (3): Differences in allele distribution and genotype frequency of ICAM-1 E469K gene polymorphism between the studied groups.

parameter	Group 1 N =35		GroupII N =35		Group III N =30		X^2	P value	OR (95% CI)
	No	%	No	%	No	%			
genoype									
EE	15	42.9	7	20.0	5	16.7	6.32	0.042*	5.57 (1.42 – 21.86)
EK	15	42.9	15	42.9	15	50.0	0.34	0.84**	
KK	5	14.2	13	37.1	10	33.3	6.46	0.039***	
allele									
E	45	64.3	29	41.4	25	41.7	6.65	0.009*	2.54 (1.29 – 5.03)
K	25	35.7	41	58.6	35	58.3	0.0	0.97**	
							7.34	0.006***	

* comparison between group I and group III ** comparison between groupII and groupIII
*** comparison between group I and group II P: Probability of error P < 0.05 significant
P> 0.05 non significant P< 0.001 highly significant CI confidence interval OR odds ratio

Table (4): Sociodemographic criteria, clinical and laboratory parameters in the group I estratified by ICAM-1 E469K gene polymorphism.

parameter	Studied groups			Test of significance	P value
	EE N=15	EK N=15	KK N=5		
Hypertension (Positive/Negative)	15/0 (100/0.0%)	11/4 (73.3/26.7%)	4/1 (80.0/20.0%)	X^2 **4.51**	**0.105**
Smoking Positive/Negative (%)	11/4 (73.3/26.7%)	9/6 (60.0/40.0%)	1/4 (20.0/80.0%)	X^2 **4.44**	**0.108**
HbA1c X ± SD (%)	10.86±1.36	10.96± 1.50	11.62 ±1.76	**Kruskal Wallis test 1.38**	**0.500**
Triglyceride (mg/dl) X ± S	216.93 ±18.62	214.0 ± 23.63	203.0±26.23	**0.94**	**0.62**
Cholesterol (mg/dl) X ± S	197.33± 40.79	198.2 ± 25.97	207.6±27.68	**0.28**	**0.86**
HDL-c (mg/dl) X ± SD	34.46 ± 4.15	32.20 ± 4.69	39.20 ± 6.53	**6.66**	**0.036**
LDL-c (mg/dl) X ± SD	144.46 ±21.83	150.93 ± 23.46	141.0±29.83	**1.02**	**0.599**
sICAM-1 (ng/ml) X ± SD	465.26 ±40.03	464.40 ± 42.09	487.8 ±38.92	**1.89**	**0.38**

P: Probability of error P> 0.05 non significant X^2=chi square

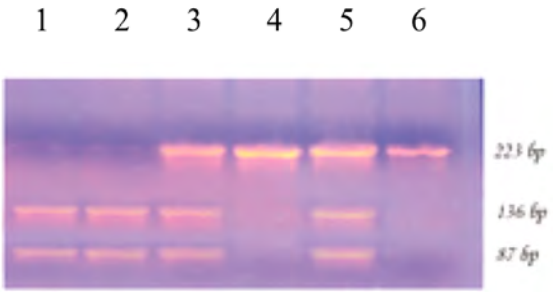

Fig. 1: Agarose gel electrophorsis 3.0% stained with ethidium bromide showing the ICAM-1 DNA product after digestion with BstUI. Lanes 1& 2: EE allelic polymorphism lane 3&5: KK allelic polymorphism Lanes 4&6: KK allelic polymorphism.

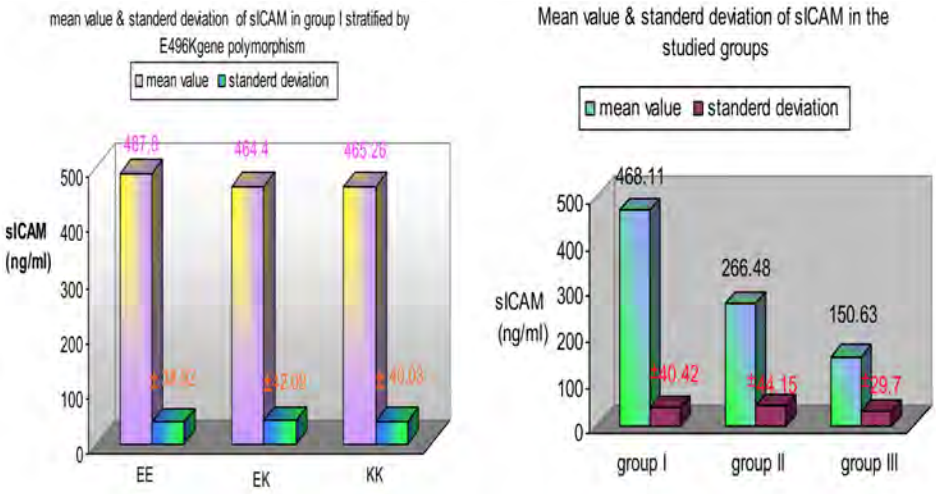

Fig. 2: sICAM-1 mean value in the studied groups & group I stratified by ICAM-1 E469K gene polymorphism.

Discussion

CAD is one of the major causes for morbidity and mortality in the developing countries[12]. One of the main and independent risk factors for the development of CAD and consequent myocardial infarction is type 2 diabetes[13]. ICAM-1 mediates the interaction of activated endothelial cells with leukocytes and plays a fundamental role in the pathogenesis of coronary atherosclerosis[14].

In the current study (table1), no statistical significant differences were found between the studied groups regarding to age and gender (p=0.691, 0.747 respectively). Also, there were high statistical significant differences between both diabetic groups and control group as regard hypertension and smoking (p<0.001 for both). While, no statistical significant differences were found between diabetic patients with MI and diabetic patients without CAD as regard hypertension and smoking (p=0.124, 0.061 respectively).

The present study (table2) showed that diabetic patients with MI had a higher statistical significant difference regarding HbA1c (10.94±1.49%) compared with diabetic patients without CAD (9.96±1.11%, p=0.001). While, no statistical significant differences were found regarding total cholesterol (200.31±32.6 mg/dl vs. 194.25±25.66 mg/dl, p= 0.338), LDL-c (150.62±26.1 mg/dl vs. 158.48 ± 20.53 mg/dl, p=0.115) levels, HDL-c (33.97±5.3 mg/dl vs. 35.11±4.03 mg/dl, p=0.260), and triglyceride levels (212.5±23.3 mg/dl vs. 208.85±17.88 mg/dl, p=0.442) between group I & group II. These results were partially in agreement with Milutinovic & Petrovic[12] who stated that their studied patients had higher total cholesterol and LDL-c levels, and lower HDL-c levels than their diabetic controls. There were no significant differences in triglyceride levels between the cases and the diabetic controls.

ICAM1 is a member of the large immunoglobulin superfamily

widely expressed at a basal level and can be upregulated by proinflammatory cytokines. Furthermore, circulating sICAM1 concentrations are increased in various inflammatory conditions, including CVD, and have been associated with future CVD risk[15].

Regarding sICAM1 level in the current study (table2, fig.2), there were statistical significant differences between diabetic patients with MI (468.11±40.42 ng/ml) and both diabetic patients without CAD (266.48±44.15 ng/ml) and the control group (150.63±29.7 ng/ml, $p<0.001$ for both). Also, a higher statistical significant difference was found between diabetic patients without CAD and the control group ($p<0.001$).

In agreement to these results, sICAM1 level increase amongst patients with coronary disease was informed by Ridker et al.,[16] & Malik et al.,[17]. The study done by El-Mesalamy et al., revealed that sICAM1 concentrations were significantly higher in the ischemic heart disease group and the diabetic patient with coronary artery disease group in comparison to those levels obtained in the healthy controls group ($p\leq0.05$). The mechanism of elevation of ICAM may be contributed to hyperglycemia, oxidative stress, and inflammation or insulin resistance [18]. On the other hand, Mohamed et al., stated that there were no significant differences in sICAM1 levels between CHD patients and control subjects[14]. This difference in results may be due to the fact that the patients in the current study had an acute state, whilst the patients in some of the previous studies had chronic CHD.

T2DM group with MI had a statistical significant higher distribution of the EE genotype and E allele frequency (42.9%, 64%) than those of T2DM without CAD group (20%, 41.7%) & the healthy controls (16.7%, 41.2%). Meanwhile, there was no statistical significance between T2DM without CAD group & the healthy control group

regarding EE genotype and E allele frequency. These results were in accordance with a study done in Caucasians in Italy[19], demonstrated a positive association between the EE genotype of the K469E polymorphism of the ICAM-1 gene and ischemic stroke. Also, the results of Yokoyama and colleagues found that the distribution of K469E polymorphism in T2DM and control subjects was not significantly different between both groups[20]. Furthermore, in a study done in Egypt by Shaker et al.,[21] diabetic patients with peripheral arterial occlusive disease including MI had a higher statistical difference in the frequency of the EE genotype (29%) compared to control group (13%). This could be explained by Yokoyama et al., [20] who stated that EE genotype of the K469E polymorphism of the ICAM-1 gene has been reported to be associated with the lower plasma fibrinogen levels in patients with T2DM.

In contrast to the present study, Milutinovic & Petrovic[1] failed to reveal a statistical differences between the EE genotype of the K469E polymorphism of the ICAM-1 gene and T2DM in patients with MI (EE=21.7%, EK=47.4%, KK=30.9%) & T2DM patients without MI (EE=19.1%, EK=50.7%, KK=30.2%). Also, McGlinchey et al.,[22] found no association between the ICAM-1 K469E polymorphism and CHD in a well-defined Irish population. Similarly, Aminian et al.,[23] found no significant differences between CHD, MI patients and controls as regards KK genotype in the studied population from Fars province, Iran.

In a study done in Egypt by Mohamed et al.,[14] ICAM-1 EK and KK variants represented 57% of the CHD patients and were associated with increased risk of disease development. In Chinese population, Zhang et al.,[15] found that the presence of KK genotype of ICAM-1 codon 469 conferred an increased risk for CHD. Whereas, a report in Chinese population demonstrated a positive associa-

tion between the KK genotype and restenosis after coronary stenting [24]. Similarly, Lu et al., [25] found that K allele frequency was higher in CHD patients than in controls, and K allele carriers develop myocardial infarction more easily.

Atherosclerosis is a multifactorial disease and evidence indicates that certain synergistic risk factors accelerate atherogenesis. In the current study by using logistic regression analysis (table3) revealed that T2DM patients with the EE genotype as well as E allel were at increased risk for MI (OR 5.57 95% CI 1.42-21.86, OR=2.54 95% CI 1.29-5.03, respectively) compared with those having the KK genotype and K allel. In the first study documenting a role of the ICAM-1 gene polymorphism in the pathogenesis of a cardiovascular disease done by Gaetani et al., [26] revealed EE genotype significantly increases the risk of PAOD (odds ratio, 3.5; 95% CI, 1.5-8.4). To the opposite of the present results, Milutinovic & Petrovic[1] demonstrated that the EE genotype was not associated with MI in subjects with T2DM (OR=1.2; 95% CI=0.7-2.0). In another study done by McGlinchey and co-workers, stated that the effects of K469E polymorphism in families with a history of coronary artery disease were investigated and no meaningful relation was found between K469E polymorphism and occurrence of coronary artery disease [22]. Aminian et al.,[23] concluded that there was no strong relation between K469E polymorphisms and occurrence of CHD and MI in the studied population from Fars province, Iran. Meanwhile, Mohamed et al.,[14] suggest that the KK and EK genotypes of the ICAM-1 gene polymorphism in codon 469 are associated with the risk for CHD development in Egyptians (OR=3.8, 95% CI: 1.7-8.5).

The results obtained by this study (table4) revealed that in T2DM with MI, stratified by ICAM-1 E469K gene polymorphism showed no statistical significant

differences regarding all studied parameters including sICAM-1 levels (fig.2) ($p<0.05$ for all). These results were in accordance, with those of Milutinovic and Petrovic [1] who found no statistical significant association between the genotypes and coronary risk factors (clinical history and laboratory data). While in a study done by Shaker et al.,[21] showed no statistical difference between the different polymorphisms and the clinical history and laboratory data among the patient groups except in those with IHD, where there was significant difference between EE and EK. Whereas, in the Han population of China, individuals with the K allele had higher plasma level of sICAM-1 than those without the K allele[24].

These discrepancies in results may be explained by variable sample sizes; also, the different populations represent different gene pools. This suggesting that gene-disease relationships can be expected to be different between populations due to the differences in a compound genetic set Moreover, in type 2 diabetic patients, ICAM-1 may have to a certain extent different import in its role in sequence of vascular harm from non-diabetic subjects[27].

Conclusion

The EE genotype was associated with MI in the present set of patients. Also, the circulating sICAM-1 was elevated in diabetic patients with MI but did not swayed by this polymorphism. Further studies enrolling larger numbers of patients from different populations are needed to confirm these findings. Hence, the K469E polymorphism of the ICAM-1 gene may be employed as a genetic indicator for MI in patients with T2DM in the Egyptian population. Economic studies are recommended for the cost benefit of using it as a screening genetic marker for diabetic patients and how far this could be used in early prophylaxis against MI.

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GENE PROMOTER 469 E/K POLYMORPHISM OF THE INTERCELLULAR ADHESION MOLECULE-1 GENE ASSOCIATION WITH MYOCARDIAL INFARCTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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CALCIMIMETIC (CINACALCET) VERSUS CALCITRIOL FOR THE CONTROL OF SECONDARY HYPERPARATHYROIDISM DUE TO CHRONIC KIDNEY DISEASSE IN CHILDREN

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Abstract

Backgroud: *one of the main problems nephrologists face when treating chronic renal failure patients is to control the increased secretion of parathyroid hormone (PTH), during dialysis and after renal transplantation. Chronic kidney disease (CKD) is the most common cause of secondary hyperparathyroidism (sHPT). The medical treatment of sHPT in maintenance hemodialysis (MHD) patients has been recently improved. Objective: this investigation aimed to evaluate the effect of calcium sensing receptor agonist (Cinacalcet) versus vitamin D metabolite (Calcitriol) on the metabolic disorders and the morphology (size) of hyperplastic parathyroid gland (PTG), in MHD children with sHPT. S**tudy design:** thirty-one MHD patients, suffering clinical, biochemical and ultasonographic manifestations of sHPT, were allocated at random, into two treatment groups. **Calcitriol group:** sixteen patients who received oral Clacitriol, 0.01-0.05 mg/kg/day and Cinacalcet group: fifteen patients who received oral Cinacalcet 30-120 mg daily. After 6 months, biochemical and sonographic assessment was repeated for both groups and data were statistically analysed. **Results:** baseline clinical, biochemical and sonographic parameters were consistent with sHPT and were similar in both groups. After 6 months of treatment, levels of serum Ca, P and CaxP were nonsignificantly different in both groups. On other hand, serum level of both intact PTH (iPTH) and ALP, as well as the percent reduction in volume of PTG, were significantly decreased*

after Cinocalcet treatment than that after Calcitrial treatment, p < 0.05. ***Conclusion:*** *1) Cinacalcet led to significant improvement in biochemical parameters (particularly iPTH and ALP) and 2) Cinacalcet can inhibit parathyroid hyperplasia with reduction of glandular volume more than that observed in Calcitriol, with nonsignificant differences of serum Ca, P and Ca x P.* ***Recommendations:*** *1) Cinacalcet therapy is superior to Caclitriol in the management of mild-to-moderately severe parathyroid hyperplasia in MHD children, 2) longer term larger clinical trials are needed to confirm these preliminary findings and to further define a more systemic approach in the treatment of sHPT.*

Keywords: *chronic kidney disease-parathyroid hyperplasia-Cinacalcet-Calcitriol- high resultion sonography.*

Introduction

Hyperparathyroidism (HPT) is an overactivity of PTG, resuling in excess production of PTH, which regulates calcium (Ca) and phosphorus (P) levels and helps to maintain these levels. Excessive PTH secretion may be due to problems in the glands themselves, in which case it is referred to as primary HPT. It may also occur in response to low Ca levels, encountered invarious situations such as vitamin D deficiency[1] or CKD, this is referred to as sHPT. In all cases the raised PTH levels are harmful to bone, and treatment is often needed[2].

Chronic kidney disease (CKD) is almost constantly associated with a systemic disorder of mineral and bone metabolism, which has been named CKD-MBD. It is manifested by either one or a combination of biochemical abnormalities, bone abnormalities and vascular or other soft tissue calcification [3]. Initially, it is characterized by a tendency towards hypocalcemia, festing normo- or hypophosphatemia, and diminished plasma Calcitriol concentration, together with a progressive increase in plasma intact parathyroid hormone (iPTH) and the development of osteitis fribrosa[4].

Secondary hyperparathyroidism (sHPT) develops progressively during the course of CKD. Phosphorus retention and reduced

synthesis of 1,25 vitamin D result in low calcium levels, which causes a feedback secretio of iPTH. Long-term hyperstimulation of PTG enhances cell proliferation resulting first in diffuse polyclonal hyperplasia and finally in monoclonal nodular hyperplasia[5]. Patients are considered to have severe sHPT when serum P, Ca, the Ca x P product (CaxP) and iPTH levels can no longer controlled by conventional therapies [6]. Following international guidelines, parathyroidectomy (PTX) becomes mandatory when one or more PTGs are enlarged (volume > 500mm^3), iPTH values are > 700 pg/ml and the response to conventional therapy is poor [7].

The introduction of calcium sensing receptor agonist (Cinacalcet) into clinical practice has led to its use in patients with various degrees of severity of sHPT, including those who are not candidates for PTX. Cinacalcet, an allosteric modulator of the calcium receptor (CaR), increases the sensitivity of the CaR to activation by extracellular Ca and thus suppresses PTH release. While simultaneously, lowering levels of serum P, Ca and CaxP(8). The aim of this study was to evaluate the effect of Cinacalcet versus Calcitriol on the size of PTGs, in MHD children with sHPT, in relation ot biochemical parameters.

Patients And Methods

This study was carried out in the Hemodialysis unit of Zagazig University Hospitals, during the years 2011 through 2012, on 31 MHD patients suffering sHPT, of ages reanging from 10 to 16 years, mean ± standard deviation: X±SD =13.5±3.3 years, 15 were males and 16 were females.

Inclusion criteria:

Children included fulfilled the following criteria of HPT:

1. Symptoms and/or signs of HPT[9], including: diffuse bone pain, articular tenderness, walking difficulty, muscular pain and itching.
2. Hyperphosphatemia (serum P > 6 mg/dl), hypercalcemia (serum Ca > 11mg/dl) or CaxP > 55[10].
3. Elevated serum iPTH levels > 300 pg/ml [10].
4. Rediographic signs of renal osteodystrophy[11].

5. Diffuse hyperplasia of the PTG with gland size of > 0.5 g, detected by Color Doppler (CD) ultrasonography[12].

In addition an informed consent was obtained from parent(s) of each patient, before inclusion to the study.

Methodology:

All study children were subjected to the following baseline parameters, at entry to the study and 6 months often implementation of the management protocols:

1. History-taking and clinical examination.
2. Biochemical parameters [serum P, Ca, CaxP, iPTH and alkaline phosphatase (ALP)]. The iPTH was measured using the Nicholas IRMA method.
3. Estimation of the PTG volume by CD ultrasonography[12]: patients were examined with the neck hyperextended in a supine decubitus. After positioning the patient, the enlarged PTG is examined by ultrasonic tomography. The cross-and longitudinal sections of the gland are imaged, and 3D measurements (vertical and horizontal lengths, and thickness) were made. Based on the 3D data, the estimated volume of the gland is calculated.

After baseline assessment was carried out, MHD patients were randomly allocated into 2-treatment group:

Calcitriol group: this group included 16 MHD patients. They received 0.01- 0.05 µg/kg/ 24hours, of Calcitriol orally[11].

Cinacalcet group: this group included 15 MHD children. They received initially Cinacalcet 30mg orally once daily. The dose was increased every 4weeks in 30 mg increments up to maximum dose of 150mg[13].

Statistical analysis:

Data were analyzed using student's t-test for quantitative values and chi2(?2)-test for qualitative values, and were considered significant for values of $p< 0.05$.

Results

Baseline clinical, biochemical

parameters of mineral metabolism and sonographic parameters were consistent with sHPT and were similar in both groups. The mean values of serum P, Ca and CaxP, iPTH, ALP, mean dialytic age and mean duration of dialysis are presented in Table 1.

Table (2) Summarizes the biochemical parameters (serum Ca, P, ALP, CaxP and iPTH) and the change of PTG by CD ultrasonography, after 6 months of therapy with Calcitriol or Cinacalcet. Serum Ca, P, and CaxP showed non-significant differences between both group. While serum ALP and iPTH showed significant reduction in the group of MHD patients treated by Cinacalcet than in those treated by Calcitriol. The percent reduction in volume of PTG was significantly more in Cinacalcet group than in Calcitriol group, $p< 0.5$.

Table 1: Baseline clinical, biochemical and sonographic parameters in 2 MHD patients groups (Cinacalcet & Calcitriol).

Parameter	Calcitriol group n= 16	Cinacalcet group n = 15	P-value
Mean age ± SD (years)	16±2.1	15±2.8	NS
Sex	8m/8f	8m/7f	
Mean ±SD duration of dialysis (months)	7±1.5	8±0.8	NS
Phosphorus ± SD (mg/dl)	6.53 ± 0.14	6.51±0.16	NS
Calcium ± SD(mg/dl)	10.06±0.33	9.6±0.3	NS
CaxP ± SD(mg^2/dl^2)	63±0.8	59±9	NS
iPTH ± SD (pg/ml)	897±177	838±203	NS
ALP ± SD (IU/L)	276±128	318±106	NS
Mean±SD PTG volume (mm^3)	466±49	449±86	NS

ALP, alkaline phosphatase; CaxP, calcium-phophorus product, iPTH, intact parathyroid hormone, NS, nonsignificant.

Table 2: Biochemical and sonographic parameters after 6 months treatment with Calcitriol (calcitriol group) versus treatment with Cinacalcet (Cinacelcet group).

	Calcitriol grop n = 16	Cinacalcet group n = 15	p-value
Phosphorus ± SD (mg/dl)	5.78±0.27	4.46±1.3	NS
Cacium±SD(mg/dl)	11.4±0.15	9.8±0.7	<0.05
CaxP±SD(mg^2/dl^2)	54±6	51±4	NS
iPTH±SD (pg/ml)	513±98	258±72	<0.001(S)
ALP±SD(IU/l)	338±29	223±25	0.03(S)
Reduction in volume of PTG (%)	33%	60%	0.01(S)

NS, nonsignificant; S, significant; %, percent; SD, standard deviation; mg, milligram;dl, deciliter;pg, picogram; IU, international Unit; l:liter; n, number; Cax P, calcium X Phosphous; ALP, Alkaline phosphatase.

Disscussion

Control of sHPT is one of the main targets in the management of uremic bone disease [9]. Just as better control of hyperphosphatemia in renal failure patients helps prevent the nearly universal development of sHPT, better control of hyperphosphatemia is also achieved through the control of sHPT. The agents commonly used to control HPT, in MHD patients are vitamin D metabolites [14], the calcium-sensing receptor agonists (Cinacalcet), phosphate binders, and several methods of surgical interventions[15]. Clinical and experimental data on parathyroid hyperplasia have added to therapeutic decision making. Measurement of the size of the PTGs is mandatory for the selection of the optimal therapy for sHP[9].

The two types of parathyroid hyperplasia observed in uremic patients are diffuse and nodular, with the latter developing from the former. Cells with a marked reduction of Calcitriol receptor in diffuse hyperplasia proliferate and form nodules that progress to nodular hyperplasia. Parathryroid cells in nodular hyperplasia are usually resistant to medical therapy, so it is critical to distinguish diffuse from nodular hyperplasia on the basis of size. Patients with at least one PTG larger than $0.5cm^3$, or 1.0 cm in diameter usually do not respond to medical treatment of sHPT[9]. This critical

size has a significant pathological basis. A study of resected PTG has shown that < 90% of PTGs heavier than 0.5 g has nodular hyperplasia. In future, this critical size may be reduced by vitamin D sterols and/or Calcimimetics[16].

This study was carrid out on 31 CKD children, who receive hemodialyis 3 times weekly, to evaluate the effect of Cinacalcet versus that of Cacitriol on biochemical abnormalities (hypercalcemia, hyperphosphatemia and high CaxP and ALP and on the abnormally increased size of the PTG, due to sHPT.

By ultrasonic examination, a normal PTG measures ~ 6x5x2 mm[12]. In this study CD ultrosonography of the PTG in two-treatment groups, found that the volume of PTG is 466 mm3 and 449 mm3 in Calcitriol and Cinacalcet group, before treatment, with non-significant difference. This indicated that there is PTG diffuse hyperplasia. Nagano et al stated that sHPT, characterized by hyperplasia of the PTG is one of common consequences of CKD and PTG hyperplasia is positively associated with the magnitude of circulating PTH. In addition, PTG hyperplasia is rarely reversible and often develops to nodular hyperplasia, which is refractory to medical treatement[17]. A similar result on adult patients, suffering CKD and on MHD, reported a tendancy for patients with more enlarged glands to have a higher PTH level. In addition, this study reported positive correlation of PTG volume and each of Ca, P, CaxP and iPTH serum levels[18].

Secondary HPT represents an adaptive response to the progressively impaired control of Ca, P and vitamin D in CKD. It is characterized by parathyroid hyperplasia and excessive synthesis and secretion of PTH, resulting in excessive bone resorption, soft-tissue and vascular calcification[19].

In this study, baseline biochemical parameters (serum levels of Ca, P, CaxP, ALP) showed siginificantly higher values than the normal values cited in the textbooks. Consequences of hyperphosphatemia in CKD include sHPT, metastatic calcification, and

osteitis fibrosa cystica. Hyperphosphatemia also contributes to the progression of renal failure [20].

In this study, CKD children on MHP were allocated at random into two treatment groups which either received Calcitriol or Cinocalcet, in the proper dose for 6 months. Then after this follow-up period, patients of each group were reassessed, biochemical parameters measured and the size of PTG calculated after CD U/S.

Levels of serum Ca, ALP and iPTH, in patients on Cinacalcet were significantly lower than that in patients on Calcitriol. Similar results were reported in other studies on adult populations [6,13,18,19,20,21]. Cinacalcet, the allosteric modulator of the Ca-sensing receptor (CaR), lowers both serum Ca and P concentrations in addition to, suppressing immunoreacitve PTH secretion. This highly reproducible and persistent effect may be due to an improved bone turnover and thus an increased ion uptake into the bone, but potentially also by effects on the ions efflux from the intracellular into the extracellualr compartment[22]. On the other hand, Poon (2005) stated that vitamin D sterols and phosphate binder are used to treat HPT. However, common adverse effects of these medications is hypercalcemia, which contribute to vascular and soft-tissue calcification[13].

Parathyroid gland volume is a key marker for the severity of sHPT and increased secretion of iPTH. High-resolution CD sonography is the only tomographic technique able to measure accurately volumetric varations in PTG. A hypoechoic gland with 2 diameters > 5mm is generally considered to be hyperplastic[6].

More than 80% of glands with an estimated volume > 0.5 cm3 proceed to nodular hyperplasia. Therefore, PTG volumetry is important for predicating the effetiveness of medical therapy. Superior results are obtained in patients with one nodular hyperplastic gland. This is one of the major revisions in the new guidelines. Thus, three or more glands, one of the criteria for refractory

response in the former guidelines, is now changed to "two or more "[23].

Enlargement of the PTG markedly increases the capacity for PTH production. Basal Ca-independent (non-suppressible) PTH secretion that parallels the increased gland size becomes an important factor in elevated PTH levels when the PTGs are 50-100 times their normal size. Moreover, as CaR and vitamin D recptor (VDR) expression are reduced in the course of hyperplasia, the PTGs become increasingly resistant to regulation by Ca and Calcitriol[19].

In this study, MHD children with sPTH due to mild-to-moderate parathyroid hyperplasia, received medical therapy in the form of either Cincalcet or Calcitriol. After 6 months of follow-up the mean volume of PTG was reduced by 60% in Cinacalcet group and by 33% in Calcitriol group, with significant difference. Nearly similar results were obtained by other studies on adult CKD patients[7,8,13,18,19,21,23]. Furthermore, Meola et al[6] concluded that cinacalcet, in combination with conventional treatments, led to an improvement in biochemical and clinical parameters of sHPT and reduced glandular volume in patients with severs sHPT.

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CALCIMIMETIC (CINACALCET) VERSUS CALCITRIOL FOR THE CONTROL OF SECONDARY HYPERPARATHYROIDISM DUE TO CHRONIC KIDNEY DISEASSE IN CHILDREN

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ADDITION OF SERUM URIC ACID LEVEL TO UTERINE ARTERY DOPPLER FOR EARLY PREDICTION OF PRE-ECLAMPSIA IN PRIMIGRAVIDA

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Abstract

Background: *pre-eclampsia is a common cause of both maternal and perinatal morbidity and mortality in both developed and developing countries. It is the second leading cause of maternal mortality; maternal complications include eclampsia, coagulopathy, HELLP syndrome, shock, and death.* ***Objective:*** *To assess the performance of uterine artery Doppler in conjunction with estimation of serum uric acid level as a predictor for pre-eclampsia. As we considered the possibility of a combination of serum uric acid level with Doppler sonography being able to improve the prediction of Pre-eclampsia.* ***Material and Method:*** *A prospective study of 60 primi pregnant women between 16-20 weeks gestation who were attending at the Antenatal Care Clinic, in obstetric and gynecologic department of Said Galal and El-Hussein hospitals, faculty of medicine Al-Azhar University, was conducted. Doppler flow velocity waveform for the presence of diastolic notch and serum uric acid level of all subjects to be related with the pregnancy outcome* ***Results:*** *Analyses of the diagnostic performance of the diastolic notch in predicting pre-eclampsia were as follows: 66.6% sensitivity, 96.2% specificity, 40% PPV, 94% NPV, 91% accuracy. Analyses of the diagnostic performance of the serum uric acid level in predicting pre-eclampsia were as follows: 83.3% sensitivity, 92.5% specificity, 55.5% PPV, 98% NPV, 91.6% accuracy.while study analysis of the diagnostic performance of the diastolic notch of the uterine artery in conjunction with High uric acid level, with the 94% confidence interval (CI) in the parenthesis, in predicting*

Introduction

Pre-eclampsia, (PE) a serious pregnancy specific disorder, characterized by proteinuria and hypertension befor and after the 20th week of gestation is still the main cause of maternal and neonatal morbidity and mortality. It occurs in 3-5% of all pregnancies.[1-3] Pre-eclampsia and intrauterine growth restriction remains important causes of maternal and neonatal complications and death.[4-6] PE is the second leading cause of Maternal mortality constituting 12% to 18% of pregnancy related maternal deaths[4]. PE is known as 'the Disease of multiple theories'. Among them genetic, immunological, circulatory factors, uterine vascular changes and endothelial dysfunction are important.[7] In the developing countries, the incidence is expected to be higher, Predisposing factors are nulliparity, black race, maternal age below 20 or over 35 years, low socioeconomic status, multiplegestation, hydatidiform mole, polyhydramnios, non immune fetal hydrops, twins, obesity, diabetes, Chronic hypertension and underlying renal disease.[7] Maternal complications include the HELLP syndrome (hemolytic anemia, elevated liver enzymes, low platelet count), eclampsia, coagulopathy, Stroke and death.[8,9] Pre-eclampsia is diagnosed when the blood pressure at or above 140/90 mmHg occurring on two occasions at least 6 hours apart, Associated with proteinuria greater than 300mg/24 hours or greater than 1 gm/l in a random sample, after and also befor 20 weeks of gestation.[10] Inability of the uterine artery to increase dilatation with advancing gestational age, like in the normal pregnancy, caused by the pathology of the uterine arterial wall was found In the women with pre-eclampsia.[11] Failure of spiral artery remodeling as reflected by enhanced uterine artery resistance has been linked to adverse pregnancy outcomes.[12] The human embryo undergoes interstitial implantation by invading the maternal decidua at the blastocyst stage.[3] Reduced placental size or defective syncytiotrophoblast formation may result in reduced production of placenta-derived proteins, such as pregnancy associated plasma protein A (PAPP-A).[13]

The uteroplacental circulation can be assessed by means of Doppler ultrasonography of the uterine arteries.[12] this procedure has been reported in numerous studies to be a promising technique for predicting the level of risk for pre-eclampsia.[13-16] large number of studies have investigated the potential of second-trimester uterine artery Doppler studies as a screening tool for pre-eclampsia and fetal growth restriction.[17]

High resistance index in the uterine artery demonstrated by the Doppler study was proposed to predict the pre-eclampsia; however it was found to have a low predictive value with varying cut-off levels reported in the studies.[18-24]

One of the most widely studied Doppler indices is the pulsatility index (calculated as the peak systolic flow minus the end diastolic flow divided by the mean flow). An increased pulsatility index has been associated with an increased risk for pre-eclampsia and intrauterine growth restriction.[15-16] The presence of an early diastolic notch in the waveform has also been shown in several studies to be associated with adverse outcomes.[16-17].

Because of this limitation, association between the diastolic notch of the uterine artery and the development of pre-eclampsia was explored.[18,25], a large number of maternal serum markers have been investigated for the prediction of PE, but their use as single screening test has also been disappointing [32].

The hyperuricemia is believed to result from the decreased renal excretion That occurs as a consequence of the pre-eclampsia but this result is probably also increased production secondary to tissue ischemia and soluble uric acid impairs nitric oxide endothelial cells. Hyperuricemia induce endothelial dysfunction and may induce hypertension and vascular disease. Previous studies have failed to identify an independent marker in maternal plasma, or an easy to measure physiological parameter which would substantially improve the screening efficacy of Doppler sonography.[2,3] We considered the possibility of a combi-

nation of this technique as serum uric acid level with Doppler sonography being able to improve the prediction of Pre-eclampsia.

Patients & Methods

A total number of 60 primigravida women (sample size) according to the designe of this study at 16-20 weeks of pregnancy were selected randomly on the basis of availability according to inclusion and exclusion criteria. The study population was the pregnant women who were attending at the antenatal Care Clinic in obstetric and gynecologic department of Said Galal and El-HusseIn hospitals faculty of medicine Al- Azhar University From 1 April 2011 to 31 March 2012. Exclusion criteria:

1. Patients having other complications or associated disease.
2. Pregnant woman having history of chronic hypertension, diabetes, renal disease.
3. Multiplepregnancies,
4. Patient with any acute or chronic illness.
5. Patient with symptoms of urinary tract infection
6. Non primigravida patients.

Inclusion criteria :

1. Primigravida patients
2. Age from 19-24 years
3. No any medical disease that may predispose to PE as chronic hypertension and renal diseases.

Before being admitted to the clinical study, the patients consent to participate, after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to them. Informed verbal consent was taken from all patients. At the time of registration, a detailed medical and obstetrical history was taken, General, systemic and obstetric examinations were carried out on the same day. These patients were under regular follow up until delivery. Specific note was made of the development of pre-eclampsia. During antenatal period the selected patients were on normal diet and requested to fast overnight (10-12 hours) then, 5 ml of venous blood was collected into a test tube. Serum was separated rapidly after centrifuging for 10 minutes at a rate of 3000 rpm at 40 C and then Serum uric acid level is detected. Rele-

vant investigations were done with blood to exclude diabetes mellitus, any renal disease and hyperlipidemia.

Data were shown as mean and standard deviation. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, of the serum uric acid level in the prediction of pre-eclampsia was studied. Statistical analyses were performed. A Doppler ultrasonography machine used in all patients was Semens ultra sound with 5 MHz convex probe and pulse wave filtered at 100 MHz. At first, the number of fetus, gestational age, gross anomalies and position of the placenta were evaluated. Circulation flow of the uterine artery was examined. The presence or absence of the notch seen in the artery during diastole was recorded bilateral uterine arteries were studied in each subject.

The presence of diastolic notch was recorded only but the results regarding the diastolic notch were not recorded in the antenatal record file in the obstetric care. Every subject was advised to be followed-up according to the appointments and to come to the hospital immediately if abnormal symptoms, such as generalized edema, Headache, blurred vision or epigastric pain, existed. All subjects were then followed through the whole pregnancy period to see whether pre-eclampsia developed. Any subject who was found to have Hypertension and positive for urine protein would be hospitalized for close observation. The demographic and obstetric data were shown as mean and standard deviation. Sensitivity, specificity, positive predictive Value (PPV), negative predictive value (NPV), accuracy, of the Diastolic notch of the uterine artery in the prediction of pre-eclampsia was studied. Statistical analyses were performed.

Results

In this study, 60 pregnant women were included at their 16-20 weeks without any complication or any known risk factors for developing pre-eclampsia. The study subjects were in the age range of 19-24 years (mean 21.5±5) all of them was primigravida.

There were 6 subjects (study), (10%) found to develop pre-eclampsia including 4 cases (6.6%) of mild pre-eclampsia and 2 cases (3.4%) of severe pre-eclampsia.

The rest 54 patients remained normotensive (Controls). The prevalence of PE among the study group was 11%. Comparison between control and study groups as regard age and BMI showed no significant difference between the two groups.

The SBP (mean±SD, mmHg) were 102±11 vs. 143±11.DBP (mean±SD, mmHg) 68±9 vs. 109±10 and MAP 83±9 vs. 119±8 respectively in Control and PE subjects. The entire BPs was higher in PE cases and the differences with the Control were highly significant ($p<0.001$). The diastolic notch of the uterine artery was found in 7 subjects (11.5%); 6 cases found in unilateral uterine artery and 1 cases found in bilateral Uterine arteries. Of these 7 cases, 4 cases developed pre-eclampsia. There were 2 cases that developed pre-eclampsia without demonstrated diastolic notch as shown in table 3.

Study analysis of the diagnostic performance of the diastolic notch of the uterine artery, with the 94% confidence interval (CI) in the parenthesis, in predicting pre-eclampsia was as follows: 66.6% sensitivity, 96.2% specificity, 40% PPV, 94% NPV, 91% accuracy. As shown in table 4.

High uric acid level was found in 9 subjects (15%), 5 cases developed preeclampsia. There was 1 case that developed pre-eclampsia without demonstrated high uric acid level. as shown in table 5 .

Study Analysis of the diagnostic performance of the High uric acid level, with the 94% confidence interval (CI) in the parenthesis, in predicting pre-eclampsia was as follows : 83.3% sensitivity, 92.5% specificity, 55.5% PPV, 98% NPV, 91.6% accuracy. As shown in table 4. In our study, the diastolic notch of the uterine artery was found in conjunction with High uric acid level in 5 patients (8.3%), of this 5 cases, 4 cases develop pre-eclampsia. There were 2 cases that developed pre-eclampsia

without demonstrated diastolic notch in conjunction and high uric acid as shown in table 6.

Study analysis of the diagnostic performance of the diastolic notch of the uterine artery in conjunction with High uric acid level, with the 94% confidence interval CI, in predicting pre-eclampsia were as follows: 66.6% sensitivity, 96.2% specificity, 66.6% PPV, 96.2% NPV, 93.3% accuracy. As shown in table 4.

Table 1: Comparison between control and study groups as regard age and BMI.

	Age (year)	BMI KGlM2
Control group n=54	21±1	21.7±2
PE group n=6	17.9±0.9	22.4±4

Table 2: Comparison between control and study groups as regard clinical parameters.

	SBP	DBP	MAP
Control group n=54	102±11	68±9	839±
PE group n=6	143±11	109±10	119±8

Table 3: Diastolic notch of the uterine artery as predictor of pre-eclampsia.

Diastolic notch of the uterine artery	Present PE	Absent PE	Total
Present	4	3	7
Absent	2	51	53
Total	6	54	50

Table 4: Comparison between Diastolic notch of the uterine artery and uric acid level as predictor of pre-eclampsia.

	Sensitivity	Specivicity	PPV	NPV	accuracy
Diastoli-c notch of the uterine artery	66.6%	96.2%	40%	94%	91%
High uric acid level	83.3%	92.5%	55.5%	98%	91.6%
Diastolic notch of the uterine artery+high uric acid level	66.6%	96.2%	66.6%	96.2%	93.3%

Table 5: High uric acid level as predictor of pre-eclampsia.

High uric acid level	Present PE	Absent PE	Total
present	5	4	9
absent	1	50	51
total	6	54	60

Table 6: Diastolic notch of the uterine artery and uric acid level in conjunction as predictor of preeclampsia.

Diastolic notch of the uterine artery+high uric acid level	Present PE	Absent PE	Total
Present	4	2	6
Absent	2	52	53
Total	6	54	50

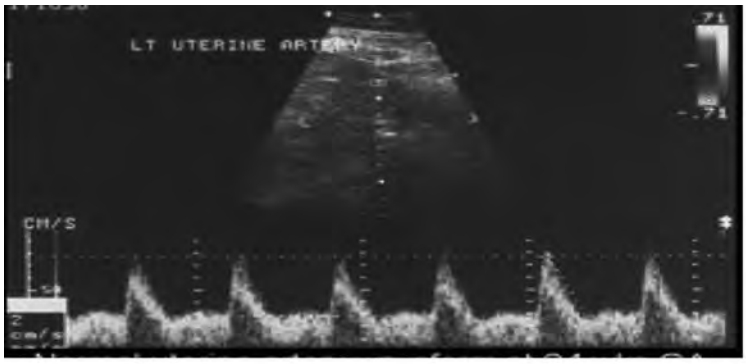

Figure 1: Left uterin artery waves at 20 weeks pregnancy.

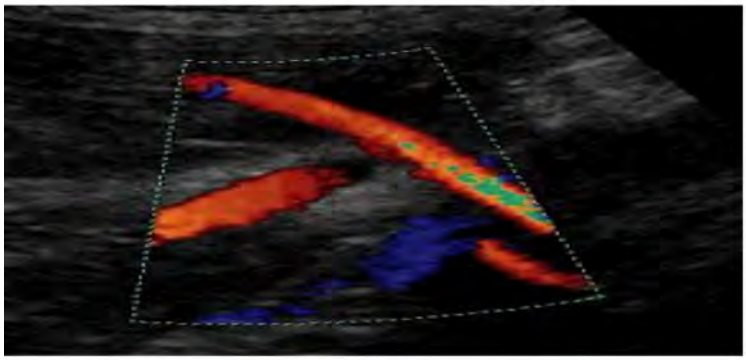

Figure 2: Insonation of the uterine artery at the crossover with the iliac artery.

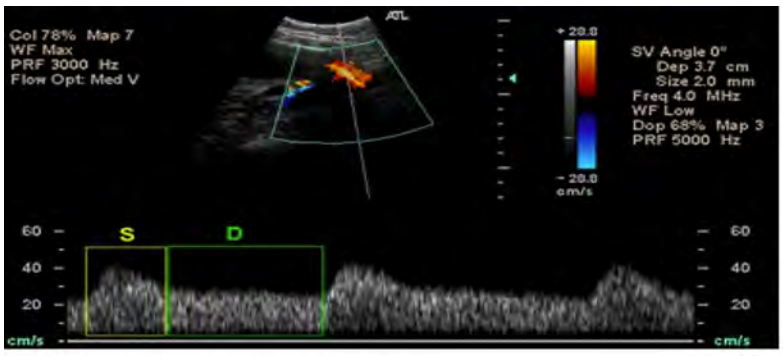

Figure 3: Normal uterin artery blood flow .

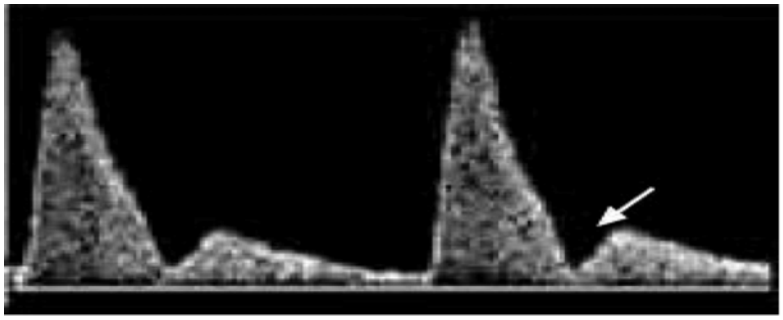

Figure 4: Uterine artery Doppler ultrasound scan showing early diastolic notch (arrow). The presence of diastolic notches is associated with an increased risk of pre-eclampsia and IUGR.

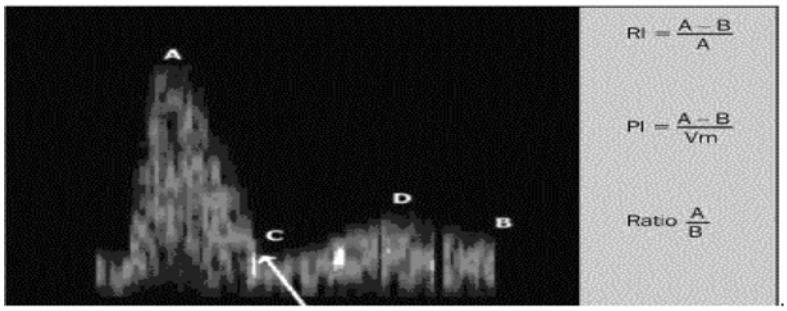

Figure 5: (RI, resistance index-PI, pulsatility index-A, systolic peak-B. end-diastolic-Vm, mean velocity-C, start of diastole-D, maximum diastole.).

Discussion

The study showed that most of the study. Subjects were in the age range of years 19-24 years (mean 21.5±5). Moreover all of them had no past medical history and all of ou r study subjects were primigravida. The incidence of pre-eclampsia in our study was 10%. Using the diastolic notch of the uterine artery, although having high sensitivity and specificity, 66.6% and 96.2% respectively, were achieved in predicting the disease, this sign provided the wide range of 95% CI of the sensitivity. Additionally, whoever the PPV was found to be relatively high. High sensitivity and specificity but relatively high. PPV of the

diastolic notch of the uterine artery in predicting the development of pre-eclampsia found in this study were in contrast to the data found in the previous studies Thawalwong et al, reported that the diastolic notch of the uterine artery yielded 78.6% sensitivity, 89.0% specificity but only 13% PPV in the prediction of pre-eclampsia.[31] in Bower et al.,[26] reported that the diastolic notch of the uterine artery yielded 82.0% sensitivity, 86.9% specificity but only 12% PPV in the prediction of pre-eclampsia. High sensitivity of 77.7% with low PPV of 29.0% were also reported in the study of Harringtonet al.,[27]

However, these data were not agreed with the other study, which found only 22.0% sensitivity but relatively high specificity and PPV, 97.0% and 35.0% respectively. this low sensitivity, but high PPV was probably explained as the criteria used in the prediction of pre-eclampsia in this study were both the high resistance index of the uterine artery and the presence of the diastolic notch of the uterine artery.[28] Iron et al[29] studied in women with the first gestation at the gestational age of 18 and 24 weeks and found that using the diastolic notch of the uterine artery in the prediction of pre-eclampsia yielded decreasing sensitivity from 50.0% to 26.0% and increasing specificity from 57.0% to 87.0% with advancing gestational age. The PPV was very low at both gestational ages, 5.0% and7.0%.whereas the NPV in this study was 94%, which was close to those in other studies, and indicating that most pregnant women with absent diastolic notch of the uterine artery wouldn't develop pre-eclampsia.

The strength of this study was that only one ultrasound machine used for the whole study, resulting in less variation caused by the machine. The subjects were blinded to the Doppler findings. In our study, high uric acid level was found in 10 subjects (16%), 7 cases developed pre-eclampsia. There were 3 cases that developed pre-eclampsia without demonstrated high uric acid level. Study analysis of the diagnostic performance of the high uric acid level, with the 94% confidence interval (CI) in the pa-

renthesis, in predicting pre-eclampsia were as follows: 83.3% sensitivity, 92.5% specificity, 55.5% PPV, 98% NPV, 91.6% Accuray. In our study, the diastolic notch of the uterine artery was found in conjunction with high uric acid level in 5 patients (8.3%), of this 5 cases, 4 cases develop pre- eclampsia. There were 2 cases that developed pre-eclampsia without demonstrated diastolic notch in conjunction and high uric acid. By Study analysis of the diagnostic performance of the diastolic notch of the uterine artery in conjunction with High uric acid level, with the 94% confidence interval (CI) in the parenthesis, in predicting pre-eclampsia were as follows: 66.6% sensitivity, 96.2% specificity, 66.6% PPV, 96.2% NPV, 93.3%. By analysis of this result, it was proved that using the diastolic notch of the uterine artery in conjunction with estimation of serum uric acid level increased the PPV for prediction of pre- eclampsia with high accuracy. Also, Antsaklis et al.,30 revealed that these predictive methods could increase the PPV for prediction of pre-eclampsia.

Conclusion

1. Although both of uterine artery Doppler and uric acid level has role in prediction of pre-eclampsia with high sensitivity and specificity. Whoever each one separately has relatively high positive predictive value.

2. Uterine artery Doppler and uric acid level together has higher positive predictive value with high accuracy and can be used in conjunction together as screening for prediction of pre-eclampsia at 16-20 weeks pregnancy and as early as possible in first trimester later on.

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THE RELATION BETWEEN VISCERAL ADIPOSITY INDEX, VIRAL LOAD AND EARLY VIROLOGICAL RESPONSE IN CHRONIC HEPATITIS C TREATED BY PEGYLATED INTERFERON AND RIBAVIRIN IN EGYPT

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Abstract

Hepatic steatosis has been associated with liver damage in patients with chronic hepatitis C (CHC). Visceral adiposity index (VAI), is a new marker of adipose dysfunction in CHC. ***Aim of the Study:*** *to assess hepatic steatosis and VAI association with host and viral factors and its link to both histological findings and Early virological response (EVR).* ***Patients and Methods:*** *One hundred consecutive Egyptian CHC patients were evaluated by liver biopsy and anthropometric and metabolic measurements, including VAI using waist circumference, body mass index, triglycerides, and high-density lipoprotein cholesterol. All biopsies were scored by one pathologist for stage of fibrosis, grade of inflammation and graded for steatosis, which was considered moderate to severe if ≥30%. Results: one hundred patients were studied, 74 patients were males, 24 patients had no steatosis and 10 patients had steatosis ≥30%, no significant difference in the grade of inflammation and stage of fibrosis in relation to severity of steatosis. VAI is more in females and significantly related to the presence (p=0.001) but not the severity (p= 0.378) of steatosis. On multivariate regression analysis, the risk factors independently linked to severity of steatosis (≥30%) were; triglycerides (beta coefficient= 2.890, p = 0.005), ALT level (beta coefficient = 2.303,*

P= 0.001), HCV RNA level (beta coefficient = 2.398, P = 0.022), stage of fibrosis (beta coefficient= 2.639, p = 0.011) and necroinflammatory activity (beta coefficient = 1.705, p = 0.002) but not EVR (beta coefficient=1.792, p= 0.097). Multiple linear regression analysis revealed that VAI score was independently associated with female gender and ALT level (beta coefficient -3.761, P= 0.001). Multivariate regression analysis showed that TG and ALT were independently linked to moderate to severe necroinflammatory activity and EVR. ***Conclusion:*** *In Egyptian CHC patients, VAI score is independently related to female gender, ALT level and the presence of steatosis. Severity of steatosis is independently associated with TG, ALT, viral load, necroinflammatory activity and stage of fibrosis but not EVR. Both moderate to severe necroinflammatory activity and EVR were independently linked to TG and ALT levels.*

Introduction

Hepatitis C virus (HCV) infection is a serious worldwide problem. It has been estimated that there are over 170 million HCV infection worldwide with an increasing incidence of new infections, 3-4 million every year .[1]

In Egypt, the estimated adjusted national prevalence rate of chronic hepatitis C virus infection is 9.8% in 2008,[2]. The current optimal therapy for patients with chronic HCV infection is the combination of Peg-Interferon and Ribavirin. [3,4]

Several important unmodifiable predictors of poor response to antiviral therapy, such as HCV genotype, high viral load, advanced fibrosis on pretreatment liver biopsy were reported.[5]. Potentially modifiable factors such as obesity, hepatic steatosis, and insulin resistance (IR) have been shown to be important predictors not only of disease progression but also of response to antiviral therapy.[6]

Obesity and metabolic syndrome are associated with hepatic steatosis and more severe fibrosis in chronic hepatitis C [7]. Hepatic steatosis is a common histopathological finding occurring in more than 50% of patients with chronic HCV. Both host and viral factors

have been demonstrated to play an important role in its development.[8] Visceral Adiposity Index (VAI), a new index of both fat function and distribution, appears to be independently associated with steatosis and necroinflammatory activity in genotype 1 chronic active hepatitis C patients and has direct correlation with HCV viral load. [9]

The aim of the study was to assess the presence and severity of hepatic steatosis and VAI and their relation to the histological, host and viral factors and relation to Early Virological Response to treatment of chronic hepatitis C patients.

Patients and Methods

This study included 100 consecutive chronic HCV Egyptian patients scheduled for combined PEG-INF and RBV therapy at Cairo Fatemic Hospital. All patients were subjected to clinical assessment and obesity indices including weight, height, waist circumference and laboratory investigations including: Complete blood count (CBC), Liver biochemical profile, Prothrombin % and AFP.

Inclusion criteria according to the Egyptian Ministry of Health include : age from 18 to 60 yrs, detectable HCV RNA, Neutrophil count ≥ 1500 / cmm, platelet count ≥ 100.000 / cmm, Hb ≥ 12 gm/dl, while exclusion criteria include decompensated liver disease, HCC, autoimmune liver disease, thyroid dysfunction, psychological disorders, drug addicts and solid organ transplantation. [10]

Percutaneous needle liver biopsies were obtained using Medax Poggio rusco needle, 16 G x 200 mm single step biopsy needle under ultrasonic guidance and complete aseptic technique according to Lindor, et al., [11]

Histological evaluation of the grade of inflammation and the stage of fibrosis were evaluated according to the Metavir scoring system, [12]

Severity of steatosis was graded according to the percentage of hepatocytes affected as absent (<5%), mild (5-30%), moderate (>30-60%) or severe (>60%).[13]. Patients were classified according

to steatosis severity into absent or present, also, classified as group one (absent to mild degree: steatosis < 30%) and group two (moderate to severe degree: steatosis ≥ 30%)

Body mass index (BMI) was calculated in every patient and classified as: normal weight (18.5 - < 25kg / m^2), overweight (25 - < 30 kg/ m2), obese (>30kg / m2). Waist circumference (WC) was measured using tap measure.(14)

Visceral adiposity index score was calculated by computer system using the following formula and was differentiated according to sex:

$$\text{Males : VAI} = \frac{WC}{39.68 + (1.88 \times BMI)} \times \frac{TG}{(1.03)} \times \frac{1.31}{HDL}$$

$$\text{Females : VAI} = \frac{WC}{39.68 + (1.89 \times BMI)} \times \frac{TG}{0.81} \times \frac{1.52}{HDL}$$

VAI= Visceral Adiposity Index, WC= Waist Circumference,

TG=Triglyceride, HDL = high density lipoprotein. **(15)**

Patients received treatment in the form of PEG-INFa-2b: 1.5 mg SC/kg once weekly and ribavirin according to the body weight (13-15 mg/kg). Or PEG-INFa-2a: 180 mg SC once weekly regardless of weight and ribavirin according to the body weight 1000mg/day for patients <75 Kg, and 1200mg/day for those >75 Kg.

Follow up and monitoring response to antiviral therapy was done 1, 2 and 4 weeks after initiation of therapy then every 4 weeks. During each follow up, signs and symptoms of possible adverse effects were evaluated and routine laboratory studies were performed.

Quantitative HCV RNA by Real time polymerase chain reaction (RT- PCR) was performed after 12 weeks of therapy to determine early virological response (EVR) loss of HCV RNA).

Statistical methods: results were expressed as means ± standard deviation of the means or number (%). Comparison between the two groups was performed using unpaired t test. Comparison between categorical data was performed using Chi square test. Univariate and multivariate linear regression analysis was used to test the association of risk factors with VAI, degree of steatosis, degree of necroinflammatory activity and

EVR. The data were considered significant if p values was ≤ 0.05 and highly significant if p< 0.01. Statistical analysis was performed with the aid of the SPSS computer program (version 17 windows).

Results

This work was conducted on 100 consecutive chronic HCV Egyptian patients. Seventy four patients were males and age ranged from 18 to 60 years.

The majority of the patients (73%) were overweight and obese. VAI is more in females than males and the mean of all biochemical tests was within normal range except ALT which is mildly elevated as shown in table (1).

Fig (1) and Tables (2, 3) showed the histopathological features of the liver biopsies of the studied patients, most of them had mild disease where 76% of patients had mild to moderate steatosis and 24% of patients had no steatosis, 97% of patients had mild to moderate necroinflammation and 85% had stages 1 and 2 fibrosis and on comparing the patients with different grades of steatosis there is no significant difference in the grade of inflammation and stage of fibrosis between patients with and without steatosis and patients with steatosis <30% and steatosis ≥ 30%.

Tables (4, 5, 6) showed the factors that can predict the presence and severity of steatosis and found that Visceral Adiposity Index is significantly higher in patients with steatosis than patients without steatosis (5.27 ± 3.60 versus 3.38 ± 1.44 p= 0.001**), also, patients who had steatosis had higher BMI and waist circumference but not reaching the significant level. Table (6) showed that lower patient' height, higher waist circumference and HDL were the significant factors that can predict advanced steatosis (≥ 30%). Table (7); on multivariate regression analysis, the risk factors independently linked to severity of steatosis (≥ 30%) were; triglycerides (beta coefficient = 2.890, p = 0.005), ALT level (beta coefficient = 2.303, P= 0.001), HCV RNA level (beta coefficient = 2.398, P = 0.022), stage of fibrosis (beta coefficient = 2.639, p = 0.011) and necroinflammatory activity (beta

coefficient = 1.705, p= 0.002), but not EVR (beta coefficient=1.792, p= 0.097).

Table (7): Multivariate regression analysis of the factors related to Visceral Adiposity Index showed that male gender (beta coefficient -4.29, P= 0.001) and ALT level (beta coefficient -3.761, P= 0.001) were significantly associated with VAI score; the negative beta coefficient means that value of the outcome (VAI) decreases with presence of male gender and high ALT, so, VAI are more in females and low ALT, however these factors were insignificant on the univariate analysis.

In table (8); Univariate and multivariate analysis of risk factors associated with Necroinflammatory activity and EVR showed that TG (beta coefficient=1.322, p=0.019), and ALT (beta coefficient=1.846, p= 0.001) were independently linked to moderate to severe necroinflammatory activity on multivariate logistic regression analysis and only ALT was significant on Univariate analysis (beta coefficient= -1.207, p=0.021). EVR was achieved in 94 (94 %) of patients, triglycerides (beta coefficient= 2.89, p= 0.005), fibrosis (beta coefficient= 1.872, p= 0,014), and ALT level (beta coefficient= 3.761, p= 0,001), were the factors that was independently linked to EVR by multivariate logistic regression analysis.

It is important to mention that when dependent factor is significantly related to the independent factor by both univariate and multivariate regression analysis, this means that both factors are significantly related when compared alone and is still having the same significance even after introducing the other parameters. In case of significant multivariate regression analysis but not univariate, this means that the effect of the two factors is not significant when studied alone, but when you put other parameters it gains significance.

In multiple linear regression analysis; positive beta coefficient means that mean value of the outcome increases with presence of risk factor (if dichotomous) or as independent continuous variable

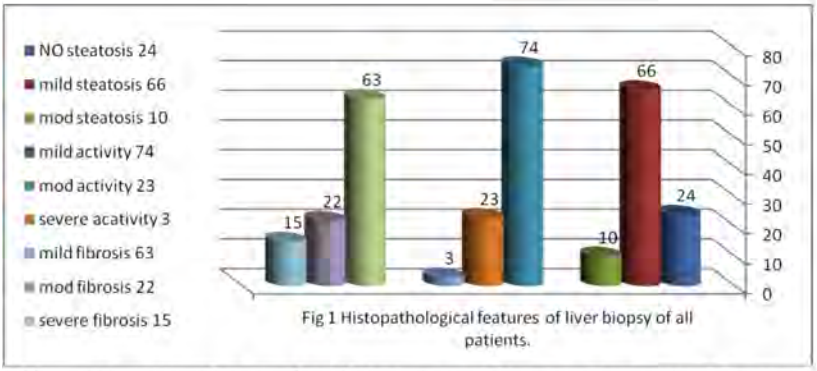
increases. Negative beta coefficient mean value of the outcome decreases with presence of risk factor (if dichotomous) or as independent continuous variable increases [21].

Table 1: obesity indices and biochemical markers of the all patients.

Characteristics	Study group (n= 100)
Weight (kg)	76.25 ± 10.51
Height (cm)	168.07 ± 7.82
Waist circumference (cm)	99.16 ± 15.36
BMI (kg/m^2) < 25	27 (27%)
BMI (kg/m^2) 25-30	73 (73%)
VAI all	4.81 ± 3.31
VAI female (n= 26)	5.92 ± 4.02
VAI male (n= 74)	4.42 ± 2.96
TG (mg/dl)	119.76 ± 54.01(up to 150mg/dl)
HDL (mg/dl)	44.23 ± 15.12(> 40mg/dl)
Glucose (mg/dl)	96.62 ± 19.53(70-110mg/dl)
Creatinine (mg/dl)	0.92 ± 0.37(up to 1 mg/dl))
Albumin (gm/dl)	4.35 ± 0.41(3.5-4.5gm/dl))
Total bilirubin (mg/dl)	0.75 ± 0.29(up to 1mg/dl)
ALP (U/l)	107.90 ± 64.54(up to115U/l)
ALT (U/l)	44.68 ± 42.3(up to 40U/l)
AST (U/l)	36.06 ± 6.86(up to 37U/l)
AFP (ng/dl)	5.83 ± 5.12(up to 13.6ng/dl)
Log HCV (IU/l)	5.23 ± 0.76

Values are expressed as mean ± SD or number (%). VAI = Visceral Adiposity Index.
TG = triglyceride, HDL = High Density Lipoprotein, ALP = Alkaline Phosphatase,
ALT = Alanine Transaminase, AST = Aspartate Transaminase, AFP = Alpha Feto Protein

Fig (1): Histopathological features of liver biopsy of all patients.



Values are expressed as number (%).F1 = portal fibrosis without septa, F2 = portal fibrosis with rare septa, F3 = numerous septa without cirrhosis,

Table (2): Comparison between histopathological features of the studied group classified according to the presence of steatosis.

Characteristics	No steatosis (n= 24)	steatosis (n= 76)	P value
Activity			
A1 (n= 74)	18 (75%)	56 (73.68%)	0.899
A2 (n= 23)	5 (20.83%)	18 (23.68%)	
A3 (n= 3)	1 (4.17%)	2 (2.63%)	
Fibrosis			
F1 (n= 63)	14 (58.33%)	49 (64.47%)	0.862
F2 (n= 22)	6 (25%)	16 (21.05%)	
F3 (n= 15)	4 (16.67%)	11 (14.47%)	

P<0.05= significant

Table (3): Comparison between histopathological features of the patients according to the degree of steatosis.

Characteristics (Total, n=100)	Steatosis < 30% (n= 90)	Steatosis ≥ 30% (n= 10)	P value
Activity			
A1(74)	68 (75.56%)	6 (60%)	
A2 (23)	19 (21.11%)	4 (40%)	0.364
A3 (3)	3 (3.33%)	0 (0%)	
Fibrosis			
F1 (63)	58 (64.44%)	5 (50%)	
F2 (22)	18 (20%)	4 (40%)	0.347
F3 (15)	14 (15.56%)	1 (10%)	

P<0.05= significant

Table (4): Comparison between obesity indices and laboratory results in patients with and without steatosis.

Characteristics	Absent steatosis (n= 24)	Present steatosis (n= 76)	P value
BMI	26.69 ± 4.01	27.10 ± 3.11	0.608
Weight	77.00 ± 11.46	76.01 ± 10.27	0.691
Height	170.04 ± 8.16	167.45 ± 7.66	0.158
Waist circumference	96.63 ± 13.83	99.96 ± 15.82	0.356
VAI	3.38 ± 1.44	5.27 ± 3.60	0.001**
TG	102.88 ± 32.82	125.10 ± 58.31	0.079
HDL	47.00 ± 12.02	43.36 ± 15.95	0.306
Glucose	93.63 ± 20.86	97.57 ± 19.14	0.392
Creatinine	0.88 ± 0.16	0.94 ± 0.41	0.539
ALP	112.54 ± 78.40	106.43 ± 60.03	0.688
AST	35.13 ± 7.70	36.36 ± 6.60	0.446
ALT	42.29 ± 13.13	45.44 ± 48.17	0.753
Total bilirubin	0.71 ± 0.22	0.76 ± 0.31	0.389
WBCs	6.48 ± 2.92	5.97 ± 1.80	0.305
Hb	14.08 ± 1.31	14.16 ± 1.48	0.810
Platelets	209.79 ± 56.22	211.83 ± 54.55	0.875
Prothrombin %	88.78 ± 11.54	89.48 ± 9.75	0.770
Log HCV	5.36 ± 0.50	5.20 ± 0.81	0.688
AFP	5.46 ± 5.39	5.95 ± 5.06	0.393

**p< 0.001= highly significant.

Table 5: Factors differentiating between patients with no or mild steatosis and patients with moderate steatosis.

Characteristics	Steatosis < 30% (n= 90)	Steatosis ≥ 30% (n= 10)	P value
BMI	26.93 ± 3.25	27.66 ± 4.10	0.508
Weight kg	76.51 ± 10.24	73.90 ± 13.09	0.459
Height cm	168.60 ± 7.74	163.30 ± 7.24	0.041*
Waist circumference (cm)	98.13 ± 12.84	108.40 ± 29.36	0.044*
VAI	4.91 ± 3.42	3.93 ± 2.06	0.378
TG	122.83 ± 54.99	92.20 ± 35.22	0.089
HDL	43.04 ± 10.97	54.90 ± 34.41	0.018*
Glucose (mg/dl)	95.68 ± 18.13	105.10 ± 29.27	0.149
Creatinine (mg/dl)	0.94 ± 0.38	0.77 ± 0.13	0.164
ALP (U/l)	109.47 ± 64.30	93.70 ± 68.42	0.466
AST (U/l)	35.89 ± 7.05	37.60 ± 4.88	0.457
ALT (U/l)	45.07 ± 44.65	41.20 ± 5.1	0.786
Total bilirubin(mg/dl)	0.76 ± 0.30	0.67 ± 0.23	0.361
WBCs (10^3/l)	6.08 ± 2.17	6.21 ± 1.63	0.850
ANC (10^3/l)	3.30 ± 1.40	3.37 ± 1.28	0.884
Hb	14.06 ± 1.35	14.82 ± 2.03	0.114
Platelets (10^3/l)	212.53 ± 55.70	200.60 ± 45.40	0.515
Prothrombin %	89.23 ± 10.08	90.07 ± 11.55	0.815
Log HCV (IU/L)	5.23 ± 0.77	5.18 ± 0.66	0.838
AFP ng/l	5.71 ± 5.19	6.91 ± 4.46	0.484

. *p< 0.05= significant.

Table (6): Univariate and multivariate regression analysis of risk factors associated with presence of steatosis and steatosis ≥ 30% in the studied group by linear regression model.

Factors	Presence of steatosis				Steatosis ≥ 30%			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	β co-efficient	P value	β co-efficient	P value	β co-efficient	P value	β co-efficient	P value
TG	-0.030	0.809	-0.457	0.718	-0.811	0.455	2.890	0.005**
Glucose(mg/dl)	0.030	0.804	0.642	0.644	0.276	0.806	1.946	0.069
ALT (U/l)	-0.010	0.939	-0.149	0.889	-0.203	0.766	2.303	0.001**
HCV RNA	-0.034	0.786	0.006	0.996	-0.291	0.792	2.398	0.022*
Fibrosis	-0.062	0.607	-0.879	0.561	-0.506	0.644	2.639	0.011*
Inflammatory activity	0.142	0.274	1.405	0.209	0.723	0.295	1.705	0.002**
EVR	0.157	0.212	1.666	0.312	0.442	0.697	1.792	0.097

Table (7): Univariate and multivariate analysis of risk factors associated with Visceral Adiposity Index in the studied group by linear regression model.

Factors	Visceral Adiposity Index			
	Univariate analysis		Multivariate analysis	
	β co-efficient	P value	β co-efficient	P value
Male sex	1.072	0.454	-4.29	0.001**
TG	17.527	0.998	--	--
HDL	-21.203	1.000	--	--
Glucose(mg/dl)	17.396	0.999	--	--
ALT (U/l)	-0.228	0.873	-3.761	0.001**
HCV RNA	16.796	0.999	--	--
Fibrosis	17.477	0.999	--	--
EVR	17.385	0.999	--	--
Activity	17.619	0.998	--	--

Table (8) : Univariate and multivariate analysis of risk factors associated with Necroinflammatory activity and EVR in the studied group by linear regression model.

Factors	Necroinflammatory activity				EVR			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	β co-efficient	P value	β co-efficient	P value	β co-efficient	P value	β co-efficient	P value
TG	-0.335	0.586	1.322	0.019*	-0.365	0.743	2.890	0.005**
HDL	-20.171	1.000	--	--	-18.627	1.000	--	--
Glucose(mg/dl)	-0.057	0.946	1.099	0.178	-18.706	0.999	--	--
ALT (U/l)	-1.207	0.021	1.846	0.001**	-1.661	0.131	3.761	0.001**
HCV RNA	0.878	0.171	0.336	0.566	-18.651	0.999	--	--
Fibrosis	0.773	0.187	0.405	0.442	0.901	0.311	1.872	0.014*
EVR	-0.791	0.474	1.792	0.097				

NS= p> 0.05= not significant. *p< 0.05= significant. **p< 0.001= highly significant

Discussion

In Egypt, the estimated adjusted national prevalence rate of chronic hepatitis C virus infection is 9.8% in 2008[2], one of the characteristic histological features of chronic hepatitis C is hepatic steatosis which is found in more than 50% of patients with chronic hepatitis C genotype 1, steatosis appears to be due to the co-existence of Non-Alcoholic Steato-Hepatitis (NASH) and increased body mass index (BMI)[8]. Visceral Adiposity Index (VAI), a new index of both fat function and distribution, appears to be independently associated with steatosis and necroinflammatory activity in genotype 1 chronic active hepatitis C patients and has direct correlation with HCV viral load[9]. Genotype 4 is the most common genotype in Egyptian chronic hepatitis C patients.

The aim of this study was to assess the presence and severity

of hepatic steatosis and the relation between VAI, histological finding and HCV viral load and its impact on Early Virological Response (EVR) in Egyptian chronic hepatitis C patients.

Around three fourths of patients (73%) were overweight and obese this is similar to the study of Petta et al., 2010.[9] Females have higher VA Index than males. Most of the patients had mild disease.

In this study, VAI can predict the presence but not the severity of steatosis, while the patient's height, waist circumference and HDL level can predict the advanced steatosis (≥ 30%), the patients who has shorter height; wider waist circumference and higher HDL were more prone to have advanced steatosis. While other parameters as liver function tests and CBC can't differentiate. The same findings were detected by Petta et al,[9] who found that the steatosis was independently associated with VAI score in Genotype 1 CHC. On the other hand, In a recent study by Vongsuvanh, et al.,[16] they studied the ability of VAI to differentiate between simple steatosis and Non Alcoholic Steato-Hepatitis (NASH) as a marker of inflammation, they examined 190 adults with biopsy-proven NAFLD (non alcoholic fatty liver disease) and 129 controls. All had anthropometric and metabolic profiling. VAI was calculated and abdominal fat was quantified by magnetic resonance imaging (MRI) in 38 patients. They found that in NAFLD, VAI is not associated with steatosis, inflammation or fibrosis.

In this study, ALT level cannot differentiate between patients with steatosis <30% and those with steatosis ≥30%, this is similar to Castera et al.[17] who had the same finding while, while Cholet et al.[19] found that serum ALT was often elevated in patients with steatosis >30% in nearly a linear relationship.

In table (2 and 3), there is no significant correlation between the presence and severity of steatosis and both the degree of inflammation (p=0.364) and stage of fibrosis (p=0.347) Castera et al.[17] had the same finding, on the other hand Wyatt et al.[18] found a sig-

nificant association between the score of steatosis and stage of fibrosis (r=0.29, P<0.01), also, Cholet et al.[19] found that increasing steatosis was associated with moderate to severe histological activity and severe fibrosis.

VAI is significantly higher in patients having hepatic steatosis than those without (table 4), while patient's height, waist circumference and HDL level have a significant associated with the severity of steatosis (table 6). On multivariate regression analysis, the risk factors independently linked to severity of steatosis (≥ 30%) were; triglycerides, ALT level, HCV RNA level, stage of fibrosis and necroinflammatory activity but not EVR (table 7); the significant correlation between HCV RNA Level and steatosis conforms to Patton et al.[20] who found that serum HCV RNA levels were associated with severity of steatosis in G3 CHC. In comparison to Petta et al.[7] who found that, no correlation between severity of steatosis & and serum HCV RNA levels in G1 CHC.

In this study, simple comparison failed to proof a significant difference between severity of steatosis and stage of fibrosis and degree of necroinflammatory activity (table 3); on using multivariate regression analysis this significant correlation was confirmed (table 6). Similarly, Wyatt et al.[18] found a significant association between the score of steatosis and stage of fibrosis (r = 0.29, P <0.01) and Cholet et al.[19] found that steatosis was associated with moderate to severe histological activity and severe fibrosis was associated with steatosis. However, Castera et al., found no significant difference regarding grade and stage of chronic hepatitis among patients without and with various grades of steatosis[19].

Factors associated with VAI score: ALT level (P= 0.001) & female gender (P= 0.001, negative beta coefficient with male gender) were associated with higher VAI score on multivariate analysis; however these factors were insignificant on the univariate analysis. No association was found between severity of fibrosis and VAI, Petta et al.[7] found no correlation between severity of fibrosis and

VAI score in G1 CHC also, he found that moderate to severe necroinflammatory activity is independently associated with VAI score in G1 CHC. In this study, no significant correlation was found between HCV RNA level and VAI score, while Petta et al,[7] found that high HCV RNA level was associated with higher VAI score in G1 CHC.

This study had shown that no significant correlation between EVR and VAI score, this conforms to Petta et al.[7], who has shown that VAI had a non significant trend for predicting failure of SVR achievement after standard antiviral therapy in G1 CHC.

Conclusion

In Egyptian CHC patients, VAI score is independently related to female gender, ALT level and to the presence of steatosis. Severity of steatosis is independently associated with TG, ALT, viral load, necroinflammatory activity and stage of fibrosis but not EVR. Both moderate to severe necroinflammatory activity and EVR are independently linked to TG and ALT levels. These results suggest that lifestyle modifications and weight reduction may improve the hepatic pathology related to chronic hepatitis C and hence the response to treatment.

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THE RELATION BETWEEN VISCERAL ADIPOSITY INDEX, VIRAL LOAD AND EARLY VIROLOGICAL RESPONSE IN CHRONIC HEPATITIS C TREATED BY PEGYLATED INTERFERON AND RIBAVIRIN IN EGYPT

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EFFECT OF PEG INTERFERON/RIBAVIRIN DOSE REDUCTION ON THE RESPONSE OF CHRONIC HEPATITIS C IN EGYPT

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Abstract

To clarify the impact of adherence to treatment on the response, we treated 800 chronic hepatitis C patients with pegylated interferon (peg-IFN) and ribavirin for 48 weeks at Shebin Elkom teaching hospital, and evaluated the adherence to the treatment. The early virologic response(EVR) was 89%, 31.6%, and 24.2% in patients given ≥90%, 80%-90% and <80% dose peg-IFN respectively, and was 87.4%, 66.7%, and 65.5% in those given ≥90%, 90%-80%, and <80% dose ribavirin, respectively. Sustained virological response (SVR) rate was 66.1%, 61%, and 31.3% in patients given ≥90%, 80%-90%, and <80% dose peg-IFN respectively, and was 66.3%, 59.2%, and 33.1% in those given ≥90%, 80% -90% and <80% dose ribavirin, respectively. EVR sharply fell from 89% to 31.6-24.2% when exposure to peg-IFN was below 90% in the first 3 month of treatment whereas it decreased in a stepwise manner as for ribavirin. Therefore, ≥90% of peg-IFN and >80% ribavirin are desired to achieve high SVR in the treatment of chronic hepatitis C.

Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide. It is estimated that approximately 170-210 million individuals, i.e. 3% of the world population, are chronically infected with HCV[1]. The prevalence varies markedly from one geographic area to another, in Egypt, the estimated adjusted national prevalence rate of

chronic hepatitis C virus infection is 9.8% in 2008 [2,3].

Chronic HCV is a slowly progressing disease that may take several decades before it results in cirrhosis[4], and standardized mortality ratios for liver-related death are 16 to 46 fold higher in infected individuals than in the general population [5,6].

The combination of pegylated interferon (IFN)- and ribavirin is the approved and well accepted standard-of-care for chronic hepatitis C, the primary goal of HCV therapy is to cure the infection, which results in eliminating detectable circulating HCV after cessation of treatment. Sustained virological response (SVR), is defined as an undetectable HCV RNA level (<50 IU/ml) 24 weeks after treatment withdrawal[4]. The response to combination therapy depends on several factors including HCV genotype, baseline HCV RNA levels and the dose and duration of therapy, and the host factors, such as body mass index, age, insulin resistance, gender, and the characteristics of liver disease, including levels of ALT, GGT, the stage of fibrosis or co-infection with another hepato-tropic virus or with HIV [7].

Anemia is a frequent complication of peg interferon plus ribavirin therapy for which ribavirin dose reduction is the primary management strategy. However, lower SVR rates have been reported in patients who undergo ribavirin dose reduction. Therefore, many clinicians have concerns to improve anemia related symptoms while maintaining ribavirin dose. Reduced ribavirin exposure may be correlated with increased rates of relapse. For most therapeutic regimens, treatment success is correlated highly with drug exposure. Drug exposure depends on the pharmacokinetic properties of a drug and the ability of a patient to adhere to the drug regimen, as well as the amount of drug administered over a specific period of time.

Combination therapy yields SVR occurs more frequently in patients who are able to maintain near full doses of these medications and is reduced substantially in patients who require reduction

in the doses of these medications. In contrast, dose reduction does not appear to influence adversely SVR in patients with genotype 2 or 3[8,9].

Adherence to therapy is one of the most important factors associated with the success of antiviral treatment. The definition of adherence used here is the "80/80 rule", that is, patients who receive more than 80% of the medication and are treated for more than 80% of the planned duration of treatment are considered adherent. One of the first studies investigating the effect of adherence in PEG-IFN/RBV treatment demonstrated that patients who fulfilled the 80/80 rule had a 63% SVR compared to 52% of those with less than 80% adherence[8]. Another study showed that a cumulative ribavirin dose of more than 60% is important to achieve an SVR [9]. While other study concluded, to prevent relapse, ≥3.0 g/kg of RBV should be administered. Higher dose of RBV may be beneficial in patients <60 years. [10]. In the IDEAL trial, PEG-IFN-α2a and PEG-IFN-α2b combination with RBV were compared. Although the EOT response was lower with PEG-IFN-α2b, higher relapse rates were observed with PEG-IFN-α2a.Therefore, the rates of SVR did not differ between the two types of PEG-IFN[8].

Failure of treatment put both physical and economic impact on the patient and community. Thus, it is crucial to understand the mechanisms of non response and relapse to overcome it and identify predictors of response to treatment, to improve the efficacy of interferon therapy[11].

AIM : This study aimed at finding out the effect of total dose of peg-interferon and Ribavirin treatment on the response of chronic hepatitis C in Egypt.

Materials and Methods

This retrospective study was done by collecting and analyzing data of naive chronic HCV patients, who were treated in Shebein El-Kom teaching hospital, Hepatology unit as a part of the Egyptian Ministry of Health project for treatment of chronic HCV between March 2009 and December 2010. Eight hundreds consec-

utive adult patients with chronic HCV were included in this study. Inclusion criteria were, Age >18 years, CHC with HCV RNA PCR positive and liver biopsy, a neutrophil count >2.000 /mm^3, a platelet count >100.000/mm^3., a hemoglobin level >13 gm/dl in males and 12 gm/dl in females; normal serum creatinine level, negative for HBs -Ag, ANA titer less than 1/80. Patients were excluded if they had liver decompensation, any other cause of liver disease, chronic renal disease, Ischemic cardiovascular disease, organ transplantation, clinically relevant depression, uncontrolled diabetes or severe hypertension, hemolytic anemia or received antiviral or immunosuppressive therapy within the last 6 months.

All patients provided written informed consent to treatment. Patients were received either peg interferon alfa2a 180 or peg Interferon alfa -2b 1.5ug/kg / body weight subcutaneous once weekly. The dosage of ribavirin was determined by body weight (1000 mg/day in patients < 75 Kg, 1200 mg/day in patients ≥ 75 kg). The recommended duration of treatment was 48 weeks. Patients were re-assessed weekly during the first month and every month thereafter during treatment. Complete blood count, aminotransferases, serum creatinine and routine laboratory tests results were checked weekly in the first month and then monthly thereafter or more frequently if necessary.

As regard to interferon dose, the patient was given the full recommended dose as long as ANC (Absolute Neutrophil Count) above 750 /mm^3 and platelet count above 50000/mm^3 and was given half dose if ANC fall between 750 /mm^3 and 500 /mm^3 or platelet count was > 25000/mm^3 and < 50000 /mm^3 and stop interferon dose if ANC below 500 /mm^3 or platelet count below 25000/ mm^3.

Patients received the full recommended dose of ribavirin according to body weight as long as hemoglobin level >10 gm/dl and decreased by 200 mg if hemoglobin falls below 10 gm/dl and stop ribavirin dose if hemoglobin falls below 8.5 gm/dl.

Patients who achieved end of therapy (ETR) response were identified by negative HCV RNA PCR at 48 weeks of treatment. Follow up of those patients by PCR at 72 weeks to identify sustained virological responders SVR (PCR negative); and relapsers (PCR positive).

Statistic analysis:

Baseline and follow up demographic, and laboratory data were collected. IBM SPSS Statistics 17 program was used for data analysis. Dichotomous or categorical variables were presented as number and percentage. Continuous variables were presented as mean ± standard deviation. Univariate analysis by using Pearson Chi square test was used to compare dichotomous or categorical variables, and two tailed t test for continuous variables. Significance was considered at P value 0.05 or less.

Results

This study was done on 800 consecutive chronic hepatitis C naive patients in Shebin Elkom teaching hospital, treated by peg interferon & ribavirin on weight base 1000-1200mg/day for 48 weeks. The patients were 421 (52.6%) males and 379(47.4%) with mean age 45.4 years range from 18-60. The demographic characters and laboratory findings are shown in table 1 and 2.

Table 3 : shows the impact of Ribavirin dose reduction or discontinuation on the response. >90% compared to <80% of ribavirin dose associated with EVR 87.4% Vs 65.5%, ETR 91% Vs 79.3% and while SVR 66.3% Vs 33.1%. Relapse rate increases from 7.3% to 32.8% with >90% and <80% of ribavirin dose respectively.

Table 4: shows that the reduction of Peg-interferon to < 90% sharply decreases the response; EVR to - 31.6% instead of 89% with > 90% of interferon dose. And SVR decrease from 66.1% to 31.3% with Peg interferon dose reduction from >90% to <80% respectively. Relapse rate increase 5 folds from 5.6% to 28.6 with >90% and <80% of total dose respectively.

Table 5: shows that there is

a highly significant statistical difference between the effect of dose reduction to < 80% Peg interferon compared with ribavirin on EVR 24.2% Vs 65.5% (P=0.001), while SVR shows no difference 31.3% Vs 33.1% (p=0.5).

The relapse rate is high with ribavirin dose reduction while early non response is high with peg interferon dose reduction < 80%.

Table 1: Title

	Minimum	Maximum	Mean	Std. Deviation
Wt.	48	115	84.83	15.37
BMI	18	35	25.83	4.51
Age	18	60	45.36	10.07
S.Creatinin	0.50	1.50	1.10	4.47
Alb	3.50	5.13	4.15	1.46
Alp	90	413.25	207.21	42.3
AST	23	212	50.88	35.6
ALT	17	232	63.15	65
T.Bil	0.50	1.4	1.12	.63
Hb	12	18.3	14.28	1.26
WBC	3000	12750	6458.78	1834.4
ANC	1753.2	8485	2550.52	903.3
Plt	100	583.30	206.41	61.7
%PC	72	100	83.66	8.26
TSH	1.10	4.7	1.890	1.49
HCV RNA	1260	2125000	353302.11	48249.6
AFP	5.20	89.7	6.59	8.17

Table 2 :

		N (800)	%
Sex	**Male**	421	52.6
	Female	379	47.4
Glu	**ND**	633	79.1
	D	167	20.9
ANA	**-ve**	782	97.8
	+ve	18	2.2

Table 3 :

STUDY GROUP		RBV		P VALUE
		RESPONDER	NON RESPONDER	
12 week	>90%	623/713(87.4%)	90/713(12.6%)	0.019
	90-80%	2/3(66.7%)	1/3(33.3%)	
	<80%	55/84(65.5%)	29/84(34.5%)	
24week	>90%	525/551(95.3%)	26/551(4.7%)	0.006
	90-80%	46/73(63%)	27/73(37%)	
	<80%	33/56(58.9%)	23/56(41.1%)	
48 week	>90%	365/401(91%)	36/401(9%)	0.003
	90-80%	106/121(87.6%)	15/121(12.4%)	
	<80%	65/82(79.3%)	17/82(20.7%)	
72 week	>90%	354/382(92.7%)	28/382(7.3%)	0.002
	90-80%	77/87(88.5%)	10/87(11.5%)	
	<80%	45/67(67.2%)	22/67(32.8%)	
TOTAL	>90%	354/534(66.3%)	180/534(33.7%)	0.001
	90-80%	77/130(59.2%)	53/130(40.8%)	
	<80%	45/136(33.1%)	91/136(66.9%)	

Table 4:

STUDY GROUP		INF		P VALUE
		RESPONDER	NON RESPONDER	
12 week	>90%	666/748(89%)	82/748(11%)	0.001
	90-80%	6/19(31.6%)	13/19(68.4%)	
	<80%	8/33(24.2%)	25/33(75.8%)	
24week	>90%	560/603(92.9%)	43/603(7.1%)	0.001
	90-80%	28/35(80%)	7/35(20%)	
	<80%	16/42(38.1%)	26/42(61.9%)	
48 week	>90%	360/389(92.5%)	29/389(7.%)	0.001
	90-80%	131/149(87.9%)	18/149(12.1%)	
	<80%	45/66(68.2%)	21/66(31.8%)	
72 week	>90%	339/359(94.4)	20/359(5.6%)	0.002
	90-80%	97/121(80.2%)	24/121(19.8%)	
	<80%	40/56(71.4%)	16/56(28.6%)	
TOTAL	>90%	339/513(66.1%)	174/513(33.9%)	0.001
	90-80%	97/159(61%)	62/159(39%)	
	<80%	40/128(31.3%)	88/128(68.8%)	

Table 5

DOSE EXPOSURE		<80%		P value
		RBV	INF	
WEEK	12	55/84(65.5%)	8/33(24.2%)	0.001
	24	33/56(58.9%)	16/42(38.1%)	0.005
	48	65/82(79.3%)	45/66(68.2%)	0.07
	SVR	45/136(33.1%)	40/128(31.3%)	0.5

Discussion

This study was done on 800 consecutive naive patients with CHCV, treated by Peg interferon & ribavirin the mean age was 44.5 years with 421 (52.6%) males & 379 (47.4%) female. The results of the study showed the impact of total ribavirin dose exposure reduction on the response. While the patients who received more than 90% of their recommended ribavirin dose achieved SVR by 66.3% and who exposed to 80-90% of dose achieved SVR by 59.2%, the patients who exposed to less than 80% of ribavirin dose only achieved response by 33.1% (p value <0.001). This coincides with Mc Hutchison, et al.,[8] who said, there is some evidence that dose reductions negatively affect SVR rate in patients with genotype 1 HCV infection.

The present study found that severe reduction of ribavirin dose (< 80%) during the first 3 months leads to sharp decease EVR, 65.5% versus 87.4% in patients exposed to >90% of ribavirin dose (P=0.001).

Regarding the impact of dose reduction on different type of response, In this study, patients who exposed to less than 80% of Ribavirin dose achieved 65.5% and 79.3% in EVR and ETR respectively, while patients who exposed to less than 80% of INF dose achieved response by only

24.2% and 68.2% in EVR and ETR respectively (p value 0.003) and this shows the great impact of either ribavirin or INF dose reduction or discontinuation in the first 12 weeks of therapy.

This is similar to, Sethi, et al., who concluded that ribavirin dose reductions during the first 12 weeks of treatment were associated with a decreased rate of early virologic response (EVR), defined as undetectable or at least a 2-log reduction in HCV RNA level after 12 weeks of therapy[12].

For most therapeutic regimens, treatment success is correlated highly with drug exposure. Drug exposure depends on the pharmacokinetic properties of a drug and the ability of a patient to adhere to the drug regimen, as well as the amount of drug administered over a specific period of time[9.12]. Regarding the impact of Ribavirin dose reduction on different types of virological response, In this study, patients who exposed to less than 80% of Ribavirin dose, achieved EVR by only 65.5%, compared with 87.4% response if the patients get >90% of ribavirin dose the and SVRachieved in 66.3% of the patients receiving >90% of the total ribavirin dose compared to 33.1% response if the dose decreased <80% which is highly significant statistically (P=0.001). Bronowicki et al., reported that, the patients who interrupted ribavirin dosing or who missed >14 days of ribavirin during the first 12 weeks of treatment had SVR of only 9% and discontinuing ribavirin permanently reduced SVR to only 3%[13]. Neither EVR nor SVR was affected adversely by mild-moderate reductions in ribavirin dose, but SVR rates were reduced in patients who received less than 60% of their cumulative ribavirin dose over the 48 weeks[9].

Adherence to therapy is one of the most important factors associated with the success of antiviral treatment. The definition of adherence used here is the "80/80 rule", that is, patients who receive more than 80% of the medication and are treated for more than 80% of the planned duration of treatment are considered adherent. One of the first studies investigating the

effect of adherence in PEG-IFN/ RBV treatment demonstrated that patients who fulfilled the 80/80 rule had a 63% SVR compared to 52% of those with less than 80% adherence [8]. Another study showed that a cumulative ribavirin dose of more than 60% is important to achieve an SVR[9].

Data mining analysis revealed that time to HCV RNA negativity, age and total RBV dose was associated with relapse. To prevent relapse, ≥3.0 g/kg of RBV should be administered. Higher dose of RBV may be beneficial in patients <60 years [14].

However, a weight-based dose of ribavirin (12-15 mg/kg) may be preferred, especially in difficult to treat patients and in Response-guided Therapy (RGT) treatment approaches [15,16].

Also regarding the impact of INF dose reduction on different types of virological response, In this study, patients who exposed to less than 80% of INF dose only achieved response by 24.5%, 75%, 63% and 31.2% in the 12^{th}, 24^{th}, 48^{th} and 72^{th} weeks respectively and this was in agreement with Fried et al.,[17] who reported that Interferon dose discontinuation resulted in a loss of SVR in all except 12%. While the patients who received more than 90% of their recommended INF dose achieved SVR by 66.1% and who exposed to 80-90% of dose achieved SVR by 61.0%, the patients who exposed to less than 80% of INF dose only achieved response by 31.3% (p value <0.001). This means that SVR decreased by the half (31-33% versus 66%) when the patients exposed to <80% compared to >90% of the total dose of peg interferon or ribavirin. Makoto et al.,[14] reported that the SVR rate in patients received ≥80% of peg-IFN dose was significantly higher than that in those received <60% and 60%-80% of the dose (P<.001 for both). They found that patients given ≥80% dose of scheduled peg-IFN were more likely to achieve SVR by 7.7-fold (95% CI; 1.926-30.798, P = .004) than those given 60%-80% dose, this is similar to our results but this 7.7 fold is more than our results 2 fold because they selected elderly females with high viral load and the old age and

high vireamia decrease the SVR.

In this study, when comparing the effect of peg interferon to ribavirin dose reduction, there was a high significant difference between EVR (24.2% Vs 65.5%) (p value 0.001), and no significant difference on ETR (68.2% Vs 79.3%) or SVR (31.4% Vs 33.2%) when patients had received less than 80% of interferon or ribavirin dose, respectively. Early dose reduction to <80%, deteriorate the EVR than sharply with peg interferon than ribavirin, this co insides with Afdhal et al.,[18] who reported that adherence to RBV and interferon dosage was critical during the first 12 weeks of therapy but not so crucial after 12-20 weeks. Makoto et al.,[14] reported that SVR rate sharply fell when exposure to peg-IFN was below 80% whereas it decreased in a stepwise manner as for RBV, In contrast, RBV should be administered as much as possible within the planned dose. To accomplish this, RBV dose should be reduced by 200-mg decrements when anemia appears, and restored to the previous dose when anemia improves. Higher than standard dose RBV given together with standard dose peg-IFN may increase SVR rate(19),.However, safety issues such as severe anemia are the major concern for this approach. Although the use of erythropoietin contributes to maintain RBV dose, the effect on SVR has not been shown[18]. which is in agreement with our results.

In the present study patients exposed to <80% 0f the total expected dose of ribavirin or peg interferon showed a high relapse rate, ETR 79.3% and 68.2%, while SVR 33.1% and 31.3 for ribavirin and peg interferon consecutive, this is similar to results of Hiramatsu, et al.,[20] who reported that RBV dose reduction raised relapse rate in a dose-dependent manner. Ribavirin significantly accelerates the second/third phase of HCV clearance in patients treated with peg interferon, and treatment with peg interferon and ribavirin enhances the rates of EVR, ETR, and SVR relative to that with peg interferon alone[17]. The impact of ribavirin dose reduction on SVR rate may be minimal if patients complete treatment and maintain greater

than 60% of their planned ribavirin dose. Reduced SVR rates were observed in patients who completed treatment, but with decreased exposure to ribavirin, in clinical trials with peg interferon alfa-2a and ribavirin. The data suggest that reduced ribavirin exposure may be correlated with increased rates of relapse[21]. A RBV dose of 15 mg/kg would be ideal, although higher doses are associated with higher rates of anemia [22]. When combined with PEG-IFN α-2a, a RBV dose of 1000 mg if <75 kg or 1200 mg if ≥75 kg is recommended for HCV G1 patients while a flat dose of 800 mg RBV was initially suggested for patients with HCV G2 and 3[23].

Conclusion

Early reduction of either peg interferon or ribavirin dosing, sharply lower the virologic response.

Maintaining peg interferon dosing >90% and ribavirin >80% of the total recommended dose, is essential to achieving high SVR .

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EFFECT OF PEG INTERFERON / RIBAVIRIN DOSE REDUCTION ON THE RESPONSE OF CHRONIC HEPATITIS C IN EGYPT

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AUTOGENOUS NON-VASCULARIZED FIBULAR AND CANCELLOUS BONE GRAFT FOR TREATMENT OF NON-UNITED FEMORAL FRACTURES WITH SEGMENTAL BONE LOSS

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Abstract

Purpose: *To evaluate outcome following using of autogenous non-vascularized fibular and cancellous bone graft for non-united femoral fractures with segmental loss.*

Methods: *A total of 14 patients with non-united femoral fractures with segmental loss at Mansoura University Hospital between 2008 and 2011, using autogenous non-vascularized fibular strut, cancellous bone grafting, dynamic hip screw (DHS) or intra-medullary nail (IMN) fixation.*

Results: *The mean follow-up period was 37 months. Union was achieved in all 14 cases by an average time of 22 weeks.*

Conclusion: *Surgical use of a non-vascularized autogenous fibular strut, cancellous grafts and fixation appears a suitable treatment option for non-union with segmental bone loss of femoral fractures.*

Key words: *Fibular and cancellous bone graft, segmental loss, non-united*

Introduction

Despite recent advances and improved primary fracture treatment protocols, post-traumatic non-union of long bone remains a persistent and often difficult management problem [(1)]. Non-unions of the femur regardless of the anatomic site are most commonly due to extensive comminution and segmental bone loss [(2)]. There are numerous reported methods of

operative treatment of these non-unions for providing stability and union. Bone defects, osteopenia and poor bone quality at the non-union site after repeated surgeries and widening of the canal as well as thin cortex in the proximal and distal femoral regions render the treatment of femoral non-unions difficult [3].

Orthopedic surgeons in developed countries and some specialized centers have the option of choosing vascularized bone grafts, bone transport, allogenic bone grafts, bone substitutes and several other means to treat such conditions. However, in developing countries or some hospitals where such facilities or expertise may not be readily available, the surgeon has to depend on other techniques of treatment [4].

In this study, we reviewed the results of non-vascularized fibular strut for non-union of the femur including subtrochanteric, diaphyseal and distal femoral areas with segmental bony loss.

Patients and Methods

Between February 2008 and February 2011, 14 patients with femoral non-union were treated with autogenous non-vascularized fibular and cancellous bone grafts. There were 8 males and 6 females. The average age was 42.9 (range, 23 - 65) years. There were 4 subtrochanteric, 3 diaphyseal and 7 supracondylar femoral non-unions.

Three patients with subtrochanteric non-union have associated with loss of bone stock from failed previous operations. One of them (case 1) had failed four operations before definitive fixation and graft (Figures 1 - 7). One patient with subtrochanteric non-union had severe osteoporosis and loss of bone stock after varus osteotomy. Diaphyseal non-union in 3 patients was due to failure of previous open reduction and internal fixations.

Suprachondylar non-union in 7 patients were due to severe comminuted fractures, gun shots (case 2) (Figures 8 - 11) or implant failures. Knee motion in such non-union usually associated with stiffness and malalignment. Preoperative average range of knee

motion was 25° (range 5-90°) and the average alignment in the anatomic axis was 10° of the varus (range, 5 of the valgus to 15 of the varus).

Two dynamic condylar screws, 1 condylar plates and 1 interlocking nail were applied for the subtrochantric femoral non-unions; 2 interlocking nails and 1 locked plate were applied for the diaphyseal femoral non-unions; and 2 interlocking nails, 3 dynamic condylar screws were applied for supra-chondylar femoral non-unions (table 2). Every patient had 1 or 2 non-vascularized autogenous bone graft from same side or both sides with supplementary autogenous cancellous bone graft from the anterior iliac crest.

The patients were made sit in the bed on the first postoperative day. Passive range-of motion exercises of knee were started on the second postoperative day. Patients were mobilized with Crutches with touchdown weight bearing in the first week. After discharge from the hospital, these patients were called for follow up clinically and radiological at 2,6,12,18 and 24 weeks and then every 3 months post-operatively. During these visits clinical and radiological union was assessed. Clinical union was considered satisfactory if progressively increasing strength provided by the mineralization process made the fracture site stable and pain-free. Radiological union was considered satisfactory when plain x-rays showed bone trabeculae crossing the fracture site. Full weight bearing was allowed when radiological signs of union were present.

Results

The average duration of follow up after repair of the non-union was 18.2 (range 8 - 36) months. All non-unions had healed by the time of follow-up: 12 patients with short bone loss (average 7 Cm) had early union in an average of 6 months (range 4-10 months) and the other 2 patients with long bone loss (average 11.5 Cm) had delayed union in an average of 11.5 months (range 11-12 months). The 7 distal femoral non-unions healed within average of 8 (range 4-11) months; the range of knee motion improved from an average of 25° before surgery to an

average of 60° (range, 15-100°) after surgery and knee alignment improved from a preoperative average of 10° 0f the varus to an average of 4° of the valgus (range; 3° of the varus to 8° of the valgus). Functional outcome: 12 patients good with full weight bearing, 1 patient fair, walk with caliper, 1 patient Poor, walk with brace. The results are summarized in Table 2.

Table 1: Preoperative clinical data of the patients.

Case No	Age (yr)	Sex	Initial event	Non-union site	No. of prior surgeries	Non-union duration (mon)
1	35	F	Closed Fracture	Subtrochantic	4	36
2	40	F	Closed Fracture	Subtrochantic	2	6
3	26	M	Closed Fracture	Subtrochantic	1	8
4	57	F	Fracture from Gun shot	Subtrochantic	1	7
5	65	M	Closed Fracture	Diaphyseal	2	16
6	60	F	Closed Fracture	Diaphyseal	2	5
7	39	M	Fracture from Gun shot	Diaphyseal	1	13
8	30	M	Fracture from Gun shot	Supracondylar	3	14
9	43	F	Closed Fracture	Supracondylar	1	14
10	55	M	Closed Fracture	Supracondylar	2	11
11	56	M	Closed Fracture	Supracondylar	1	17
12	23	M	Closed Fracture	Supracondylar	2	9
13	49	F	Closed Fracture	Supracondylar	1	18
14	52	M	Closed Fracture	Supracondylar	1	10

Table 2: postoperative data of the patients.

Case No	Type of implant used	Operative condition	Pieces of fibular strut	Time of union (mon)	Duration of follow up (mon)	Outcome
1	DCS	Bone loss 10 cm	2	10	12	Good, FWB
2	CP	Bone loss 4 cm	2	8	24	Good, FWB
3	ILN	Bone loss 5 cm	1	6	36	Good, FWB
4	DCS	Bone loss 11 cm	1	12	16	Fair, walk with caliper
5	ILN	Lateral cortex Bone loss 4 cm	1	6	8	Good, FWB
6	ILN	Bone loss 5 cm	1	10	11	Good, FWB
7	LP	Bone loss 5 cm	2	8	14	Good, FWB
8	ILN	Bone loss 10 cm	2	6	14	Good, FWB
9	DCS	Lateral cortex Bone loss 6 cm	1	7	30	Good, FWB
10	ILN	Bone loss 6 cm	1	4	28	Good, FWB
11	DCS	Medial cortex Bone loss 12 cm	1	11	16	Poor, walk with brace
12	CP	Cortical defect 5 cm	1	9	18	Good, FWB
13	DCS	Cortical defect 6 cm	2	8	10	Good, FWB
14	CP	Cortical defect 4 cm	1	6	12	Good, FWB

DCS: dynamic compression screw, ILN: interlocking nail, CP: condylar plate, LP: locked plate, FWB: full weight bearing

Case 1: female patient aged 35 Y with comminuted sub-trochanteric fracture from motor car accident.

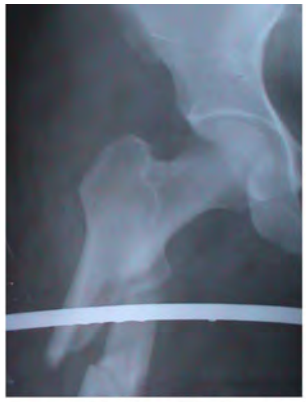


Figure (1): Primary Fracture

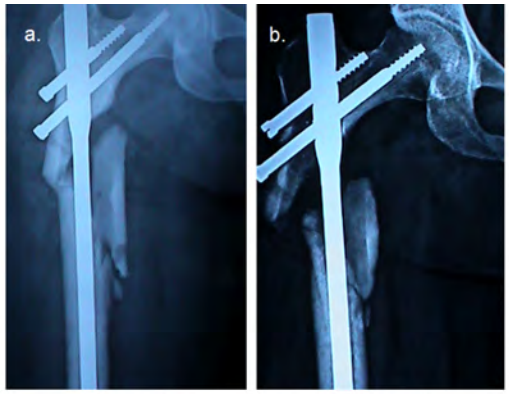


Figure (2): Fisrt operation (reconstruction nail)
a. early postop. b. 6 months postop

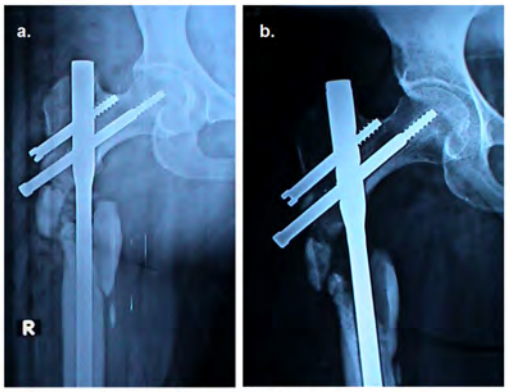


Figure (3): Second op. (grafting over the nail)
a. early postop. b. 6 months postop

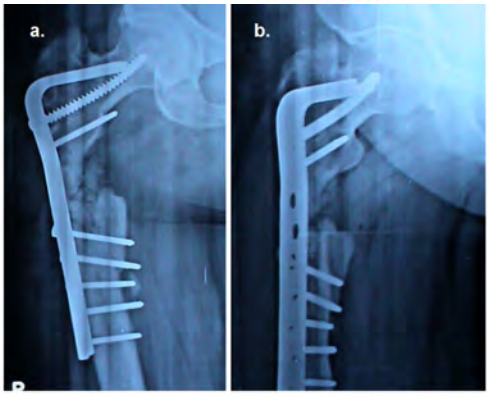


Figure (4): third op (replacement of the nail by CP & grafting)
a. early postop b. 6 months postop

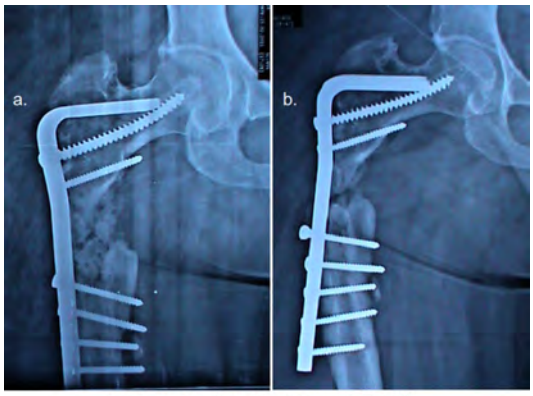
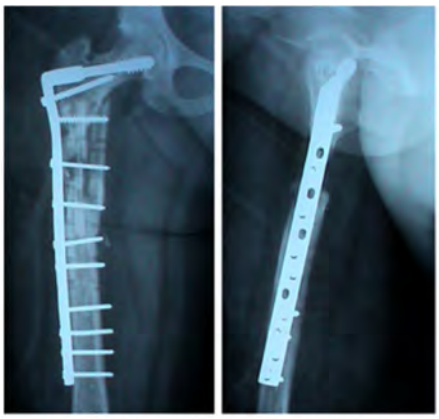


Figure (5): Fourth op (Regrafting over the CP)
a. early postop. b.6 months postop



Figure(6): Replacemen of CP with DCS & double fibular struts & cancellous bone graft (AP & Lat views)

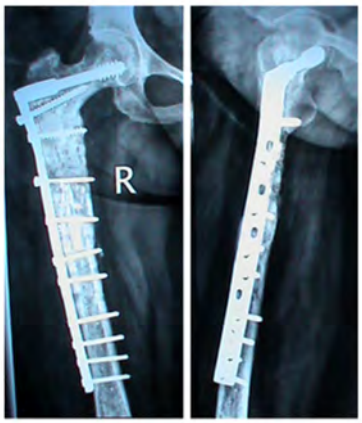
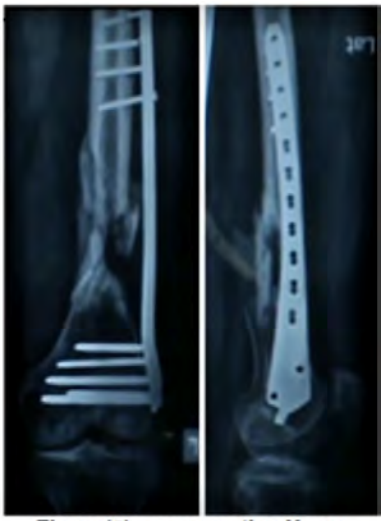


Figure (7): One year postop (AP & Lat)

Case 2: Male patient aged 30 Y with comminuted surpracndylar fracture from gun shots.



Figure(8): preoperative X-ray (non-united fracture femur)

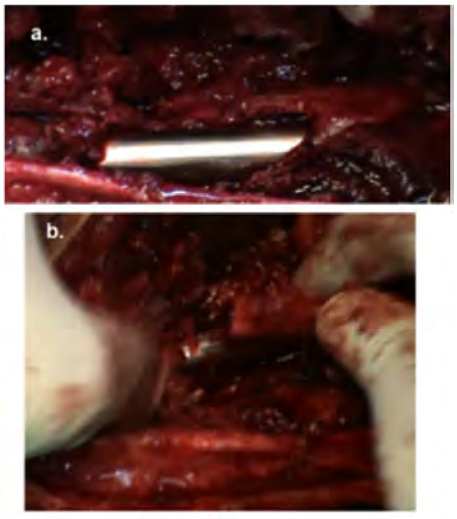


Figure (9):
a. Intraoperative view after replacement of the implant with ILN (note ILN appears through bone defect)
b. Application of the fibular strut

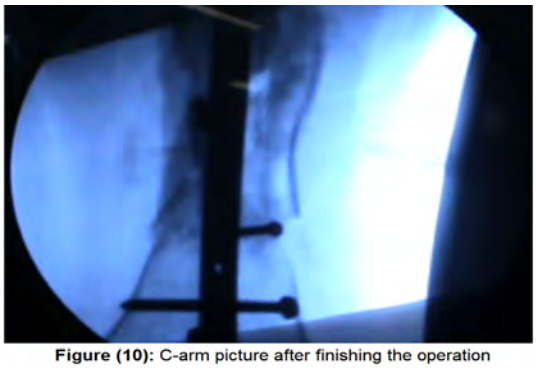
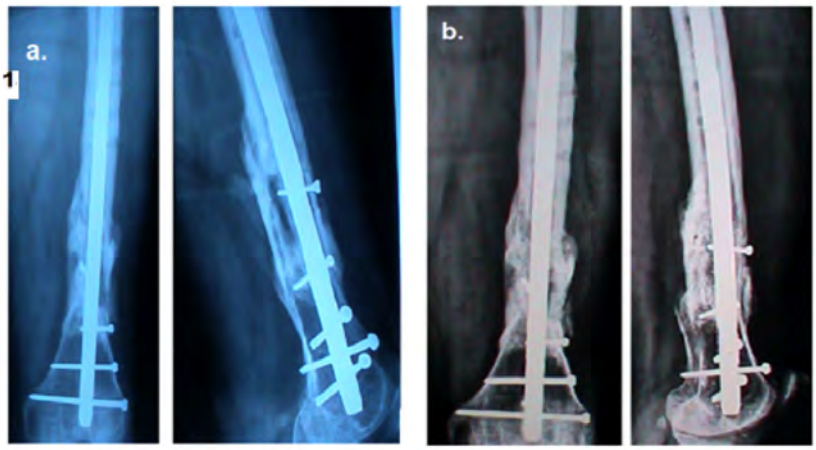


Figure (10): C-arm picture after finishing the operation



Figure(11):
a. 3 months postop **b. one year posto**

Discussion

The factors contributing to non-union are divided into three categories : deficiencies in vascularity, chondro-osseous response and stability[5]. Repeated surgeries increase the possibility of infection, devascularization of bone fragments; and loss of bone stock because of prior implant disuse osteopenia. Fragments often have poor bone quality that makes stable fixation and healing difficult. In the proximal and distal femoral regions, a widened canal, thin cor-

tex and poor bone stock also make rigid fixation difficult and when there is non-union of these femoral fractures, treatment becomes more difficult[6,7].

In 1999, Chapmann et al. reported a series of treatments using single and double plate fixation combined with autogenous bone grafting for suprachondylar non-union. Results of rigid fixation and autogenous bone grafts were successful and average time of union was 8 months, with only 2 cases with delayed union. The spirit of medial buttressing was similar to that for the 7 distal femoral non-unions in the current study [2].

Locked intra-medullary nailing remains the gold standard treatment for fractures of the femur at the isthmus [8]. In general, non-union of the femoral shaft is rare, and up to 99% of femoral shaft fractures achieve an eventual union following intra-medullary nailing [8]. However, its application is limited in subtrochantric area because of inadequate stability. Once non-union develops, the loosened implant can precipitate a condition of bone loss. A better option is the additional healing potential and mechanical support is provided by our technique. The non-vascularized autogenous fibular strut graft acts as an adjunct support to the femur weakened from osteopenia or bone defects [8].

Vascularized fibula combined with cancellous graft provides probably best means of dealing with fractures with segmental bone loss. However, in our environment the expertise and facilities required for this operation are not available or are in short supply. Autogenous cancellous graft is osteogenic and osteoinductive but not osteoconductive [8]. The main drawback of cancellous grafting is the finite number of harvest sites and volume available as well as high failure rate when used alone. Donor site morbidity is another problem. Allografts have no problem of donor site morbidity but can transmit infections and require tissue banks which are not available in our hospital and have lower graft

incorporation rates compared to autografts [9].

In this study, all non-unions were successfully healed. The complications included one wound superficial infection, two cases with delayed union, 4 cases with donor site pain and one case with stress fracture in a piece of fibular strut which is said to be common when the grafts are greater or equal to 12 cm long[10]. For these difficult femoral non-unions with bone defects, poor bone quality combine with insufficient local stability due to repeated surgeries or at the proximal and distal femoral regions in our study, the use of autogenous strut fibular grafts, autogenous cancellous grafts and internal fixation achieved osteoconduction, osteo-induction and stable reduction. Strict adherence to the principles of non-union treatment including good reduction, sufficient strut bone grafting and firm stabilization of the fragments is a good alternative for the treatment of difficult femoral nonunions [11,12].

Conclusion

This series demonstrated that the usage of a non-vascularized autogenous fibular strut graft and cancellous grafts and internal fixation appears a suitable treatment option for non-union with segmental bone loss of femoral fractures in developing countries.

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AUTOGENOUS NON-VASCULARIZED FIBULAR AND CANCELLOUS BONE GRAFT FOR TREATMENT OF NON-UNITED FEMORAL FRACTURES WITH SEGMENTAL BONE LOSS

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RESULTS OF METAL-ON-METAL BEARING SURFACES FOR ARTHROPLASTY IN POST-TRAUMATIC ARTHRITIS OF THE HIP

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Abstract

Total hip arthroplasty (THA) outcomes for posttraumatic arthritis after acetabular and proximal femoral fractures have yielded inferior results compared to primary non traumatic THA. Recently, improved results have been demonstrated using cementless acetabular rand femoral reconstruction. The use of Large Femoral Heads increased stability in addition to increased range of motion of the hip beside advantage of metal on metal bearing surfaces. 10 patients underwent THA for posttraumatic arthritis after acetabular and proximal femoral fracture. Harris Hip score increased from 38 to 84 points. 3-year survival without revision, loosening, dislocation, or infection as an end point was 90%. Survival for aseptic acetabular loosening was 90%. Despite obvious challenges, advances in fracture management and cementless fixation in THA demonstrate improved results for posttraumatic arthritis following acetabular and proximal femoral fractures.

Key words : *Cementless arthroplasty; posttraumatic arthritis; acetabular fracture; hip; total hip arthroplasty.*

Introduction

The treatment of acetabular fractures is complicated and depends on numerous factors including age, functional status, fracture pattern, degree of displacement, associated injuries, and surgical experience. Indications for operative management of acetabular fracture are rapidly expanding and evolving[1,2] anatomic reduction and internal fixation

afford the best opportunity for joint preservation and minimize the risk of posttraumatic osteoarthrosis, However, even modern fracture management using improved surgical techniques and near-anatomic reduction yields an incidence of posttraumatic arthrosis approaching 20% to 30%. The severity of the initial injury, missed or untreated osteochondral defects, femoral head avascular necrosis, poor surgical technique, and subsequent surgical complications all contribute to this [1].

This is prospective study for evaluation of the clinical and radiological results of metal on metal THR in patients with posttraumatic arthritis. The study was designed to primary assess functional outcome, early complications and not the longevity of the reconstruction. This study was carried on in Arthroplasty unit Mansoura University Hospital, Egypt in period from November 2007 till January 2010. The patients were identified from arthroplasty clinic, Mansoura university hospital according to inclusion criteria which include any patients with post-traumatic hip disease other than pyogenic infection. The follow up of the cases ranged from 33 months to 60 months.

This study included 9 patients (10 hips), Age ranged from 18 to 54 years with a mean age of 36 years; they were 6 males and 3 females; one cases was bilateral affection, 5 cases with right hip affection and 3 cases with left hip affection out of these cases 6 hips were acetabular side affection 4 hips with femoral side affection (2 hips with non-united femoral neck fracture and bilateral subtrochanteric fracture). Preoperative radiological evaluation included plain AP., lat. and Judet oblique views, and C. T. scan. Patients were followed up both clinically and radiologically by plain X-rays. Operative data, early and late postoperative complications were recorded.

Methods:

The procedure was done through the posterolateral approach of the hip. Implants used: XL-head cementless metal on metal (MOM) and Standard hydroxyapatite coated cementless stem.

Postoperative all patients received intravenous third generation cephalosporin for 7 days postoperatively and Fractionated heparin for 2 weeks and 5 weeks for high risk patients. Patient controlled analgesia was continued for the first 36-48 hours. Immediate hip and knee flexion, rapid foot pumps, and deep breathing exercises are emphasized to minimize thromboembolic and pulmonary complications. Walking is started on the first postoperative day. Weight-bearing as tolerated is allowed, and all primary cases were instructed to be full weight bearing immediately after the operation, and the supporting method was used for balance only.

Postoperative Radiological Evaluation :

To assess the biomechanical reconstruction of the hip, the following measurements were made on the AP radiograph of the pelvis as described originally by Johnston and Larson[4] and applied to THR by Russotti and Harris.[5]

- The femoral offset
- The hip lever arm ratio (abductor moment arm divided by body moment arm) for both hips.
- Acetabular inclination was determined in relation to the inter-teardrop line (ideally 45°).
- The hip axis length was measured preoperatively and postoperatively.[6]

After discharge :

All patients had clinical (Harris hip score was used for clinical evaluation of patients at 6 weeks, 6 months, and last follow up.) and radiological evaluation at regular periods during their follow up for Component position or migration, Presence of heterotopic ossification, Osteolysis and loosening. Evidence of radiolucent lines of greater than 1 mm was recorded for the acetabular socket using the zone of DeLee and Charnley adding a fourth zone for the ischium.[7]

Results

The result was assessed clinically using Harris hip score (Table 1) and found that 90% had excellent to good score with 10% poor score with no statistical difference between male and female

or unilateral and bilateral cases or preoperative indication , Preoperative limb length discrepancy (LLD) ranged from 0 to 4 cm and Postoperative limb length discrepancy ranged from 0 to 1 cm, there were three cases with intraoperative cracking and fissuring of the acetabulum and calcar which were treated conservatively by delayed weight bearing, there was one case with posterior dislocation due to abnormal positioning which was treated by closed reduction and traction, and one case with aseptic loosening 2 years postoperative and revised with single stage revision of acetabular cup, case with common peroneal nerve injury which recovered 8 weeks postoperative, there were no thromboembolic complications nor postoperative periprothetic fractures.

Table (1): Overall results according Harris hip score .

	Frequency	Percent
poor	1	10.0
good	3	30.0
excellent	6	60.0
Total	10	100.0

Table (2) : Values of preoperative and postoperative HHS.

.	N	Minimum	Maximum	Mean	Std. Deviation
pre_HHS	10	32	43	38.40	3.806
Final_HHS	10	30.00	94.00	84.2000	19.45251
Valid N (listwise)	10				

Case No.1

1. 32 years old male
2. Fracture femoral neck treated by multiple cancellous screws since 5years
3. Limited abduction
4. 6 cm LLD
5. MOMTHR on 3-2008
6. Intra-operative:

a. Deficient post &sup aspect of acetabulum ttt by posterosuperior autograft from excised head and fixation with screws and washers

b. Fissuring of calcar ttt by screw

c. Large cup (60mm)

7. HHS
   - Preoperative: 35
   - Postoperative: 94

Preoperative AP pelvis & Lat RT hip &clinical Views

inal follow up AP pelvis & Lat RT hip &clinical Views

Case No.2

- 33 years old male
- Bilat. subtrochanteric fracture femur treated by bilat. condylar plate since 4 years
- Bilateral Anavcular necrosis
- Removal of plate since 1.5 year
- Staged MOMTHR
  - Rt in 5-2007
    - Preoperative: 35
    - Postoperative: 97
  - Lt in 5-2008
    - Preoperat

Preoperative AP& froglat. Views of pelvis

Final follow up AP& froglat. Views of pelvis

Final follow functional outcome

Discussion

Total hip arthroplasty in the setting of an acetabular and femoral fractures remains a difficult reconstructive dilemma. Historically, this population has inferior outcomes when compared with patients with primary osteoarthritis. We hypothesized that preoperative factors including infection and nonanatomic restoration of hip center would predict a poor outcome of THA after acetabular fracture. In addition, optimizing the anatomy of the reconstructed hip center at reconstruction is paramount. We identify the true acetabulum using our preoperative template and standard intraoperative revision landmarks, which include the location of the pubis, teardrop, ilium, and ischium after extensive releases and removing all scar debris and hardware that will be in directly affect with acetabular placement.

Using large femoral heads is supposed to increase these functional abilities, besides it provides better stability and decreases dislocation rate. Large femoral head which couldn't be used with metal on polyethylene bearings due to higher wear rates became available with MOM bearings.

There was only one case of aseptic loosening resulting in survival rate of 90%. The low rate of aseptic acetabular loosening in this series corroborates findings in other recent series where cementless acetabular fixation was also used. Weber et al cited 87% survival with aseptic acetabular loosening at 10 years in 1998[1]. Bellabarba et al described 97% survival at 10 years in 2001[1], and Berry et al described 93% survival at 10 years in 2002 [2].

Component loosening is also related to activity level and age, both problematic factors in posttraumatic patients. The average age of patients in this study was 52 years, which is younger than those who customarily undergo THA for nontraumatic arthritis. Monoarticular disease and young age of patient permit a high activity level that may eventually manifest as osteolysis or loosening.[9] Still there is no long term follow up studies about stemmed XL-head metal-on-metal hip arthroplasty, however, early result are

promising. McMinn et al[10] reported excellent early results of XL-head MOM THA with no early failure.

Conclusion

The large - head metal on metal total hip replacement has excellent medium term results and Large head diameter offers excellent range of motion and decreases dislocation rate. Aseptic loosening was one of the leading causes of failure in metal on metal bearing surface in total hip replacement. Longer term follow up is required to asses wear and survivorship of metal on metal implants.

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RESULTS OF METAL-ON-METAL BEARING SURFACES FOR ARTHROPLASTY IN POST-TRAUMATIC ARTHRITIS OF THE HIP

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GENETIC DISSIMILAR LYMPH NODE METASTATIC COLORECTAL CARCINOMA : AN IMMUNOHISTOCHEMICAL STUDY

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Abstract

Background/Aim: The current concept of metastasis proposes that rare cells within primary tumors acquire metastatic capability via sequential mutations, suggesting that metastases are genetically dissimilar from their primary tumors. This study investigated the changes, if any, in the level of expression of a well defined panel of apoptosis markers between the primary colorectal cancer and the corresponding synchronous lymph node metastasis from the same patient. Materials and methods: pathological morphology and immunostaining of p53 and Bcl-2 analyses were carried out to meet that goal. Lymph nodes from 36 cases of colorectal cancer radical resection specimens were studied. Results: Metastatic tumors were significantly more diffusely positive for p53 than the primary tumors (p=0.0001). No significant difference was found between the lymph nodes and the primary tumors in Bcl-2 positivity (p=0.538). Conclusions: Lymph node metastatic colorectal carcinoma have a tendency of being less differentiated, a higher incidence of diffuse p53 positive staining, and near-similar incidence of bcl-2 positivity in comparison to their primary tumors suggesting a more aggressive biological behavior in comparison to their primary ones.

Introduction

The colorectal cancer incidence is 9.4% of all cancer incidence in males and 10.1% in females. It kills approximately 500,000 people world wide each year[1&2]. The search for novel molecular prognostic markers could be used to identify groups of patients with differing relative risks of

recurrence and improve patient stratification for adjuvant treatment. P53 and Bcl-2 (both regulators of cell apoptosis) have been considered to be involved in the initiation and progression of the colorectal tumorigenic process, respectively[3]. Although p53 expression is abnormal in more than 50% of colorectal carcinomas, data on the prognostic role of the intensively studied p53 tumor suppressor gene are contradictory[4]. Bcl2 oncoprotien expression in colorectal carcinoma has been demonstrated as abeing a favorable prognostic factor and associated with less aggressive tumor behavior and or may reflect different stages of tumor progression[5].[8,9&10] reported that P53 overexpression was more frequently seen in adenocarcinoma than in mucus secreting cells. No mucinous structures was encountered with bcl-2 positivity[11,12&10].

The dissemination of cells from a primary tumor, resulting in the progressive growth of metastatic carcinoma in distant sites, including bone, lungs, liver and brain, is the most common cause of death of colorectal cancer patients. Studies on animal and human cancer cell lines, combined with clinical observations, suggest that metastasis is not a random event.

Metastasis to regional lymph nodes is an important prognostic factor and is used for the staging of colorectal cancer. Five-year survival rates drop from 80 percent in patients with tumor-node-metastasis (TNM) stage II disease (who have no lymph-node metastases) to 45 to 50 percent in those with TNM stage III disease (in which lymph-node metastases are present) [6].

Many studies have discussed the genetic alterations in colorectal carcinoma (CRC) in relation to vascular and lymphatic invasion, lymph node involvement and metastatic potential.[7] They found a reciprocal relationship between bcl-2 reactivity and P53 over expression, suggesting a strong correlation between the bcl-2-positive / p53-negative subgroup with negative lymph node status, implying a less aggressive pathway.

A greater understanding of the metastatic phenotype from cellular and molecular analyses will provide a rationale approach for controlling this cancer. To date, studies of p53 and Bcl-2 expression in CRC have focused on their expression in primary tumors.

Currently, there are no data regarding the expression of these markers in the corresponding metastatic lymph nodes. Therefore, our goals were to compare the expression of p53 and Bcl-2 in primary CRC and their corresponding lymph node metastases and to evaluate the relation between these markers in primary tumors and lymph node metastases.

Materials and Methods

The draining lymph nodes were examined for tumor metastasis comparing the histological type and the degree of differentiation with the primary tumor, 36 cases were positive for tumor infiltration.

From each tumor block and positive draining lymph nodes 6 coated slides 4 ? thick sections. Monoclonal antibodies for p53, and Bcl-2 (Dako, Glostrup, Demark) were applied using the avidin-Biotin complex (ABC) Santa Barbara, CA). Slides were deparaffinized and blocked for endogenous peroxidase for 20 minutes. Antigen retrieval was performed using Biogenex Antigen Retrieval Citra solution in a 90 O C 30 minute with blocking by normal horse serum for five minutes at 37 O C. The monoclonal antibody was applied over night in humid medium at room temperature, followed by a biotinylated secondary antibody for 15 minutes at 37 o C then the ABC complex for 15 minutes at 37 o C (Vectastain Elite ABC Kit from Vector Laboratories, Burlingame, CA). 3,3 Diaminobenzidine (DAB) for 20 minutes at room temperature was used as chromogen with Meyer's hematoxylin as counterstain. In negative control slides the monoclonal antibody was replaced by diluted normal bovine serum.

Interpretation of immunohistochemical staining:

Staining results were interpreted according to the percent of stained tumor cells (<25%,

25-50%, 50-75% and >75%) and the specific localization of the stain within the cell (Nuclear "N" or Cytoplasmic "C").

Statistical Analysis :

Data were analyzed for significance by Chi-square analysis of 2 x 2 contingency tables by the Kwikstat computer program (Texasoft software)[13]. P-value is considered significant when $p \leq 0.05$. When any of the data is lower than 5, the (p value) is expressed as Yates Chi Square.

Results

Thirty six cases with metastatic colorectal carcinoma were included in the present study, 19 females and 17 males with mean age of 44 (±14.4) years. Sixteen cases had tumor in the rectum, 10 cases in the left side and 10 cases in the right side of the colon.

The pathological examination of the primary tumors revealed 26 (72.2%) cases had adenocarcinoma, 6 (16.6%) had MUC and 4 (11.1%) had SIG. Lymph node metastatic tumors exhibited similar histological pattern to their primary ones except in few cases where 2 primary ADC gave rise to two MUC, 2 primary MUC gave rise to one ADC and one to SIG and 2 primary SIG gave rise to one ADC and another one to MUC (Table 1). Pathological types were compared between primary and metastatic tumors by chi-square test for independence, it was found that there is very low probability that the two types of tumor types are independent (chi-square=25.0, p = 0.0001).

From the 36 stained lymph nodes, 18 (50%) were diffusely p53 positive (Fig. 2), 4 focally stained and 14 were negative. Metastatic tumors were significantly more diffusely positive for p53 than the primary tumors (Fig.1) (chi-square = 29.2, p = 0.0001) (table 2).

Only 35 out of 36 Lymph node samples were analysed for the expression of Bcl-2. From the 35 stained lymph nodes 11 (31.4%) were positive (Fig.4) in comparison to 16 positive primary tumours (Fig.3), however, the difference did not reach statistical significance (chi-square = 7.96, p=0.538).

Table 1 : Pathological findings of CRC of primary tumour and lymph node metastasis.

		LYMPH NODES		
		Adenocarcinoma No.=26	Mucinous No.=7	SIG No.=3
PRIMARY TUMOR	**Adenocarcinoma No.=26**	**24**	**2**	**0**
	Mucinous No.= 6	**1**	**4**	**1**
	SIG No.=4	**1**	**1**	**2**

Table 2: p53 staining pattern in 36 lymph node metastatic tumors in Comparison to their primary ones.

		LYMPH NODES		
		Diffuse No.= 18	**Focal No.= 4**	**Negative No.= 14**
PRIMARY TUMOR	**Diffuse No.= 15**	**15**	**0**	**0**
	Focal No.= 7	**3**	**1**	**3**
	Negative No.= 14	**0**	**3**	**11**

(chi-square=25.0, p = 0.0001).

Table 3 : Bcl-2 staining pattern in 35 lymph node metastatic tumors in Comparison to their primary ones.

		LYMPH NODES			
		Negative No.= 24	<25 No.= 2	>25 No.= 5	>75 No.= 4
PRIMARY TUMOR	**Negative No.= 19**	**15**	**1**	**2**	**1**
	<25 No.= 9	**6**	**0**	**2**	**1**
	>25-<75 No.= 5	**2**	**1**	**1**	**1**
	>75 No.= 2	**1**	**0**	**0**	**1**

(chi-square= 7.96, p=0.538).

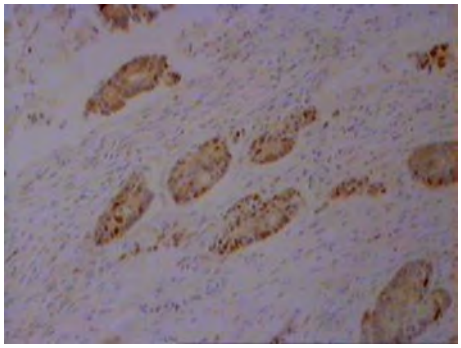

Fig. 1 : Focal positive nuclear reaction for P53 in primary CRC (immunohistochemical staining. original magnification x 100).

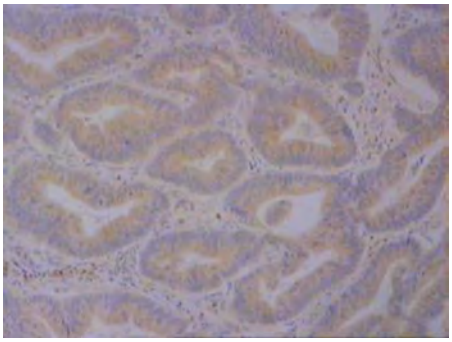

Fig. 3 : Positive cytoplasmic bcl-2 immunostaining (>75%) of colorectal adenocarcinoma control (immunohistochemical staining.original magnigication x 200).

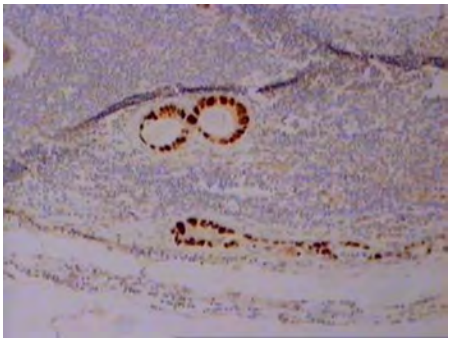

Fig. 2 : Diffuse positive nuclear reaction for P53 in lymph node metastatic CRC (immunohistochemical staining. original magnification x 100).

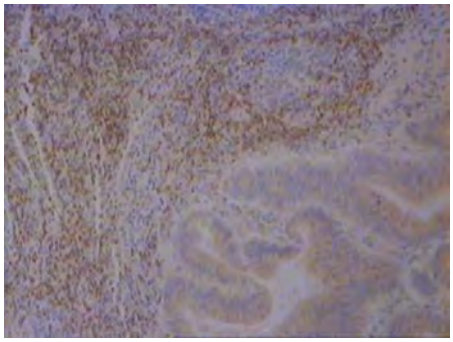

Fig. 4 : Positive cytoplasmic bcl-2 imunostaining (>75%) of metastatic colorectal adenocarcinoma in lymph node. (Immunohistochemical staining. original magnification x 200).

Discussion

Studies comparing genetic changes in metastases with those found in the corresponding primary tumors in the same patient would be informative in revealing differences between primary and metastatic lesions and thereby pinpointing genetic events that could have predisposed to metastatic spreading.

In colorectal tumorigenesis, mutation often occurs in p53 and Bcl-2 genes. p53 expression has been found to be a less favorable marker and Bcl-2 expression a more favorable marker of behavior[14,15,16&17], To date, studies of Bcl-2 and p53 expression in CRC have focused on their expression in primary tumors. Currently, there are no data regarding the expression of these markers in the corresponding metastatic lymph nodes.

The p53 tumor suppressor gene product is a participant in the cellular response to DNA damage, resulting in either G1 cell cycle arrest or cell death by apoptosis[18]. The p53 tumor suppressor gene has been studied extensively in CRC cell lines and clinical tumors[19,20]. Mutations of p53 are present in up to 50% of invasive CRC[4]. and loss of its function is associated with a high proliferation index and poor clinical outcome,[21,16&4].

In the present study, lymph node metastatic tumor showed a significantly higher percent of p53 diffuse positive staining pattern in comparison to primary ones ($p<0.0001$). These finding may indicate a poorer prognosis and that tumors with p53 over expression tend to have a higher tendency for metastasis. While[4] reported that overall survival was significantly better in patients with p53-positive carcinomas with better recurrence - free survival than in those without p53 expression,some studies found p53 positivity to be associated with a higher risk of tumor relapse. While others reported no relation to tumor recurrence[22]. These discrepancies between patient outcomes may be due to differences in the tumor location, p53 mutation site, tumor type, and response to adjuvant therapy.

bcl-2, protects cells from apoptosis, thus potentially allowing malignant cells to proliferate [23]. A number of investigators have shown that 32-85.9% of patients with invasive CRC express Bcl-2. Most of these studies concluded that Bcl-2 expression is associated with favorable clinical outcome in patients with CRC[24,25]. In the present study, Lower incidence of bcl-2 positivity (31.4%) in metastatic lymph node tumors may suggest more aggressive behavior that is in agreement with Giannoulis et al., who found that Bcl-2 levels significantly higher in patients with non-metastatic disease CRC (61%), compared to patients with metastatic disease[26]. Moreover, other studies carried out on CRC and other tumors such as breast cancer and oesophageal cancer have shown that Bcl-2 overexpression is negatively linked with lymph node metastasis and is associated with favorable outcome[24,27&28], therefore, it can be speculated that the lower expression of Bcl-2 in the metastatic lymph node specimens associated with increased risk of distant metastatic potential in colorectal carcinoma, which could indicate the necessity for more aggressive adjuvant therapy.

In summary, our results showed that metastatic colorectal carcinoma have a higher incidence of diffuse p53 positive staining, and lower incidence of bcl-2 positivity in comparison to their primary tumors; suggesting a more aggressive biological behavior and poorer prognosis in comparison to their primary ones which could indicate the necessity for more aggressive adjuvant therapy.

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IS CRYPTOSPORIDIOSIS IN HEPATIC PATIENTS A SIGNIFICANT HEALTH PROBLEM?

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Abstract

Cryptosporidium species are enteric pathogens that have emerged as significant causes of diarrhea in immunologically compromised individuals. Patients with chronic liver diseases are at risk of developing serious infections due to altered immune defenses. The aim of this study was to evaluate the incidence and clinical significance of Cryptosporidium in patients with chronic liver diseases. The study included 150 patients with chronic liver diseases and diarrhea versus fifty healthy subjects with diarrhea as control group. Stool samples were screened for the presence of Cryptosporidium by microscopic examination following the modified Ziehl-Neelsen staining method and detection of Cryptosporidium copro-antigen by ELISA. The incidence of Cryptosporidium infection in chronic liver diseases patients was 30% (45/150) versus 14% (7/50) in healthy controls. Cryptosporidium was demonstrated in the stools of eleven patients with liver cirrhosis without ascites (22%), eighteen cirrhotic patients with ascites (36%) and sixteen patients with hepatocellular carcinoma (32%). Nine patients developed hepatic encephalopathy, four cirrhotic patients with ascites and five patients with hepatocellular carcinoma. Only diarrhea was identified as precipitating factor for development of hepatic encephalopathy. Cryptosporidium was detected in 52 samples (26%) of the 200 subjects included in this study using the Cryptosporidium copro-antigen detection. Using acid-fast stain Cryptosporidium was detected in 45 samples (22.5%).

In conclusions, Cryptosporidium infection is a common cause of diarrhea in patients with chronic liver diseases and the clinical significance of this parasitic infection in chronic liver diseases needs further studies. For diagnosis of this infection, antigen detection method is more sensitive than acid fast staining method.

Key words. *Cryptosporidium, Liver diseases and diarrhea.*

Introduction

Cryptosporidium species are protozoan parasites that cause infection and diarrheal illness in a wide range of mammalian species[1]. The infection may causes a fatal illness mainly accompanied by severe diarrhea and dehydration in immunocompromised individuals, e.g. acquired immune deficiency syndrome (AIDS) or leukemia patients, malnourished children and elderly individuals. In otherwise healthy persons the infection usually results in self-limited diarrhea [2,3]. Transmission of this parasite is mainly through fecal-oral route and results from the ingestion of *Cryptosporidium* oocysts through the consumption of fecally contaminated food, water, through person-to-person spread and contact with infected animals[4]. *Cryptosporidium hominis* and *C. parvum* are the most frequently detected. *C. hominis* infections are more common in developing countries[5]. Evidence has indicated that the immune status is impaired in patients with chronic liver failure, precipitating high susceptibility to infection [6]. Chronic cryptosporidiosis can cause liver cirrhosis, and, even worse, a fatal disseminated disease during therapeutical transplantation in children with X-linked hyper-IgM syndrome [7]. Little was known about the *Cryptosporidium* infection in chronic liver diseases and hepatocellular carcinoma, so that this study aimed to elucidate the incidence and clinical significance of *Cryptosporidium* infection in patients with chronic liver diseases.

Materials and Methods

This case control study was carried out from September 2011 to October 2012. One hundred and fifty chronic liver disease cases with diarrhea (82 males and 68 females) and 50 control subjects

with diarrhea (matched, sex, and age) were recruited. Participants were recruited from 3 outpatient clinics and 2 centers in Mansoura University Hospital. The study was approved by the medical Ethics of the 1975 Declaration of Helsinki, and all participants provided written informed consent.

Diarrhea was defined as three or more unformed stools / 24 hours period. Nitazoxanide is the only broad-spectrum antiparasitic drug that has been approved in the United States for treatment of cryptosporidiosis for adults (8). In our study in addition to fluid and electrolyte replacement we used Nitazoxanide 500-mg twice daily for treatment of cryptosporidiosis cases for 3 days. We excluded all hepatic patients using Lactulose to avoid its laxative effect.

The study population was classified into 4 groups:

Group A: 50 Patients with liver cirrhosis without ascites (35 male and 15 female). Liver cirrhosis was diagnosed by, history, clinical examination, abnormal liver function tests, abdominal ultrasound and liver biopsy when present.

Group B: 50 Patients with liver cirrhosis plus ascites (32 male and 18 female). Ascites was diagnosed by clinical examination, abdominal ultrasound and abdominal CT.

Group C: 50 Patients with hepatocellular carcinoma (42 male and 8 female). The HCC was diagnosed by abdominal ultrasound, triphasic CT and elevated serum level of alpha fetoprotein.

Group D: 50 individuals with no liver diseases and complaining of diarrhea served as control group (30 males and 20 females).

All patients and controls included in the study were subjected to thorough clinical history taking stressing on the following points; age and sex, loss of weight, jaundice, vomiting, diarrhea and its characters, abdominal pain, right hypochondrial pain, dyspnea, cough and expectoration, discoloration of urine, history of drug intake as corticosteroids and cytotoxic drugs and symptoms related to other body systems affection. General and ab-

dominal examination included imaging using ultrasound scan and triphasic CT were done.

Methods.

Laboratory investigations and serological tests

HBsAg and anti-HCV antibodies were detected by enzyme - linked immunosorbent assay (ELISA). Laboratory investigations to exclude other diseases as diabetes mellitus , HIV, other parasitic diseases, bacterial causes of acute or chronic diarrhea and other malignant diseases such as leukemia, were done by using standard clinical chemistry analyzers.

Collection of fecal samples

Microscopical examination was performed on 3 stool samples from each subject to increase its sensitivity. Stool samples collected from each individual was preserved in 2 containers, one for direct examination by concentration method[9] and for staining with Modified Ziehl Neelsen stain[10]. The second container was preserved for detection of *Cryptosporidium* copro-antigen by ELISA. Fecal samples were collected in dry, clean, labeled plastic containers with tight fitting covers containing no preservatives, stool contamination was avoided. Macroscopic examination was conducted at first regarding several aspects: consistency, presence of blood and mucus and macroscopic parasitic elements. Microscopic examination was done using direct wet smear technique for the presence of cysts of parasitic protozoa [11].

Detection of *Cryptosporidium* antigen in stool by ELISA. The method was done according to the instructions supplied with the commercial ELISA kit (Ridascreen *Cryptosporidium*, C1201), R-Biopharm AG, Germany.

Statistical analysis

A computer program, SPSS for Windows (Release 11.5.0), was used for data analysis. The descriptive data was given in mean (SD). The chi-square test, Student's t-test, and Fischer's bicaudal exact test were used for the analytical assessment. The differences were considered to be statistically significant when the P-value obtained was less than 0.05.

Results

The 3 groups of patients and controls were well matched for age and sex. Their ages ranged from 40-65 years (mean 53.64 ± 7.72).

The incidence of *Cryptosporidium* infection in patients with liver cirrhosis without ascites was 22% (11/50), in patients with liver cirrhosis with ascites 36% (18/50), in patients with HCC 32% (16/50) and in the control group with diarrhea 14% (7/50). The incidence of *Cryptosporidium* infection was significantly higher in liver cirrhosis with ascites than in control group (p=0.02) (table1).

The results showed that the incidence of positive *Cryptosporidium* infection in patients with chronic liver diseases was significantly higher than in the control 30% versus 14% respectively (p=0.04).

Using acid-fast stain *Cryptosporidium* was detected in 45 samples (22.5%) out of the 200 subjects included in this study. *Cryptosporidium* infection was diagnosed in 52 samples (26%) using *Cryptosporidium* copro-antigen detection method. Comparison between results of antigen detection method and acid fast stained smears for diagnosis of *Cryptosporidium* infection revealed that additional seven positive samples were diagnosed by antigen detection method.

Clinical characteristics of *Cryptosporidium* infection in patients with chronic liver diseases showed that, the number of motion per day varied from 3 to 10 times per day. The duration of diarrhea ranged from 3 to 6 days and all patients with *Cryptosporidium* infection showed no intractable diarrhea. Diarrhea was generally associated with other signs suggesting enteric involvements, including vomiting (31.3%), abdominal pain (21.2%), flatulence (56.86%), anorexia (63.89%), tenesmus (27.3%) and lastly fever (41.47%). 4 patients with Liver cirrhosis plus ascites developed hepatic encephalopathy (grade 1-2), 5 patients with HCC developed hepatic encephalopathy (grade 1-2), while no

patients with Liver cirrhosis without ascites developed hepatic encephalopathy. After exclusion of other precipitating causes of hepatic encephalopathy, only diarrhea was identified as a risk factor for development of hepatic encephalopathy in the studied patients. The risk factors for *Cryptosporidium* infection were advanced liver diseases (34% versus 22% in patients with cirrhosis plus ascites versus cirrhosis without ascites and 32% versus 22% in patients with HCC versus cirrhosis without ascites), living in rural areas, education level, contact with farm animals and / or pet animals. However, most of these risk factors although showed high percent, yet they occurred in patients with other causes of diarrhea.

Table 1: *Cryptosporidium* infection in patients and controls.

Group	Positive cases No.	%	P value
(A)Liver cirrhosis without ascites (n =50)	11	22	A vs B =0.19
(B)Liver cirrhosis with ascites (n=50)	18	36	A vs C =0.36 A vs D =0.43
(C)Hepatocellular carcinoma (n=50)	16	32	B vs C=0.8 B vs D=0.02
(D)Controls (n=50)	7	14	C vs D=0.06

Discussion

Cryptosporidiosis is a gastrointestinal illness caused by protozoa of the genus *Cryptosporidium*[12]. To the best of our knowledge little literatures evaluated the *Cryptosporidium* infection in chronic liver diseases. This study aimed to determine the incidence of *Cryptosporidium* infection in chronic liver diseases and hepatocellular carcinoma.

Chronic diarrhea is common in patients with liver disease. Previous study reported that *Cryptosporidium* oocysts were demonstrated in the stools of 20% of patients with liver disease[13]. In Egypt Youssef et al.[14] reported that the incidence of *Cryptosporidium parvum* ranged from 0%-47%. The risk factors for this protozoa included population, ecology, and environmental findings, the authors suggested that zoonotic transmission and water transmission modalities are the principal route of infection[14]. Recently Badawy et al reported 31.1% incidence of *Cryptosporidium* parvum among Egyptian Military recruits [15].

Previous studies demonstrated that, severe liver injury and liver failure are closely associated with reduced cellular immunity[16,6]. This reduced cellular immune function could contribute to an increased susceptibility to infection in subjects with chronic liver diseases including *Cryptosporidium* infection. In this study we found that, 32% of patients with hepatocellular carcinoma and diarrhea were harboring *Cryptosporidium* in comparison to 14% in patients with liver cirrhosis without ascites and 22.2% in Liver cirrhosis plus ascites. In AIDS patients *Cryptosporidium* infection can promote disease progression and worsen the prognosis of the disease[17]. Unlike *Cryptosporidium* infection in AIDS patients, our results demonstrated that *Cryptosporidium* infection does not appear to be problematic in patients with chronic liver diseases as five patients (10%) with HCC and four patients (8%) with liver cirrhosis plus ascites developed hepatic encephalopathy (grade 1-2) and all patients were recovered from hepatic encephalopathy. Our results are in agreement with that of Hunter and Nichols, who found

that in cancer patients (other than those with haematological malignancies) cryptosporidiosis does not appear to be as problematic as it is in haematological malignancies[18].

In this study, although statistically non significant, the incidence of *Cryptosporidium* infection increased with the progression or severity of chronic liver diseases. High difference was observed between patients with liver cirrhosis without ascites (22%) versus 36% and 32% in liver cirrhosis plus ascites and hepatocellular carcinoma respectively. This may be explained by impaired cellular immune response in patients with chronic liver disease [16].

Several studies investigated the epidemiology and prognosis of *Cryptosporidium* infection in patients with malignant disease. Tanyuksel et al. reported that, *Cryptosporidium* oocysts were detected in 18 (17.0%), of 106 patients with diarrhea and various cancers[19]. On the other hand, low incidence of *Cryptosporidium* was found in an Indian study of 560 patients with cancer and diarrhea, oocysts were found in only 7 patients (1.3%). Of these seven patients, five had hematological cancers[20]. In our study a high incidence of *Cryptosporidium* oocysts was found in patients with HCC (32%). This high incidence could be explained by differences in subject selection as we selected patients with HCC only rather than hematological malignancy. Also, the high incidence of *Cryptosporidium* infections in the present study suggests that this protozoon is widespread in our environment. In addition to the opportunistic nature of this parasite that promotes its incidence among cancer patients with reduced immunity [21].

Regarding the method of detection of *Cryptosporidium* infection, in the present work, both acid-fast staining method and coproantigen detection (Ridascreen Cryptosporidium, C1201) have been used. The number of positive cases was 45 by the acid-fast method and 52 by the antigen detection test, with additional seven positive samples diagnosed by the antigen detection method. El Shazly et al found that the ZN stain had

lower sensitivity than ELISA coproantigen[22]. Al Hindi et al.[23], on using ZN stain and ELISA reported positivity of 14.9% and 16.3% respectively. On the other hand, Bialek et al found sensitivity of antigen EIA and DFA were similar (94% and 95%) respectively[24].

In conclusion: Cryptosporidium infection is a common cause of diarrhea in patients with chronic liver diseases and the clinical significance of this parasitic infection in chronic liver diseases needs further studies. For diagnosis of this infection, antigen detection method is more sensitive than acid fast staining method.

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IS CRYPTOSPORIDIOSIS IN HEPATIC PATIENTS A SIGNIFICANT HEALTH PROBLEM?

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TUBULARIZED INCISED PLATE URETHROPLASTY FOR HYPOSPADIAS REOPERATIONS : FACTORS PREDICTING THE OUTCOME

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Abstract

Objectives: *To determine the key points for a successful redo hypospadias procedure using tubularized incised plate uretheroplasty operation.*

Methods: *A retrospective review of 24 patients (Mean age: 3.5 years, range: 2-9 years) who had undergone a redo tubularized incised plate uretheroplasty operation.*

Results; *The overall complication rate was 29% (7 cases). Three patients (12.5%) had uretheral fistula, four had meatal stenosis (16.6%), and no cases of wound dehiscence or stenosis of the neourethera.The incidence of complications of tubularized incised plate uretheroplasty reoperation after failed repairs of meatal advancement and glanuloplasty procedure (4 cases), meatal based flap (14 cases), and tubularized incised plate uretheroplasty (6 cases) were 25%, 28% and, 33.3% respectively. The ultimate success rate of tubularized incised plate uretheroplasty reoperation was 100% after repair of fistula and meatal stenosis by simple fistula closure and meatoplasty procedure. Near about 90% of the patients had an excellent cosmetic outcome with a vertically oriented slit like meatus and conical glanular configuration.*

Conclusion: *Tubularized incised plate uretheroplasty is a good and safe alternative procedure for hypospadius reoperations. Many factors including type of initial repair, the integrity of the urethral plate, technical aspects of Snodgrass performance and duration of stenting have a significant effect on the outcome.*

Keywords: *Tubularized incised plate uretheroplasty. Reoperations . Hypospadias*

Introduction

Since its first description in 1994, the Snodgrass modification of the Thiersch-Duplay technique has proved to be an extremely useful and reliable method of hypospadius repair[1]. Because of the excellent results obtained with Snodgrss technique its use is extended to all types of hypospadius from penoscrotal to glanular types[2]. Failure rates in primary hypospadius repairs vary from 5%-20% depending on site of hypospadius, type of procedure and quality of available tissues for repair[3].

The tubularized incised plate uretheroplasty has gained widespread acceptance for primary and secondary hypospadias repairs. The key point in deciding its use as a secondary hypospadias operation is the quality and suitability of the urethral plate. Another challenge is lack of healthy or adequate amount of local tissue to create flaps for both replacement and / or coverage of the defective urethra and, resurfacing the penile shaft[4].

The aim of this study is to report our experience in using the TIP uretheroplasty in reoperative hypospadius repairs and detect the various factors predicting its outcome.

Patients and Methods

The medical records of 24 patients who had undergone TIP uretheroplasty hypospadius reoperation at pediatric surgery department, Madinah Munawarrah, KSA between February 2007 and December 2011 were analyzed retrospectively. Twenty-four patients were admitted for hypospadius complications (six from them were operated on for primary hypospadius repair at our institution). We classified our patients by the type of their initial hypospadius repair: meatal advancement and glanuloplasty (n = 4 patients), 'meatal-based flap procedure (n = 14 patients), and tubularized incised plate uretheroplasty (n = 6 patients). The interval from the most

recent previous surgery till the time of tubularized incised plate uretheroplasty reoperation ranged from 4 to 13 months (Mean: 7 months). Table 1 shows the indications of redo-hypospadius surgery in each group of cases and their numbers.

Technique

TIP uretheroplasty was performed by one surgical team. After deciding to proceed with a secondary tubularized incised plate uretheroplasty procedure, the previously reconstructed neourethra, including the regions with fistulas or stenotic segment, is incised entirely starting from the intact portion of the neourethera towards the glans. The penis is then degloved. Figure (1). The urethral plate is separated from the glans by longitudinal parallel incisions on either side of the urethral plate. Intraoperatively, the level of meatus was distal penile in 15 patients and coronal in 9. The urethral plate is deeply incised in the midline and tubularization is achieved with a single continuous 6/0 Vicryl suture, line over 8-10 Fr catheters. A tunica vaginalis flap is used to cover the neourethra if a well vascularized de-epithelialized skin flap is not available. The meatus is secured to the glans, the coronal margins of glanular wings are approximated by a 6/0 Vicryl suture and the skin is closed. Figure (2) All patients were admitted to the hospital for 7 days, were discharged after removal of catheter and ensure passage of good urinary stream. The follow-up patients range between 6 months and 1 year.

Results

The type of primary hypospadias repair and complications encountered after tubularized incised plate uretheroplasty reoperation are summarized in Table 1. The mean age at the time of secondary hypospadius repair was 3.5 (range: 2-9) years. The incidence of complications of tubularized incised plate uretheroplasty reoperation after failed meatal advancement and glanuloplasty, meatal based flap procedure, and tubularized incised plate uretheroplasty operations were 25%, 28.5%, and 33.3% respectively .

The overall complication rate was 29% (n : 7). Three patients

(12.5%) had fistula and four had meatal stenosis (16.6%). Simple fistula closure was performed in two patients eight months postoperatively. Two patients underwent meatoplasty procedure for meatal stenosis and the other two cases respond to regular dilatations for 2 to 3 months postoperatively. Table 2 demonstrates the type of primary hypospadias repair and complications after tubularized incised plate uretheroplasty hypospadias reoperation.

Table 1 : Indications of redo-hypospadius surgery in each category of cases.

Initial hypospadius repair	Site of meatus before reoperation and number of cases	Number of cases of fistula needs TIP redo
Meatal advancement and glanuloplasty (n: 4)	Coronal(n: 2) Subcoronal(n: 2)	N: 0
Meatal based flap (n: 14)	Subcoronal(n: 2) Distalpenile(n: 4) Midshaft(n: 4)	N: 4
Tubularized incised plate uretheroplasty (n: 6)	Subcoronal(n: 2) Distalpenile(n: 2)	N: 2

Table 2 : Incidence of complications in relation to previous repair.

Initial hypospadias repair	Complications	Incidence of complication (n:7) (29%)
Meatal advancement and glanuloplasty (n:4)	Fistula:0 Meatal stenosis: 1	25%
Meatal based flap(n:14)	Fistula: 2 Meatal stenosis: 2	28.5%
Tubularized incised plate(n:6)	Fistula: 1 Meatal stenosis: 1	33.3%

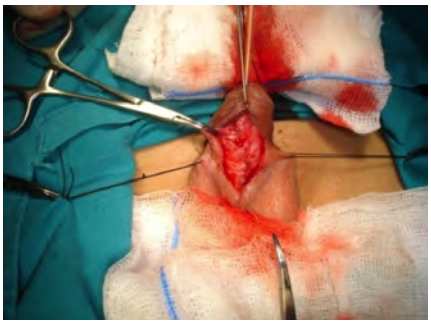

Fig. 1 : Creation of new urethral tube around catheter size 8.

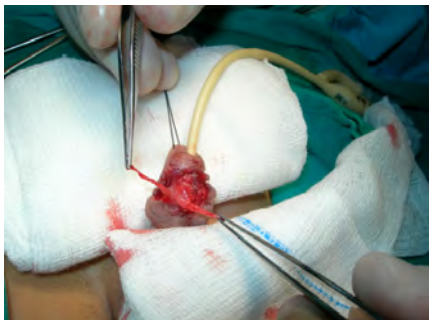

Fig. 2 : A dartos flap is used as a second layer.

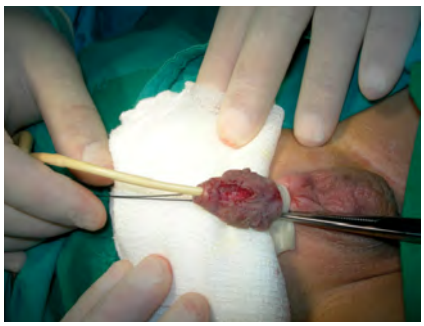

Fig. 3 : The repair is completed.

Discussion

Management of hypospadius repairs complications still a significant surgical challenge. Compromised tissue vascularity and deficient penile skin and preputual tissues create a difficult setting to reconstruct a viable ,patent neourethera.[5]

Reoperative hypospadius complication rates are higher than primary repair, because of the altered vascular supply of tissue flaps, scarring and limited hairless skin. Reoperative repair rates by using the Mathieu and onlay techniques reportedly give higher complication rates, at 24% and 14% respectively.[6]

Tubularized incised plate ure-

theroplasty provides a reliable approach for hypospadias reoperations. The successful use of tubularized incised plate uretheroplasty for reoperative hypospadias repair is supported by many reports and the complication rate varies between 0% and 26%[7].

In our series the reoperative tubularized incised plate uretheroplasty has the least complication rate (25%), if the first failed hypospadias repair was a meatal advancement and glanuloplasty procedure. This can be explained by the integrity of the urethral plate in these cases. Subsequently, the difference in outcomes of the reoperative tubularized incised plate uretheroplasty in relationship to the type of the original procedure was significant.

It was previously reported that the incidence of complications of tubularized incised plate uretheroplasty hypospadias reoperation in patients with prior incised urethral plates versus those without incision was 22% and 23%[8,9]. In our series we agree with that view although Mehmt E.,et al(2007)[10] mentioned that a previous incision of the urethral plate increases the complication rate of tubularized incised plate uretheroplasty hypospadias reoperation (28%) as compared to an unincised urethral plate (22%).

Although there is a generous vascularization of the urethral plate, the vascular integrity of the urethral plate can be disturbed by initially failed hypospadias repairs. Therefore the type of previous surgical intervention is an important factor that needs to be taken into consideration with respect to the successful outcome of tubularized incised plate uretheroplasty hypospadias operations.[8]

Many technical points during the performance of Snodgrass uretheroplasty have paramount significance on the outcome of the procedure. Snodgrass et al[1] did not sew the glans to the new meatus but on their final reviews they had mentioned that the meatus should be secured to the glans at 5 and 7 o'clock to improve cosmetic result and we agree to this concept to decrease meatal stenosis postoperatively. Another concept is to take three stitches at 4,6,8

o'clock after getting a high rate of meatal stenosis in the initial study of Mehmet et al. [10].

Meatal regression can occur if the depth of the incision in the glanular portion of the urethral plate is not adequate for adequate mobilization and tubularization of urethral plate. It is documented that if the midline incision is deepened beyond the limit of the urethral plate to improve tubularization of the urethral plate and conical configuration of glans, some bleeding occurs, and possibility of stenosis or dehiscence increases[11]. Therefore, the level of the first stitch of neourethral tube is better to be at the middle glanular level in these reoperations.

The type of covering layer over the neourethera may be derived from the dorsal preputuium (if available) or the dartos tissue. The use of the dorsal prepuce needs extensive dissection and may impair the blood supply of the skin and causes postoperative penile torsion. To avoid these problems, a covering layer from dartos tissue is modified. In the later technique, when the ventral skin is deficient, the size of the flap may be inadequate to cover the repair[8]. A new technique is to raise a lateral dartos ventral flap and cover the neourethera[5]. The last technique is associated with a low fistula rate (14.3%) and agree with our result (12.5%). Some limitation to this technique is shortness of the flap that can be solved by another flap from the other side and the two flaps can then be sutured together[12].

There are controversies around the penile skin degloving to be complete till the penoscrotal area or limited to area around the meatus. Selami S. and Warren S[13]. advised limited degloving of the penile skin so as to limit the need of a large covering layer of the neourethera and limit the use of TIP uretheroplasty to those cases without severe penile curvature On the other hand, Hmmouda H, et al.[14] Supported our view in doing complete degloving of the penile skin to provide full erection and prevent postoperative torsion.

We recommend to stent all cases of Snodgrass redo urethero-

plasty especially in patients with a poorly developed urethral plate or if there is some concern regarding the viability of the covering flap. Also, the stent keeps the dorsal midline incision stretched open and limits the chance that it may heal primarily and thereby lose the benefit of the dorsal incision.

However Borer et al.,[15] did not stent most of his repairs, and no case of uretherocutaneous fistula, urethral stricture or meatal stenosis occurred. From our experience, we think that the use of the stenting catheter shouldn't exceed 5 days postoperatively to avoid the problems of catheter blockage, bladder irritation and long hospital stay.

The overall complication rate for our secondary tubularized incised plate uretheroplasty hypospadias repair is 29% including fistula and meatal stenosis. The ultimate success rate of tubularized incised plate uretheroplasty reoperation was 100% after repair of fistula and meatal stenosis by simple fistula closure and meatoplasty procedure. Near about 90% of the patients had an excellent cosmetic outcome with a vertically oriented slit like meatus and conical glanular configuration.

Snodgrass tubularized incised plate uretheroplasty can get a very good results if the above- mentioned technical points are followed, but we think that multiple prior midline urethral plate incisions more than twice are contradiction for tubularized incised plate urethroplasty reoperations. A buccal mucosa uretheroplasty can be used for neourethral reconstruction in patients who had failed repairs after tubularized incised plate uretheroplasty hypospadias reoperation [12].

Conclusion

Tubularized incised, plate uretheroplasty is a safe and efficacious alternative procedure for hypospadias reoperations if the urethral plate has no scars and outcome is favorable if the first failed hypospadias repair is a meatal advancement and glanuloplasty procedure. Another factors like the duration of catheter usage and technique performance, have effects on the outcome.

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TUBULARIZED INCISED PLATE URETHROPLASTY FOR HYPOSPADIAS REOPERATIONS : FACTORS PREDICTING THE OUTCOME

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GASTRIC VOLVULUS IN INFANTS AND CHILDREN : DILEMMA OF MANAGEMENT

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Abstract

Purpose: *The aim of the study was to review the cases of all children who had gastric volvulus from 2006 to 2011 at a tertiary care centre in Mansoura University Children Hospital and to compare the outcome of management with the reported series on gastric volvulus in a paediatric age group.*

Materials and Methods: *This was a retrospective study of 35 children with an age range between 4 months and 3 years who were managed for gastric volvulus between 2006 and 2011. The records of these patients were reviewed for clinical features, investigations, management and outcome.*

Results: *All patients were less than 3 years of age with male preponderance (n = 35). No patients had acute presentation. The commonest symptom was abdominal distension and non-bilious vomiting. All patients were diagnosed by barium studies and were confirmed intraoperatively. The response to medical therapy was increasing in the group of low age especially before 12 months (85.7%), decreasing to (62.5%) in the group between 12 and 24 months and became 20% in the group of age more than 24 months. Out of 35 cases of chronic G.V., Hill's posterior gastropexy was performed for 20 patients with the total rate of postoperative complications reaching 20%. The most common postoperative complication in the present study was postoperative vomiting reported in two cases (10%). These two cases were treated by medical antireflux therapy and improved totally after a period varying from 2 weeks to one month.*

Conclusion: *Barium meal has been shown to be a very valuable investigation for diagnosis of cases of chronic G.V. with a sensitivity of 100% in the present study. We can state that chronic G.V. is often a radiological diagnosis that even may not be seen at laparotomy. Medical therapy should be considered as the principal item of management for cases of 'G.V. especially at the younger age group (below one year of age) Posterior gastropexy is recommended as the only line of surgical treatment required for cases of chronic G.V. in whom medical treatment fails.*

Keywords: *Gastric volvulus, Barium meal, Hill"s posterior gastropexy.*

Introduction

Gastric volvolus is abnormal rotation of one part of the stomach around another along its coronal or sagittal axis due to complete absence or weakness of its ligamental attachments. The age ranges from birth to 15 years with 15% of cases diagnosed immediately after birth, 20% in the neonatal period, 44% before the age of 1 year and 82% before the age of 5 years. The sex distribution is almost equal with a minimal predominance of males.(1)

Clinical presentations vary from acute to chronic forms. Patients with the classic acute form are characterized by the triad of Brochardt i.e. severe epigastric distension, intractable retching and inability to pass a nasogastric tube The chronic form is probably more common and should be suspected with the following pentalogy (intermittent colicky abdominal pain, non bilious vomiting, upper abdominal distension, failure to thrive and may be hematemesis). Diagnosis starts by clinical suspicion, X-ray films and barium studies.(2)

The treatment of G.V. varies with disease presentation. The acute form requires immediate surgical intervention. The treatment of chronic idiopathic GV remains controversial ranging from conservative management in the form of anti-reflux procedures up to gastropexy either alone or with fundoplication.(3) The aim of our series is to show our experience in diagnosing and treating cases of chronic G. V.

Patients and Methods

The present study included 35 cases of surgical non-bilious vomiting diagnosed as gastric malrotation admitted at the Department of Pediatric Surgery, Mansoura University Children Hospital during the period from October 2006 to February 2011. Their age groups ranged between 4 months and 3 years (mean age 18 months). Infants with medical diseases causing non bilious vomiting e.g. viral gastroenteritis, drug toxicity, gastritis, renal failure, sepsis and meningitis were excluded from the study. Patients were subjected to, proper history taking including descriptive data: name, age, sex, family history of the same condition, presenting complaints and their analysis e.g. vomiting (onset, course, frequency, color, projectile or not, relation to feeding, aggravating and relieving factors, history of medical therapy and the response in addition to other associated symptoms), analysis of GERD complications i.e. failure to thive, bleeding and respiratory symptoms. Thorough clinical examination included vital signs (pulse, temperature, body weight, signs of dehydration, general examination of head, neck, chest, abdomen and extremities) Proper Diagnostic investigation including routine laboratory investigations, plain x-ray for the abdomen and the chest and barium meal and follow through. Fig. 1 demonstrates the view of G.V. in barium meal study.

After establishing the diagnosis medical treatment was given for uncomplicated cases. This Treatment consists of positioning (sermisetting position was advised at least for one hour after every feed), frequent small feeds, dietary formulas e.g. nutrilion milk formula. Drugs included, antiemetics e.g. metclopromide, gastric regulators e.g. dormpridone, H2 receptor antagonists (H2RA) e.g. ranitidine and famotidine and protone pump inhibitors e.g. omoperazole. Mild eases of reflux are treated firstly by positioning and frequent small feeds for two weeks. If no response, antiemetics and gastric regulators are added for three months. Hill's posterior gastropexy for cases of chronic gastric volvolus not responding to medical therapy for six months. After induction of general anesthesia and

through limited supra-umbilical incision the stomach is released from the transverse mesocolon and fixed posteriortly to the ligament of Treiz by two simple vicryl 4/0 stitches to ensure gastric fixation. (Fig. 2).

Postoperative follow up included the use of analgesics for at least 48 hours to minimize postoperative pain. Different forms of non-steroidal anti-inflammatory drugs were given. Nasogastric tube drainage was continued till the postoperative ileus was resolved. Intra-venous fluids were given for about 1-2 days till oral feeding was started. Oral feedings when allowed, are started by non residue fluids for 24- then semi-solids or breast feeding when possible. The scheme of postoperative follow up sheet included essentially the analysis of postoperative vomiting, pain, in addition to recording the body weight.

Results

Table (1): Shows the descriptive data of gastric volvolus category. The largest group of age lies between 24 months and 36 months (57%). This series included 18 males and 17 females. Consanguinity was positive in 5 patients (14.28%).

The response to medical therapy was increasing in the group of low age especially before 12 months (85.7%), decreasing to (62.5%) in the group between 12 and 24 months and became 20% in the group of age more than 24 months as shown in table (2).

In the present study, out of 35 cases of chronic G. V., Hill's posterior gastropexy was performed for 20 patients with the total rate of postoperative complications reaching 20%. The most common postoperative complication in the present study was postoperative vomiting reported in two cases (10%). These two cases were treated by medical antireflux therapy and improved totally after a period varying from 2 weeks to one month.

The postoperative complications of Hill's posterior gastropexy in gastric complications of Hill's posterior gastropexy was 40%. The most common complication was secondary GER occurring in two cases (10%) as shown in table (3).

Table 1 : The descriptive data of gastric volvolus cases (total 35cases) .

		NO	%
Age	4 months-12months	7	20.0
	12months -24months	8	22.9
	24 months -36 months	20	57.1
Sex	Male	18	51.4
	Female	17	48.6
Positive family history		0	0.0
Positive consanguinity		5	14.28

Table (2): Results of medical treatment in gastric volvolus category (total 35 cases) .

	Total	Responding (15)		Not responding (20)	
		No	%	No	%
4 months-12 months	7	6	85.7	1	14
12months-24months	8	5	62.5	3	37.5
24months-36 months	20	4	20	16	80
Total	35	15	42.8	20	52.2

Table (3) : Post operative complications of Hill 's posterior gastropexy in gastric volvolus cases (8 cases) .

	Hill's posterior Gastropexy (n=20)	
	No	%
1-GIT		
(A)Early complications :		
- Upper GIT bleeding	1	5
- Prolonged ileus	0	0
- Gastric fistula	0	0
(B) Late complications :		
- Persistent vomiting	4	20
* Persistent GER	2	
- Adhesive intestinal obstruction	1	5
2- Wound complications		
* Wound infection	1	5
* Burst a bdomen	0	0
* Incisional hernia	0	0
3- Respiratory tract infections :		
- Bronchitis	1	5
- Pneumonia	0	0
4-Mortality	0	0
Total	8	40

Table (4) : The operative outcome in relation to different operative procedures of G.V. in different studies .

Study	No of patients	Operative procedure	The total of complications	postoperative persistent vomiting
1-Dilorenzo et al., (1987)	16	Anterior gastropexy	24.3%	12%
2- EI-Gohary et al., (1995)	8	Gastropexy and partial fundoplication	12.7%	5%
3-EI-halaby and Emad, (2001).	8	Anterior gastropexy	8.6%	Not recorded
4-Darani et al., (2005)	19	Posterior gastropexy	35.9%	10.53%
5- Present study (2009)	20	Posterior gastropexy	20%	10%

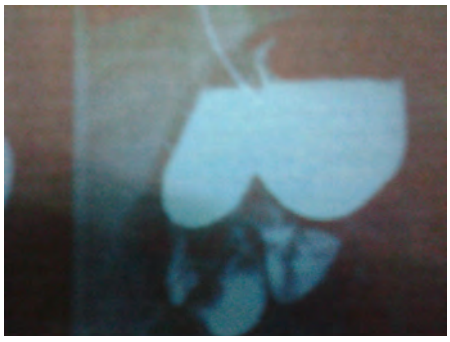

Fig. 1 : The beaking sign of organoaxial gastric malrotation.

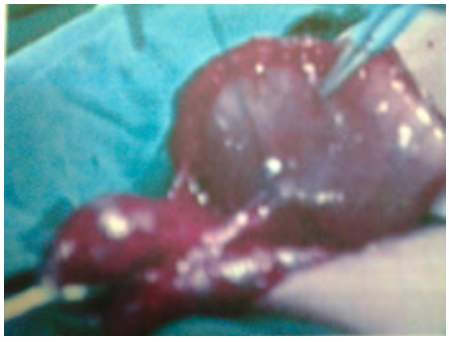

Fig. 2 : Fixation of the stomach to ligament of treitz.

Discussion

Gastric volvolus is abnormal rotation of one part of the stomach around another along its coronal or sagittal axis due to complete absence or Weakness of its ligamental attachments. The age ranges from birth to 15 years with 15% of cases diagnosed immediately after birth, 20% in the neonatal period, 44% before the age of 1 year and 82% before the age of 5 years. The sex distribution is almost equal with a minimal predominance of males.(1) The original classification of gastric volvulus was based on the axis of gastric rotation: organoaxial rotation (along the long axis extending from the hiatus of the diaphragm to the pylorus), mesentericooaxial rotation (along the short axis transecting the lesser and greater curvatures) and combined type (Fig. 33). The disorder can be divided also by degrees of rotation : complete (more than 180 degrees) and incomplete (less than 180 degrees).(4)

Gastric volvulus may be primary (35% of cases) or secondary (65% of cases). Primary gastric volvolus is caused by hyperlaxity of the peritoneal attachments of the stomach (the gastrosplenic, gastrophrenic, gastrocolic and gastrohepatic ligaments) due to abnormal fusion of the fetal mesenteries. The fixed elements besides the ligaments are the duodenum which becomes retroperitoneal at the second portion and the gastroesophageal junction at the diaphragmatic hiatus. A diaphragmatic defects such as in cases of eventration, giant hiatus hernia or left Bochdalek can lead to secondary types.(5) The clinical presentation of gastric volvulus varies widely depending on the degree of rotation of the stomach and the resulting obstruction. Patients with the classic acute form are characterized by the triad of Brochardt i.e. severe epigastric distension, intractable retching and inability to pass a nasogastric tube. These findings indicate an obstruction at the pylorus and the cardia with distension of the stomach causing vascular compromise and leading to ischemia, necrosis, perforation and possible death. Surgery must be performed immediately, The acute form is rarely seen in neonates and is not typical in infants,

however, about 70% of children present with some of these criteria. In children, symptoms include vomiting, chest pain, dysphagia, dyspepsia and acute respiratory distress.[6] The chronic form is probably more common and should be suspected with the following pentalogy (intermittent colicky abdominal pain, non bilious vomiting, upper abdominal distension, failure to thrive and may be hematemesis). Patients may show epigastric pain, nausea, vomiting, intermittent dysphagia, irritability and failure to thrive. The symptoms can appear intermittently for weeks to years. The golden standard for diagnosis of acute gastric volvulus is the findings on laparotomy. This usually will confirm the radiologic and clinical diagnosis. In cases of chronic G.V. the surgeon can notice only laxity of stomach ligaments and evidence for minor organ affection depending on the duration to the devolvulation.[7] The clinical suspicion of gastric volvulus is generally confirmed by radiological studies. The clinician must keep in mind, that the radiologic appearance varies widely with the broad spectrum of the disease. In patients with the acute presentation, plain abdominal X-ray film can reveal gastric dilatation and paucity of gas in the remainder of the intestinal tract.[8] Barium meal almost confirms the diagnosis in chronic intermittent cases but the results of this test may be normal so that it should be repeated as soon as possible after the appearance of the symptoms. In organoaxial volvulus, the greater curvature of the stomach swings anteriorly and upward. The result is an “upside-down”‘ of the stomach with the lesser curvature below the greater one. There is a low gastroesophageal junction and distortion of the duodenum with either a single or double fluid levels .In mesentericoaxial volvulus, the pylorus rotates from right to left so that the stomach lies “right-side up". In the supine position the distended stomach appears spherical, whereas in the up-right plain film, there is a double fluid levels, one at the proximal stomach and one at the distal stomach. “Beaking” is seen at the obstructed pyloric canal.[2]

Barium meal has been shown to be a very valuable investigation

for diagnosis of cases of chronic G.V. with a sensitivity of 100% in the present study and Elhalaby and Emad,(200l). These findings are higher than those reported by Mayo et al., (2001) (97%) and Dilorenzo et al., (1987) (89%)[9]. Thus we can state that chronic G.V. is often a radiological diagnosis that even may not be seen at laparotomy.

The treatment of G.V. varies with disease presentation. The acute form requires immediate surgical intervention. The first step after resuscitation is decompression of the dilated stomach to prevent gastric wall ischemia and necrosis. In children, the use of a nasogastric tube is controversial because of the risk of perforation. Laparotomy is performed with a good exposure of the operative field and intraoperative decompression of the stomach. This is followed by reduction of the stomach, resection of the necrotic portion and correction of the associated anatomic defects. The third step is prevention of recurrence by gastric fixation. Fixation is usually achieved by anterior gastropexy and/or gastrostomy. The gastrostomy tube serves for both fixation and postoperative decompression and it can be removed within 2 to 3 weeks and gastropexy acts to prevent the recurrence. In complicated cases partial gastric resection with gastroenterostomy is recommended. [10] The treatment of chronic idiopathic GV remains controversial. Conservative management in the form of positioning of the infant in the prone or upright position for at least one hour after meals and prokinetic drugs may improve the tone and strength of gastric ligamentous attachments. The success rate of the conservative therapy ranges from 70%-85% especially when the infants don't have symptoms of GERD and under 6 months of age[11]. Surgery is indicated in refractory cases of chronic G. V. with failed medical therapy especially when the infants have symptoms of GERD and after 6 month of age. Teague et al., (2000)[12] advised gastropexy and partial fundoplication as a considerable procedure in treating G.V. But, Darani et al., (2005) recommended gastropexy alone as the mode of treatment. Both procedures are accompanied by

correction of associated anomalies which may include plication of the diaphragm for repair of the paraesophageal hernia and Ladd's procedure for intestinal malrotation. Gastropexy is of three types: anterior (Boerema) gastropexy, posterior (Hills) gastropexy and fixation of the stomach during gastrostomy. In the present series, 35 cases of chronic G.V. received medical therapy with a success rate of 42.8%. These results agreed with those obtained by Darani et al., (20O5) (43%), higher than Mayo et al., (2001) (25.7%) and[13] and lower than Dilorenzo ct al., (1987) (77%) and E1-halaby and Emad, (2001) (89%). In the present study, out of 35 cases of chronic G. V., Hill's posterior gastropexy was performed for 20 patients with the total rate of postoperative complications reaching 20%. The most common postoperative complication in the present study was postoperative vomiting reported in two cases (10%). These two cases were treated by medical antireflux therapy and improved totally after a period varying from 2 weeks to one month. Thus posterior gastropexy is recommended as the only line of surgical treatment required for cases of chronic G.V. in whom medical treatment fails. These findings agree with Darani et al., (2005) and Mayo et al., (200l). On the other hand, El-Gohary et al., (l995) and[14] advised partial fundoplication as an initial procedure with gastropexy in cases of chronic G.V. In contrary to these recommendations, El-halaby and Emad, (2001) considered gastropexy in G.V. as over treatment.

Wound complications were reported in one case of Hill's posterior gastropexy (5%) in the present study. These results agreed with the common rate of wound complications reported in other series that was between 4.7-5.9%. Recurrent respiratory tract infections occurred in one case of Hill's posterior gastropexy (5%) in the present study. This rate is higher than that of Dilorenzo et al., (l987) (8.3%) and lower than that of Darani et al., (2005) (3.5%). The operative outcome in relations to different operative procedures in different series is shown in table[4].

In conclusion, barium meal has

been shown to be a very valuable investigation for diagnosis of cases of chronic G.V. with a sensitivity of 100% in the present study. Thus We can state that chronic G.V. is often a radiological diagnosis that even may not be seen at laparotomy. Medical therapy should be considered as the principal item of management for cases of 'G.V. especially at the younger age group (below 4 months of age) Posterior gastropexy is recommended as the only line of surgical treatment required for cases of chronic G.V. in whom medical treatment fails.

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GASTRIC VOLVULUS IN INFANTS AND CHILDREN : DILEMMA OF MANAGEMENT

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EVALUATION OF LOW LEVEL LASER THERAPY VERSUS SURGICAL DEBRIDEMENT IN DIABETIC FOOT ULCER

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Abstract

Objective: *to evaluate diabetic foot ulcer healing using LLLT (low level laser therapy) in comparison to traditional method of surgical debridement and frequent dressing.*

Methods: *forty patients with diabetic foot ulcers were subjected to surgical debridement, and then the patients were randomly classified in to 2 different groups of treatment. The 1st group was treated by LLLT. The 2nd group was treated by daily dressing using topical preparations.*

Results: *There was no significant difference between both groups concerning the progress of healing, but we noticed a faster healing process in the laser group with less numbers of follow up and dressing sessions.*

Conclusion: *Although our work was with a mono method (composed of treatment with laser) and the small number of patients, the cure of most diabetic feet ulcers in about 2 months gives us hopes for opening new horizons in the treatment of diabetic feet ulcers those already known to be refractory chronic ulcers.*

Introduction

There are many complications of diabetes; one of them is diabetic foot ulcer, which is an open sore or wound that most commonly occurs on the bottom of the foot in approximately 15% of patients with diabetes. Of those who develop a foot ulcer, approximately 6% will be hospitalized due to infection or other ulcer related complications. (1)

Around 7-20% of patients with foot ulcers will subsequently require amputation; as foot ulceration is the precursor to approximately 85% of lower extremity amputations in persons with diabetes. [2]

Debridement of necrotic tissue is an integral component in the treatment of chronic wounds since they will not heal in the presence of unviable tissue, debris, or critical colonization[3]. Undermined tissue or closed wound spaces will otherwise harbor bacterial growth. [4]

The effects of LLLT include wound epithelialization, reduction of edema and inflammation, and re-establishment of arterial, venous and lymph microcirculation. Increased rates of ATP, RNA and DNA synthesis are also observed. Major changes seen in wounds treated with LLLT include increased granulation tissue, early epithelialization, increased fibroblast proliferation, increased extracellular matrix synthesis and enhanced neo-vascularisation. Also it was found that laser irradiation was able to significantly enhance the healing of diabetic wounds. And has a stimulatory effect on the fibroblast cells of the diabetic wounds. [5,6]

The aim of this study is to evaluate diabetic foot ulcer healing using LLLT in comparison to traditional method of surgical debridement and frequent dressing.

Patients and Methods

Forty patients of both sexes with a diagnosis of diabetic foot ulcers either with or without signs of infection were included in this study.

The patients were either grade 1 stage A (superficial ulceration not infected) or grade 2 stage A (deep ulceration exposing tendon or capsule); according to University of Texas wound classification system [7].

We excluded the patients who had : chronic ischemia (ABI<0.9), severe septic conditions, renal failure, pregnancy, cancer or other serious diseases, inability to cooperate with the requirement of the study, recent history of alcohol or drug abuse, current thera-

py of any immunosuppressive agents or anticonvulsant.

All patients were subjected to surgical debridement then the patients were randomly classified in to 2 different groups of treatment.

Group I

It includes 20 patients treated by LLLT. They were 13 females and 7 males, all patients subjected to LLLT to the ulcer in vertical and horizontal directions.

The infrared laser was applied by scanning method to the ulcer. It was applied for 8-10 minutes (980 nm; power: 5W; 18 J/cm^2). The patient lied comfortably on the bed, and an eye goggle was given to the patient to protect the eyes from the hazard of the low level laser therapy.

After laser, hydro-active gel was applied to the ulcer and sterile dressing was applied over the gel.

The treatment was applied for 3 months, 2 setting per week, with follow up every month to detect the improvement by measuring the diameter of the ulcer and detect the healing progression.

Group II

It includes 20 patients treated by daily dressing using topical preparations. It included 10 females and 10 males.

All patients were subjected to X-ray of feet, doppler ultrasound of both legs, calculation of the area of the ulcer was done by using the graph papers to document the ulcer's perpendicular linear dimensions (typically in centimeters using graph paper); the maximum distance was length and the perpendicular distance was width[8]. The progress of healing was measured every month with recording the percent of healing, for 3 months in both groups.

Results

This study is a randomized controlled clinical trial that was carried out on 40 patients with diabetic foot ulcers, who were collected from the inpatient and outpatient clinics of vascular surgery unit in Zagazig University Hospitals. The duration of the study was 12 months, from January 2012 to January 2013.

Our results showed that the number of female was 13 patients in the laser group and 10 patients in the surgery group. And the number of male patients was 7 patients in the laser group and 10 patients in the surgery group. Also showed the median age (51 in the LLLT group, 53 in the surgery group) and duration of the disease (14.8 weeks in the LLLT group, 14.2 weeks in the surgery group). There are insignificant differences between the groups as regards sex, age and duration of diseases. As shown in table (1).

In this study the number of patients with previous foot infections was 16 in the laser group and 18 in the surgery group. The number of patients with signs of inflammation as hotness, redness, swelling, tenderness was 14 in the laser group and 11 in the surgery group. All patients had surgical debridement, but 7 patients had also minor amputation with the debridement (4 patients in the laser group and 3 patients in the surgery group).

The study shows that, the frequency of ulcer occurrence at different sites is as follow: 16 patients at the forefoot (10 patients in laser group and 6 patients in the surgery), 11 patients at the midfoot (4 patients in laser group and 7 patients in the surgery group), 4 patients at the heel (1 patients in laser group and 3 patients in the surgery group), 7 patients at the dorsum(4 patients in laser group and 3 patients in the surgery group) and 2 patients at more than one site (one patient in the laser group and one patient in the surgery group). As shown in Table (3).

Out of 20 patients subjected to LLLT, 14(70%) showed complete healing at average time of 8.5 weeks (7-10 weeks). These patients needed 14-20 sessions of laser (average 17 sessions). While in the surgical group 11 ulcers out of 20 ulcers showed complete healing at average time of 10 .5 weeks (9-12 weeks). These patients needed frequent daily or every other day dressing of the ulcers assisted by a type of offloading techniques.

There was no significant difference between both groups concerning the progress of healing.

Table (1): Shows the frequency of sex, age and duration of the disease among the two groups.

	Laser N=20	Surgery N=20	P
Sex			
Male	7 (35%)	10(50%)	0.2
Female	13(65%)	10(50%)	
Age			
Range	40-63	38-68	
$\bar{X}$±SD	51.35±7.1	53.6±8.2	0.08
Duration			
Range (weeks)	5-30	6-32	
$\bar{X}$±SD	14.8 ±7.8	14.2±8.1	0.31

Table (2): Shows foot specific history.

	Laser N =20	Surgery N =20	x^2	p
Previous foot infection	16(80%)	18(90%)	0.784	0.67
Inflammatory signs	14(70%)	11(55%)	1.452	0.48
Surgery done				
Debridement	20(100%)	20(100%)		
Amputation+debridement	4(20%)	3(15%)	3.73	0.15

Table (3): Shows the distribution of ulcer site.

Site	Laser N=20	Surgery N=20	X^2	P
Forefoot	10(50%)	6(30%)	1.67	0.43
Mid foot	4(20%)	7(35%)	1.19	0.55
Heel	1(5%)	3(15%)	1.11	0.57
Dorsum	4(20%)	3(15%)	0.24	0.88
More than one	1(5%)	1(5%)	0.54	0.76

Table (4): Shows the comparison of the healing percentage among the two groups at 1,2,3 months.

	LLLT	Surgery	P
1st month	2(10%)	0 (0%)	0.05
2nd month	8(40%)	4(20%)	0.08
3rd month	4(20%)	7(35%)	0.07

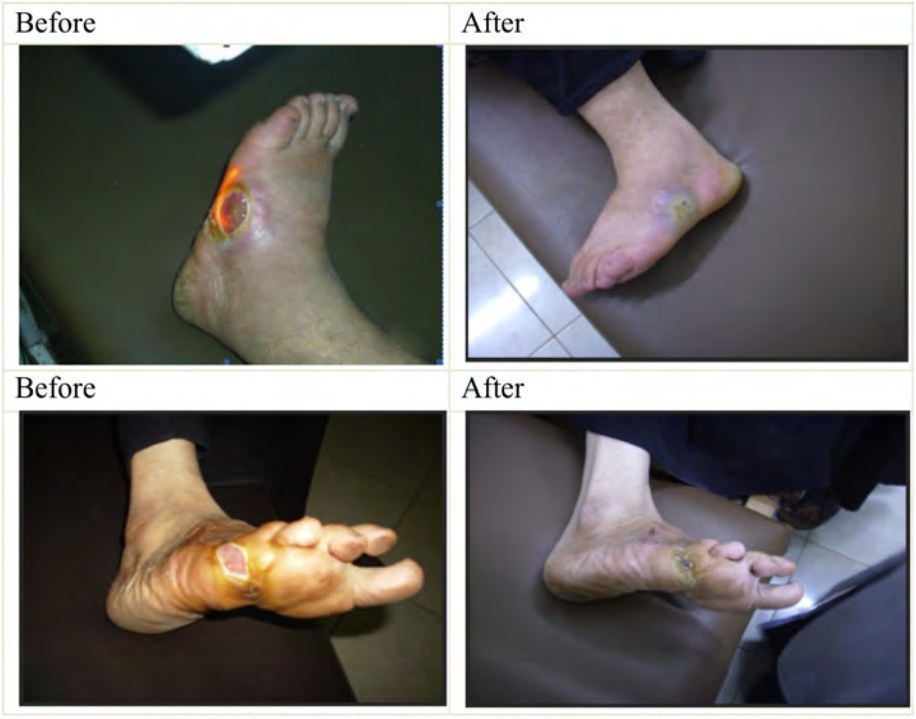

Figure (1): Before and after LLLT.

Discussion

Diabetic ulcers are the most common foot lesions leading to lower extremity amputations. Management of the diabetic foot requires a thorough knowledge of the major risk factors for amputation. The most common risk factors for ulcer formation include diabetic neuropathy, structural foot deformity and peripheral arterial occlusive disease. A careful physical examination can identify patients at risk for foot ulcers and appropriately classify patients who already have ulcers or other diabetic foot complications. Patient education regarding foot hygiene, nail care and proper footwear is crucial to reduce the risk of an injury that can lead to ulcer formation[9].

This study was carried out to evaluate the efficacy of low level laser therapy on diabetic foot ulcers, comparing the results with traditional surgical method.

Schindi et al, reported the first case of diabetic foot patient treated with low-intensity laser therapy in 1999 and proposed that this therapeutic method might constitute a useful side effect-free alternative treatment modality for the induction of wound healing of neuropathic ulcers in diabetic patients. Since then, some researches have been oriented to application of low-intensity lasers for treatment of diabetic foot ulcers[10].

Both groups underwent computer based randomization, and all the patients in both groups had no sever sepsis or chronic ischemia. we have found a good improvement in healing process in both groups. Although the results were not statistically significant, but we can notice the faster healing process in the laser group with less number of follow up and dressing sessions, as shown in table (4).

Kazemi concluded that Low-level laser therapy could be a safe and effective method for treatment of diabetic foot ulcers. This study included only 7 patients, in a study done by using laser in treatment of diabetic foot ulcer, complete recovery was achieved in all cases and there was no relapse or other problem with ulcers during average of about 6 months. There were no side-effects reported by the patients in this period. Clinical trials with higher sample size are proposed to more evaluate the efficacy of low-level laser therapy in treatment of this type of wounds. [11]

In a study that was done by Kaviani et al., Twenty-three patients with a diabetic foot wound for at least 3 months were included in this double-blind randomized clinical trial. Patients were randomized to receive placebo treatment (n = 10) or LLLT (n = 13) in addition to conventional therapy. After 20 weeks, in the LLLT group, eight patients had complete healing and in the placebo group only three patients experienced complete wound healing. The mean time of complete healing in LLLT patients (11 weeks) was less than that in placebo patients (14 weeks) though the difference was not statistically significant. The study

provides evidence that LLLT can accelerate the healing process of chronic diabetic foot ulcers, and it can be presumed that LLLT may shorten the time period needed to achieve complete healing.[12]

Cullum in a systemic review concluded that there is generally insufficient reliable evidence to draw conclusions about the contribution of laser therapy to chronic wound healing.[13]

In summary, further innovations in diabetes therapy are needed to improve treatment and its cost. LLLT therapy could provide a solution to diabetes therapy. LLLT therapy could be a future alternative in the therapy of diabetes and its complications.

Although our work was with a mono method (composed of treatment with laser) and the small number of patients, the cure of most diabetic feet in about 2 months gives us hopes for opening new horizons in treatment of diabetic foot ulcers thoes already known to be refractory chronic ulcers.

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EVALUATION OF LOW LEVEL LASER THERAPY VERSUS SURGICAL DEBRIDEMENT IN DIABETIC FOOT ULCER

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LEPTIN AND TUMOR NECROSIS FACTOR (TNF-α) LEVELS IN BENIGN VERSUS MALIGNANT ASCITES

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Abstract

Background: *Leptin is a polypeptide hormone secreted mainly by adipocytes. Tumor necrosis factor (TNF-α), a polypeptide cytokine produced during infection, injury, or invasion. These factors are strongly influenced by altered body composition, hypermetabolic diseases such as liver cirrhosis as well as by malignancy.*

Objective: *To evaluate the role of leptin and TNF-α in the differential diagnosis between sterile and malignant ascites.*

Subjects and Methods: *The study included 80 subjects. They were divided into 3 groups: 30 patients with malignant ascites (Group I), 30 patients with cirrhotic liver disease and ascites (Group II) as well as 20 subjects served as control group (Group III). All subjects were submitted to complete clinical examination including assessment of body mass index (BMI), waist to hip ratio (WHR), skin fold thickness, radiological investigations, routine laboratory investigations, measurement of serum and ascetic leptin and TNF-α, cytological and biochemical examination of ascetic fluid, Alpha-fetoprotein (AFP) and tumor markers as carcinoembryonic antigen (CEA) and CA125.*

Results: *There is a strong statistically significant positive correlation between body fat percentage (BFP) and skin fold thickness (SFT) versus serum leptin. Females had a higher levels of serum leptin compared to males with highly significant difference between them in all groups. Serum and ascitic fluid leptin levels of malignant patients are significantly lower than cirrhotic ascites. Serum and ascitic TNF-α were elevated in*

cirrhotic and malignant patients but with no statistical significance between both groups. The serum markers are more valid than ascitic fluid markers as; serum leptin is good positive, while serum TNF-α is good negative in differential diagnosis between sterile and malignant ascites.

Conclusion: *Serum leptin is an additional non-invasive investigation to help to differentiate between sterile and malignant ascites.*

Keywords: *Leptin,TNF-α,Benign ascites,Malignant ascites,Cirrhosis.*

Introduction

Leptin, a product of the obese gene, is a multifunctional hormone secreted predominantly by adipocytes. It is a 16-kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite, hunger and metabolism. It is one of the most important adipose-derived hormones[1]. It controls food intake and energy expenditure by acting on receptors in the mediobasal hypothalamus [2].

Human leptin is a protein of 167 amino acids. It is manufactured primarily in the adipocytes of white adipose tissue, and the level of circulating leptin is proportional to the total amount of fat in the body. In addition to white adipose tissue-the major source of leptin-it can also be produced by brown adipose tissue, placenta, ovaries, skeletal muscle, stomach (the lower part of the fundic glands), mammary epithelial cells, bone marrow, pituitary, and liver [3].

Mean leptin levels were more than twice as high in females as in males of corresponding weight status, especially among females of healthy weight who exhibited levels that were 5.7 times higher. Overweight individuals generally demonstrated higher circulating leptin concentrations than healthy-weight and underweight participants [4].

On the other hand leptin serum levels can be altered in various diseases [5]. One of the unconventional area in which leptin is now receiving great attention is liver diseases as several published studies have indicated that the circulating leptin levels are increasing in cirrhosis

and chronic liver injury[6].

The ascitic fluid leptin levels of cirrhotic patients with sterile ascites are on average two times higher than circulating levels of this hormone[7].

Serum leptin levels have been reported to be reduced in cancer patients[8]. Abramov et al. found low leptin levels in serum, pleural, peritoneal fluids and in Meigs' syndrome as well[9].

The elevated circulating leptin in patients with cirrhosis is most likely caused by a combination of decreased renal extraction and increased release from subcutaneous abdominal, femoral, gluteal, retroperitoneal pelvic and upper limb fat tissue areas[10].

Recently, obesity has been recognized as a risk factor of the development of chronic liver diseases caused by a variety of etiologies including chronic hepatitis C, alcohol and nonalcoholic fatty liver disease (NAFLD)[11]. Although these conditions have been associated with high serum leptin level, progression of liver fibrosis including cryptogenic cirrhosis is observed often in overweight individuals without any recognizable etiologies [12].

Factors that acutely affect leptin levels are also factors that influence other markers of inflammation, e.g., testosterone, sleep, emotional stress, caloric restriction, and body fat levels. While it is well-established that leptin is involved in the regulation of the inflammatory response, it has been further theorized that leptin's role as an inflammatory marker is to respond specifically to adipose-derived inflammatory cytokines[13].

In terms of both structure and function, leptin resembles interleukin (IL)-6 and is a member of the cytokine superfamily[14]. Circulating leptin seems to affect the hypothalamic - pituitary - adrenal (HPA) axis, suggesting a role for leptin in stress response[15]. Elevated leptin concentrations are associated with elevated white blood cell counts in both men and women[16].

Similar to what is observed in

chronic inflammation, chronically elevated leptin levels are associated with obesity, overeating, and inflammation-related diseases, including hypertension, metabolic syndrome, and cardiovascular disease. However, while leptin is associated with body fat mass, the size of individual fat cells, and the act of overeating, it is interesting that it is not affected by exercise (for comparison, IL-6 is released in response to muscular contractions). Thus, it is speculated that leptin responds specifically to adipose-derived inflammation[17].

Leptin is a pro-angiogenic, pro-inflammatory and mitogenic factor, the actions of which are reinforced through crosstalk with IL-1 family cytokines in cancer[18]. Also leptin mediated signaling pathways play an important role in cancer cell proliferation, invasion and metastasis [19].

Tumor necrosis factor -alpha (TNF-α), or cachectin a polypeptide cytokine involved in systemic inflammation and produced during infection, injury, or invasion, has proved pivotal in triggering the lethal effects of septic shock syndrome, cachexia, and other systemic manifestations of disease[20].

TNF-α was thought to be produced primarily by macrophages[21], but it is produced also by a broad variety of other cell types including lymphoid cells, mast cells, endothelial cells, fibroblasts, and neuronal cells[22].

TNF-α plays several important roles in inflammation, including activation and chemotaxis of leukocytes, expression of adhesion molecules on neutrophils and endothelial cells, and regulation of the secretion of other proinflammatory cytokines[23]. TNF-α is part of a complex network of cytokines and is capable of initiating cytokine cascades involving both synergistic and inhibitory reactions, which control the synthesis and expression of other cytokines, hormones, and their receptors[24].

Dysregulation of TNF-α production has been implicated in a variety of human diseases, including Alzheimer's disease[25], cancer[26], major depression[27]

and inflammatory bowel disease (IBD)[28]. While still controversial, studies of depression and IBD are currently being linked by TNF-α level [29].

TNF-α is a pleiotropic cytokine that induces cellular responses such as proliferation, production of inflammatory mediators, and cell death and plays a major role in the pathophysiology of septic shock and the wasting syndrome. In the liver, TNF-α is involved in the pathophysiology of viral hepatitis, alcoholic liver disease, NAFLD, and ischemia-reperfusion (I/R) injury. In the liver, TNF-α is not only a mediator of hepatotoxicity but also contributes to the restoration of functional liver mass by driving hepatocytes proliferation and liver regeneration[30].

A role for TNF-α in the pathogenesis of chronic hepatitis B and C viral infection has been suggested. Both viruses induce TNF-α expression in human liver and human hepatoma cell lines. Patients with chronic hepatitis B have elevated plasma TNF-α levels, and their peripheral blood mononuclear cells show enhanced TNF-α production in vitro [31].

Additionally, TNF-α has an important, but probably not a central, role for liver injury in alcohol-mediated liver toxicity [32].

The differential diagnosis of ascites often leads to confusion and an inability to exclude its multitude of causes in many patients [33]. Non-invasive tests such as laboratory tests ,acid-fast stain and culture of the ascitic fluid are usually insufficient for differential diagnosis of ascites [34]. Therefore invasive laparoscopy and peritoneal biopsy are necessary for the diagnosis of ascites [35].

Subjects and Methods

This study was carried out in department of internal medicine and clinical pathology, faculty of Medicine, Zagazig University.

The study included 80 subjects. They were divided into 3 groups:

1. Patients with cirrhotic liver disease and ascites (Group I):

It included 30 known patients with cirrhotic liver disease and

ascites. Mean age was (52±7), (14 male and 16 female).

The diagnosis of cirrhosis was depended on typical cirrhotic appearance, splenomegaly, esophageal varices and ascites by ultrasonography and upper gastrointestinal endoscopic evaluation with biochemical data.

The severity of cirrhosis was graded according to Child - Pugh classification, 20 patients were Child C and 10 were Child B.

2. Patients with malignant ascites (Group II):

It included 30 known patients with various types of malignant ascites (8 ovarian adenocarcinoma, 10 adenocarcinoma of colon, 7 gastric adenocarcinoma, 5 hepatocellular carcinoma). Mean age was (53±9), (13 male and 17 female).

Malignancy was documented by positive ascetic fluid cytology, presence of relevant tumor markers as CA125 and carcinoembryonic antigen (CEA) markers.

3. Control Group (Group III):

This group composed of 20 subjects, 11males and 9 females matched for age and sex with mean age (48±9).

Patients were randomly recruited from those attending the outpatients' clinic and inpatients of Zagazig University Hospitals after being informed on the purpose and procedure of the study, all subjects singed an informed consent form.

Patients were excluded from the study if they were suffering from diabetes mellitus, obese participants (BMI of 30 or more), cachectic participants (BMI of 18 or less), alcoholic, pregnancy, gastrointestinal bleeding, spontaneous bacterial peritonitis and renal failure, treatment with corticosteroids, immunosuppressive agents and oral contraceptive agents within the last 6 months.

All subjects were submitted to full clinical assessment including history taking and through clinical examination including assessment of body mass index (BMI) [weight (kg)/ high (m^2)], waist to

hip ratio (WHR) [waist circumference (cm)/hip circumference (cm)] and skin fold thickness (SFT) was measured at three different sites on the right side of the body (triceps, suprailiac and thigh) using Acc-Measure personal body fat caliper for calculation of body fat percentage (BFP) using Jackson Pollock formula[36]. Also, all subjects were submitted to the following investigations:

* Liver and kidney function tests.
* Complete blood count and ESR.
* Fasting and 2-houres postprandial blood sugar.
* Urine analysis.
* Stool analysis with occult blood test.

*** Specific laboratory investigations:**

- Measurement of serum and ascetic leptin in ng/ml using Human Leptin quantikine ELISA Kit (R & D systems, Inc., USA)[37].
- Measurement of serum and ascetic TNF-α in ng/ml using Human TNF-α ELISA kit (BioSource International Inc, USA)[38].
- Cytological and biochemical examination of ascetic fluid.
- Alpha fetoprotein in patients with suspected hepatocellular carcinoma and focal liver lesions by electrochemiluminescence immunoassay using Cobase 411 immunoassay analyzer (Roch Diagnostics, GmbH, German)[39].
- Tumor markers as CEA in gastrointestinal tract tumor and CA125 in patients of ovarian carcinoma using Cobase 411 immunoassay analyzer (Roch Diagnostics, GmbH, German)[40].

*** Radiological investigations including plain chest x-ray and abdominal ultrasonography.**

Statistical Analysis :

Statistical analysis was performed using statistical package for social science (SPSS) version 13. Quantitative data are presented as means ±SD. Qualitative data are presented as number and percentage. The unpaired t-test was used for comparison of means of the continuous variables to evaluate differences between the two groups. ROC (receiver operator

characteristic curve) was used to find out the best cut off value and to detect sensitivity and specificity. Pearson´s correlation was used for detection of relation between 2 variables. r was considered weak if <0.25, mild if ≥0.25 - <0.5, moderate if ≥0.5 - <0.75 and strong if ≥0.75. p- Value is considered significant if ≤0.05.

Results

Table (1): Correlation between serum and ascetic fluid leptin and several parameters in cirrhotic group: In cirrhotic group there was a highly statistically significant positive correlation between BFP and serum leptin.

Table (2): Correlation between serum and ascetic fluid leptin and several parameters in malignant group: In malignant group there was a highly statistically significant positive correlation between SFT and serum leptin.

Table (3): Comparison of mean ±SD between male and female as regard leptin, and TNF-α in cirrhotic group: There was a highly significant difference between male and female as regard serum leptin. Females had a higher levels of serum leptin compared to males in cirrhotic group.

Table (4): Comparison of mean ±SD between male and female as regard leptin and TNF-α in malignant group: There was a highly significant difference between male and female as regard serum leptin. Females had a higher levels of serum leptin compared to males in malignant group.

Table (5): Comparison of mean ±SD between all study groups as regard leptin and TNF-α : Serum and ascetic leptin were significantly lower in malignant group as compared to other groups. While serum and ascitic TNF-α were elevated in cirrhotic patients with sterile ascites as well as in patients with malignant ascites but with no statistical significant difference between both groups.

Table (6): Comparison between leptin and TNF-α and cytology results in malignant group: Patients of malignant group with positive cytology had a lower level of serum leptin compared to those with negative cytology.

Table (7): Sensitivity and specificity of leptin and TNF-α in differentiation between benign and malignant ascites: Serum leptin and TNF-α were more valid than ascetic fluid markers. Serum leptin is good positive with sensitivity 86% and specificity 77.3% while serum TNF-α is good negative with sensitivity 51% and specificity 76% in differentiation between benign and malignant ascites.

Table (1): Correlation between serum and ascetic fluid leptin and several parameters in cirrhotic group.

Variables	Serum leptin		Ascetic fluid leptin	
	r	p	r	p
Serum leptin	-	-	0.15	>0.05
Serum TNF-α	0.03	>0.05	0.2	>0.05
Ascetic TNF-α	-0.12	>0.05	0.08	>0.05
Age	0.2	>0.05	-0.19	>0.05
BMI	0.18	>0.05	0.07	>0.05
BFP	0.69	<0.01**	0.12	>0.05
SFT	0.19	>0.05	0.13	>0.05
Albumin	0.03	>0.05	0.15	>0.05
Child	0.19	>0.05	0.12	>0.05

**= P is significant

Table (2): Correlation between serum and ascetic fluid leptin and several parameters in malignant group.

Variables	Serum leptin		Ascetic fluid leptin	
	r	p	r	p
Serum leptin	-	-	0.19	>0.05
Serum TNF-α	0.13	>0.05	0.12	>0.05
Ascetic TNF-α	-0.14	>0.05	0.04	>0.05
Age	0.12	>0.05	-0.15	>0.05
BMI	0.11	>0.05	0.06	>0.05
BFP	0.09	>0.05	0.13	>0.05
SFT	0.66	<0.01**	0.2	>0.05
Albumin	0.18	>0.05	0.14	>0.05

**= P is significant

Table (3): Comparison of mean ±SD between male and female as regard leptin and TNF-α in cirrhotic group.

Variables	Gender		t	p
	Male	Female		
Serum leptin	2.7±0.5	4.9±1.6	4.1	<0.01**
Ascetic fluid leptin	3.2±2.6	3.5±3	0.3	>0.05
Serum TNF-α	25.9±10	26±9	0.2	>0.05
Ascetic TNF-α	27±9	22.8±9	0.79	>0.05

**= P is significant

Table (4): Comparison of mean ±SD between male and female as regard leptin and TNF-α in malignant group.

Variables	Gender		t	p
	Male	Female		
Serum leptin	1.4±0.4	1.9±0.6	3.1	<0.01**
Ascetic fluid leptin	2.2±0.7	2.1±1.4	0.3	>0.05
Serum TNF-α	32±16	25±8	1.3	>0.05
Ascetic TNF-α	30±12	22±7	1.9	>0.05

**= P is significant

Table (5): Comparison of mean ±SD between all study groups as regard leptin and TNF-α.

	Cirrhotic group (n=30)	Malignant group (n=30)	Control group (n=20)	t	p
Serum leptin	3.9±1.8	1.6 ±0.6	2 ±0.5	5.9	<0.01**
Ascetic fluid leptin	3.3±3	2.07±2	-	2.3	<0.01**
Serum TNF-α	25.7±9	29±14	15±1.8	0.8	>0.05
Ascetic TNF-α	24.4±9	25.9±11	-	0.5	>0.05

**= P is significant

Table (6): Comparison between leptin and TNF-α and cytology results in malignant group.

	cytology		t	P
	Negative	Positive		
Serum leptin	2.9±1.8	1.5±0.8	2.2	<0.05**
Ascetic fluid leptin	2.8±1.8	2.3±0.8	1.9	>0.05
Serum TNF-α	25.9±10	33±20	0.7	>0.05
Ascetic TNF-α	24.7±10	29±8	1.5	>0.05

**= P is significant

Table (7): Sensitivity and specificity of leptin and TNF-α in differentiation between benign and malignant ascites.

Variables	Serum leptin	Ascetic fluid leptin	Serum TNF-α	Ascetic TNF-α
Best cut off value	2.1	2.6	27	26
Sensitivity	86%	63%	51%	51%
specificity	77.3%	59%	76%	61%

Discussion

Leptin is one of the key adipokine regulators for inflammation and progression of fibrosis in various chronic liver diseases including Non Alcoholic Steatohepatites (NASH) [41].

Several reports demonstrated that serum levels of TNF-α and leptin were elevated in cirrhotic patients [42]. Also, the severity of liver fibrosis was associated with serum leptin and TNF-α [43].

Serum leptin concentrations are well correlated with both body mass index and total body fat in humans [44].

This is in agreement with our study, which states that the cirrhotic and malignant groups had a direct correlation between serum leptin and BMI and body fat percentage.

Several studies suggested that the physiologic correlations among

serum leptin level, sex, BMI and body fat mass (BFM) were well preserved in patients with chronic liver disease. Patients with alcoholic liver cirrhosis had higher leptin levels. In early stages of liver disease, leptin levels and absolute leptin levels are higher than in normal subjects. However, in advanced stages of the disease the significant decline in leptin levels and similar levels of leptin expressed in relation to BFM compared to control subjects predominantly represent the expression of fat mass[45].

McCullough et al. found higher leptin levels between cirrhotic females than cirrhotic males [46].

In our study, serum leptin levels were significantly higher between females than males in all groups. In addition, serum leptin was higher in cirrhotic females and males than control and malignant group.

These concepts are important in cirrhosis, because cirrhosis have gender - dependent alternations in body composition and sex steroids [47].

Since BMI and BFM values do not differ according to sex and presence or absence of cirrhosis or malignancy so that changed serum leptin levels may not be simply dedicated to BFM or malnutrition status in cirrhosis or malignancy.

Cirrhosis is usually accompanied by anorexia and increased energy expenditure [48].

Liver cirrhosis is frequently associated with hypermetabolic syndrome that leads to increased energy expenditure [49].

The majority of cirrhotic patients unintentionally follow a low caloric diet, a fact that is attributed to various side-effects observed in cirrhosis. Loss of appetite, which is currently attributed to the presence of cytokines such as TNF-α, or alcohol-induced anorexia, is the most common reason [50].

The well-recognized hyperdynamic circulation in cirrhosis leads to a systematic vasodilatation and to an expanded intravascular blood volume. As a direct

effect, a higher heart blood volume and therefore a greater use of macro- and micronutrients is one of the most common causes of high energy expenditure and demand. Among cirrhotic patients, 34% are considered hypermetabolic with resting energy expenditure 120% of the expected value [51]. Elevated pro-inflammatory and anti-inflammatory cytokine levels point to a cytokine-driven hypermetabolism in cirrhosis [52].

Anorexia-cachexia syndrome appears to be multifactorial, often associated with the underlying disease process, and related to both peripheral and central neurohormonal signals regulating both appetite and energy expenditure. Inflammatory cytokines, such as TNF-α, IL-1, IL-6, and interferon- (IFN) γ have been postulated to play a key pathogenic role in the decreased food intake and increased energy expenditure seen in most chronic conditions associated with the anorexia and cachexia syndrome (ACS) [53].

Also, There is an evidence suggests that the central effects of leptin in suppressing appetite[54] and increasing energy expenditure via activation of Pro-opiomelanocortin (POMC) neurons is at least partially dependent upon inflammation [55].

In patients with liver cirrhosis of either alcoholic or viral origin leptin serum levels are inappropriately elevated, although the relationship with body fat mass is preserved [56].

Leptin serum levels increase as liver function worsens, reflecting a loss in the capacity to down regulate energy expenditure [56]. It is generally accepted that cirrhotic patients are wasted and may be over hydrated, thus the excess in the extracellular fluid that occurs in all stages of liver disease causes weight to height parameters such as BMI to be increased [48].

In our study we did not depend on BMI alone, but we measured the body fat percentage which could give us a more accurate reflection of wasting and malnutrition and we had found that serum leptin preserved a significant correlation with the body fat percentage.

MacCullough et al. reported modestly elevated circulating leptin levels in patients with alcoholic cirrhosis and they suggested that elevated serum leptin levels in cirrhosis might be responsible for the high prevalence of malnutrition among cirrhotic patients [46].

Campillo et al. studied serum leptin levels in alcoholic liver cirrhosis and concluded that there is a gender difference in regulation of serum leptin level increasing more in male patients. As Insulin level is the best determinant of leptin level in female patients while inflammatory state related to alcoholic hepatitis seems to have a greater influence in male patients. Although leptin levels positively correlated with resting energy expenditure (REE) in female patients, there is no evidence that leptin reduces caloric intake and fat stores in these patients [57].

However, in a study done by Nakamuta et al. about serum leptin level in non¬alcoholic patients showed that, the serum leptin level of patients with non-alcoholic liver diseases was not elevated. On the other hand, the serum leptin level of patients with alcoholic cirrhosis has been reported to be elevated. The difference in the serum leptin level of patients with nonalcoholic liver disease and that of patients with alcoholic cirrhosis may be due to difference in factors such as the level of cytokines or sex steroids and/or nutrition. Furthermore, it is likely that leptin is cleared in part by the portosystemic circulation through the liver [58].

In our study, we observed that the circulating leptin levels were increased in cirrhosis caused by viral hepatitis without sever energy malnutrition state. In this study, BFP was found to be associated with serum leptin levels of controls. Serum leptin levels among cirrhotic and malignant patients were different despite having similar BFP. So we conclude that leptin production may differ among healthy and cirrhotic patients as well as malignant patients.

The obese patients with NASH without fibrosis show an over expression of proinflammatory and

proapoptotic genes. Also, the NASH patients with fibrosis show an over expression of fibrogenic genes, including the leptin receptors Ob-Rb2 [59].

Henriksen et al. suggested that the elevated circulating leptin in patients with alcoholic cirrhosis was most likely caused by combination of decreased renal extraction and increased release from fat tissue areas [10].

In our study, we exclude cases with renal impairment to avoid accumulation of leptin in serum. Also we use three different sites of skinfold thickness measurement to calculate BFP and exclude cases of diabetes, hypothyroidism, and patients on contraception or corticosteroid treatment in the past 6 months.

The increase in leptin production that occurs during infection or inflammatory processes strongly suggests that, this adipokine is a part of cytokines network which governs inflammatory /immune response and host defense mechanisms. Indeed, leptin plays a relevant role in inflammatory processes involving either innate or adaptive immune response. Several studies have implicated leptin in the pathogenesis of autoimmune inflammatory conditions such as encephalomyelitis, type I diabetes, bowel inflammation and also articular degenerative diseases such as rheumatoid arthritis and osteoarthritis [60].

In the present study, we found that the ascitic leptin in cirrhotic patients was significantly higher than the malignant group. But, there was no significant correlation between ascitic and serum leptin.

Our findings are consistent with a study conducted by Giannini et al. which reported that in cirrhotic patients with sterile ascites, ascitic fluid leptin levels are remarkably higher than the serum levels. The ascitic fluid leptin levels are on average two times higher than circulating levels of this hormone [7].

Previous studies have shown that TNF-α is elevated in the ascitic fluid of cirrhotic patients with spontaneous bacterial peritonitis

(SBP)[61], and this cytokines likely derived from peritoneal cells[62].

Subclinical activation of ascitic fluid defense mechanisms from previous silent colonization by bacteria has been suggested in cirrhotic patients[63]. Indeed, higher baseline serum concentrations of TNF-α and IL-6 have been observed in cirrhotic patients with sterile ascites than in patients without ascites[64]. In addition, cirrhotic patients who subsequently develop SBP appear to have higher serum TNF-α concentrations and polymorphnuclear (PMN) cell counts in the sterile ascitic fluid than patients who do not [63].

Leptin secretion from adipocytes may be enhanced by cytokines released as a part of the inflammatory or fibrogenic process. Alternatively, as suggested, cirrhotic patients may simply exhibit decreased hepatic clearance of this protein [65].

TNF-α is also a major inducer of leptin[54], moreover, it has recently been shown that TNF-α polymorphisms represents a susceptibility genotype for insulin resistance and steatohepatites [66].

A study by Moradi et al. showed that the patients with ovarian cancer have elevated levels of IL-6 and TNF-α in serum and ascitic fluid [67].

This is in agreement with our study, which has shown significantly elevated serum TNF-α in the cirrhotic and malignant groups as compared to control group. There was no significant difference between ascitic TNF-α in the cirrhotic and malignant groups.

On the other hand, serum leptin levels have been reported to be significantly lower in patients with inflammatory states such as cancer[68] despite correction for body fat. These low levels of leptin, however, are not associated with greater appetite or lower energy expenditure, as might be expected. Disturbances in the feedback mechanism in the hypothalamus and/or release of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, are thought

to be responsible for cachexia in this setting [69].

Abramov et al. found low leptin levels in serum, pleural, peritoneal fluids in Meigs' syndrome as well serum levels were increased following surgical therapy of the tumor along the resolution of ascitic and pleural fluids. Suggested that mediators of the tumor tissue inhibit leptin secration and excision of the tumor tissue increase leptin levels [9].

Also, independently from the site of gastrointestinal tract, serum leptin concentration in advanced gastrointestinal cancer is lower than controls and it is not a determinant factor in weight loss [70].

Some available studies have shown that leptin stimulates ovarian cancer cell growth at least in vitro. That may be due to the role of these cytokines in malignant ascites[71]. As leptin increase the growth and migration and was antiapoptotic for cholangiocarcinoma cells, moreover, the loss of leptin function reduced the development and growth of cholangiocarcinoma [72].

In the present study, serum leptin levels of malignant patients are significantly lower than control and cirrhotic groups; also ascitic fluid leptin was significantly lower in malignant patients than in cirrhotic patients.

On other hand, we found that, the serum markers are more valid than ascitic fluid markers. As the sensitivity and specificity of serum leptin were 86 % and 77.3% and for serum TNF-α 51% and 76% respectively. Serum leptin emerges to have higher sensitivity and specificity than serum TNF-α. So, serum leptin is good positive while serum TNF-α is good negative in differentiation between benign and malignant ascites.

Summery

Our study has demonstrated that, females had a higher levels of serum leptin compared to males with highly significant difference between them in all groups. There is a strong statistically significant positive correlation between BFP and SFT versus serum leptin. Also, that serum and ascitic fluid

leptin of cirrhotic patients with sterile ascites are significantly higher than malignant patients. Serum and ascitic TNF-α were elevated in cirrhotic and malignant patients but with no statistical significance between both groups. The serum markers are more valid than ascitic fluid markers; serum leptin is good positive, while serum TNF-α is good negative. So, serum leptin appears to be more sensitive and specific as a marker in differential diagnosis between benign and malignant ascites.

Recommendation

The use of serum leptin as an additional non-invasive investigation to help to differentiate between sterile and malignant ascites. Large scale studies of serum leptin and TNF-α are necessary to verify and insure accuracy and possibility of using leptin and TNF-α as markers to differentiate between sterile and malignant ascites.

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LEPTIN AND TUMOR NECROSIS FACTOR (TNF-α) LEVELS IN BENIGN VERSUS MALIGNANT ASCITES

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POSSIBLE ROLE OF NITRIC OXIDE IN HEPATIC INJURY SECONDARY TO RENAL ISCHEMIA-REPERFUSION (I/R) INJURY

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Abstract

Objectives: *this work studied the possible role of nitric oxide (NO) in the pathogenesis of hepatic injury 2ry to renal I/R injury.* ***Methods:*** *48 Sprague-Dawley rats were divided into 4 equal groups; sham-operated, I/R injury group, I/R injury group with administration of L-arginine; 300 mg/kg IV 20 min before ischemia), I/R group injury with administration of N- omega-nitro-L-arginine methyl ester (L-NAME); 50 mg/kg in IV 20 min before ischemia). Kidney functions tests (serum creatinine and BUN), liver enzymes (ALT, AST) and malondialdehyde (MDA), catalase, reduced glutathione (GSH) and NO were measured 2 hrs after ischemia in liver tissues. Histopathology (H & E stain) of the liver was examined by a specialized pathologist.* ***Results:*** *compared to sham group, I/R group showed significant increase in kidney function tests and liver enzymes but without any change in liver histopathology ($p<0.001$). L-arginine significantly worsened the liver enzymes and histological parameters ($p \leq 0.028$) with improvement of the kidney functions tests ($p \leq 0.007$). Moreover, administration of L-NAME caused significant and marked worsening of liver enzymes and liver histopathology ($p \leq 0.008$). Compared to sham group, liver tissues from I/R group showed significant increase in MDA and NO with decrease in GSH ($p \leq 0.001$). L-arginine significantly decrease GSH and increase NO and MDA (p? 0.001), while, L-NAME group significantly decreased NO and GSH and increased MDA ($p<0.001$).* ***Conclusion:*** *Endogenous NO might have protective effect against hepatic injury induced by renal I/R injury and this probably might be due to increased formation of reactive oxygen species.*

Introduction

Renal ischemia-reperfusion injury (I/R) is an inevitable consequence of kidney transplantation and also results from systemic hypoperfusion with subsequent circulatory resuscitation and local renal hypoperfusion following partial nephrectomy and aortic cross-clamping[1]. Ischemia-reperfusion injury is a complex phenomenon that induces cell damage through a bi-phasic process. Ischemia initiates the injury by deprivation of the energy needed to maintain ionic gradients and homeostasis, which may ultimately, leads to cellular dysfunction and death. Reperfusion exacerbates this damage triggering an inflammatory reaction in which participate oxygen free radicals, endothelial factors and leukocytes[2]. Reactive oxygen species (ROS) and nitric oxide (NO) play an important role in mediating cell damage during I/R injury[3,4].

Liver and kidney are important regulators of body homeostasis and are involved in excreting the toxic products of metabolism and exogenous drugs[5]. Recent studies have suggested cross-talk between the liver and kidneys and found that any injury to either of them may affect the other. Liver injury is one of the distant-organ damages induced by kidney I/R. It has been demonstrated that renal IR injury causes a significant increase lipid peroxidation[6,7] and decrease in antioxidant enzyme activities8 in liver tissues. Recently, Kadkhodaee and his associates demonstrated significant decrease in liver GSH, as well as a significant increase in proinflammatory cytokines; TNF-α and IL-10 concentrations in liver after renal I/R injury [9].

Nitric oxide (NO) is an important mediator of the physiological and pathological processes in renal I/R injury[10-12]. There remains continuing uncertainty about the role of nitric oxide in renal I/R injury with theoretical and experimental evidences offering support for both toxic and protective role. Some studies have reported that NO induces cellular cytotoxicity and tissue injury via lipid peroxidation, DNA damage, and proapoptotic effects, which are part of I/R injury[13,14]. On the other hand, many studies

have demonstrated that increased NOS activity is associated with reduced I/R injury and increased blood flow in the ischemic region[15]. To the best of our knowledge, no study investigated the role of NO in pathogenesis of the hepatic injury secondary to renal I/R injury. So, the aim of this experimental study was to test if there a role for NO in pathogenesis of hepatic injury secondary to renal I/R injury in Sprague-Dawley rats.

Materials and Methods

Experimental animals :

This study was conducted on 48 adult male healthy Sprague Dawley rats weighing between 250-300 grams aging 4-6 months. Animals were bred and housed in the animal house of the Medical Experimental Research Center (MERC), Mansoura University, at a temperature of 20°C, fed a standard laboratory chow and had a free access to tap water. The protocol was approved by our local ethics committee.

Experimental design :

Rats were randomly allocated into 4 groups (12 rats each): Group I (sham); was subjected to all experimental procedure without any renal ischemia. Group II (control); was subjected to all experimental procedure with renal ischemia. Group III (L-arginine-treated): as control group with administration of L-arginine 300 mg/kg/bw in 0.5 ml saline via penile vein 20 min before ischemia. Group IV (L-NAME-treated): as control group with administration of N- omega-nitro-L-arginine methyl ester (L-NAME) 50 mg/kg/bw in 0.5 ml saline via penile vein 20 min before ischemia.

Renal I/R injury model

The rats were obtained from cages, kept for 30 seconds in a glass container containing a piece of cotton soaked with 10 ml of halothane. Then animals were maintained on sodium thiopental at a dose of 12 mg/100 g body weight injected intraperitoneally. After anesthesia, the animal was fixed in the supine position on a thermoregulated heating board to maintain body temperature at 37°C. To induce renal warm ischemia followed by reperfusion, a midline laparotomy was done, then both renal pedicles were

exposed and clamped for 45 min using a vascular Bulldog clamps. 2 hrs after release of vascular clamps (reperfusion), blood was collected for determination of blood urea nitrogen (BUN), creatinine and liver function tests. Moreover, kidney and liver tissues were harvested and prepared for histopathological examination and assay of NO and oxidants and antioxidants.

Biochemical assay :

Blood concentrations of creatinine, BUN, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by commercially available kits (Fortress diagnostics, UK).

Histological procedures :

After formalin fixation (10% phosphate-buffered) and dehydration, paraffin-embedded renal and hepatic sections (4μm) were stained by hematoxylin and eosin. Histopathology for all tissues was evaluated per section in at least 10 randomly selected non-overlapping fields at x 400 magnifications of the sections. Kidney tissues were evaluated for the presence of congestion. Tubules were evaluated for the presence of degenerative changes (vacuolization), tubular dilatation, luminal debris and cast formation, and loss of brush borders from proximal tubules. Liver sections were evaluated for the presence of congestion, cellular degenerative changes, cytoplasmic vacuolization and leukocyte infiltration.

Estimation of lipid peroxidation and antioxidant enzymes :

Liver was removed and kept in cold conditions (precooled in Petri dish inverted on ice). It was cross-chopped with surgical scalpel into fine slices in chilled 0.25-M sucrose and quickly blotted on a filter paper. The tissue was minced and homogenized in 10-mM Tris-HCl buffer, pH 7.4 (10% w/v), with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for assays of lipid peroxidation (MDA content) and endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GSHPx) by commercially available kits (Biodiagnostic Company, Dokki, Giza, Egypt).

Determination of nitric oxide level :

Nitric oxide (NO) easily breaks down in the presence of free radicals, hence nitrite levels were measured as a level of NO inactivated due to superoxide radical. Nitrite was estimated colorimetrically with the Griess reagent in homogenate at 540 nm. Nitrite is determined from the standard curve obtained using sodium nitrite as standard. The resulting equation is used to calculate the unknown sample concentrations (Biodiagnostic Company, Dokki, Giza, Egypt).

Statistical analysis :

The results were expressed as mean ± SD. One away ANOVA test with Tukey's posthoc test was used to find statistical difference among various groups. The null hypothesis was rejected at the 0.05 level of significance. SPSS 16.0 software was used for data analysis.

Results

Renal functions (serum creatinine and BUN) and liver functions (SGPT and SGOT)

Compared to sham group, serum creatinine and BUN were significantly high in control and all studied groups, 2 hrs after reperfusion ($p<0.05$). Compared to control group, serum creatinine and BUN were significantly attenuated in L-arginine group and enhanced in L-NAME group. Regarding, liver enzymes SGOT and SGPT, all groups showed significant increase in their serum level when compared to sham group ($p<0.05$). Compared to control group, SGPT and SGOT were significantly high in L-arginine and L-NAME group. Moreover, SGPT and SGOT were significantly high in L-NAME group compared to L-arginine (table 1).

Kidney and liver histopathological examination :

Tables 2 and 3 show the results of kidney and hepatic damage score. Compared to sham group, the weight of kidney was significantly high in control ischemic and L-NAME groups at 2 hrs after reperfusion. Also, kidney congestion, tubular vacuolization and dilatation, luminal casts and debris and loss of brush border were significantly high in all

studied groups compared to sham group. L-arginine group showed significant attenuation of tubular injury compared to control group. However, L-NAME group showed significant worsening of tubular injury at 2 hrs after reperfusion. Figure 1 shows samples of kidney tissues from different groups.

Compared to sham group, the weight of liver was significantly high in control ischemic and L-NAME groups at 2 hrs after reperfusion. Also, liver vacuolization and leucocyte infiltration were significantly high in ischemic control group at 2 hrs after reperfusion compared to sham group. Fatty degenerative changes, vacuolization and leucocyte infiltration were significantly high in L-arginine group compared to control group. Also, compared to control group the liver damage score was significantly high in L-NAME group compared to control ischemic and L-arginine group. Figure 2 shows samples of liver tissues from different groups.

Nitric oxide (NO), oxidative stress (MAD), and antioxidant (GSH, catalase) markers in liver tissues

The concentration of NO was significantly high in liver tissues of control ischemic group compared to sham group ($p<0.05$). Compared to control group, NO concentration was significantly low in L-NAME group and significantly high in L-arginine group ($p< 0.05$) (fig. 3A).

Compared to sham group, MDA was significantly high in liver tissues of all studied groups ($p \leq 0.001$). Compared to control group, MDA was significantly high in L-arginine group and L-NAME groups and it was significantly high in L-NAME group compared to L-arginine ($p < 0.001$) (fig.3B).

Compared to sham group, GSH and catalase were significantly low in liver tissues of all studied groups ($p \leq 0.01$). Compared to control group, GSH was significantly low in L-arginine and L-NAME groups ($p<0.001$) and but catalase showed no significant difference with control group (fig. 3C, D).

Table (1): Effect of renal ischemia on kidney functions (serum creatinine, BUN), liver functions (SGOT and SGPT)

	Sham group	**Control** group	**L-arginine** group	**L-NAME** group
Renal functions				
Serum creatinine (mg/dl)	0.475 ± 0.069	0.667* ± 0.13	0.577 ± 0.028	0.79*#$ ± 0.033
Serum BUN (mg/dl)	48.50 ± 5.63	82.15* ± 12.39	57.600*# ± 14.69	94.63*#$ ± 5.37
Liver functions				
SGOT (iu/ml)	318.75 ± 89.53	352.25* ± 144.35	543.00*# ± 111.13	797.00*#$ ± 153.01
SGPT (iu/ml)	139.75 ± 16.25	146.00* ± 65.61	221.00*# ± 49.92	395.00*#$ ± 80.44

All data expressed as Mean ± SD. One way ANOVA with Tukey's posthoc test
* significant vs sham group significant, # significant vs control group,
$ significant vs L-arginine group ($p < 0.05$). BUN= blood urea nitrogen

Table (2): Kidney histopathological damage score in different groups .

Group	**Kidney Weight (g/100g b.w)**	**Congestion**	**Tubular vacuolization and dilatation**	**Luminal debris and casts**	**Loss of proximal tubules brush borders**
Sham	0.42 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control	0.65 * ± 0.01	1.00 * ± 0.00	0.88 * ± 0.11	1.00 * ± 0.00	1.00 * ± 0.00
L-arginine	0.47 # ± 0.11	0.41 *# ± 0.16	0.14 * # ± 0.02	0.33 *# ± 0.26	0.41 *# ± 0.03
L-NAME	0.94*#$ ± 0.08	1.16*#$ ± 0.12	1.20 *#$ ± 0.01	1.69 *#$ ± 0.16	1.26 *#$ ± 0.14

All data expressed as Mean ± SD. One way ANOVA with Tukey's posthoc test
* significant vs sham group significant, # significant vs control group,
$ significant vs L-arginine group ($p < 0.05$).

Table (3): Liver histopathological damage score in different groups .

Group	Liver Weight (g/100g b.w)	Congestion	Vacuolization	Fatty degenerative changes	Leucocyte infiltration
Sham	3.29 ± 0.52	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control	4.97 * ± 0.91	0.00 ± 0.00	0.143* ± 0.04	0.00 ± 0.00	0.143 * ± 0.04
L-arginine	5.01 # ± 0.03	0.16*# ± 0.01	0.16* ± 0.01	0.16*# ± 0.01	0.16 *# ± 0.01
L-NAME	7.21*#$ ± 1.04	0.32*#$ ± 0.02	1.00*#$ ± 0.00	1.00 *#$ ± 0.00	1.16 *#$ ± 0.14

All data expressed as Mean ± SD. One way ANOVA with Tukey's posthoc test
* significant vs sham group significant, # significant vs control group,
$ significant vs L-arginine group ($p < 0.05$).

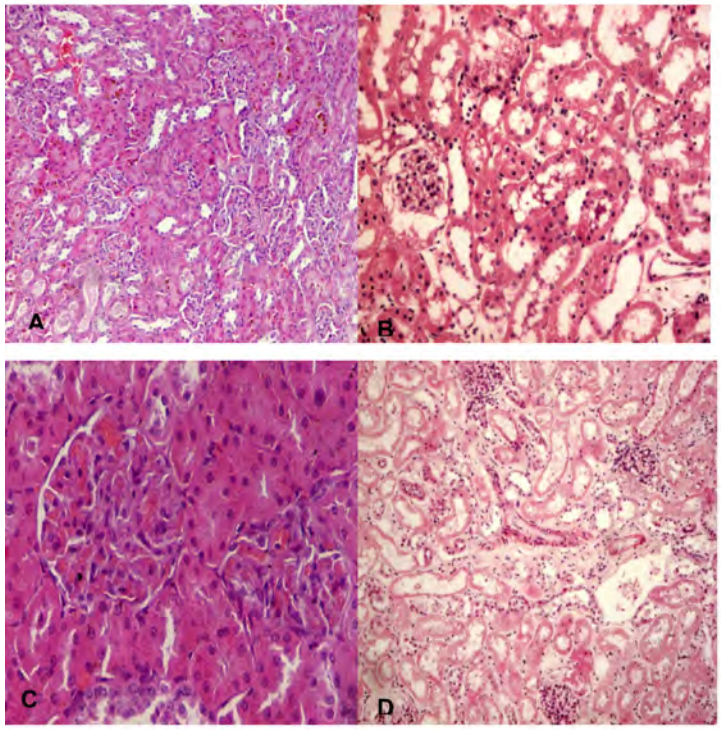


Fig. (1): Kidney specimens. (A)= normal kidney cortical area (H&E X200) (sham group); (B)= kidney with mild degeneration in the form of vacuolar changes in addition to focal areas of ischemia especially in the tubules (H&E X400) (control group); (C)= kidney with mild degeneration (H&E X400) (L-arginine group) , and (D) = kidney with more ischemic changes preserving the glomeruli more than the tubules (H&E X400) (L-NAME group).

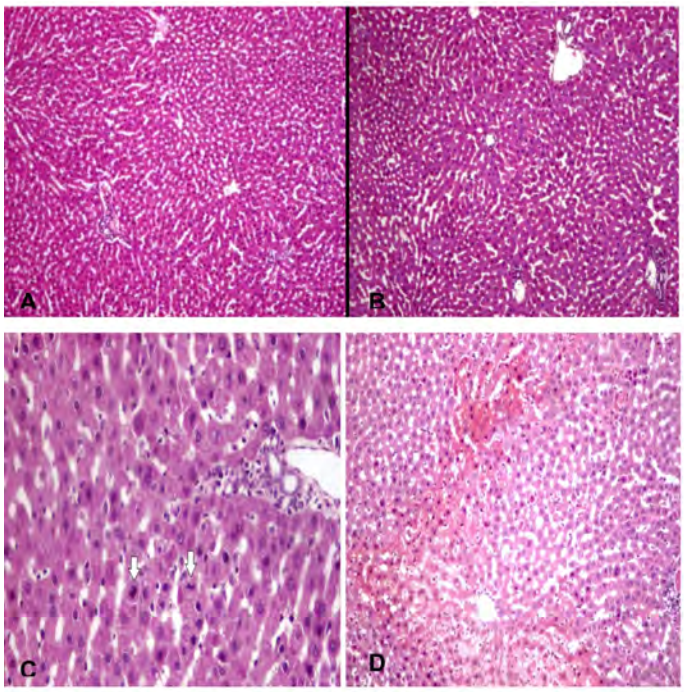
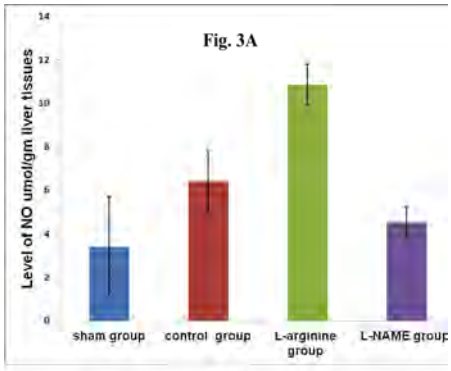
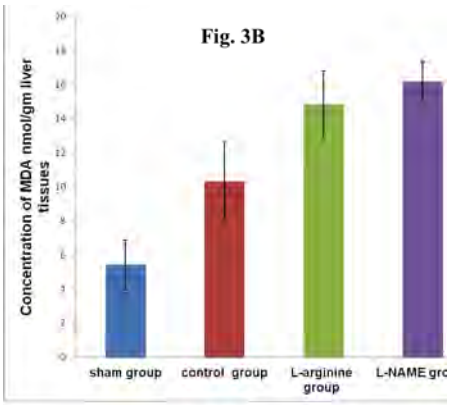
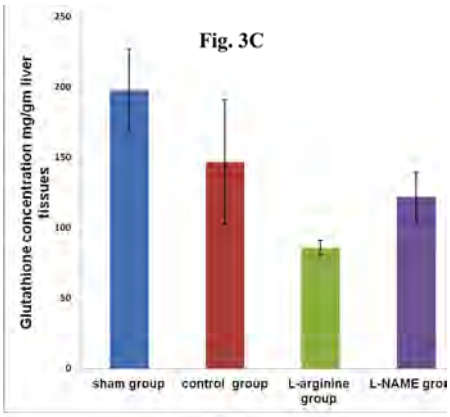


Fig. (2): Specimens of liver tissues. (A)= normally appeared liver architecture (H&E X200) (sham group); (B) = Normal liver with the characteristic pattern of the hepatocytes trabeculae between central veins and portal areas (H&E X200) (control group); (C) = Liver with focal degenerative changes in the form of hyperchromatic large nuclei and frequent mitosis (white arrows) (H&E X400) (L-arginine group); (D) = liver with focal ischemic changes mainly in zone 3 with areas of haemorrhage (H&E X200) (L-NAME group).







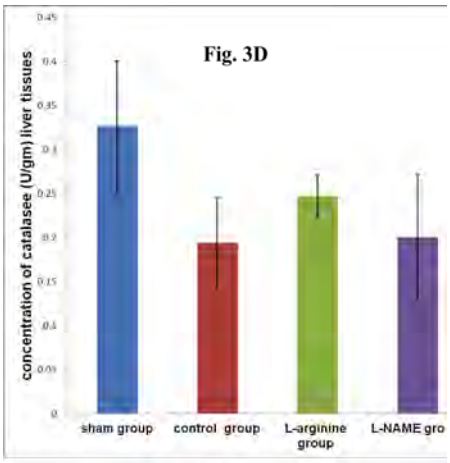


Fig. (3) : Oxidative stress markers in liver tissues from different group. A= concentration of NO, B= concentration of MDA, C= concentration of reduced glutathione, D = concentration of catalase enzyme* significant vs sham group significant, # significant vs control group, $ significant vs L-arginine group ($p<0.05$).

Discussion

Renal I/R injury is a common clinical problem that encountered in many conditions such as transplantation, partial nephrectomy, sepsis, hydronephrosis, or elective urological operations. Although, most research studies in this area has focused on the local renal response to this injury[16,17], recent studies emerge to investigate the remote effects of renal I/R injury on distant organs such as liver, brain and lungs[18]. In the present study, we investigated the role of NO in pathogenesis of hepatic injury secondary to renal I/R injury. We found that both exogenous administration of L-arginine (precursor of NO) and inhibition of formation of NO by L-NAME caused worsening of liver enzymes and liver morphology. Moreover, L-NAME effect was marked than L-arginine. These findings suggested that the endogenous NO has a protective effect against hepatic injury induced by renal I/R injury, while exogenous NO by L-arginine worsens the hepatic injury induced by renal I/R injury.

In the present study, we found that bilateral 45 min ischemia caused renal injury at the level of functions and morphology as evidenced by significant increase in serum creatinine and BUN as well as histopathological damage score in control group compared to sham group 2 hrs after reperfusion. These findings are in agreement with our previous studies[19]. Administration of L-arginine improved both renal functions and morphology, while administration of L-NAME caused more worsening in renal functions and histopathological damage score. These findings in agreement with our previous studies and concluded that exogenous NO has a protective effect against renal I/R injury. The beneficial effect of the NO precursor L-arginine on the course of I/R injury provides evidence of a functional NO deficiency. Decreased NO in the course of ischemic acute renal failure has been described in a variety of experimental models[20,21]. So, exogenous NO supply provided protective effect on renal functions and morphology.

Regarding hepatic injury secondary to renal I/R injury, we

found significant increase in liver enzymes but with little changes in hepatic morphology 2 hrs after reperfusion in control ischemic group. The minimal changes in liver morphology might be due to early scarifying of rats. These findings are in line with other studies[9,22]. Wang et al in 60 min unilateral ischemia model demonstrated significant elevation in liver enzymes; SGPT and SGOT and liver histological damage score 1, 4, and 8 hrs post-reperfusion and the significant change was at 4 hrs after reperfusion[22]. Moreover, they demonstrated significant elevation of MPO enzymes in early hours of reperfusion with the maximal elevation at 1 hr of reperfusion and suggested early migration and activation of neutrophils and in liver tissues. Also, liver histology on their study showed lymphocyte, monocyte, and especially neutrophil infiltration was observed in the liver central zone after renal I-R injury[22]. On the other hand, Kadkhodaee et al., investigated the effect of different periods (30 min, 45 min and 60 min) of bilateral renal ischemia on liver functions and histology. They concluded that minimum 45 min ischemia; 60 min reperfusion is needed to study the effect of renal ischemia on liver functions and histology[9]. That is why in our study we choose the model of bilateral renal ischemia for 45 min and 2 hrs reperfusion. Our results demonstrate impairment of liver functions and histology by renal ischemia for 45 min and reperfusion for 2 hrs. However, Serteser et al., 2002 concluded that 30 min renal ischemia, 60 min reperfusion is sufficient to elicit hepatic injury. This might be explained by difference in animal species used in each study. The present study and that of Kadkhodaee et al.,[9] used rat model but Serteser et al.,[8] used a mice model.

The main issue of the present study was to investigate the role of NO in pathogenesis of hepatic injury induced by renal I/R injury. This was done by administration of NO precursor (L-arginine and non-selective NOS inhibitor L-NAME) 20 min before renal ischemia. We found inhibition of NOS by L-NAME caused more significant damage in liver functions and histology. Unfortunately, NO

precursor L-arginine caused also more significant impairment of liver functions and histology. These findings suggested that the endogenous NO released in liver has a partial protective effect against hepatic injury secondary renal I/R. In the present study we found elevation of hepatic NO in I/R group compared to sham group. This elevation was significantly attenuated in L-NAME group and increased in L-arginine group. These findings suggested that endogenous production of NO might be one of endogenous adaptive mechanisms that are triggered in liver to partially protect it against injury secondary to renal I/R injury. Inhibition of this production by L-NAME worsens the liver injury secondary to renal I/R injury. Moreover, enhancement of this production by exogenous NO precursors e.g. L-arginine also cause worsening of the hepatic injury secondary to renal I/R injury.

It is believed that I/R injury induces inflammatory response, which elicits tissue damage in a number of organs in which reactive oxygen (ROS) and nitrogen species play a key role in the pathophysiology of renal IR injury[23,24]. So, the question here, does ROS has a role in this action of NO or not? We found in the present study significant elevation in MDA (marker of lipid peroxidations) in both L-arginine and L-NAME groups; however the elevation was significantly high in L-NAME. On the other hand, reduced glutathione was significantly low in both L-arginine and L-NAME groups compared to control group. But, catalase showed non-significant change in both L-arginine and L-NAME groups. These findings are in agreement with Yildirim et al.,[6]; Vaghasiya et al.[7] who demonstrated significant increase lipid peroxidation secondary to renal I/R injury. Also, Serteser et al.,[8] demonstrated significant decrease in antioxidant enzyme activities in liver while Kadkhodaee and his associates9 demonstrated significant decrease in liver GSH secondary to renal I/R injury. Although, the present study demonstrated beneficial role for endogenous NO, and possible role for ROS in mediating the injurious role for exogenous NO precursor NO, some questions are not answered in this study.

For example are there mediators such as IL1beta, IL-10 or TNF-α involved in this process or not? What is the source of ROS in liver is it from leucocytes infiltrating the liver tissues? The questions will be considered in further studies.

Conclusion

We concluded that liver is subjected to injury secondary to renal I/R injury. The endogenous NO has a protective effect against hepatic injury secondary to renal I/R injury. Moreover, exogenous NO has a protective effect against renal I/R injury, but it worsens hepatic injury secondary to renal I/R injury.

Acknowledgment:

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POSSIBLE ROLE OF NITRIC OXIDE IN HEPATIC INJURY SECONDARY TO RENAL ISCHEMIA-REPERFUSION (I/R) INJURY

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INTERLEUKIN 28B POLYMORPHISM PREDICTS TREATMENT OUTCOME AMONG EGYPTIAN PATIENTS INFECTED WITH HCV GENOTYPE 4.

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Abstract

Introduction: *Egypt has the highest prevalence of HCV worldwide (15%) with a high morbidity and mortality from chronic liver disease, cirrhosis, and hepatocellular carcinoma. The most prevalent genotype is 4, which responds less successfully to treatment with pegylated interferon & ribavirin for 48 weeks as it is effective in eradicating virus in only 40 to 50% of infected individuals. The Aim of this study was to investigate the role of genetic factor of IL-28B on responding to available treatment to HCV. Thus may help to provide a new guideline for treatment of HCV and reduce the cost-effective for interferon therapy.*

Methods: *HCV patients were genotyped as CC, CT, or TT at the polymorphic site, rs12979860 in unrelated case control of Egyptian population with HCV. The patients classified into two groups as follow:1st group; Chronic HCV patients with sustained virological responder to peg interferon regimen (SVRs) (N=87), 2nd group; Chronic HCV patients non responding to peg interferon regimen (non responders (NRs)) (N=135).*

Results: *In Egyptians, the CC IL-28B polymorphism was associated with the responding to peg interferon therapy. The frequency of allele C in SVRs was 77.6% and allele T 22.4% compared with 32.6% and 67.4% in NRs group. The carriage for at least one allele T of IL-28B locus was the strongest pretreatment predictor of NRs (Odds ratio, 3.75 with 95% confidence interval (2.308, 6.1067) and the risk ratio was*

1.6026, CI 95% (1.3402, 1.9163).

Conclusion: *The results suggest that host related factors, in particular IL28B play a crucial role in the treatment of HCV with Peg-interferon-α and it may predicts the treatment outcome.*

Introduction

Hepatitis C is a global health problem which affects a significant proportion of the world, accounting for 3% of the total population[1]. Egypt has the highest prevalence of hepatitis C virus (HCV) infection of any country in the world, with a seroprevalence ranging from 10% in children to 45% in adults[2]. More than 90% of HCV infections in Egypt involve genotype 4[2]. Several immunological factors have been implicated in determining disease outcomes in HCV infections[3]. Approximately 30% of individuals clear the infection naturally, whereas the remaining 70% develop chronic disease that may result in liver cirrhosis (LC) and/or hepatocellular carcinoma (HCC)[4].

The current standard-of-care for chronic HCV infection comprises weekly injections of Peg-interferon-α (peg-IFN α) in combination with daily oral ribavirin for 24 - 48 weeks[5,6]. Approximately 45% of patients infected with the most common form of HCV (genotype 1) achieve a sustained viral response (SVR) with this treatment. The effectiveness of this combination therapy has two main phases: a rapid first phase decline, referring to the viral decline during the initial day (s) after onset of treatment, and a slower second phase decline, which is usually defined as the reduction of HCV RNA levels from the second to the fourth week[7]. These phases are assumed to reflect, respectively, the antiviral action of interferon (first phase) and the loss of infected hepatocytes (second phase)[8].

Nevertheless, the current standard of care for CHC using pegylated (Peg) IFN plus ribavirin is expensive, is effective in only a certain proportion of patients who have CHC, and has many unpleasant adverse effects[9]. Thus, an understanding of the difference in host resistance to HCV

infection and in response to treatment would be clinically important and may lead to novel therapeutic interventions.

Several factors have been linked to the therapeutic response of patients who have CHC, including viral factors [10], host factors [11], metabolic factors [12], histological factors [13], types of regimen [9], and duration of infection [14]. Among these factors, viral kinetics following antiviral therapy has become widely accepted in both clinical trials and daily practice and increasingly recognized as the most outweighing predictor of sustained virological response (SVR) to IFN-based therapy [15].

Recently, several genome wide association studies (GWAs) have revealed that single nucleotide polymorphisms (SNPs) within or adjacent to IL28B predict spontaneous clearance of HCV[16,17] as well as the likelihood of SVR following therapy for chronic hepatitis C in different Western populations[18-20]. The IL28B gene encodes for interferon-λ3 (IFN-λ3), which constitutes the IFN- λ family together with IFN-λ 1 (encoded by IL29) and IFN-λ 2 (encoded by IL28A). The IL28A, IL28B, and IL29 genes are located on chromosome 19 (19q13) [21]. Several SNPs have been identified in the promoter region of IL-28B, some of these markers had highly predictive of SVR in HCV genotype 1 infected patients, i.e. rs12979860 and rs 8099917 markers. The present study was designed to investigate the association between IL28B-related SNP, rs12979860, as predictive marker for treatment outcome of HCV in Egyptian patients.

Patient Selection

This study is a part of the project on the liver disease Funded by the Mansoura university post graduate & research affairs in which 222 consecutive adult patients with chronic hepatitis C genotype 4 were included. The study was performed among patient attending outpatient clinic of Tropical Medicine Department receiving pegylated interferon therapy and ribavirin for 48 weeks. The study conducted between 2009 and 2012 in Mansoura. The inclusion criteria were as follows:

patients above the age of 18 years with positive HCV RNA in serum and elevated alanine aminotransferase (ALT) levels at least 6 months before the inclusion, chronic hepatitis confirmed by histological examination, body mass index (BMI) below 30 kg/ m^2. The exclusion criteria were as follows: decompensated liver cirrhosis, autoimmune liver disease, uncontrolled thyroid disease, alcohol abuse, liver cancer, hepatitis B virus or HIV coinfection, any severe chronic disease, hemochromatosis, and immunosuppressive therapy. Patients were treated for 48 weeks with standard of care medication: PegIFN α-2a 180 μg subcutaneously once a week (Hoffmann-La Roche, Basel, Switzerland); or PegIFN α-2b 1.5 μg/kg subcutaneously once a week (Schering-Plough Co, MSD, United States) plus oral ribavirin (1.000 mg/ day for patients with body weight < 75 kg; 1.200 mg/day for patients with body weight > 75 kg) for 48 weeks. Patients were classified into the following two groups based on treatment outcome: sustained virological responders (SVRs) and non- responders (NRs). SVRs had no evidence of viraemia at 24 weeks after completion of IFN therapy, whereas NRs were still viraemic at this stage. All subjects in the present study received a detailed explanation, and all signed a written informed consent. This study was approved by the local Ethical Committee, and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Efficacy Assessments:

Serum HCV RNA levels were measured by real time polymerase chain reaction assay (ABI 7300 ; limit of detection 15 IU/mL) at weeks 12, 24, and 48 during treatment and at week 72 after treatment. The primary end point was SVR, defined as undetectable HCV RNA at week 72[7].

Quantitative PCR:

Total cellular and viral RNA was isolated using RNeasy Mini columns (QIAGEN) followed by one step RT-PCR (Applied Biosystems; Foster City, CA), then quantited by 7300 ABI real time PCR using the DyNAmo HS SYBR Green qPCR Kit). The viral load was quantited against standard curve.

HCV Genotyping

Because the currently recommended treatment durations and ribavirin doses depend on the HCV genotype, HCV genotyping is recommended for patients considering antiviral therapy[22]. HCV genotyping was determined by VERSANT HCV Genotype Assay (LiPA), (Bayer Corporation, Tarrytown, NY, USA). The Amplicor HCV kit and the LiPA were performed according to manufactures' instructions.

IL28B GENOTYPING :

Only 222 patients have been genotyped for IL-28B rs12979860 polymorphism.The genomic region associated with HCV response[18] contains several highly correlated SNPs around the IL28B gene. We selected the most strongly associated SNP, rs12979860, located upstream of this gene for genotyping in our cohort using restriction fragment length polymorphism (RFLP) procedure[23] with some modification. DNA from peripheral blood mononuclear cells was isolated using the QIAamp DNA mini kit (Qiagen) and quantified on a GENWAY 6105 spectrophotometer. DNA samples were then genotyped for the IL28B rs12979860 polymorphism. PCR primers were designed to amplify products of exactly 151 pb using Primer3 (v. 0.4.0) (http://frodo.wi.mit.edu/) software to generate more appropriate allele-discriminating DNA fragments.

A DNA fragment of 151 bp containing the C/T polymorphism of IL28B rs12979860 was amplified using the forward primer 5'-GGTCGTGCCTGTCGTGTACT-3' and reverse primer 5'-AGGCTCAGGGTCAATCACAG-3' to generate 151 bp.

We carried out PCR using 1 ng DNA, 0.2 mM of each primer, 1X reaction buffer, 200 mM dNTPs, 2.5 mM MgCl2, and 1 unit of Taq DNA polymerase in 25-ul reactions. PCR amplification was done using a block thermal cycler (DNA Engine, MJ Research) programmed 35 cycles of 94 C for 1.0 min, 59 C for 30 s and 72 C for 30 s following a 10 min Taq activation step.

The PCR products (5 ul) was then electrophorsed using 1.5 agrose gel stained with ethidi-

um bromide to check a PCR reaction. Ten microliter of remaining PCR products were digested using 10x NE buffer and 2.0 U of appropriate restriction enzymes (Hpy166II). The reaction mixture was incubated at 37℃ for 4 hours according to manufacturer's instructions (New England Biolabs, Beverly, MA, USA). This enzyme will cut PCR product at T allele.

The resolved PCR digest was then visualized by staining with ethedium bromide on 2% agarose gels and examination under UV light. The 100 bp ladder was used as molecular size markers. The T allele (wild allele) was identified by the presence of two fragments 105 and 30 bps. Whereas C allele (rare allele) was identified by the presence of one fragment of size 135 bps figure 1. The 100 bp ladder was used as molecular size markers.

Liver Biopsy:

Liver biopsies were obtained with an 18-gauge or larger needle with a minimum of five portal tracts and were routinely stained with hematoxylin-eosin stain. Biopsies were interpreted according to the scoring schema developed by the METAVIR group. Needle liver biopsy specimens were examined by a pathologist unaware of the laboratory results. METAVIR score was used to stage fibrosis (F0-F4). Fibrosis was scored on a 5-point scale: F0, no fibrosis; F1, portal fibrosis alone; F2, portal fibrosis with rare septae; F3, portal fibrosis with many septae; F4, cirrhosis [24].

Statistical Analysis:

Statistical analysis was performed using the standard statistical software SPSS 10.0 (Chicago, IL). application program. Frequency tables were calculated to obtain information about the occurrence of the different risk factors and polymorphic variants between the three groups. The Hardy-Weinberg equilibrium was calculated to describe the relationship between gene frequency and genotype frequency. Genotypes were compared by using the Chi-square test or Fisher's exact test for categorical data and one-way analysis of variance (ANOVA) non-parametric test for continuous data. P values less than 0.05 were considered statistically significant.

Results

Descriptive Results.

We performed genotyping for IL28B rs12979860 in a total of 222 Egyptian with chronic active hepatitis C treated with combined peginterferon alpha and ribavirin. Our Cohort included 87 patients responded to treatment, and 135 non responders. The clinical characteristics of our study cohort of Egyptian patients with chronic active hepatitis C are summarized in Table 1. Eighty-seven patients (39.2%) responded successfully to HCV treatment (i.e. achieved SVR). The stage of fibrosis was not significantly associated with treatment failure.

IL28B rs12979860 Polymorphism

From 245 patients with HCV, we successfully genotyped 222 patients for IL28B rs12979860 Polymorphism using RFLP analysis procedure (figure 1).

The remaining 23 patients have been excluded from this study due to failure of DNA extraction for this group. The genotype frequencies were in Hardy-Weinberg equilibrium in both groups (P>0.05), and the polymorphism information content were 29% and 34% in SVRs and NRs groups respectively. The homozygosity were 66.7% and 49.6% among SVRs and NRs groups respectively Table 2.

The proportions of rs12979860 CC, CT, and TT genotypes were 59.7%, 34.5%, and 5.8% among patients with SVR, versus 7.4%, 50.4% and 42.2% among those with treatment failure (NRs). The frequency of wild type allele C was 77.6% in SVR while allele frequency of rare allele T in NRS group was 67.4%.

The association of rs12979860 SNP genotypes with the HCV peg-IFN-α/RBV treatment response have been evaluated. Overall, allele T carriers had a significantly higher risk of treatment failure than patients carrying the C genotype, Odds ratio (OR) 3.75 (CI, 95% 2.3,6.1) and P<0.0001 (Table 3). This suggests that this rs12979860 SNP may predict treatment failure before peg-IFN-α/RBV therapy. The odds and the risk of carriage for each genotype was also significantly different in

SVR group compared to NR group Table 3. p values were also significant after Bonferroni correction.

By univariate analysis, the minor allele of IL28B (p<0.0001), high serum level of HCV-RNA (p = 0.035), and advanced fibrosis (p = 0.02) were associated with NVR. By multivariate analysis, the minor allele of IL28B (OR = 20.83, 95%CI = 11.63-37.04, p <0.0001) was associated with NVR independent of other covariates.

Table (1) : Clinical and laboratory characteristics of two groups of patients with HCV.

	SVRs (n=87)	NRS (n=135)	P value
Mean age (SD)	42 (15.4)	44(13.4)	*N*
Gender n (%) male	66 (75.9)	108 (80%)	*N*
ALT IU/L (SD)	65 (9.5)	62 (11.5)	*N*
AST IU/L (SD)	55 (7.8)	59 (10.4)	*N*
HCV viral Load IU/ml(SD)	127,200	101,200	<0.05
Fibrosis stage n(%)			
2	51 (58.6)	71 (52.6)	*N*
3	36 (41.4)	64 (47.4)	*N*

SVRs = sustained virological response NRS = Non –responders

Table (2) : Distributions of the IL28B rs12979860 polymorphism among HCV patients.

	SVRs	NRs
Wild type (CC)	52 (59.77)	10 (7.41)
Heterozygous (CT)	30 (34.48)	68 (50.37)
Rare allele (TT)	5 (5.75)	57 (42.23)
Allele frequencies		
C	77.6%	32.6 %
T	22.4 %	67.4 %
PIC	0.29	0.34
Homozygosty	66.7 %	49.6%
PD	0.51	0.56
Hardy-Weinberg		
x2	0.059	2.89
P	0.81	0.089

PIC =polymorphism information content PD=Power of Discrimination

Table (3): Association between IL28 rs12979860 SNP and interferon response in HCV patients.

	OR	95 % CI		Chi-square	*P*
		lower	high		
Overall T	3.7546	2.3084	6.1067	28.51	<.0001
CT	1.9284	1.1058	3.3629	4.79	0.028625
TT	11.9846	4.5645	31.4667	33.18	<.0001

OR= Odds ratio

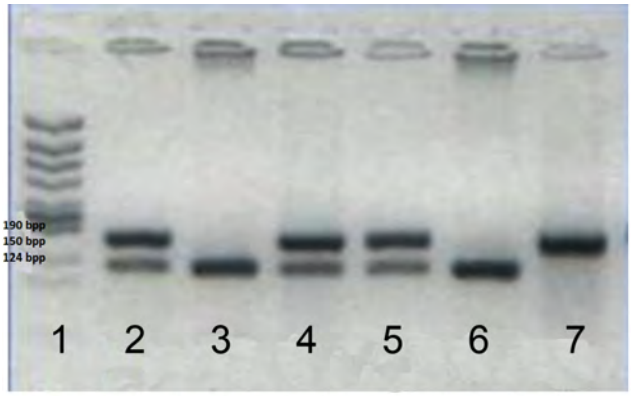

Figure 1 ethidium bromide stained agarose patients with different genotypes of IL-28B rs12979860
1=HAEIII DNA marker, 2,4,5=CT, 3,6= TT, 7=CC

Discussion

Various viral and host factors determine the outcome of IFN plus ribavirin therapy [25]. Ethnic differences in the response to IFN therapy and in the rate of spontaneous clearance in chronic hepatitis C suggest the influence of genetic factors[26]. Nowadays, several studies have reported that genetic variants near IL-28B are associated with response to treatment of chronic HCV infection with a combination of PEG-IFN and RBV[16,17]. The current study was conducted to explore the impact of the IL28B variations (rs12979860) on the therapeutic outcome in the Egyptian population infected with HCV genotype 4.

Previous studies have revealed a substantial impact of genetic polymorphisms near the IL28B

gene, including rs12979860 on the outcome of treatment with PEG-IFN-a and ribavirin in patients with chronic hepatitis C as well as on the outcome of acute hepatitis C in genotype 1 in Caucasians [18]. Ge et al.(2009) reported that among Caucasian patients with G1, the rs12979860 (3 kilobases upstream of the IL-28B gene) wild CC genotype was an independent predictor favoring SVR [18]. Also, McCarthy et al. (2010) demonstrated a similar finding with respect to off-treatment viral loads in Caucasian patients with G1 [27].

In the current study, we provide evidence for association of IL28B gene on the natural course and the success of Peg-IFN-α and ribavirin treatment outcome of HCV in Egyptian. In our data, the frequency distribution of rs 12979860 SNP in patients with chronic HCV who achieved SVR was 59.7%, 34.5% and 5.8% for C/C genotype, CT and TT genotypes respectively. However, in NRs, the frequency distribution of rs 12979860 SNP was 42.2%, 50.4% and 7.4% for T/T genotype, CT and CC genotypes. The frequency of T allele (rs12979860) was significantly higher among individuals with persistent HCV than those with cleared infection. The C allele had higher SVR rates while the carrying one or two T alleles had about 4 fold risk of failure to PEG-IFN-α and ribavirin treatment. This is in accordance with several global studies revealed that the C/C genotype is the major player in drug induced viral clearance, while both the C/T and T/T genotypes have a poor association with clearance rates [28-30].

The mechanism by which polymorphisms within this locus influence the outcome of therapy has not been established. One of the polymorphisms in strong linkage disequilibrium with the two SNPs is a missense substitution within the IL-28B coding region. Recently, Urban et al. (2010) reported that there was no difference in antiviral potency between wild-type IL-28B and amino-acid substituted variant in vitro using an HCV replicon system. On the other hand, it has been reported that genetic variation in the IL-28B locus is associated with expression

levels of IL-28B (plus IL-28A) IFN in peripheral blood mononuclear cells [19,20], although findings by Ge et al. (2009) are contradictory[18].

The rs12979860 allele is 3 kb upstream from the IL28B locus which also contains several genes, including IL28A and IL-29 [31]. It is likely that the SNP may also affect the function of other genes in the locus. Indeed, it has been reported that this variant is associated with increased serum IL-29 and IL-28A/B levels and the resolution of HCV infection [32]. These results suggest that the variations upstream of IL28B gene may have an impact on the expression and production of all IFNs, which may explain, at least in part, their profound association with the outcomes of HCV infection.

On the other hand, the IL-28B variant has recently been reported to be associated with treatment response following liver transplantation in patients infected with HCV [33,34]. These findings suggest that the IL-28B polymorphism may be associated with innate as well as adaptive immunity.

Potentially, this genotype could be associated with a weaker antibody response and a bias toward both innate and adaptive cell mediated immunity [35]. Interestingly, Zhang et al (2012) [36] demonstrate that IL28B inhibits HCV replication in three independent HCV models. Loss-of-function studies on the inhibition of the JAK-STAT pathway suggest that the suppression of HCV by IL28B is predominantly mediated by this pathway. Therefore, it is important to further elucidate the mechanism by which the gene variants regulate the expression of IFNs in HCV infection.

In conclusion, the data from the present study confirmed the association of C allele of rs12979860 in Egyptian patients with HCV and response to treatment with peginterferon and ribavirin combination therapy. Further studies will be needed to investigated the other SNPs closed to rs12979860 in promoter region of IL-28B gene in more large cohort (s). This will allow investigat-

ing whether this association is true or due to population stratification.

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Conflict of interest :

The authors who have taken part in this study declare that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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INTERLEUKIN 28B POLYMORPHISM PREDICTS TREATMENT OUTCOME AMONG EGYPTIAN PATIENTS INFECTED WITH HCV GENOTYPE 4.

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CLINICAL VERSUS LABORATORY PARAMETERS FOR PREDICTION OF DISEASE PROGRESSION IN SURGICAL NECROTIZING ENTEROCOLITIS

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Abstract

Aim: *To evaluate our experience in management of surgical NEC and whether clinical or laboratory parameter can predict the progression of disease in surgical NEC*

Patient and Methods: *Retrospective study was cared on 30 patients with surgical necrotizing enterocolitis in the form of abdominal wall erythema, positive paracentesis, abdominal lump and bowel perforation were included, patients developing enterocolitis after surgery were excluded. The patients were evaluated with clinical, laboratory and radiological parameters .These include age at presentation, stage, symptoms and sign at presentation with laboratory parameters of blood counts, pH, base deficit, platelet counts, electrolytes and random blood sugar levels. A comparison was done between clinical and laboratory parameters, and between survival and non survival patients and also between operated and non operated patients.*

Results: *There were 25 males and 5 females patients with mean birth weight of 1.9 kg. Age at presentation ranged from 3 to 20 days (mean: 9.3 days). Mortality was 33.3%.The neonates with severe disease had abdominal distention with wall erythema .43.3% of patients had shock at the time of presentation, also it was observed that neonates with acidosis and higher base deficit and had progressive fall in platelets counts had more chance of requirement of laparotomy. There was no statistical difference in birth weight, gestational age, total leukocyte count, serum electrolytes, blood sugar and other measured parameters.*

Also neonate not presenting with shock were more likely to survive.

Conclusion: *Clinical parameter assessment still remains the best to predict the disease progression in surgical necrotizing enterocolitis .*

Key words: *necrotizing enterocolitis, laboratory parameters, peritoneal drain*

Introduction

Necrotizing enterocolitis (NEC) is an acquired disease of the gastrointestinal tract that affects premature neonates in 90% of cases, with improvement of prenatal and neonatal care in the past two decades, more and more premature and critically ill neonates are surviving, therefore, the number of cases of ENC is increasing[1]. It is becoming the commonest neonatal surgical emergency. The risk factors are prematurity and low birth weight with their inherit immaturity of body systems including the pulmonary, gastrointestinal, and immune systems[2]. secondary risk factors are prematurity and early feeding of these neonates, especially feeding with hyperosmilar formulas. The diagnosis of the NEC become an easy task with the awareness of the disease and its commonly used triad of abdominal distention, intolerance to feedings, and increased gastric aspirate with or without bloody stools and with or without pneumatosis intestinalis[3]. various clinical and laboratory parameters used to decide surgical interventions in cases of NEC, surgical intervention is only indicated in about 40 to 50% of cases, depending upon the criteria used[4]. Identifications of such criteria can predict the progression of the disease need for laparotomy in cases managed with peritoneal drainage and ultimate outcome of patient. And also add a new dimension to the existing management protocol and helps in early recognition of deteriorating patient[5].

Patients and Methods

The study was retrospectively carried out at the department of pediatric surgery in zagazig university hospitals from January 2008 to December 2009. 30 patients was admitted during this time were included in the study. They had clinically proven

diagnosis of surgical NEC and fulfilled one or more criteria of surgical NEC in the form of clinical or radiological evidence of bowel perforation, positive paracentesis, abdominal wall erythema indicating bowel gangrene, presence of abdominal lump and patients not responding to conservative management with falling general condition. Patients with post operative NEC were excluded from the study.

The patients qualified for the study were evaluated with, clinical, laboratory and radiological parameters. the clinical parameters include gestational age, birth weight, and modified bell's stage of the disease at time of presentations. the laboratory parameters include, serial blood count, platelet count, electrolytes, blood PH, base deficit, blood culture, and random blood sugar levels. The radiological parameters include serial abdominal x-ray taken at 12 hour intervals. comparison was done from the data collected between (1) patients with primary peritoneal drainage versus patients with peritoneal drainage followed by laparotomy. (2) operated versus non operated patients [3] bell's stage 2 versus stage 3 patients[4] survivors and non survivors. Survivor was defined by patients who were alive at one month post discharge from the hospital.

Statistical Analysis

The data collected were analyzed using mean standard deviation (SD) for continuous data and percentages for categorical data. To compare two groups for categorical data, chi-square test was used. While for comparison of continues data. Mann-whitney test was used. $p>0.05$ was considered statistically significant.

Results

he demographic counts were as follow. There were 25 males (83.3%) and 5 females (16.7%). Birth weight was in range 1-3.5 kg (mean 1.9%kg). Age at presentation ranged from 3 to 20 days (mean 9.3 days). mean age for presentation for preterm patients was 9.1 days, while for full term patients was 9 days. Gestational age at time of presentations was 28 to 41 weeks (mean 34.12 weeks). The majority of patients

had presented with feed intolerance and abdominal distention. The patients with severe disease had abdominal distention with wall erythema. 50% of patients presented by shock at the time of admission. 5 of 30 patients were treated conservatively without any drain, they initially showed features of abdominal wall erythema and distention, they were stable and had good general condition, they were observed to improve in 48 hours.

The clinical data of patients managed by PD versus PD followed by laparotomy was compared, to give idea based on the parameters observed whether we can predict the need for laparotomy in patients managed by PD. patients who required laparotomy following peritoneal drain, had lower PH (P=0.059) and higher base deficit (P=0.39), they also had progressive fall in platelet counts but I did not reach to statistical significance (p=0.373) due to small sample size. There was no difference in birth weight, gestational age, total leukocyte count, serum Na+ and random blood sugar levels. Presence of shock at presentation, presence of positive blood culture. In our study we studied laboratory parameters to see there was difference in any parameter between operated and conservatively managed patients. All patients were operated were found to have either intestinal gangrene or perforation. And there is progressive increase in total leukocyte count, progressive fall in platelet count and PH were observed in operated cases. No significant difference in serum Na+ and blood glucose level between two groups.

In our study there is 20 survivors and 10 non survivor table (3) the survivor had higher mean birth weight (p=0.650) and gestational age (p=0.105) as compared with non survivor but there is no statistical significance may be due to small sample size. 80% of non survivor patients were presented by shock as compared with 25%in the survivor group. But there is statistical significance between the two groups, which include PH (P=0.03), base deficit (p=0.02), and number of patients presented with shock (p=0.002). patients who died remained more acidotic and

had higher base deficit as compared with survivor. laboratory parameters was studied to predict the progression of the disease table (4) in which there is correlation between Bell's stage II and stage III in which there is severe decrease in the platelet count in stage III with progression of the disease and there is higher base deficit in stage III. there is no difference in other laboratory parameter between two stages.

Table (1): Correlations of parameters between patients managed with peritoneal drain only and patients requiring salvage laparotomy.

	P D only (n=20)	**P D + LAP (n=5)**	**P value**
Gestational age	34.2±5	34.03±1.7	0.653
Age at presentation (wks)	10.5±4	5.7±5	0.273
Birth weight (kg)	1.8±0.5	2.05±0.2	0.253
Total leukocyte count	11.300±5.200	13.500±6.200	0.256
Platelet concentration	125.125±80.600	60.500±60.500	0.372
P H	7.3±0.05	7.22±012	0.0592
Serum Na+	137.6.5±6.7	135±8.5	0.621
Base deficit	6.2±2.5	11±4.5	0.0332
Random blood sugar	115±20	110±15	0.951
Positive blood culture	10 (33.3%)	2 (40%)	1.000
Shock	7 (35%)	2 (40%)	1.000

Table (2): Comparison between operated and non operated patients.

	Operated (n=10)	**Non operated (n=20)**	**P value**
Platelet concentration	80.750±22.700	125.600±80.200	-0.432
Total leukocyte count	15.300±3.200	10.500±2.500	0.085
Serum Na+	132±8.3	137±6.1	0.231
P H	7.2±0.07	7.33±0.06	0.142
Base deficit	8.6±4.3	6.5±3.7	0.261
Random blood sugar	105±30.5	110.5±25	0.653
Survival	5 (50%)	15 (75%)	0.103

Table (3): Correlations of parameters between survivor and non survivor.

	Survivor (n=20)	Non survivor (10)	P value
Gestational age (wks)	36.5±4.3	33.3±4.02	0.105
Birth weight (kg)	1.9±0.56	1.75±0.7	0.650
Total leukocyte count	11.500±5.700	13.300±5100	0.521
Platelet concentration	129.200±80.500	90.600±56.500	0.214
P H	7.3±0.05	7.2±0.10	0.003
Base deficit	5.21±2.4	11.23±4.2	0.002
Random blood sugar	98.5±27.43	115.4±27.2	0.120
Positive blood c/s	10(33.3)	5 (50%)	0.321
Shock	5 (25%)	8 (80%)	0.002
Stage II	7 (35%)	0	0.132
Stage III	13(65%)	10 (100%)	

Table (4): Laboratory parameters to predict the progression of the disease in NEC.

	Stage II (n=7)	Stage III (n=23)	
Platelet concentration	179.000±60,500	105.000±65,000	0.051
Total leukocyte count	10,500±2,700	12,000±5,200	0.76
Na+	134±5.2	135±7.0	0.381
Base deficit	3.3±2.5	8.2±4.3	0.122
Random blood sugar	90±15.4	110±25.4	0.124
P H	7.3±0.32	7.25±0.09	0.023
Survived	7 (100%)	13 (56.5%)	0.123

Discussion

NEC is the most common gastrointestinal medical and /or surgical emergency occurring in neonates. It accounts s for 1-5 of nursery admission[6]. The advances in modern neonatal intensive care units have resulted in better survival of both term and preterm infants. As a result of improved survival in low birth weight babies the incidence of NEC has increased[7]. NEC continues to present a diagnostic challenge to clinicians. Especially, early detection of intestinal necrosis requiring surgical treatment is still a key problem. The initial clinical manifestations of NEC are non specific and indistinguishable from other gastrointestinal disorders and sepsis[8] we conducted a retrospective

study in our pediatric surgical unit to know whether clinical or laboratory parameters can predict the progression of the disease in surgical NEC patients. and to know which patient need for peritoneal drain and patient required laparotomy and salvage surgery. Extensive studies have been done in the past regarding the severity indices and monitoring of NEC patients who are treated conservatively[9]. In our study, the patients managed by peritoneal drainage was 20(66.6%) and patient managed by peritoneal drainage and followed by laparotomy was 5 patients (16.6%) there is no significant difference between two groups in gestational age, age at presentations, and birth weight. but there statistical significance in P H and base deficit, in which patients managed by laparotomy more acidotic and with higher base deficient. In other series the favorable course in a patient with NEC with perforation managed with PD is to have a controlled fistula which gradually resolves over a period of time[10] in our study there is comparison between operated and non operated groups of surgical NEC, in which 20 patients not operated and 10 patients was operated, the operated patients was more acidotic and with higher base deficit, 5 patients (50%) from operated patients was survived and 15 patients (75%) from non operated group was survived ,also there higher increase in platelets concentration and decrease in total leukocyte count in non operated group, there is no significant deference in random blood sugar in both groups. Random blood sugar may be normal, elevated or decrease in NEC.This depend on the degree of the sepsis. Few studies report that random blood sugar decrease in patient with positive blood culture with G-negative bacilli,other cases of normoglycemia seen in patients with mixed infections and patient with hyperglycemia is seen in patients with E-coli sepsis[11]. In our study we do comparison between survivor and non survivor patients ,in which 20 patients (66.7%) was survived and 10 patients (33.3%) non survived. non survived patients had lower birth weight and lower gestational age but without statistical significance. 5 patients (25%) from survived group was presented by shock and 8 patients

(80%),in other series presentation of shock was (23.5%) in survivor and (90%) in non survivor, this means that presentation of shock is a grave prognostic factor for progression of the disease, also non survivor group more acidotic with higher base deficit . in the present series comparison was done between laboratory parameters of Bell's stage II and Bell's stage III, in which bell's stage III had lower PH, higher base deficit and high total leukocyte count .Bell's stage II had higher platelet concentration. All patients with Bell's stage II was survived but only (56.5%) of Bell's stage III was survived these results are compatible with results of other series. Mitul parikh[5], showed that 100% of patients with Bell's stage II was survived and 54.5% of Bell's stageIII was survived. Overall no single laboratory parameter can predict the progression of the disease in NEC but clinical parameters associated with laboratory parameters can give a good idea about progression of the disease in NEC. Recently there is non invasive markers (intestinal fatty acid binding protein I-FABP) which can diagnose and predict progression of the disease in NEC [12] but still under trial.

Conclusion

There is no single parameter can predict the progression of the disease in surgical NEC. And clinical parameters assessment still remains the best to predict the disease progression in surgical necrotizing enterocolitis.

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CLINICAL VERSUS LABORATORY PARAMETERS FOR PREDICTION OF DISEASE PROGRESSION IN SURGICAL NECROTIZING ENTEROCOLITIS

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AUDITORY MEMORY IN BLIND

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Abstract

Sixty Blind children aging 6-16 years were included in this study. All subjects were submitted to history taking, basic audiological evaluation and memory tests (recognition memory, memory for content, memory for sequence). Their results were compared to age normative data of age matching sighted children. It was found that blind children significantly outperformed the sighted children.

Introduction

Humans have a remarkable capacity to adapt to changes in environmental input, such as those associated with sensory deprivation. Blind individuals need to extract crucial spatial information from available sensory modalities as audition and touch to function in their surroundings. Auditory modality is of particular importance as it provides critical information for interacting with the environment and is essential for mediating important signals as those indicating a possible danger. In order to fully use auditory modality as a means of detecting potentially important signals, one has to efficiently react to changes in the auditory environment. Being very dependent on this modality, it is expected that the blind are superior to the sighted in various auditory functions [1].

A substantial amount of research studies have suggested that in absence of visual stimulation, auditory advantages develop that exceeds those of sighted individuals[2]. It has been argued however that advantages do not develop at all levels of auditory function; rather the early blind (EB) typically outperform sighted

counterparts on auditory tasks that require complex perceptual discriminations. For instance, the EB consistently show better performance than SC individuals on tasks that examine auditory memory [3], verbal memory[4] and auditory attention[5] but have comparable auditory detection thresholds and intensity discrimination[6].

The aim of the present study is to compare memory skills in blind and sighted individuals to ascertain whether visual deprivation is primarily responsible for any observed differences.

Methodology:

Subjects:

The study group consists of sixty normal hearing blind children aging 6 to 16 years with different causes and duration of blindness. They were divided to 3 subgroups (I: 6-8 years, II: › 8 -12 years, III : › 12- 16 years).

Methods:

All children participating in the study will be subjected to:

1. History taking including full medical and audiological history.
2. Otological examination.
3. Basic audiological evaluation:-
   a. Pure tone audiometry including air and bone conduction.
   b. Speech audiometry including Speech Reception Threshold (S.R.T) using Arabic bisyllabic words, and speech discrimination test using Arabic Phonetically Balanced Kinder Garten (PBKG) words [7].
   c. Immittancemetery including tympanometry and acoustic reflex threshold.
   d. Arabic Memory tests for recognition, content and sequence[8] .

Recognition memory test:

The subject was instructed to raise his hand each time he hears a repeated word in the same list.

Scoring for each list was done by subtracting wrong responses from eleven. Final scoring is reached by calculating the mean of the five lists.

Memory for content test:

The subject was instructed to repeat the whole list he had just heard irrespective of its sequence. The highest number of words the candidate could memorize was taken as his score.

Memory for sequence test:

The subject was asked to repeat the list he heard in the same order. Scoring was done on" all" or "none" basis. The list sequence was considered wrong with any wrong word arrangement. If the score of the memory for sequence test was six this means that candidate was able to repeat six words in the same order correctly.

Results

Table (1) : Mean, standard deviation of the study group and its comparison with the normative data for recognition memory test.

	Study		Control		t	P
	Mean SD	Range	Mean SD	Range		
I	10.13 (0.4)	9.2-11	8.8 (0.8)	7.6-10	11.04	0.00
II	10.4 (0.4)	9.8-11	9 (0.6)	7.9-10	11.8	0.01
III	10.7 (0.3)	10-11	10.2 (0.6)	9-11	4.9	0.00

The table shows significant difference between the study and the control group.

Table (2) : Mean, standard deviation of the study group and its comparison with the normative data for memory for content test:

	Study		Control		t	P
	Mean (SD)	Range	Mean (SD)	Range		
I	5.7 (0.7)	5-7	3.8 (0.6)	3-6	10.8	0.01
II	5.9 (0.8)	5-8	4.5 (0.8)	3-6	6.7	0.00
III	6.7 (0.67)	6-9	6.05 (0.6)	5-7	3.8	0.00

The table shows significant difference between the study and the control group.

Table (3) : Mean, standard deviation of the study group and its comparison with the normative data for memory for sequence test:

	Study		Control		t	P
	Mean (SD)	Range	Mean (SD)	Range		
I	5.2 (0.6)	4-6	4 (0.7)	3-5	7.4	0.00
II	5.6 (0.7)	5-8	5 (1.1)	3-7	2.8	0.00
III	6.8 (0.8)	6-8	5.6 (0.7)	4-6	5.9	0.02

The table shows significant difference between the study and the control group.

Discussion

In memory tests the blind tests scores were significantly higher than the study group in recognition memory, memory for content and memory for sequence, this goes with results of Roder and Neville 2003 who have shown longer short-term memory spans in the blind in tasks that require recall of items in their correct order, such as the digit-span or the word-span task [9]. Our results also goes with that of Raz et al. 2007 compared the performance of 19 congenially blind subjects and individually matched sighted controls in two types of memory tasks: item memory and serial memory. Overall, recall probabilities are higher in the blind across all item positions, but the serial position curves' structure is practically the same in the sighted and blind. Thus, it seems that the advantage of blind in item recall is not a result of a specific advantage in remembering the first words in the list, or the most recent words. Rather, the blind recall better all words, irrespective of their serial position. This suggests that the blind may represent item lists as chains of words, in their correct order ("chaining"), perhaps by generating associations between adjacent items. Thus, recalling an item increases the probability of recalling its following item (in all list positions). Also, the blind recalled markedly more words in sequences compared with their sighted peers [10].

The superior memory and specifically the serial memory is attributed to the fact that in the absence of vision, perception of space is likely to be highly depen-

dent on memory. Sighted people mostly code spatial information in the form of a global, externally based representation In contrast, the blind tend to code spatial information (especially of large spaces) in the form of a local, sequential representation based on routes. This may be a natural consequence of the fact that the path traveled by a blind person cannot be apprehended at a glance (e.g., from a mountaintop) but rather must be constructed serially out of segmented inputs from each location along the path. The blind also seem to adopt a serial strategy when encountered with a (small-scale) spatial imagery task compared with a more global map-like representation of the sighted [11].

Another situation that requires extensive use of serial memory strategies by the blind is the identification of objects that are distinguishable from one another only by their visual properties (such as different brands of yogurts that differ only in their color or written tag). According to their own reports, in order to correctly choose a desired item, the blind typically place such items in a fashioned order and give them ordinal tags, such as "the third item on the left" (thus, they use verbal labeling to define ordinal relationships among items within the scene) [12].

In this sense, our results are a classical case of "practice makes perfect" because the blind constantly use serial memory strategies in everyday circumstances, they develop superior serial memory skills that can also be used when required to recall a list of words as in the present study.

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COMPARISON OF PTEN EXPRESSION AND PTEN MUTATIONS IN BENIGN VERSUS MALIGNANT BREAST LESIONS

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Abstract

Purpose: *Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is one of the most frequently mutated human tumor suppressor genes. The present study aims at investigating role of PTEN mutation in breast carcinogenesis by analyzing PTEN mutation spectrum and PTEN protein expression in selected benign breast lesions versus breast cancer as regards comparison between PTEN immunohistochemical expression and PTEN mutations in studied cases.*

Patients and Methods: *Fifty cases of breast lesions including 30 non-consecutive retrospective selected breast carcinoma (10 cases intraductal breast carcinoma (DCIS). 20 cases of invasive breast ductal carcinoma) and 20 cases of benign proliferative breast lesions (10 fibroadenoma, 10 fibrocystic disease). 10 cases of non- neoplastic breast tissue adjacent to selected lesions were taken as control. Cases were collected in the period 2004-2010, selected from files of pathology department, faculty of medicine- Benha University and Egyptian national cancer institute (NCI). Results showed highly significant inverse correlation between PTEN immuno/expression and types of the disease ($P<0.01$). There is highly significant inverse correlation between PTEN expression and IDC grade, pathologic stage ($P<0.01$) as well as significant inverse correlation with lymph node status ($P<0.05$). Similarly, there is highly significant inverse correlation between inactivated/ lost PTEN DNA and tumor type as well as DCIS grade ($P<0.01$). There is significant inverse correlation between lost PTEN DNA and tumor grade& lymph node status, ($P < 0.05$).*

Conclusion: *PTEN is a negative cell cycle regulator and it is involved in early breast carcinogenesis by inactivated/ lost its expression as examined by both immunohistochemistry and PCR testing with significant values as regard tumor type, grade, lymph node status which can be used during therapy and patient's follow up.*

Key words: *Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), Duct carcinoma in situ (DCIS), tumor suppressor gene (TSG), invasive duct carcinoma (IDC), polymerase chain reaction (PCR).*

Introduction

The human breast presents many benign lesions that involve both the glandular and stromal tissues. These include fibrocystic disease, benign proliferative breast lesions, and other non-neoplastic lesions[18]. Fibrocystic changes affect more than 50% of women during their lifetime that composed of cystic dilation of ducts, apocrine metaplasia of ductal epithelium, fibrosis, adenosis, and intraductal epithelial proliferation [19,37].

The multistep model of breast cancer progression suggests a transition from normal epithelium to invasive carcinoma via intraductal hyperplasia and in situ carcinoma [10]. These precursor lesions are currently defined by their histological features. Ductal carcinoma in situ (DCIS) is a pre-invasive lesion with diverse histological morphologies and molecular alterations. The risk of DCIS progressing to invasive carcinoma is not well defined and biomarkers are capable of stratifying the most aggressive from the more benign forms of the disease are currently lacking[37]. Apocrine change or differentiation is characterized by cells that are larger than usual and have abundant, granular eosinophilic cytoplasm, distinct cell borders, and luminal apical blebbing. Nuclei are eccentrically located, variable in size and either vesicular or hyperchromatic, usually with prominent nucleoli. A second type of apocrine cell has a pale, foamy cytoplasm caused by numerous vesicles. The nuclei are similar to those of the first type [15]. Apocrine change occurs in a spectrum of benign lesions in the female breast and is also demon-

strated in a subgroup of in situ and invasive carcinomas [30].

Cells of apocrine carcinoma show strong cytoplasmic staining with antibody to gross cystic disease fluid protein-15, cytokeratin AE1, CEA, androgen receptor and c-erbB-2 oncoprotein. Vimentin and S100 are negative [3].

In 1997, phosphatase and tensin homologue deleted from chromosome 10 (PTEN) was identified as a tumor suppressor gene located on chromosome 10q23.3[25]. It encodes a lipid phosphatase that functions in the phosphatidylinositol 3-kinase (PI3K) signaling cascade. A broad spectrum of somatic PTEN deletions in primary human tumors has been reported, including missense mutations, null mutations, and truncations[14].

About 50% of women with germ-line mutations of PTEN gene (at 10q23), develop breast cancer. However, 29-48% of breast cancers display loss of heterozygosity in 10q23, and about 40% of breast cancers show a decrease or absence of PTEN protein levels at the time of diagnosis. Promoter hypermethylation has been identified as an alternative mechanism of tumor-suppressor gene inactivation, but its importance in PTEN silencing in sporadic breast carcinoma is unknown[17].

Aim of the Work

This study aims at evaluating role of PTEN in selected examined benign and malignant breast lesions as regards comparison between PTEN immunohistochemical expression and PTEN mutations by methylation/ inactivation in studied cases of breast lesions.

Materials and Methods

- **Clinical investigations and tissue samples:**

This study was based on 50 cases of breast lesions including 30 non-consecutive retrospective selected breast carcinoma (10 cases intraductal breast carcinoma (DCIS) and 20 cases of invasive breast ductal carcinoma) and 20 cases of benign proliferative breast lesions (10 fibroadenoma, 10 fibrocystic changes). 10 cases of non- neoplastic breast tissue adjacent to selected lesions were taken as control. Cases were collected in

the period 2004-2010. They selected from files of pathology department, faculty of medicine/ Benha University and Egyptian national cancer institute (NCI). Cases were selected according the availability of clinical and follow-up data. Staging was carried out according to the TNM classification system (Singletary, et al; 2003) into 10 cases of SII, 6 cases of SIII, 4 cases of SIV. Lymph node-positive cases were 15 and lymph node-negative cases were 5 cases.

Grading of DCIS was done according to four-tiered system: low (low cytonuclear grade), intermediate (intermediate cytonuclear grade), high (high cytonuclear grade, but not pure comedo i. e. not predominantly solid architecture or 50% ducts without necrosis) and very high (high-grade DCIS of 50% solid architecture with ducts bearing comedo-type necrosis). This classification system showed a strong relationship with development of ipsilateral recurrence according to Pinder, et al; 2010. Invasive breast cancers were classified based on Elston-Ellis classification system, grades I to III [13] which was performed by Muggerud, et al; 2010. Each specimen was assessed for tumor extension by inked specimen margin as well as extension into peri mammary tissue. Only patients with primary breast cancer who had not undergone previous irradiation or chemotherapeutic treatment were included in the study. Two experienced pathologists blindly and independently confirmed the histological diagnosis of each breast lesion and agreed on the grading. Normal breast tissue neighboring to tumor area in the examined specimens was used as control. Formalin - fixed, paraffin-embedded breast tissues were used. Three sections of 4 micron thickness were obtained from each case. One section was H & E stained for diagnosis and grading. Other sections were mounted on positively-charged slides, immunohistochemically stained with antibodies against PTEN (Clone 28H6, diluted 1 : 100, Novocastra Laboratories Ltd) using the Ultra Vision Detection System (Anti-polyvalent, HRP/DAB, ready-to-use, Lab Vision corporation).

Immunohistochemical staining : Paraffin-embedded tissue sections, 3-4 micron thick were mounted on positively- charged slides and heated at 60°C for 30 minutes then deparaffinized and rehydrated through a series of xylene and alcohol before staining. After antigen retrieval with microwave treatment in 10mM citrate buffer (Neo-Markers, Cat. ? AP-9003), pH 6.0, endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 minutes. Sections were washed 3 times with cold 0.01 M phosphate buffered saline (PBS). After blocking with 10% normal rabbit serum, sections were incubated with polyclonal antibody against PTEN. Slides were incubated for 1 hour with PTEN antibody. The prepared DAB-substrate-chromogen solution was applied and incubated for 5-15 minutes until color intensity has been reached. Lastly, sections were counterstained with Mayer's hematoxylin. Dako positive control slides including sections of a human normal breast tissue for positive expression of PTEN. Negative control was done by replacing the antibody by normal non-immune serum.

Interpretation and evaluation of PTEN immunohistochemical staining : PTEN positive cells showed cytoplasmic and/or nuclear brown stain. The surrounding microenvironment served as positive control and internal reference for semi-quantitative scoring of the degree of immunoreactivity in tumor cells. Specially, the cytoplasmic and/or nuclear immunoreaction was scored based on the intensity, ++ = positive (equal in intensity to normal epithelial cells), + = positive with reduced intensity as compared with normal epithelial cells, and a -ve = negative (no immunoreaction) [32].

PCR methodology:

All exons of PTEN gene were amplified by PCR with DNA extracted from 50 of human breast cancers and adjacent breast tissues, PCR products were then sequenced for mutation methylation screening.

Pyrosequencing :

A total of 1 µg of DNA was bisulphite converted using the MethylEasy™ HT Kit for Centrifuge Human Genetic Signatures, North Ryde, New South Wales,

Australia) according to the manufacturer's instructions. Quantitative DNA methylation analysis of the bisulphite treated DNA was performed by pyrosequencing or, in case of several sequencing primers, by serial pyrosequencing[36]. Oligonucleotides for PCR amplification and pyrosequencing were synthesized by Biotez (Buch, Germany) and sequences are given in Additional file 1. Quantitative DNA methylation analysis was carried out on a PSQ 96 MD system with the Pyro-Gold SQA Reagent Kit (Pyrosequencing, Biotage, Uppsala, Sweden) and results were analyzed using the Q-CpG software (V.1.0.9, Pyrosequencing AB). Unmethylated commercial DNA (Qiagen, Valencia, CA, USA) and mixed human lymphocyte DNA (Promega, Madison, WI, USA) was analyzed in parallel to define the technical background. CpG- values for tumor and normal tissue samples are given in Additional file.

DNA extraction: Proteinase K (Sigma) (20 mg/ml); 10-40 µl was added to the cell lysate. After incubation at 55°C overnight, the DNA was extracted with phenol/ chloroform and precipitated with cold ethanol. DNA was dissolved in water and the concentration was determined from its optical density.

cDNA synthesis and real-time PCR analysis

QRT-PCR was performed on 10 DCIS, 20 invasive duct carcinomas, 10 cases fibroadenomas and 10 cases fibrocystic disease. cDNA was synthesized in a total volume of 20 µl with 100 ng total RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems,) and used as template for real-time PCR analysis with the TaqMan Gene Expression Assay for PTEN gene(Hs00559473-s1, Applied Biosystems,) on an ABI Prism 7900HT sequence detector system (Applied Biosystems). Universal human reference RNA (Stratagene, La Jolla, CA, USA) was used to generate standard curves. Each sample was run in triplicate. Relative gene expression levels were determined using the standard curve method and normalized to the reference gene. [24,8]

Steps : 10 micron thick sections were used for DNA extraction and reaction was performed as described by (34). The integrity of DNA was confirmed by amplification of each sample with microsatellite primers. The presence of PTEN DNA was evaluated by PCR using degenerate primers for the detection of any known PTEN type. In this study we used a universal PTEN primer pair (termed "consensus primer"). All PCR reactions were performed in a total volume of 20 µl. PCR mixture contained 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 200 pmol each primer, 2 U Taq DNA polymerase and 100 ng DNA. The cycling conditions were 94°C for 45 s, 58°C for 45 s and 72°C for 45 s for 35 cycles. PCR products were electrophoresed on 1.5% agarose gel and visualized with ethidium bromide staining. The commercially provided positive controls for the identification of PTEN by Maxim Biotech, Inc (San Francisco, Calif, USA) were used as a positive control. Human liver tissues were tested as negative controls and were consistently negative.

Statistical analysis :

Statistical analysis was performed using the SPSS (8.0 for windows) software package according to Pearson's correlation coefficient. Correlation between several variables was computed using Fisher's exact test. P value less than 0.05 (<0.05) was considered significant and <0.01 was highly significant.

Results

A- immunohistochemical results: All examined 10 cases (100%) of fibrocystic changes showed positive PTEN expression, similarly examined 10 fibroadenoma cases (100%) showed positive PTEN expression(++).

- PTEN expression was seen in 8 cases (80%) out of 10 DCIS cases. Seven cases out of 20 cases of IDC cases (35%) showed positive PTEN immunostaining with reduced + positivity, this revealed highly significant inverse relationship between PTEN expression and the tumor type (P<0.01).

* In relation to the tumor grade, 3 cases (75%) of low/ intermediate grade of DCIS,

showed + PTEN expression. Among 6 cases of high grade DCIS, 5 out of 6 cases DCIS (83.3%) showed + PTEN expression, but this relationship is statistically insignificant (P> 0.05).

As regard IDC, 4 out of 6 GI cases (66.7%) showed + PTEN expression, 3 out of 12 GII cases (25%) showed +PTEN expression, conversely, all grade III cases showed negative PTEN expression. Thus, PTEN positivity was inversely correlated with tumor grade with statistically highly significant relation (P< 0.01).

* Stage II cases showed positive PTEN expression in 6 out of 10 (60%), stage III showed positive PTEN expression in one out of 6 (16.7%), and stage IV cases showed positive PTEN expression in 0 out of 4 (0%), This revealed a highly significant inverse relationship between PTEN expression and the tumor stage (P<0.01).

- Concerning to lymph node status, 4 out of the 5 lymph node negative cases (80%) showed positive PTEN expression, while it was seen in only 3 out of 15 lymph node positive cases (33.3%). This relationship was statistically significant (P<0.05). (Table 1).

B- Results of PCR for PTEN DNA (Table 2): By PCR, PTEN DNA was detected in all control cases, in all cases of fibroadenoma (10 cases) and fibrocystic disease (10 cases) (Fig 4) out of the 50 breast cases examined. Correlating the clinical aggressiveness with PTEN DNA detection, six cases of 10 DCIS cases (60%) were positive for PTEN DNA, while it was positive in 2(10%) cases of 20 IDC cases (Fig 5). This is a highly statistically significant relationship (p<0.001).

In relation to tumor grade, IDC cases showed PTEN DNA in 2 cases out of 20 cases IDC (10%) that wer of GI. Conversely, GII and GII cases were completely lost PETEN DNA positive pattern, this inverse relation with tumor grade was statistically significant (P< 0.05).

In relation to tumor TNM stage; Cases with stage II IDC showed preserved PTEN DNA in only 2 cases out of 10 (20%), while SIII and SIV cases showed lost PTEN

DNA in all cases examined. This relation was statistically non- significant (p> 0.05).

In relation to lymph node status: two case of node negative cases showed Positive PTEN DNA expression out of 5 cases examined (20%) while all node positive cases showed lost PTEN DNA in a statistically significant relationship (P< 0.05).

Table (1): correlation between PTEN immunoexpression and clinicopathological data in examined patients.

	Total	PTEN expression			P value
		Negative	+	++	
Type					
Fibrocystic changes	10	0	4(40%)	6 (60%)	< 0.01
Fibroadenoma	10	0	2(20%)	8(80%)	
DCIS	10	2	8	0	
IDC	20	13	7	0	
DCIS grade:					> 0.05
Low-Intermediate grade	4	1(25%)	3(75%)	0	
High grade	6	1(16.7%)	5(83.3%)	0	
IDC grade:					< 0.01
G I	6	2 (33.3%)	4(66.7%)	0	
G II	12	9 (75%)	3 (25%)	0	
G III	2	2 (100%)	0	0	
Pathologic stage.					< 0.01
Stage II	10	4(50%)	6(60%)	0	
Stage III	6	5(83.3%)	1(16.7%)	0	
Stage IV	4	4(100%)	0	0	
Lymph node status					< 0.05
Node negative	5	1(20%)	4(80%)	0	
Node positive	15	12(66.7%)	3(33.3)	0	

NB: Highly significant inverse correlation between PTEN expression and types of the disease ($P < 0.01$). There is highly significant inverse correlation between PTEN expression and IDC grade, pathologic stage ($P < 0.01$) as well as significant inverse correlation with lymph node status ($P < 0.05$).

Table (2): correlation between PTEN PCR expression and clinicopathological data in examined patients.

CLINICOPATH. VARIABLE	PTEN-PCR -VE	PTEN-PCR +VE	TOTAL	P VALUE	CHI-SQ
Type					
Fibrocystic changes	0(0 %)	10(100%)	10	P<0.01	13.29
Fibroadenoma	0(0 %)	10(100%)	10		
DCIS	4(40%)	6(60%)	10		
IDC	8(80%)	2(20%)	20		
DCIS grade:					
Low-Intermediate grade	0(0%)	4(100%)	4	P < 0.01	5.6
High grade	4(? %)	2 (? %)	6		
IDC grade:					
G I	4(? %)	2 (? %)	6	P<0.05	5.3
G II	12(100 %)	0(0%)	12		
G III	2(100%)	0(0%)	2		
Pathologic stage.					
Stage II	8(80 %)	2(20 %)	10	P>0.05	7.6
Stage III	6(100%)	0(0 %)	6		
Stage IV	4(100%)	0(0 %)	4		
Lymph node status					
Node negative	3(60 %)	2(40 %)	5	P<0.05	0
Node positive	15(100%)	0(0 %)	15		0

NB: There is highly significant inverse correlation between preserved PTEN DNA and tumor type as well as DCIS grade ($P < 0.01$). There is significant inverse correlation between preserved PTEN DNA and tumor grade& lymph node status, ($P < 0.05$).

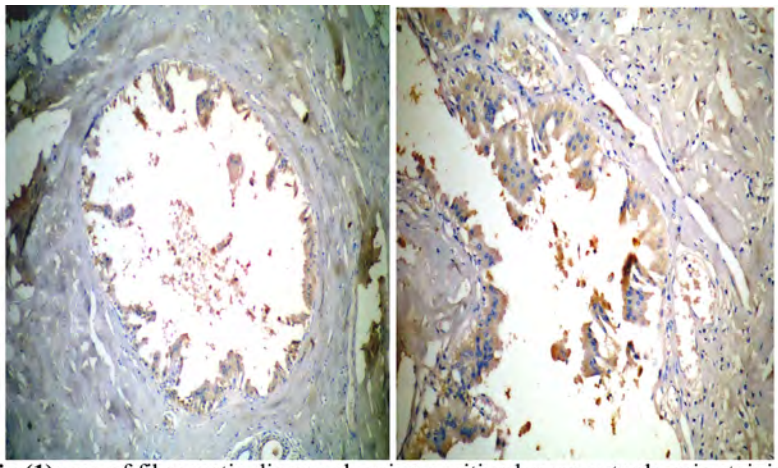

Fig (1): ase of fibrocystic disease showing positive brown cytoplasmic staining of ductal cells for PTEN ++ (streptavidin/biotin DAB, x400).

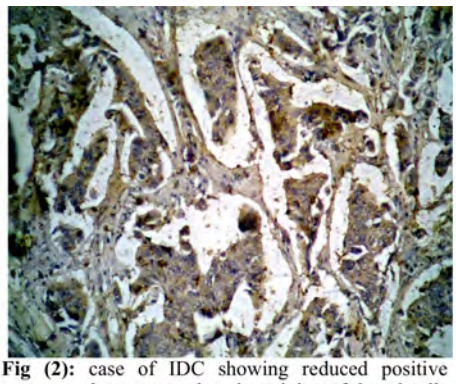

Fig (2): case of IDC showing reduced positive brown cytoplasmic staining of ductal cells for PTEN + (streptavidin/biotin DAB, x400).

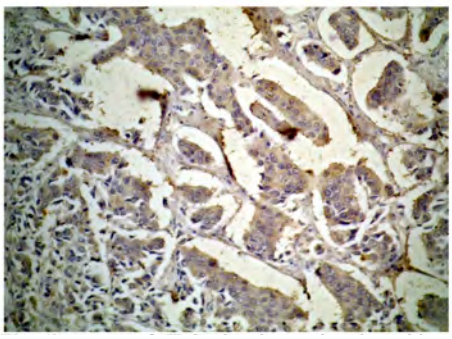

Fig (3): case of IDC showing reduced positive brown cytoplasmic staining of ductal cells for PTEN + (streptavidin/biotin DAB, x400).

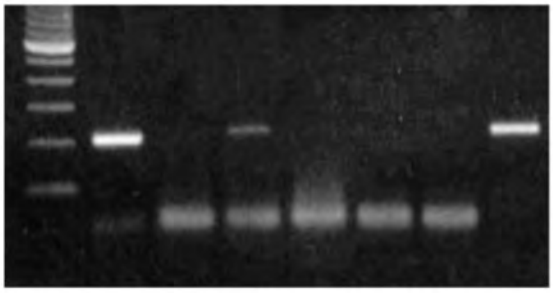

Fig (4): PCR for PTEN DNA in different examined breast cases. Lane 1, DNA marker (DNA ladder 100 bps); Lane 2, 4 positive samples. lines 3, 5, 6 (negative samples), lane 7 (negative control), and lane 8 (positive control).

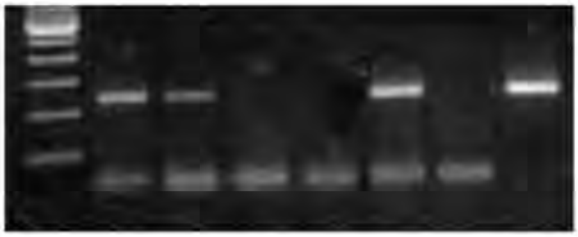

Fig (5): Results for PTEN DNA in different examined cases. Lane 1, DNA marker (DNA ladder 100 bps); Lane 2, 3,6 positive samples. lines 4, 5 (negative samples), lane 7 (negative control), and lane 8 (positive control).

Discussion

Phosphatidylinositol 3-kinase (PI3K)/AKT pathway aberrations are common in cancer. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) proteins is a negative regulator of Akt pathway, leading to suppression of apoptosis and increased cell survival. PTEN catalyzes the conversion of the membrane lipid second messenger PIP3 to PIP2 and is therefore a key mediator of the AKT/PKB pathway. In breast cancer, there is emerging evidence suggesting that loss of function of PTEN not only plays a role in tumorigenesis, but also it may be a key event in resistance to targeted therapy, its role as a tumor-suppressor gene has been adequately substantiated, and homozygous mutations have been demonstrated in familial and sporadic cancers [1,35].

Here we report PTEN expression in non-neoplastic and neoplastic tissues obtained from breast tissues; immunohistochemical staining of examined breast lesions showed highly significant inverse correlation between PTEN expression and types of the disease (P<0.01), all 10 cases of fibrocystic changes (100%) showed positive expression of PTEN ,all 10 cases (100%) of fibroadenoma showed positive PTEN expression. Examined cases of DCIS showed positive PTEN expression in 80% of cases, similarly 7 out of 20 cases IDC (35%) showed (+) PTEN expression, these results was in agreement with Frattini, et al; 2007[27] who reported that Normal PTEN expression was documented in non- neoplastic colonic and breast disorders mainly in non specific colitis as well as benign lesions as colonic adenomas without high dysplastic features, while loss of PTEN protein expression was found in 74% of studied colorectal carcinoma patients.

Highly significant inverse correlation (p<0,01) between PTEN expression and IDC grade was found, 4 out of 6 cases (66.7%) GI cases showed (+) PTEN expression, grade II cases showed (25%) (+) PTEN expression while all grade III cases were negative for PTEN immunostaining. Similarly, highly significant inverse correla-

tion between PTEN expression pathologic stage ($P<0.01$) was reported, this was consistent with results done by Rita et al; 2010 who found that PTEN immunoexpression is a strong predicator for tumor progression as it is markedly under expressed in poorly differentiated cases of lung carcinomas and gastric carcinomas as well as associated with high pathologic stage. Significant inverse correlation between PTEN expression and lymph node status ($P<0.05$) was reported as it was found that among 5 cases with no lymph node metastasis, 4 cases (80%) showed (+) expression of PTEN, 3 cases (33.3%) out of 12 cases examined with lymph node metastasis showed (+) PTEN expression In parallel to the current results, Khatib et al, 2011[23] reported that there was loss of PTEN expression in patients with breast cancer which was statistically significant ($p < 0.05$), but in converse to the current study no significant correlation between PTEN loss and grade or stage was found. Moreover, Julio et al; 2012[21] hypothesize PTEN expressions have prognostic significance in breast cancer; there was positive correlation between the IHC results and the RT-PCR values PTEN. Tumors with homozygotic deletion of PTEN expressed little or no mRNA or protein, and both parameters were correlated with poor patient's outcome.

Naguib et al, 2008 explained that frequent occurrence of PTEN loss in tumors as in colorectal cancers and pancreatic adenocarcinoma is associated with poor patient's outcome and poor response to tumor therapy. Moreover, malignant cases expressing PTEN are always surrounded by lymphoid and stromal cells which are expressing PTEN. The normal stromal cells exhibit strong nuclear and cytoplasmic staining. The adenoma cells appear to have reduced nuclear but retained cytoplasmic staining, whereas the invasive cancer cells have lost both nuclear and cytoplasmic PTEN expression.also showed an association of loss of PTEN expression with Dukes' stage and colorectal location, indicating a worse prognosis.

In breast cancers, many authors judged that expression of

PTEN protein is lost / or reduced. Somatic mutations are, however, rarely found. Our study was therefore designed to determine if differential methylation of the PTEN promoter region has a role in the transcriptional inactivation of the gene in invasive breast carcinomas as compared with non-malignant lesions. We investigated PTEN promoter inactivation methylation including mRNA levels of PTEN. This study, a methylation-specific PCR assay, was carried out with methylated specific primers designed in a region with scarce homology with the psiPTEN pseudogene. Expression was analyzed by real-time PCR. A total of 20 samples of invasive human breast cancer, 10 cases of DCIS, 10 cases of fibrocystic diseases, 10 cases of fibroadenoma and 10 control cases were studied for methylation of the PTEN promoter by methylation-specific PCR and compared to PTEN protein expression by immunohistochemistry. We found by PCR that PTEN DNA was detected in all cases control group and in all cases of fibroadenoma (10 cases) and fibrocystic disease (10 cases) out of the 50 breast cases examined. Correlating the clinical aggressiveness with PTEN DNA detection, six cases of 10 DCIS cases (60%) were positive for PTEN DNA, while it was positive in 2(10%) cases of 20 IDC cases with highly statistically significant relationship ($p<0.001$), this result suggests that PTEN mutation by inactivation loss may be an early event in neoplastic progression in the breast carcinoma as found in other carcinomas like vulval and cervical carcinomas in situ progression to invasive carcinomas, this result was in agreement with results done by Li et al., 1997[25] who found PTEN gene lost function by methylation was seen in 75% of invasive vulval carcinomas versus in situ cases, In relation to tumor grade, IDC cases showed PTEN DNA in 2 out of 20 cases IDC (10%) that were of GI. Conversely, GII and GII cases were completely lost PTEN DNA positive pattern, this inverse relation with tumor grade was statistically significant ($P< 0.05$). Cases with stage II IDC showed preserved PTEN DNA in only 2 cases out of 10 (20%), while SIII and SIV cases showed lost PTEN DNA in all cases examined. This was statistically non- significant

(p> 0.05), these results were consistent with Phuong et al., 2011 [31] who concluded that PTEN mutations by methylation were integrated in breast carcinogenesis, moreover, cases with decreased and lost PTEN expression as detected by PCR method were resistant to Tamoxifen therapy. Chuan-Yong et al., 2008[11] found that abnormal expression of PTEN protein is usually found in gastric cancer and related to tumor differentiation, infiltrating depth, lymph node metastasis and pTMN staging. PTEN may play an important role in the occurrence and development of gastric cancer. PTEN protein expression phenotype can be considered an indicator for the pathophysiological behavior of gastric cancer. These results coincide with the present results. In relation to lymph node status: two case of node negative cases showed Positive PTEN DNA expression out of 5 cases examined (20%) while all node positive cases showed lost PTEN DNA in a statistically significant relationship (P< 0.05). this coincide with Antonia et al., 2000[4] who stated that PTEN mutations can be involved in clinicopathological parameters as regard tumor staging and lymph nodal deposits in many cancers as vulval carcinomas and breast carcinomas. Chuan-Yong et al., 2008[11] concluded that inactivation of PTEN induces infiltration and metastasis of tumors. PTEN restrains attack and metastasis of tumor cells by regulating matrix metalloproteinase (MMPs) and vascular endothelial growth factor (VEGF). Abnormal expression of PTEN protein increases synthesis of MMPs and VEGF, thus leading to attack and metastasis of tumor cells. PTEN can also selectively increase dephosphorylation of focal adhesion kinase (FAK) to reduce cell transference by phosphated FAK. Besides, PTEN protein and tensin have a homologous sequence. Tensin is a cell matrix protein, which participates in adhesion to cells and extracellular matrix (ECM). These results can support our results as regard lost PTEN DNA in all cases with high/ grade DCIS, similarly increased tumor grade, tumor stage, lymph nodes invasion in cases with lost PTEN gene expression as detected by either Immunostaining or by PCR method.

We concluded that PTEN gene mutations by inactivation as well as decreased versus lost PTEN protein immunoexpression are common events in early breast carcinogenesis and correlating with other well-established prognostic factors of this malignancy. Additionally, PTEN gene mutations are found in cases with DCIS and coincide with their progression to invasive carcinoma.

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COMPARISON OF PTEN EXPRESSION AND PTEN MUTATIONS IN BENIGN VERSUS MALIGNANT BREAST LESIONS

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LAPAROSCOPIC RECTOPEXY FOR COMPLETE RECTAL PROLAPSE

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Abstract

Background: *Controversies regarding the management of rectal prolapse continue to stimulate interest in the study of its surgical treatment, as there is no single operation that is suitable for every patient with prolapse. With the evolution of laparoscopic surgery, both laparoscopic resection rectopexy and laparoscopic rectopexy with sutures or with mesh without resection has been described.*

Patients and Methods: *This study included forty patients with complete rectal prolapse. Patients were divided into two groups; Group (A); were operated by laparoscopic rectopexy without resection, 8 patients were operated by suture rectopexy, and 12 patients were operated by mesh rectopexy. Group (B) [20 patients]; were operated by laparoscopic sigmoidectomy and suture rectopexy for patients who have redundant sigmoid colon.*

Results: *Constipation improved in 76% of patients and with more improvement in patient with resection rectopexy than with mesh or suture rectopexy. Fecal incontinence improved in 75% of patients regardless of which method was used. No recurrence was found in any of our patients post-operatively during the period of follow up.*

Conclusion: *Laparoscopic surgery for rectal prolapse is a technically feasible method which resulted in improved constipation and incontinence in the great majority of patients and eliminate the prolapse with nearly no recurrence.*

Introduction

A number of surgical procedures have been developed to treat rectal prolapse, but there is still no agreement on the operation of choice. Because recurrence is generally lower with the abdominal approach, it is considered by some surgeons as the treatment of choice for rectal prolapse and resection rectopexy seems to be the best procedure as regards functional outcome. However, the need for a laparotomy wound represents a potential source of significant mortality and morbidity, which minimizes the role of transabdominal approaches in older, debilitated patients[1].

Since its first description in 1991, laparoscopic colorectal surgery has met with considerable reservations by the surgical community. A number of factors are responsible for this. First, the learning curve is longer than for laparoscopic cholecystectomy, and surgeons require additional training. Second, very little long term data exist for patients who have undergone laparoscopic surgery to prove its appropriateness. Third, cost effectiveness of the laparoscopic technique in comparison with the open technique is still in dispute. These concerns, however, are beginning to be resolved. Laparoscopic surgery for colorectal disease has now become a variable alternative to the traditional open technique[2].

With the evolution of laparoscopic colorectal surgery, the laparoscopic mesh fixation rectopexy and the laparoscopic suture rectopexy without the use of mesh, have been the more common procedures used. With improvement of laparoscopic experience, a few cases of laparoscopic resection rectopexy have been reported[1].

This study was aimed for evaluation of the outcome of both operations as regard; complications, recurrence and functional results were recorded.

Patients and Methods

This study included 40 patients that had been subjected to the following: Clinical history: including symptoms of the prolapse (as prolapsed mass, mucous discharge, bleeding per rectum or anal pain), constipation or difficult

evacuation and fecal incontinence using the scale described by Browning and Parks[1] four grades of anal continence were given to describe the severity of incontinence: Grade I : Continence to flatus, liquid and solid stool. Grade II : Incontinence is to flatus, but continent to liquid. Grade III: Incontinence is to flatus and liquid stool. Grade IV : Incontinence is to flatus, liquid and hard stool (continuous fecal leakage). Also any past history of anal operation or obstetric trauma in females was considered.

Clinical examination: including general examination to assess fitness for the operation and local examination Fig (1) which was including Inspection for prolapsed mass with or without straining, patulous anus, scar of a previous operation or mucous discharge or pruritis and palpation for resting tone (external sphincter integrity), squeeze tone (internal sphincter integrity), sphincter relaxation during straining, ano-rectal ring, pin prick touch for examination of perianal skin sensation, ano-cutaneous reflex, rectal mass or rectocele.

Pre-operative investigations: including routine investigations to assess the patients' fitness for general anesthesia, barium enema which was done for detection of redundant sigmoid colon or other associated lesions and sigmoidoscopy which was performed for detection of prolapsing mass especially during straining and exclusion of associated lesions.

Pre-operative preparations: All patients received standard bowel preparation and preoperative antibiotic prophylaxis. In our study patients was divided into two groups: Group A; patients operated by laparoscopic rectopexy alone using either suture or mesh rectopexy for those with normal sigmoid colon. Group B; patients with redundant sigmoid colon operated by laparoscopic sigmoidectomy and suture rectopexy.

Operative procedures: Patients position: Patients are placed in lithotomy position with the head down tilt. Trocar placement: Supra-umbilical incision is made for CO_2 insufflation by Veress needle. The main working port is 10mm trocar placed low

and lateral in the right iliac fossa. A third 5mm trocar is placed in the mid-clavicular plane on the right side, few centimeters below the umbilicus; this is the second working trocar. A fourth 5mm trocar is placed in the anterior axillary line few centimeters below the umbilicus, used by the assistant for traction on the left side. An optional 5mm port can be placed in the midline suprapubic region if needed. Laparoscopic rectopexy: Fixation is now carried by either; Suture rectopexy: The rectum is held on light tension using the laparoscopic Babcock forceps and two or three 2-0 non absorbable (Polypropylene) sutures are placed anchoring the mesorectum to the fascia just below the sacral promontory Fig (2). Mesh rectopexy: A strip of polypropylene mesh about 6 x10 cm tightly rolled, introduced like a cigarette, is inserted into the abdomen in the presacral space and attached to the presacral fascia and sacral promontory periosteum using non absorbable 2-0 (Polypropylene) or delayed absorbable (polyglactin 910) stitches. After determing that the mesh was firmly attached to the sacrum, the rectum was held under tension and the mesh was fixed to the seromuscular lateral wall of the rectum using three non absorbable 2-0 (Polypropylene) or delayed absorbable (polyglactin 910) stitches. Colopexy: It is an additional step, which was performed by fixing the stretched pelvic colon to the peritoneum and other abdominal wall layers in the left iliac fossa through small 3-4cm incision in the left iliac fossa Fig (3). Laparoscopic assisted resection rectopexy: The rectum is held stretched tight in a cephalad direction to choose the appropriate level of resection at the pelvic brim. The peritoneum over the mesorectum is then divided at this level, and the avascular plane is developed between the rectum and mesorectum. The rectum is divided first, with a 30mm to 35mm endoscopic stapler (Endo GIA II Autosuture European Services staple length, 3.8mm). Usually two serial applications of the bowel stapler are required to traverse the rectum. The divided proximal end of the colon is now held with atraumatic grasper and delivered via a 40 to 50 mm muscle splitting incision in the left iliac fossa. The specimen of the colon is divided

50mm above the skin, and the anvil of a 33mm circular stapler (Premium Plus CEEA-Auto Suture Company) is secured with a 2-0 (Polypropylene) purse string suture. The shaft of the circular stapler is passed per rectum, and the spike was brought through the stapled rectal stump adjacent to the staple line. The anvil is then docked onto the spike, and the anastomosis is done under direct vision. With use of a two or three 2-0 non absorbable (Polypropylene) sutures are placed anchoring the mesorectum to the fascia just below the sacral promontory, just distal to the anastomosis. The anastomosis is checked with a rigid sigmoidoscope for homeostasis and integrity Fig (4).

Post-operative care: Oral intake is started when there is good intestinal sounds, usually on the next morning starting with liquids in cases of rectopexy alone, while in cases of resection rectopexy patients are kept nil by mouth till the third post-operative day. During the next two weeks post operatively, non-stimulant laxatives are used along with instructions to avoid excessive straining at defecation, thereafter the use of laxatives is determined according to the degree of recovery of bowel function. Defecation is resumed in the normal sitting position.

Surgical outcome: The operative technique is assessed, evaluating the following parameters: feasibility and easiness of the technique, operative time, time for passage of flatus, postoperative hospital stay and time to return to normal activity.

Follow Up: Patients are followed up postoperatively for a period of two years to evaluate the following parameters: improvement or persistence of symptoms of prolapse, recurrence and its type (complete or partial), changes in constipation and difficult evacuation, changes in anal incontinence if it was present preoperatively and effect on sexual function by asking about; impotence and retrograde ejaculation in males. All of the above collected data are subjected to analysis to obtain the relevant results.

Results

All patients underwent laparos-

copic repair (rectopexy or resection rectopexy) and were followed up for a period of two years. The preoperative, operative and postoperative data were collected, analyzed and the following results were obtained. The mean age of patients in our study was 47.1 (range 17-66) years and male to female ratio was 11-9. The most common presenting symptoms were the prolapsing mass (100%) and anal pain (70%), while the incontinence represented the least incidence (30%). Two patients of all 18 female patients (11.1%) had multiple vaginal deliveries with a prolonged last labor, while one patient (5.5%) had multiple vaginal deliveries with a history of perineal tear Fig (5).

One patient (2.5%) had a history of perineal repair for rectal prolapse 30 years ago (the nature of the operation was not specified) followed by recurrence two months later, while four patients (10%) had history of anal fistula operation 5years ago. The most common findings on patients examination was the prolapsing mass on straining (100%), with 14 patients (35%) have patulous anus, 12 patients (30%) have poor sphincter tone, while scar of fistula operation, 14 patients, represented the least incidence (10%).

Both laparoscopic rectopexy and resection rectopexy were successfully performed and completed in all patients Table (I). Group (A) patients [20 patients]; were operated by laparoscopic rectopexy without resection, 8 patients were operated by suture rectopexy, and 12 patients were operated by mesh rectopexy. Group (B) patients [20 patients]; were operated by laparoscopic sigmoidectomy and suture rectopexy for patients who have redundant sigmoid colon.

All the 40 patients (100%) who were complaining of prolapsed mass improved postoperatively, 2 patients (7.14%) of the 28 patients (100%) who were complaining of anal pain preoperatively persisted postoperatively and 3 patients (20%) of the 15 patients (100%) who were complaining of bleeding per rectum pre-operatively persisted post-operatively Table (II).

Totally 25 patients (62.5%) were complaining from constipation or difficult evacuation preoperatively; 11 patients [out of the 25 patients (i. e.; 44%)] were in group (A) and operated by laparoscopic rectopexy alone and 14 patients [out of the 25 patients (i.e.; 56%)] were in group (B) and operated by laparoscopic resection rectopexy. Constipation improved in 19 patients (76%); 7 patients [out of the 11 patients (i. e.; 63.6%)] were operated by laparoscopic rectopexy alone (group A) and 12 patients [out of the 14 patents (i.e.; 85.7%)] were operated by laparoscopic resection rectopexy (group B); and persisted in 6 patients (24%). New onset constipation developed in 2 patients from group (A) who were improved by fiber enriched diet and mild laxative Table (III).

12 patients (30%) were complaining from incontinence preoperatively. Fecal incontinence improved in 9 patients [out of the 12 patients (i.e.; 75%)], and persisted in 3 patients [out of the 12 patients (i.e.; 25%)]. One patient showed only a mild improvement, while there was a marked increase in continence in eight of the nine patients who suffered from either grade IV or grade III incontinence before operation. The other 28 patients were continent before surgery and remained so afterward during the period of follow up Fig. (6). The effect on sexual and urologic functions was not found in any of our patients postoperatively during the period of follow up. There was no recurrence found in any of our patients post-operatively during the period of follow up.

Table (I): Surgical parameters.

Surgical Parameters items	Rectopexy		Resection Ectopexy	
	Range	Mean	Range	Mean
Operative time in minutes	90-210	149	180-330	256
Time for passage of flatus in days	1-3	1.9	2-4	2.5
Postoperative hospital stay in days	2-5	2.6	5-9	7
Return to normal activity in days	10-15	12.8	14-21	17.65

Table (II): Post-Operative Symptomatic Outcome.

Symptoms	Total Number and percentage of patients complaining	Number and percentage of patients persisted post-operatively	Number and percentage of patients improved post-operatively
Prolapsed mass	40 (100%)	0 (0%)	40 (100%)
Anal pain	28 (70%)	2 (7.14%)	26 (92.86%)
Bleeding/ rectum	15(37.5%)	3 (20%)	12 (80%)

Table (III): Constipation and symptoms of difficult evacuation

Constipation	Number of patients	Percentage of patients (%)
Total patients complaining pre-operatively	25	100%
Patients improved post-operatively	19	76%
Patients persisted post-operatively	6	24%
Newly complaining patients post-operatively	2 (out of 40 patients)	5%

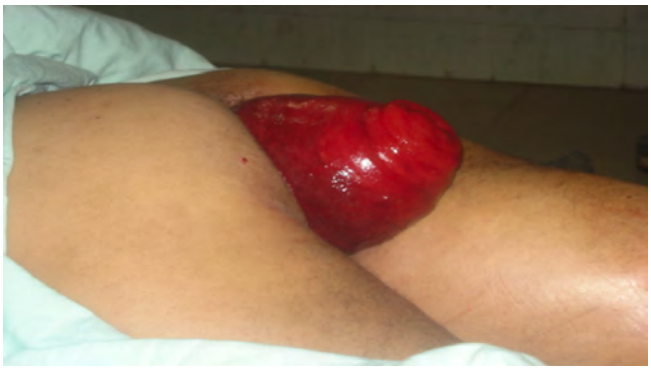
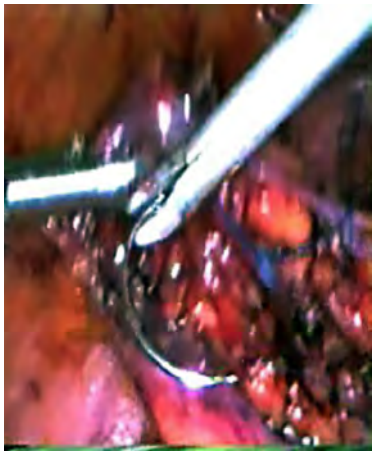
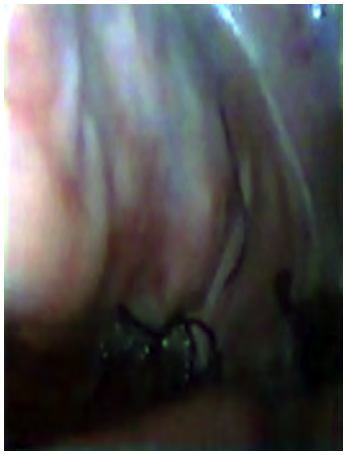


Fig (1): Complete rectal prolapse (PRE-OPERATIVE).

Fig (2): Laparoscopic Suture Rectopexy.



(a) Suture rectopexy



(b) Completing suture rectopexy.

Fig (3): Laparoscopic Mesh Rectopexy (Presacral placement of the mesh).

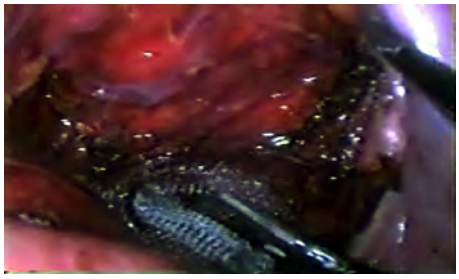
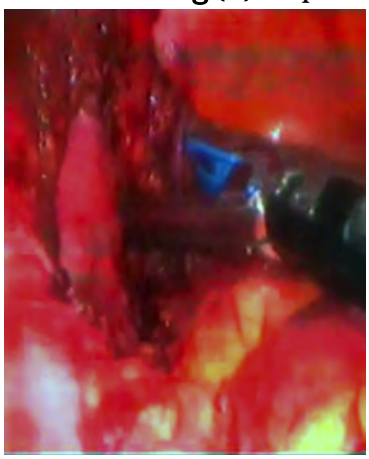
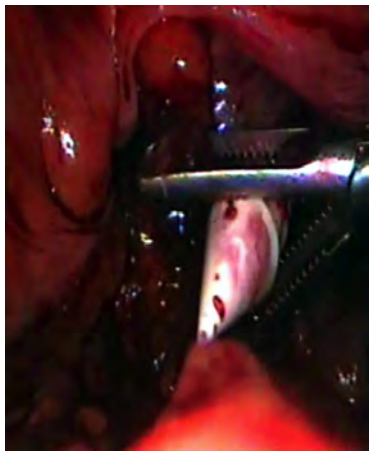


Fig (4): Laparoscopic Resection Rectopexy.



(a) Application of the endoscopic stapler.



(b) Circular stapler passing per rectum.

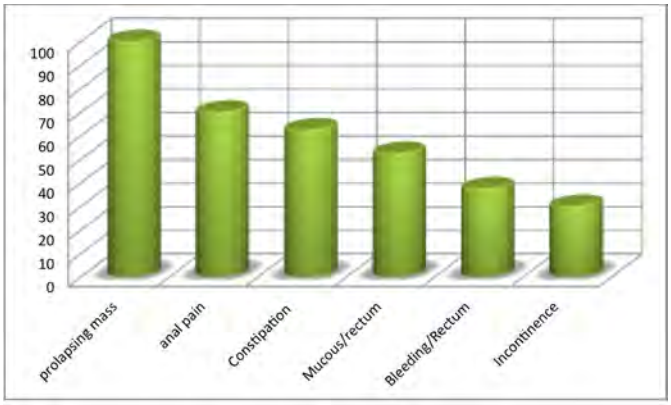


Fig (5): Percentage of the presenting symptoms.

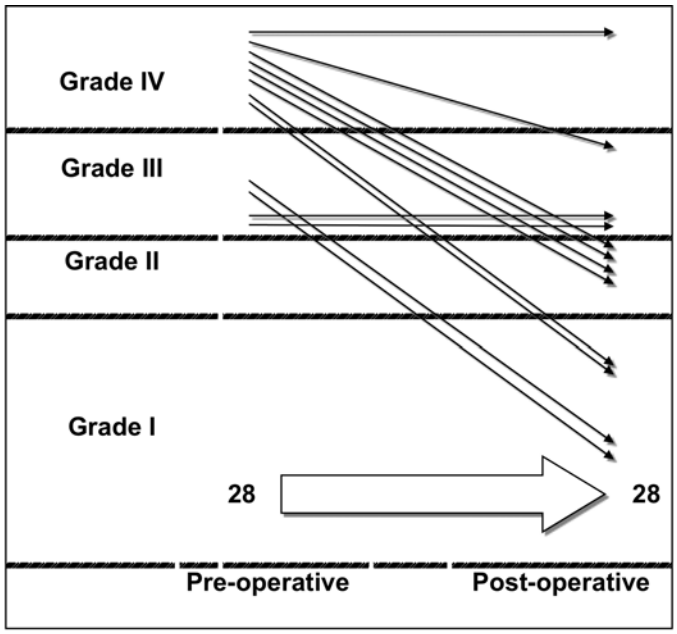


Fig (6): Changes in the continence grading pre and post-operatively.

Discussion

Although considerably in excess of 100 different surgical techniques have been reported for the treatment of rectal prolapse, there is a common consensus that transabdominal fixation procedures, with or without resection, offer better functional results and lower recurrence rates. Despite

this, perineal procedures, which can be performed under spinal or epidural anesthesia, are often given preference in very old and high-risk patients. The development of minimally invasive surgery has changed the scene as laparoscopic rectopexy or resection rectopexy can be accomplished safely[4].

Rectal prolapse tends to occur most commonly in elderly, as the mean age of patients in most studies varies from 61 years (range 24-94 years)[6], 62 years (range 23-88 years)[7], 72 years (range 37-89 years)[3], to 73 years (range 57-86 years)[8]. However, in our study it tends to occur in younger group as the mean age was 47.1 years (range 17-66 years), and this is in correspondence to some authors, 45.8 years (range 25-82 years)[9], and 51.5 years (range 20-87 years)[10]. Also, rectal prolapse has been reported to occur more commonly in females with a female to male ratio varying from 4:1[9], to 17:1[7] and 29:1[6]. However, in our thesis rectal prolapse tends to occur more slightly in male patients with a female to male ratio of 1:1.22.

The associated functional disorders in this thesis were mainly constipation or symptoms of pelvic outlet obstruction (62.5%) and incontinence represented only (30%), and this is in correspondence to some authors; (50%) with constipation and (20.8%) with incontinence[11], and (52.5%) with constipation and (45%) with incontinence[5]. However, other authors reported higher association of complete rectal prolapse with incontinence rather than with constipation; as it was (66.7%) with incontinence and (46.7%) with constipation[6], and (53.7%) with incontinence and (37%) with constipation and outlet obstruction[12].

Laparoscopic rectopexy (suture or mesh) and laparoscopic assisted resection rectopexy are successfully feasible with the following surgical parameters in this study: The mean operative time was 149 (range 90-210) minutes in rectopexy and 256 (range 180-330) minutes in resection rectopexy, however a reduction in operating time is noticed with increasing our experience. These results were similar to other series; as the

mean operative time was 258 (range 150-380) minutes in resection rectopexy[7], 143 (range 90-300) minutes in suture rectopexy and 215 (range 180-260) minutes for resection rectopexy[10]. Moreover, it was 126 (range 92-175) minutes in rectopexy and 222.8 (range 145-330) minutes in resection rectopexy[5], and 150 (range 90-295) minutes for rectopexy and 255 (range 180-360) for resection rectopexy[13].

The mean postoperative hospital stay in our study, it was 2.6 days (range 2-5 days) in rectopexy and 7 days (range 5-9 days) in resection rectopexy. This was in accordance with the results of other studies; 3 days (range 1-5 days) in rectopexy and 6.1 days (range 3-13 days) in resection rectopexy[5], 4 days (range 2-10 days) in rectopexy[9]. The mean time to return to normal activity in our study it was 12.8 days (range 10-15 days) in rectopexy and 17.65 days (range 14-21 days) in resection rectopexy, and this was similar to other study; 14 days (range 12-21 days) following both rectopexy and resection rectopexy[13].

In this study; totally 25 patients (62.5%) were complaining from constipation or difficult evacuation pre-operatively; 11 patients [out of the 25 patients (i.e.; 44%)] were in group (A) and operated by laparoscopic rectopexy alone and 14 patients [out of the 25 patients (i.e.; 56%)] were in group (B) and operated by laparoscopic resection rectopexy. Constipation improved in 19 patients (76%); 7 patients [out of the 11 patents (i.e.; 63.6%)] were operated by laparoscopic rectopexy alone (group A) and 12 patients [out of the 14 patents (i.e.; 85.7%)] were operated by laparoscopic resection rectopexy (group B); and persisted in 6 patients (24%). These figures mean that improvement in pre-operative constipation was more with resection rectopexy than with mesh or suture rectopexy[11].

As regarding incontinence in our study 12 patients (30%) were complaining from incontinence pre-operatively. Fecal incontinence improved in 9 patients [out of the 12 patients (i.e.; 75%)], and persisted in 3 patients [out of the 12 patients (i.e.; 25%)]. This was in accordance with the results of other studies; 75% of patients presenting with fecal incontinence

were improved by laparoscopic rectopexy whatever the procedure used[1], incontinence was improved or completely removed in 64% of patients[7]. Moreover, incontinence was improved in 77.8% of patients[8], in 70 % of patients[6], and improved in 83.3% of patients regardless of which procedure used[5].

As regarding recurrence in our study no recurrence was found in any of our patients postoperatively during the period of follow up. This was in correspondence to many authors; there have been no recurrences following laparoscopic rectopexy without resection[9], no recurrence was detected for laparoscopic suture rectopexy without resection[3], no recurrence was recognized regarding laparoscopic surgery for complete rectal prolapse whatever the procedure used[7].

Conclusion

Laparoscopic surgery for rectal prolapse is a technically feasible method which resulted in improved constipation and incontinence in the great majority of patients and eliminate the prolapse with nearly no recurrence. The addition of laparoscopic colopexy helps to decrease the recurrence rate. Laparoscopic surgery for rectal prolapse has the advantages of minimally invasive surgery including the shorter hospital stay, less pain, early recovery and return to work.

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LAPAROSCOPIC RECTOPEXY FOR COMPLETE RECTAL PROLAPSE

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EVALUATION OF ANTI-HEPATITIS A VIRUS IMMUNOGLOBULIN M IN URINE SAMPLES FOR RAPID DIAGNOSIS OF HEPATITIS A IN CHILDREN

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Abstract

Children are the most frequently infected group by Hepatitis A virus (HAV). Viral hepatitis infections are frequently observed in preschool age, among schoolchildren and young adults, and within closed institutions . Frequently, it is difficult to collect blood samples, especially from infants, children, and individuals to whom access is limited. Urine samples are easier to collect, the collection method is not invasive, and collection does not require qualified staff. In addition, urine samples can be tested without previous concentration or treatments by using a class-specific antibody capture assay .The main goal of this study was to test the feasibility of using urine as a specimen for detection of anti-HAV antibodies IgM for diagnosing HAV infections. A correlation of 90.78% between the test results of urine and serum samples was obtained. The levels of anti-HAV immunoglobulin M (IgM) antibodies capture enzyme-linked immunosorbent assays for hepatitis A were performed on paired serum and urine specimens collected from hepatitis A patients (n = 100) and healthy individuals (n = 50). Hepatitis A patients seropositive for anti-HAV IgM showed 95.65% uropositivity. No false-positive reactions were observed in control groups. The uropositivity of anti-HAV IgM persisted during the convalescent phase of the disease. Using seroreactivity as a "gold standard," the sensitivity and specificity for anti-HAV IgM, tests with urine as a specimen were found to be 95.65 and 76.47%. Urine appears to be comparable to serum for diagnosis of recent infection with hepatitis A.

Introduction

The immune response against HAV consists initially of immunoglobulin M (IgM) antibodies, which is detectable at the time of jaundice appears. It is therefore important in laboratory diagnosis of HAV. The appearance of IgM is followed 1-3 weeks later by the production of Immunoglobulin G (IgG) antibodies, which provides lifelong protection[1].

Blood samples are of prime importance in biochemical testing and in seroimmunological diagnosis. Collection of blood specimens, however, is cumbersome on account of the need for sterile equipment and trained staff. In many developing countries, the use of disposable syringes, needles, and gloves is not regularly practiced, rendering the subjects at risk for infections. A slippery vein or improper judgment of the location of a vein gives rise to untoward reactions. To circumvent the need for blood samples, the potential of alternative body fluids such as saliva and urine for detection of immunoglobu lins against various microbial agents has been investigated[2,3].

Urine is a body fluid with low concentrations of immunoglobulins. It has been postulated that large macromolecules such as immunoglobulin M (IgM) antibodies cannot pass through the glomerular filter under normal conditions. However, monomeric IgM proteins (67,000 kDa) have been detected in postrenal sources and not in the glomerular filter[4,5]. The utility of urine for diagnostic testing has been reported for many viral infectious diseases[6,7]. Particularly for hepatitis A, Joshi et al. have found that urine appears to be comparable to serum as a clinical specimen for the diagnosis of recent and past infections[1].

Among the assays employed for detection of salivary or urinary antibodies against infectious agents, antibody class capture assays were preferred to conventional assays(8,9). The capture assays have been reported to be dependable due to their abilities to capture specific immunoglobulin even at low levels and to establish specificity in the initial stage of the assay. Immunoglobulin M (IgM) and IgG capture radioimmunosorbent assays have been demonstrated to

detect urinary and salivary anti-hepatitis A virus (anti-HAV) antibodies[4]. An IgG capture enzyme-linked immunosorbent assay (ELISA) has been attempted for detection of antibodies to respiratory syncytial and influenza A/Taiwan (H1N1) viruses in urine[10]. However, satisfactory use of IgG capture ELISA for detection of salivary and urinary antibodies against human immunodeficiency virus (HIV) types 1 and 2 has been described[8,11]. This assay appeared to be a promising alternative to conventional tests for use as a new epidemiological tool for surveillance purposes[12].

Materials and Methods

Paired serum and urine samples were collected from 100 jaundiced children "patients group" and 50 apparently healthy children "control group", no history of recent illness was reported for control group. All cases were selected form Pediatric Outpatient Clinic , Al-noor specialist Hospital from Feb. 2010 to Octob. 2010. The patients were clinically examined for characteristic symptoms and signs and elevated serum liver enzymes "ALT & AST " levels . Patients group included 65 males and 35 females, with age range "5 to 11 years", control group included 24 males and 26 females with similar age range. Prior to sample collection, informed consent was sought from their parents. All serum and urine specimens were stored in aliquots at -20 and -70℃, respectively until processed for ELISA.

A class-specific capture ELISA was used to detect anti-HAV IgM antibodies in both serum and urine samples. The cutoff value was determined by adding 1/10 of the mean optical density (OD) from duplicate determinations with a known positive control sample to the mean OD from triplicate determinations with a known negative control sample[13]. Specimens with absorbance value less than and greater than the cutoff value were considered negative and positive, respectively[14].

The highest 10 urine samples concentrations were collected and tested at 1,2 and 3 months after the onset of the disease to compare it with blood samples at the same intervals.

Twenty positive urine samples were used to evaluate the level of anti-HAV IgM antibodies after storage under refrigeration (4℃) at 48 to72 h after collection.

Another 20 positive urine samples were used to evaluate the stability of anti-HAV IgM antibodies stored at -70℃ for 6 months.

Statistical analysis was performed by using the Statistica statistics package. The Kolmogorov-Smirnov test, the Student t test, analysis of variance, and Fisher's exact test were used to analyze the data.

Results

A total of 150 children (100 patients and 50 control) were included in this study. The median ages of the patients were 7 years (age range, 5 to 11 years, 65% males) and for control were 6 years (with similar age range, 48% males) .

Serum and urine samples were collected at the same time from all cases, and levels of serum IgM, and urinary IgM against HAV were measured using ELISA. Serum HAV IgM was positive for all patients, while urinary HAV IgM was positive for only 91 (91%) cases. There was no statistically significant difference between the percent positivities of serum and urine samples (P>0.05). Both serum and urinary HAV IgM were negative for all control cases (Tab.1).

The sensitivity and specificity of the urine-based ELISA were 82.59 and 90.21%, respectively. A good correlation (90.27%) between the results of the urine and serum assays was obtained. The positive and negative predictive values were 91.48 and 84.52%, respectively, which is an acceptable proportion between positive and negative results and between results for infected and healthy individuals.

Twenty urine samples were used to evaluate the stability of anti-HAV IgM antibodies stored at -70℃ for 6 months . At 6 months, 9 out of the 20 urine samples stored at -70℃ tested negative, indicating loss of IgM during storage. Studies on the effects of

freezing and storage conditions on the stability of urine samples have been reported .

Twenty samples were tested after storage under refrigeration (4℃), all 20 were found to be positive without any loss of anti-HAV IgM activity at 48 to72 h after collection.

Samples were collected and tested at 1,2 and 3 months after the onset of the disease to evaluate the kinetics of urinary anti-HAV IgM. The median value OD value of the serum samples collected at one months postinfection was 0.826, higher than that for the urine samples (0.597). Also, for the specimens collected one month later, the median OD value for the serum samples was higher value (0.294), than the median OD value for the urine samples (0.162). At 3 months postinfection, all of the serum samples tested positive, while seven (70%) out of ten urine samples tested negative. The OD median values for the urine samples collected at 1.2 and 3 months postinfection were significantly different ($P < 0.01$), these results indicating persistence of anti-HAV IgM for prolonged periods during the convalescent phase of the disease

The results for the urine and serum samples from healthy children, which were used as negative controls, demonstrated that using urine samples did not decrease the specificity of the ELISA. These results provide evidence that support the use of urine samples for rapid diagnosis of hepatitis A .While the results of hepatitis cases also suggest the usefulness of urine samples in achieving a quick response for interpretation of cases.

Tab.1: Comparison of paired serum and urine specimens for anti-HAV IgM.

	Serum		Urine	
	No .positive/ no. tested (% positive)	**OD (mean±SD)**	**No .positive/ no. tested (%positive)**	**OD (mean±SD)**
Patient group	100/100(100)	0.983 ± 0.037	91/100(91)	0.604 ± 0.049
Control group	0/50(0)	0.058 ± 0.017	0/50(0)	0.043 ± 0.014

Discussion

Hepatitis A virus infections are frequently observed in preschool age, among schoolchildren and young adults, and within closed institutions. Frequently, it is difficult to collect blood samples, especially from infants, children, and individuals to whom access is limited. Urine samples are easier to collect, the collection method is not invasive, and collection does not require qualified staff. In addition, urine samples can be tested without previous concentration or treatments by using a class-specific antibody capture assay.

The class-specific antibody capture ELISAs was used to detect anti-HAV IgM antibodies in both serum and urine samples. Use of urine specimens in anti-HAV IgM ELISA correctly identified 91% of anti-HAV IgM-seropositive hepatitis A patients and did not produce any false-positive reactions in control groups. The sensitivity of the test using urine may have been equivalent to that of the test used for serum. However, the urine samples classified as negative for anti-HAV IgM. Thus, the absence of IgM in 9% of urine samples indicated the absence of filtration, local synthesis, or transudation of IgM in urine or its presence below the detection limits of the IgM ELISAs employed.

Chitambar et al (1996)[14] explain low sensitivity of they test that could have been caused by the following reasons. (i) Urine samples may not have been collected at the optimal time (the anti-HAV IgM kinetics in urine are not well known). (ii) There may have been immunoenzymatic reaction inhibitors present in the urine, due to chemicals, drugs, or toxic products which normally are excreted in urine. (iii) Differences in the amounts of liquids ingested promote fluctuation in the immunoglobulin concentrations in urine.

While Ireland, and Nicholson (1996)[15] explain false-positive results that are probably due to the effect of the pH of the urine, bacterial contamination, or the presence of sediments in the urine of the individuals tested. All of these could interfere with or block the immunochemical reaction in the ELISA.

Many types of microorganisms multiply rapidly in urine at room temperature. For practical purposes, urine specimens need to be processed rapidly or stored refrigerated during the time before analysis is performed[1]. In order to avoid the problem of bacterial contamination, we preferred to perform tests on fresh samples and store them in aliquots at -70℃. The stability of urine anti-HAV IgM after storage was examined. The percent positivity for anti-HAV IgM in urine specimens declined significantly to 55% (11 of 20) ($P < 0.05$, chi-square test) 6 months, indicating loss of IgM during storage. Twenty samples were tested after storage under refrigeration (4℃). All samples were found to be positive without any loss of anti-HAV IgM activity at 48 to 72 h after collection.

The highest levels of anti-HAV IgM antibodies in serum are reached during the acute phase of HAV, and the antibodies often disappear 3 or 4 months after the onset of the illness. However, Raymond, S. K. (1992)[16] demonstrated that anti-HAV IgM antibodies may persist for more than 6 months in 25% of patients. Our results have shown that the level of anti-HAV IgM antibodies seems to decrease gradually, but it decreased faster in urine than in serum. It is remarkable that the anti-HAV IgM levels for Ten patients were higher in their urine than in their serum at the beginning of the disease. Nevertheless, this anti-HAV IgM reactivity in urine decreased significantly at 6 months postinfection ($P<0.01$). Further studies of this topic with more specimens may be needed.

Finally, the usefulness of urine as a specimen for diagnosis of hepatitis A, could be confirmed in large-scale epidemiological studies. If it stands the test of large sample sizes, this may find several applications in routine surveillance, epidemiological investigations, and hepatitis A vaccination programs.

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IMPACT OF HEPATOCELLULAR CARCINOMA AND LOCAL ABLATIVE TREATMENT ON THE OUTCOME OF ENDOSCOPIC PROPHYLACTIC BAND LIGATION FOR ESOPHAGEAL VARICES IN CIRRHOTIC PATIENTS

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Abstract

Hepatocellular Carcinoma (HCC) is a common cause of death in patients with cirrhosis. Moreover, the presence of HCC influences either early rebleeding or mortality in these patients. Prophylactic therapy for varices is needed to reduce death from esophageal varices and to offer some survival benefit to patients with HCC. The aim of this study was to evaluate outcome of endoscopic prophylactic band ligation in patients with post hepatitis C liver cirrhosis with large esophageal varices in comparison to patients with post hepatitis C liver cirrhosis associated with hepatocellular carcinoma and relation to local ablative treatment in hepatocellular carcinoma group.

Method: *Prophylactic endoscopic band ligation was done to eighty patients with liver cirrhosis and large esophageal varices of which 60 patients had HCC. Band ligation was done until complete eradication of varices. 40 patients with HCC had Percutaneous ethanol injection for HCC Treatment was repeated until complete tumor necrosis was confirmed by contrast computed tomography (CT) scanning. Serum vascular endothelial growth factor was measured in all groups. The therapeutic efficacy and complications were compared between groups.*

Results: *The endoscopic recovery was significantly higher in the cirrhotic patients (gp I) 95% compared to both HCC treated (gpII) and HCC*

(gpIII) untreated with mean number of sessions needed to eradicate the varices was 4.45±1.15. In HCC treated group the mean number of sessions needed to eradicate the varices was 5.35±1.4 with endoscopic recovery in 75% of patients. While in HCC untreated patients the endoscopic recovery occurred only in 5% of patients with mean number of sessions 6.2±1.28. The mortality rate in HCC treated patients was 5% due haematemesis. While, in HCC untreated patients the mortality rate was 55%. The cause of mortality was due to haematemesis in 35% of patients and in 20% due to liver cell failure complications.VEGF correlate with the severity of both HCC and esophageal varices.

Conclusions: *early local treatment of HCC improves the endoscopic prophylactic band ligation outcome with improvement of survival and less complication than HCC not candidate for treatment.*

Introduction

Most cirrhotic patients develop esophageal varices, with lifetime incidence as high as 80-90%[1]. Bleeding from esophageal varices remains one of the life-threatening complications in liver cirrhosis. Universal screening upper endoscopy is recommended for patients with cirrhosis to evaluate for the presence of esophageal varices[2].

Hepatocellular Carcinoma (HCC) is a common cause of death in patients with cirrhosis. Moreover, the presence of HCC influences either early rebleeding or mortality in these patients[3]. Prophylactic therapy for varices is needed to reduce death from esophageal varices and to offer some survival benefit to patients with HCC. However, only a few clinical studies have compared the response to prophylactic band ligation in relation to local treatment of HCC. The aim of the Current study was to evaluate outcome of endoscopic prophylactic band ligation in patients with HCC and the effect of local ablative treatment.

Methods

Patients

Included (80) patients suffering from post-hepatitis C virus cirrhosis with large esophageal varices with no history of bleeding or prophylactic treatment from were une 2010- December 2012. They

were prospectively evaluated and randomized into three groups: **Group I :** 20 patients post hepatitis C virus cirrhosis with large esophageal varices who underwent prophylactic endoscopic band ligation. **Group II :** 40 post hepatitis C virus cirrhosis with large esophageal varices and hepatocellular carcinoma who underwent prophylactic endoscopic band ligation and PEI for HCC. **Group III :** 20 post hepatitis C virus cirrhosis with large esophageal varices and hepatocellular carcinoma who underwent prophylactic endoscopic band ligation but not candidate for PEI. Before treatment, informed consent was obtained from each patient.

Inclusion Criteria : included post hepatitis C virus cirrhosis with large esophageal varices, patent portal vein & hepatic veins. No extrahepatic metastasi, compensated cirrhotic liver (Child A or B only), the international normalized ratio I.N.R less than 2 and platelets count not less than 50.000-mm^3. Exclusion Criteria included isolated fundic varices with or without with esophageal varices ,associated organ failure (heart or renal failure),other causes of hepatitis: hepatitis B virus or autoimmune hepatitis, Child C cirrhotic patients,Inferior vena caval , portal vein or hepatic veins thrombosis. Patients with extrahepatic metastasi, Bleeding tendancy with I.N.R more than 2, and/ platelets count less than 50.000-mm^3, Patients receiving ß-blockers.

The severity of the varices was classified according to the criteria for endoscopic assessment of esophageal varices proposed by the Japanese Research Society for Portal Hypertension: small (F1), moderate (F2), huge (F3)[5]. All patients underwent prophylactic endoscopic band ligation at three weeks interval till obliteration of varices or reduction to grade I.

Technique of percutaneous alcohol ablation :

All patients were fasting before the procedure, ablation was performed on an outpatient bases and the patients were hospitalized for 2 hours after procedure for possible complications. All complications encountered during or after the session were recorded. Treatment was performed with the

patient under conscious sedation and induced by the administration of diazepam 0.03-0.1 mg/kg/IV every 30 minutes or midazolam at dose of 2-5 (up to 10) mg I.V. After cleansing the skin with povidone iodine and alcohol, local anesthesia was achieved by using 3 ml of 2% lidocaine. To achieve complete tumor necrosis, homogenous diffusion of absolute alcohol throughout the lesion was essential. Typically, one injection at a dose of 5-10 ml absolute alcohol was given during each treatment session. Absolute alcohol was slowly injected until the echogenic area appearing immediately after injection covered the entire tumor. After the injection was complete, the needle was left in place for 1-2 min to prevent reflux of alcohol into the peritoneal cavity.

Post-procedure Assessment : Evaluation of the patients after the procedure was done by: Conventional abdominal US regarding the focal lesion noting the post session size, echogenicity, Doppler examination, presence of local or primary recurrence (recurrence in the same lesion) or secondary recurrence or De novo lesions (recurrence elsewhere in the liver).

Also examination for the development of PV thrombosis or not, development or increase of ascites and the development of any complications as hematoma or abscess formation and their aspiration.

Triphasic spiral CT:

Contrast enhanced CT images were obtained after four weeks from the treatment session. The response to treatment was rated as complete when CT scans showed no contrast enhancement inside the lesion in the arterial phase. The response was rated as partial when CT scans showed areas of enhancement within the boundaries of the original lesion in the arterial phase. Also the diameter of the HCC lesion after ablation was measured and primary recurrence or De novo lesions were recorded.

Post treatment follow - up :

Follow up of the patients was done for about six months with special emphasis on recurrence of HCC, any remote complication related to procedure, development of

liver decompansation (ascites, jaundice, encephalopathy, bleeding tendency), haematemesis, or death.

Endoscopic Band Ligation :

After local application of lidocaine and midazolam (Dormicum), as sedative at dose of 2-5 (up to 10) mg I. V., an endoscope (model 1T20 Olympus Optical, Tokyo) was introduced, A complete upper endoscopy is performed before placement of the band ligation device onto the endoscope. In the elective setting, this allows assessment of size and number of variceal columns and the presence of other pathology (e.g., gastric varices, portal hypertensive gastropathy, gastric antral vascular ectasia). prophylactic banding of large varices particularly when they are at highest risk of hemorrhage i.e., presence of red wale[5].

Then ligation was carried out by placing a single or multiple rubber band over a varix each time the endoscope was inserted. As many bands as possible (average, 3 to 5 bands, with fewer in later sessions) were placed in the lower 5 to 7 cm of all variceal columns. Each residual varix was ligated distally and proximally to accelerate obliteration. Patients underwent regular EVL without drug therapy such as beta-blockers until variceal obliteration. Endoscopic recovery is achieved when varices were obliterated or were reduced to a size of grade 1.

Follow-up :

Patients were asked to record all symptoms, such as hematemesis, melena, chest pain, fever, and dysphagia. The presence of ulcers, esophagitis or strictures was noted on endoscopic examination.

Assessment of VEGF:

Boster's human VEGF ELISA Kit was based on standard sandwich enzyme-linked immunesorbent assay technology. Human VEGF specific monoclonal antibodies were precoated onto 96 well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with buffering solution TMB.

Statistical analysis:

It was carried out via both Statistical package for social Science (SPSS) version 17 program on windows XP. Qualitative data were represented in the form of number and frequency. Kolmogrov-smirnov test was used to test normality of quantitative data. Based on its results, quantitative data were either represented in the form of mean ± standard deviation (mean±SD) if normally distributed or median (range) if non-normally distributed. Chi square test was used to compare qualitative data; on the other hand, Student's t test, one way analysis of variance (ANOVA), Mann-Whitney and Kruskal-Wallis tests were used to quantitative data. Whereas, Spearman's rank test was used to determine correlation between variables.

All tests were considered significant if p value ≤ 0.05.

Results

The difference between general features of both groups was statistically not significant.

There was no significant difference between the three groups regarding serum albumin bilirubin, AST,ALT,INR and α-fetoprotein.

Early complications of PEI included fever and abdominal pain which occurred in 4 (10%) and 17 (42%) patients respectively. Late complications included abscess , portal vein thrombosis and ascites which occurred in 6(15%),1(2%) and 2(5%) patients respectively. The liver transaminases (ALT,AST) showed mild elevation 1 month after the procedure with mean 64.3U/l, 64.3U/l and ranging from 48-98 U/l, 44-65 U/l respectively.

This scattered plot is showing a significantly higher VEGF in HCC untreated (P<0.001). 6 patients (30%) of HCC treated group (gpII) suffered from dysphagia compared to 7 patients (35%) of HCC untreated group (gpIII). Ulcer was found in 7 patients (35%) in HCC treated group (gpII) (gp I), while it was found in 7 patients (35%)of HCC untreated group (gpIII). Chest pain was complicating post endoscopy in10 patients of groupII compared to 8 patients of group III. The incidence of haematemesis was higher in HCC untreated group (gp III) 9 patients (45%)

compared to 4 patients (10%) of HCC treated group (gpII) P=0.005. There was no statistically significant difference regarding dysphagia ,chest pain or ulcer between the two groups.

Dysphagia was found in 4 patients (25%) of cirrhotic group (gp I) and in 6 patients (30%) of HCC treated group (gpII). Ulcer occurred in 2 patients (25%) in cirrhotic group (gp I), while in HCC treated group (gpII)it occured in 6 (15%) of patients. 4 patients of HCC treated group (gpII) (10%) had Haematemesis ,while non of the cirrhotic patients had.There was statistically no significant difference regarding haematemesis dysphagia or ulcer between the two groups.

The rate of endoscopic recovery in cirrhotic group (gp I) was 95% (19 patients), while in HCC treated (gp II) was 75%(15 patients).There was no significant difference between cirrhotic group (gp I) and HCC treated (gp II) regarding endoscopic recovery.

The rate of recovery in HCC treated (gpII) was 75%(15 patients) compared to 5% (1 patient) in the HCC untreated (gp III). There was statistically significant difference between the two groups P<0.001.

Mortality occurred in 2(5%) cases of group II due haematemesis. Mortality occurred in 11(55%) cases of group III of which 7 due haematemesis, 4 due liver cell failure.

Table 1 : General features and Child score of the studied groups

Item	*Group I*		*Group II*		*Group III*		*P value*
Age	*54.8(47-65)*		*57.8(46-68)*		*58.1(47-67)*		*0.11 (N.S)*
Gender	No.	%	No.	%	No.	%	
Male	13	67%	22	55%	14	70%	*0.61 (N.S)*
Female	7	35%	18	45%	6	30%	
Child-Pugh	*N'*	*%*	*N'*	*%*	*N'*	*%*	*N.S(0.48)*
Child A	8	40%	12	30%	9	45%	
Child B	12	60%	28	70%	11	55%	

-The difference between general features of both groups was statistically not significant.

Table 2: Comparison of laboratory results of the studied groups before procedures:

Item	*Group I*	*Group II*	*Group III*	*P value*
Albumin(g/l)	3.45±0.6	3.74±0.53	3.46±0.47	*0.064*
Bilirubin(mg/dl)	1.73±0.71	1.52±0.59	1.43±0.56	*0.245*
AST(U/l)	54.1(39-87)	47.2(40-62)	46.5(37-60)	*0.268*
ALT(U/l)	67.9(44-120)	58.5(45-91)	55.8(45-73)	*0.457*
I.N.R	1.43±0.31	1.45±0.42	1.63±0.42	*0.191*
Platelets x10^6	83.65 (60-110)	98.2 (52-230)	90.55 (52-200)	*0.412*
α-fetoprotein (up to 10ng)	*Median (min.-max.)*	*54.85 (5 – 200)*	*74.8(5 – 600)*	*0.53*

Table 3: Complications related to PEI and liver enzymes (after1month) in Group II (40 patients).

Item		*Group II(40 patients) N'(%)*
Early complications	*fever*	*4(10%)*
	Abd. pain	*17(42.5%)*
Late complications	*abscess*	*6(15%)*
	PVT	*1(2.5%)*
	Ascites	*10(25%)*
liver transaminases (1 month)		*Mean (min-max)*
ALT(U/l)		*64.3(48-98)*
AST(U/l)		*50.5(44-65)*

Table 4 : Endoscopic complications in HCC treated group (gp II) and HCC untreated group (gp III):

complication	*Gp II N'(%)*	*Gp III N'(%)*	*P value*
Dysphagia	6 (15%)	7 (35%)	*0. 15*
Ulcer	6 (15%)	7 (35%)	*0.15*
Chest pain	10(25%)	8(40%)	*0.37*
Haematemesis	4 (10%)	9 (45%)	*0.005*

- *PVT in group III 5 (25%) of patients*

Table 5 : Endoscopic complications in cirrhotic group (gp I) and HCC untreated group (gpIII):

complication	*Gp I N' (%)*	*Gp III N' (%)*	*P value*
Dysphagia	1 (5%)	7 (35%)	***0.048***
Ulcer	2 (10%)	7 (35%)	***0.13***
Chest pain	6(30%)	8(40%)	***0.48***
Haematemesis	1(5%)	9 (45%)	***0.003***

Table 6 : Endoscopic recovery in cirrhotic group (gp I) and HCC treated group (gp II):

Groups (20 pt.)	*Recovery*	*N' of sessions needed until recovery*	*P value*
	N'(%)	*Mean ±SD*	
Cirrhosis (gp I) (20 pt)	***19(95%)***	***4.45±1.15***	***P=0.016***
HCC treated (gp II)(40 pt)	***30(75%)***	***5.35±1.4***	

Table 7 : Endoscopic recovery in HCC treated group (gp II) and HCC untreated group (gp III):

Groups	*Recovery*	*N' of sessions*	*P value*
	Yes N'(%)	*Mean ±SD*	
HCC treated (gp II)	***30(75%)***	***5.35±1.4***	***<0.001***
HCC untreated (gp III)	***1(5%)***	***6.2±1.28***	

Table 8 : Mortality rate in both in HCC treated group (gp II) and HCC untreated group (gp III).

Item	*Gp II N'(%)*	*Gp III N'(%)*	*P value*
Mortality rate:	***2(5%)***	***11(55%)***	***P<0.001***
Cause of death:	***2(5%)***		
• *Haematemesis*		***7(35%)***	***0.007***
• *LCF related*	***0***	***4(20%)***	***0.059***

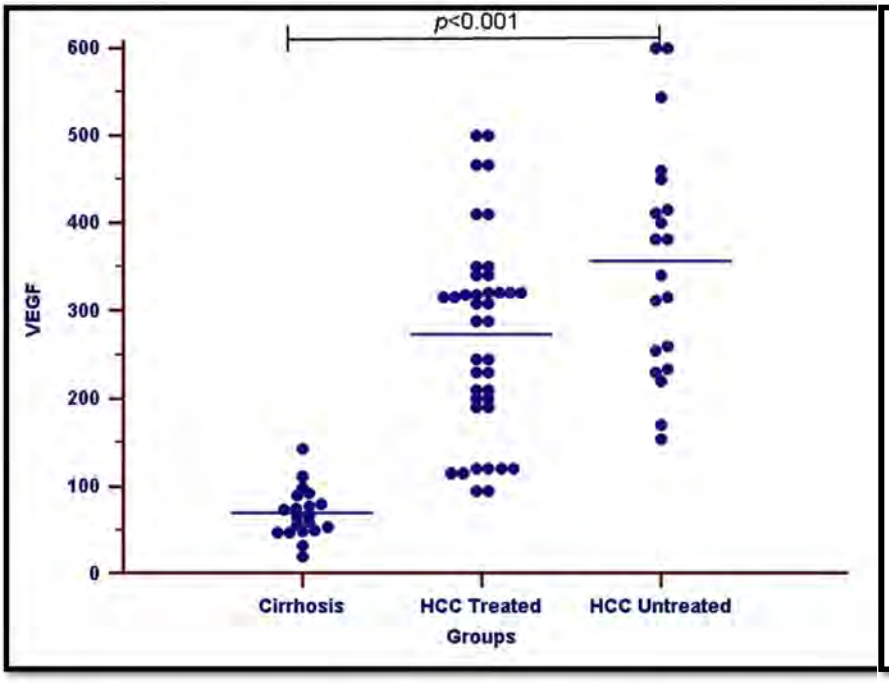

Fig. 1 : Serum Vascular Endothelial Growth Factor (VEGF) (Mean ± SD) : The serum VEGF in cirrhotic group was 69.3±28.3 pg/ml ,while in HCC treated group was 273.15± 114.82 pg/ml and in HCC untreated was 356.65 ± 132.26 pg/ml.

Disscussion

HCC is the fifth common cancer and the third most common cause of mortality from cancer worldwide[6]. Most patients with HCC have advanced liver cirrhosis[7]. Esophageal varices are a complication in 20% to 50% in cirrhotic patients[7]. Approximately half of cirrhotic patients die either directly or indirectly from hemorrhage of esophageal[8]. The successful control of massive bleeding is the major goal of the emergency management of such patients [9].

Bleeding from esophageal varices (EV) in patients with liver cirrhosis and liver cirrhosis accompanied by hepatocellular carcinoma (HCC) is one of the serious complications that endangers patient survival[10]. The risk of primary bleeding from EV for cirrhosis patients accounts for 12-30% 2-6 and the mortality ranges between 30% and 70%[11]. Hemorrhage from esophageal varices, in addition to hepatic failure and growth of the tumor itself, is a major cause of death in patients with early stage HCC [12].

The aim of this study to evaluate outcome of endoscopic prophylactic band ligation in patients with post hepatitis C liver cirrhosis with large esophageal varices in comparison to patients with post hepatitis C liver cirrhosis associated with hepatocellular carcinoma and relation to local ablative treatment in hepatocellular carcinoma group.

Prior to the procedure, all patients were subjected to full medical history taking and complete physical examination, laboratory evaluation including bilirubin, AST, ALT, serum albumin, prothrombin time, serum α fetoprotein, imaging studies as abdominal ultrasound with doppler examination and triphasic CT of the liver and finally fine needle aspiration cytology (FNAC), true-cut biopsy from focal hepatic lesion or MRI (if the results of spiral CT are non-conclusive). Evaluation of the patients after the procedure was done by: i) Laboratory investigations: liver assessment tests (as before procedure) were performed to all cases one month after treatment.

ii) Imaging studies as conventional abdominal US regarding the HCC lesion noting the echogenicity, presence of recurrence also examination for the development of ascites and the development of any complications as hematoma or abscess formation.

iii) Triphasic spiral CT: Contrast enhanced CT images were obtained after 1 month and 6 months from the end of sessions. The absence of enhancement in the arterial phase denotes complete ablation and when enhancement occurs within the boundaries of the tumor this denotes partial ablation.

iv) Upper GI endoscopy with prophylactic band ligation for large esophageal varices every 3 weeks until endoscopic recovery is achieved when varices were obliterated or were reduced to a size of grade 1. V) Measurement of serum vascular endothelial growth factor. Vi) Percutanous ethanol injection for patients of groupII.

In the current study, no significant differences were observed between both groups with respect to the following baseline characteristics: patient age and sex and Child-Pugh class (table 1).

The liver biochemical profile, platelet count and alpha fetoprotein in our study (performed before the procedures) showed no significant changes between the groups (table 2) with mild elevation in liver enzymes 1 month after procedure (table 3).

Alpha fetoprotein mean was 54.85 (ranging from 5to 200) in groupII and 74.8 (ranging from 5 to 600) in group III. This was in agreement with Lawrence, (1996) who stated that not all hepatomas secrete AFP and approximately 30% of the HCC patients had normal AFP levels[13].

Complications of PEI in group II were minor and mainly transient it included early complications occurring from time of session till 1 week after ,as abdominal pain and fever which was found in 17 patients (42.5%) and 4 patients (10%) respectively. Late complications(after more than 1 week) as abscess and ascites occurred in 6 patients (15%)

and 10 patients (25%) respectively.

This is in agreement with Tapani et al., (1996) who reported the incidence of abdominal pain in 48% of cases (15 patients). Also, Immediate pain during the treatment was related to the ethanol dose and increased significantly with increasing doses[14] .

Another late complication is portal vein thrombosis occurred in 1patient (2.5%). Portal vein thrombosis (PVT) may have occurred due to low portal flow, infections, inflammatory diseases and the intervention that was done[15]. PVT has conventionally been considered a relative or absolute contraindication to therapeutic intervention, because of the potential risk of hepatic insufficiency resulting from ischemia following the procedure [16,17].

Hepatocellular carcinoma (HCC) is characterized by a high vascularity. Tumour angiogenesis, the development of new vasculature from previous existing blood vessels, is a major requirement for tumour growth and metastasis and is regulated by pro- and anti-angiogenic factors [18]. Physiologically, anti-angiogenic molecules outweigh the angiogenic molecules and angiogenesis does not occur. Under certain conditions such as tumour formation or wound healing, the positive regulation of angiogenesis predominates and the endothelium becomes activated [19].

In the current study the serum VEGF level in the studied group was 69.3±28.3pg/ml in group I (cirrhotic), 273.15 ± 114.82 pg/ml(HCC treated)and 356.65 ± 132.26 pg/ml in group III(HCC untreated).Vascular endothelial growth factor was significantly higher in the group III(figure 1).

In HCC patients, VEGF was increased more than in the cirrhotic. This may be due to their production by HCC cells for the formation of tumour vessels 20.

Rupture of esophageal varices with severe gastrointestinal hemorrhage is one of the most serious complications of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) complicating LC. It is well-

known that EV bleeding is usually a terminal event in unresectable HCC [21].

The present study looks at the success of breaking the varices cluster and the rate of rebleeding and complications in patients of LC and LC accompanied by HCC, subject to prophylactic ligation and the effect of PEI on it. Prophylactic band ligation was done for large esophageal varices in all patients every 3 weeks till endoscopic recovery. Endoscopic recovery is achieved when varices were obliterated or were reduced to a size of grade 1.

The endoscopic recovery was significantly higher in the cirrhotic patients (gp I) 95% compared to both HCC treated (gpII) and HCC (gpIII) untreated with mean number of sessions needed to eradicate the varices was 4.45±1.15. In HCC treated group the mean number of sessions needed to eradicate the varices was 5.35±1.4 with endoscopic recovery in 75% of patients. While in HCC untreated patients the endoscopic recovery occurred only in 5% of patients with mean number of sessions 6.2±1.28.

In a study by Lay et al.,(1997) the success rate in eradication of varices with EVL in cirrhotic patients was 100%. The mean number of sessions needed to eradicate the varices was 3.6 ± 1.7 for EVL.

In the current study, patients in group II were followed up for 6 month period during which one patient died. In a study by Ohnishi et al, (1996)[22] in which survival rate for large HCC after 1 year was 100%. Also, Guglielmi et al, (2003)[23] in their study had found the survival rate of patients after treatment were 87% after 1 year. Survival was significantly related to Child-Pugh class After 3 years survival was 83% in Child-Pugh A cirrhotic patients and 31% in Child-Pugh B patients.

In conclusion, the current results indicate that prevention of bleeding with prophylactic band ligation contributed to an improvement in the survival period for cirrhotic patients with HCC side by side to treatment of HCC itself, which can be achieved by periodic

monitoring of cirrhotic patients for detection of HCC at an early stage with for proper management. And successful ablation of HCC improves the outcome of endoscopic prophylactic band ligation which becomes equivelant to cirrhosis.

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IMPACT OF HEPATOCELLULAR CARCINOMA AND LOCAL ABLATIVE TREATMENT ON THE OUTCOME OF ENDOSCOPIC PROPHYLACTIC BAND LIGATION FOR ESOPHAGEAL VARICES IN CIRRHOTIC PATIENTS

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EFFECTS OF SOME ANTIOXIDANTS ON ACUTE FLUORIDE-INDUCED RAT KIDNEY DAMAGE

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Abstract

A single oral dose of sodium fluoride (NaF) in aqueous solution was given to male Wistar rats. Twenty-four-hours urine samples were collected and examined to evaluate fluoride-induced acute renal damage. The following parameters were measured in 24 h urine: N-acetyl-β-D-*glucosaminidase (NAG) and creatinine (CR). Fluoride exposure produced specific changes of these parameters. NaF at 25 mg/kg produced significant impairment of glomerular function and renal tubules damage which is manifested by decreased urinary CR excretion (P<0.05) and increased NAG isozyme activity (P<0.01), respectively. This may be related to its known ability to initiate free radical formation or direct damaging effects on the lysosomal membrane. This renal tubular damage as well as the glomerular dysfunction effects of NaF were ameliorated by either pycnogenol (PYC) or ascorbic acid which are effective natural free radical scavengers.*

Introduction

High concentration of fluoride is noxious to the health of humans and animals. There are many reported patterns of fluoride toxicity in the world. These include endemic fluorosis that is related to high concentration of fluoride in drinking water[1]. The fluoride concentration reaches as high as 100 ppm in some countries, industry related air pollution [2], clinically used fluorinated anaesthetics[3], and the misuse of fluoride containing consumer items, particularly the oral hygiene products[4]. Death has been also recorded due to excessive flu-

oride intake either from drinking highly fluorinated water[5] or in the industrial workplace[6].

The kidneys are primary organs concerned with excretion and retention of fluoride and thus are generally involved in fluoride intoxication[7]. This toxicity can vary from sub-clinical to overt clinical impairment[8] and there is now growing evidence to suggest that fluoride intake provokes nephrotoxic changes in the human[9,10] and animals[11,12]. Histopathological studies have revealed that acute and chronic fluorosis does lead to subtle renal damage, which is manifested by degeneration of tubular epithelia, extensive vacuolisation and necrosis in renal tubules, hypertrophy of glumeruli and interstitial nephritis [12,13].

Fluoride has been observed to disrupt kidney lysosomes and cause the release of N-acetyl-β-D-glucosamindase (NAG) isozymes in urine after acute and chronic exposure to high fluoride concentration in animals[14,15] and in patients operated under fluorinated anaesthetics[16]. The lysosomes are considered as very sensitive organelles to free radicals and oxidative stress perturbations[17] and have been implicated in cellular injury induced by free radicals [18]. Fluoride has the ability to generate reactive oxygen species (ROS)[19] and to increase renal and other tissues lipid peroxide levels in animal[20] and human serum[10].

Recently, some researchers have reported that low molecular weight antioxidants, β-carotene, vitamin C and E, glutathione (GSH) and superoxide dismutase (SOD) have the ability to inhibit the lipid pro-oxidant activity of fluoride[21,22]. It has also been reported that free radical scavengers like vitamin C and pycnogenol (PYC) can efficiently prevent, reduce or even reverse the toxic effects of fluoride[23,24,25].

Ascorbic acid (vitamin C) is an antioxidant[26] found at high levels in a variety of tissues and it has an effective free radical scavenging power over a wide variety of free radicals[27,28].

Pycnogenol (PYC), a blend of

flavonoids, has been recognised as a potent scavenger of ROS (superoxide, hydroxyl and peroxyl radicals) and reactive nitrogen species (RNS) (nitric oxide and peroxynitrite radicals) which are the most important free radicals in biological environment[24]. Several plant flavonoids have been indicated to protect lysosomes against oxidative damage by their free radical scavenging activity as well as by a direct action on the lysosomal membrane making it more resistant to oxidative attack[29].

Aim of the Study

We assessed the in vivo effects of the natural antioxidants (ascorbic acid and PYC) in the prevention of fluoride induced acute renal toxicity in the rat.

Materials and Methods

Forty-four male Wister rats (200-250 g) were divided into four groups (11 in each group). A 24-h urine samples were collected for providing baseline values. The first group received single dose of NaF (25 mg/kg i.p), the second and third groups were given ascorbic acid (250 mg/kg) and Pycnogenol (25 mg/kg) i.p three times daily (for one day). Then these groups were challenged with 25 mg/ kg NaF one hour after the first dose of scavengers has been injected. The last group served as a control and received only equivalent volume of saline. All the doses were given in 0.5 ml saline. Twenty-four hour urine samples were collected following the drug administrations and urinary creatinine (CR) and NAG isozyme were assessed. The two other groups were given only equivalent doses of ascorbic acid and Pycnogenol.

Ascorbic acid used in this study was purchased from Sigma Aldrich Co. while PYC was provided by Horphage Research, Geneva, Switzerland.

Animals housing and urine samples collection:

Male Wister rats were housed in a single stainless steel metabolic cage, which allowed the collection of urine free of faeces in a disposable plastic beaker. The animals were kept in a temperature-controlled room with illumination cycles of 12 hours/day and they had free access to water and food. Twenty-four hours urine

samples were collected, starting on the day before drug treatment (for baseline determination) and continued along the study. The last urine sample was collected 24 hours after the last dose of the tested drug had been given. The urine samples were centrifuged at 1000 rpm at 4 ºC for 10 min and the fresh supernatants were used for NAG and CR levels determination.

Assay of N-acetyl-β-D- glucosamindase isozymes activity:

The enzyme activity was estimated by simple and rapid colorimetric method[30] based on the hydrolysis of substrate, p-nitrophenyl N-Acetyl β-D glucosaminide (Sigma Aldrich), by NAG (Sigma Aldrich) in an acidic pH (5.0).

A 100 µl (in duplicate) of samples was mixed with 1.0 ml buffer-substrate (3.0 mM substrate in acetate buffer (0.1 N sodium acetate and 0.1 N acetic acid, pH 5.0) for test and with 1.0 ml buffer only for reagent blank in ordinary test tubes and incubated for 15 min at 37ºC. Then 2.0 ml of 1.0 M sodium carbonate pH (11.0) was added to all tubes to terminate the reaction and develop the yellow colour. The colour absorbance was measured at 405 nm wavelength in Shimadzu UV-spectrophotometer against reagent blank.

Calculation of NAG isozymes activity:

The above measures were converted to NAG isozymes units by direct calculation from standard curve prepared in the same way by using (0.00078- 0.0125 units) N-acetyl- β-D- glucosamindase (Sigma Aldrich) (r = 0.999, fig. 1). NAG isozymes activity was expressed as enzyme units. One enzyme unit had the ability to hydrolyse 1 nmol of substrate/min/mg protein. The intra-assay variation of NAG isozymes activity was 3.8% and the inter-assay variation was 6.4%.

Determination of urine creatinine (CR):

The most widely used methods for CR determination are based on Jaffe reaction[31], the reaction occurs between CR and picrate ion formed in alkaline medium; an orange-red adduct develops which

is followed photometrically at 500 nm.

Thirty micro litre of urine was diluted to 3.0 ml of water (1:100 v/v), vortex mixed with 1.0 ml picric acid of 40 mmol/l (Sigma Aldrich) followed by 1.0 ml of 750 mM NaOH. A 3.0 ml of standard solution (1.13 mg/100ml creatinine sulphate, Sigma) and 3.0 ml H2O (reagent blank) were treated in the same way, and then allowed to stand for 15 minutes and read at 500 nm. The intra-assay variation of CR assay was 3.1% and the intra-assay variation was 7.3%.

$$\text{Urinary CR(g/l)} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 1.13$$

Statistical analysis:

Results were expressed as mean ± SD (standard deviation). All statistical analyses of the results were carried out on a PC by computer using Prism 4.0 software. Linear regression analysis was used to examine the correlation coefficients, and Analysis of Variance (ANOVA) was employed to determine the effect of variables on the dependent factor studied. A P value less than 0.05 was considered statistically significant.

Results

A significant impairment of glomerular function and renal tubular damage was observed by 25-mg/kg NaF which was manifested by decreased urinary CR excretion (9.35 ± 1.4 vs. 6.08 ± 1.24, $P<0.05$) and increased NAG isozyme activity (0.87 ± 0.2 vs. 2.08 ± 0.8, $P<0.01$). This renal tubular damage as well as the glomerular dysfunction effects of NaF were prevented by either PYC or ascorbic acid (Fig. 3 and 4).

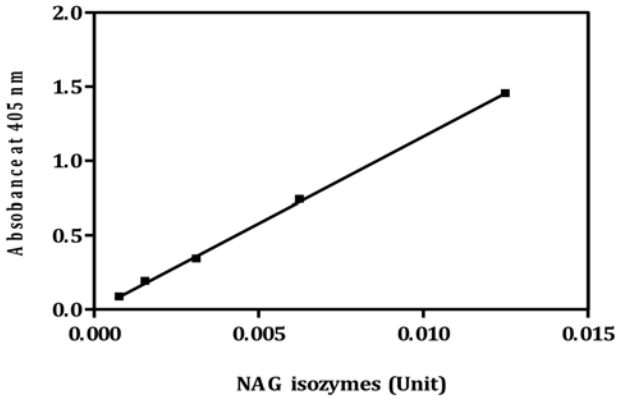

Fig. 1: Standard curve for NAG isozymes activity (r = 0.999). One enzyme unit had the ability to hydrolyse1 nmol of substrate/ min/mg protein.

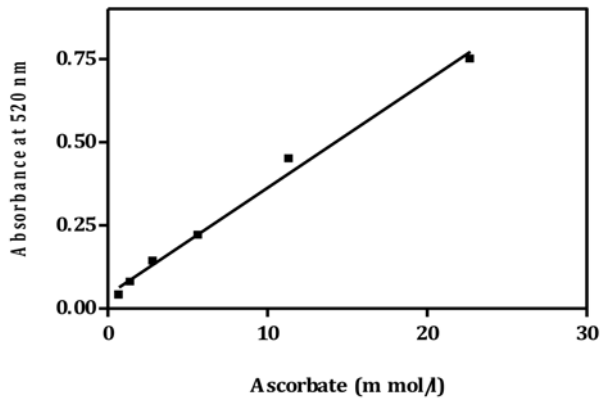

Fig. 2: A standard curve for measuring plasma and tissue ascorbic acid concentrations (r = 0.991).

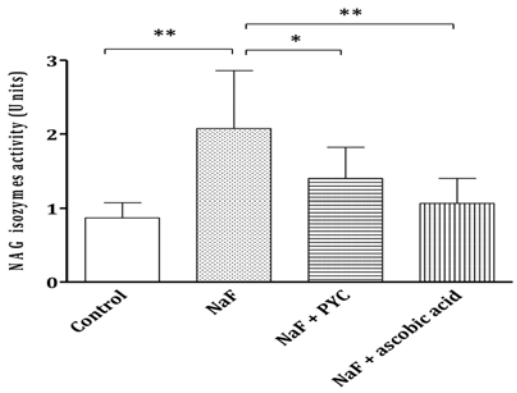

Fig. 3: Urinary NAG isozymes level 24 hr following NaF (25-mg/kg i.p) with and with out PYC (25 mg/kg three times) or ascorbic acid (250 mg/kg three times). Mean ± SD, n= 8, *P<0.05, **P<0.01 compared to NaF treated group.

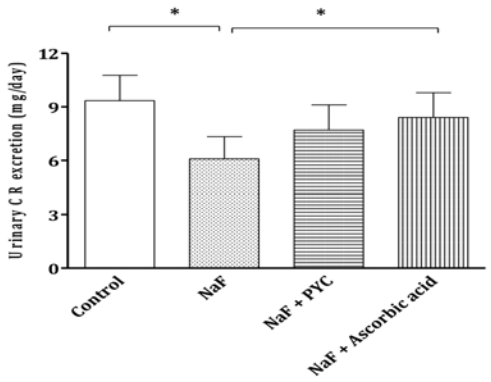

Fig. 4: A 24 hr urinary CR excretion following NaF (25-mg/kg i.p) with and with out PYC (25 mg/kg three times) or ascorbic acid (250 mg/kg three times). Mean ± SD, n= 8, *P<0.05 compared to NaF treated group.

Discussion

The potential for health effects of fluoride exposures on renal function is enhanced because of selective absorption by the kidney and the kinetic of fluoride distribution and excretion. Furthermore, the tissue-to-plasma fluoride concentration ratios for soft tissues are highest in the kidneys (Table 1). Therefore kidney is considered as a target organ for any adverse effects of fluoride. Moreover, in conditions like renal insufficiency the risk of fluorosis is increased as a result of increased plasma fluoride level[32].

In experimental animals, transitory renal dysfunction has been observed such as polyuria, and proximal tubular necrosis following acute single dose exposure to fluoride in rats which has been suggested due its rapid elimination[11,15].

Histological studies have shown extensive necrosis and vacuolisation of the renal proximal tubules following acute single dose of fluoride[12,33] in rats and rabbits. This tubular necrosis has been followed by complete regeneration almost at 7 days[33] after fluoride exposure. It has also been reported that ingestion of excess fluoride facilitates calcium oxalate crystalluria and promotes the formation of bladder stones in rats [34]. There is also clinical evidence suggesting that exposure to high fluoride provokes nephrotoxic changes in human cells[14] and may result in renal failure in patient with chronic fluorosis[35].

Urinary lysosomal enzymes are

reliable biomarkers of nephrotoxicity and they are useful for early diagnosis of tubule damage induced by drugs and chemicals. Fluoride has been observed to target kidney lysosomes and to increase the release of NAG in urine in human following fluorinated anaesthetics[36] and in experimental animals exposed either to acute dose of fluoride[15,37], or chronic high fluoride concentration in drinking water[38]. The increase in urinary NAG isozyme was of significant correlation with the dose of fluoride administered [37]. The early changes of urinary NAG isozyme activity in the absence of other biochemical changes like blood urea nitrogen (BUN), creatinine (CR) and CR clearance (biomarkers of glomerular function) [39] or less pronounced than changes in NAG activity[15] may reflect that the effect of fluoride on glomerular function is less severe than proximal tubules.

Antioxidants and antioxidant enzymes protect living organisms against the attack from ROS. An adequate daily intake of the individual antioxidants is therefore important to protect the cells against oxidative damage[40]. Ascorbic acid is a potent reducing agent and has the ability to scavenge free radicals[28,41].

Ascorbic acid, similar to the dose that we used (or higher) has been found to reduce resuscitation fluid volume requirements as well as severity of respiratory dysfunction in thermally injured patients[42] and has also been found to reduce kidney enlargement, glomerular volume and albumin clearance in diabetic rats[43]. Moreover, it was reported to reduce other pathological as well as xenobiotic induced toxic effects in animals in which free radicals has been incriminated [44]. Ascorbate has been shown to have a pro-oxidant activity in the presence of transition metal ions in vitro, in which it has been shown to reduce metal ions (like ferric to ferrous) which drives the production of the most serious ROS via the so-called Fenton reaction and formation of dehydroascobate and lipid pro-oxidants [45,46]. However, the in vitro pro-oxidant activity of ascorbate is unlikely relevant to the in vivo situations, even in the presence of iron overload[47,48].

Ascorbate has also been reported to augment the pro-oxidant activity of fluoride under in vitro conditions[49]. Based on these reports, we assessed the protective ability of ascorbate against fluoride induced renal failure in vivo along with PYC. Since both ascorbic acid and PYC are natural antioxidants and can be derived from food, therefore it is worthwhile evaluating the potential of ascorbic acid and PYC to prevent fluoride induced renal toxicity. The present study clearly shows that administration of PYC or ascorbic acid with NaF significantly ameliorated fluoride induced renal tubular damage, decreased urinary NAG isozyme level, and improved glomerular function (increased urinary CR excretion). This withstanding ability shown in animals treated with ascorbic acid or PYC may be related to their free radical scavenging activity and inhibition of oxidative stress perturbation and is consistent with in vitro studies[50], which have shown that PYC and other antioxidants can stabilize renal lysosomes against fluoride insult. It is also noteworthy that the capability of ascorbic acid to resist fluoride induced renal toxicity is consistent with recent reports that have observed that ascorbic acid can ameliorate fluoride induced embryotoxicity[23] and can reverse fluoride induced damage to reproductive organs[51] and also, liver and gastrocnemius muscle toxicity[52]. The present study strongly suggests that ascorbic acid and PYC may have a potential as therapeutic agents to protect against fluoride induced renal toxicity.

Conclusions

The results of this study indicate that lipid per-oxidation pathway is implicated in fluoride induced lysosomal damage. We concluded that fluoride induced renal lysosomal damage is amenable to a blockade by free radical scavengers. The natural antioxidants (PYC and ascorbic acid) efficiently attenuated fluoride induced acute nephrotoxicity, which raises the significant role of dietary antioxidants in the amelioration of general fluorosis in people continuously exposed to high fluoride concentrations.

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EFFECTS OF SOME ANTIOXIDANTS ON ACUTE FLUORIDE-INDUCED RAT KIDNEY DAMAGE

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ENDOSCOPIC NASAL SEPTOPLASTY

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Abstract

Objectives: *Septoplasty has been used for decades to correct nasal airway obstruction. The aim of this study is to evaluate efficacy of endoscopic septoplasty compared with traditional septoplasty.*

Subjects & Methods: *This study was carried on patients complaining of nasal obstruction and presented to the ENT outpatient clinic in Benha university hospital. Out of these patients 40 cases were diagnosed to have nasal septal deviations to significant degree during the period from July 2010 to April 2011. 20 patients underwent endoscopic septoplasty and the rest underwent traditional septoplasty. Pre and postoperative evaluations were done in the form of history taking, clinical examination and nasal endoscopy. Endoscopic septoplasty was done for different types of septal deformities to show the technique & to compare efficacy with traditional septoplasty.*

Results: *It was found that endoscopic septoplasty is more effective than traditional septoplasty in treating nasal obstruction, correction of posterior deviations and septal spurs and prevention of contact between septum and turbinates.*

Key Words : *Endoscopy, Endoscopic septoplasty, Nasal Septum.*

Introduction

Surgery on a deviated nasal septum has seen several modifications since its inceptions, starting from radical septal resection to mucosal preservation and subsequent preservations of the possible septal framework (Freer, 1902; Metzenbaum, 1929; Galloway, 1946; Cottle et al., 1958; Maran, 1974).

Recently, the emphasis has been on conservation of the septal framework rather than resection, as the former gives

rise to lesser complications, allows concomitant rhinoplasty or a revision surgery later and moreover conservative surgery can be safely performed in children, without fear of a possible poor development of the midface.(Nayak et al., 1998).

The application of endoscopic techniques to the correction of septal deformities was initially described in 1991 by both Lanza et al. and by Stammberger. In 1993, Lanza et al. described a detailed endoscopic approach to the treatment of isolated septal spurs and septal deviation. The advantages of this technique are: (Hwang et al., 1999)

- Better visualization, particularly to posterior septal deformities.
- It improves surgical transition between septoplasty and sinus surgery.
- Minimal dissection results in less postoperative oedema, less need for packing less post operative adhesion, and decreased hospital stay period with early resumption of normal routine activity.

Could be a part of rhinoplasty.

Revesion could be done.

No limitation of age.

It is used an effective teaching tool.

Less postoperative adhesion.

Subjects and Methods

After approval of the relevant ethical committee, this prospective study was conducted on 40 patients presenting to ENT outpatient clinic at Benha University Hospital complaining mainly of nasal obstruction due to deviated nasal septum during the period from July 2010 to April 2011. They were divided into 2 groups:

Group A : 20 patients underwent endoscopic septoplasty.

Group B : 20 patients underwent traditional septoplasty.

Half of each group septal splints were applied after correction of the deviation.

All operative and non operative procedures were explained in full detail to the patients, who provided informed consents. Each patient was subjected to a preoperative protocol that included a thorough history taking, general and full ENT examination, coronal paranasal sinus CT to exclude

other pathology (Fig. 1), nasal smear for eosinophilia to exclude nasal allergy, nasal endoscopic examination and active anterior rhinomanometry.

Steps of endoscopic septoplasty:

Nasal decongestant drops e.g. oxymetazoline Hcl were used one hour before operation. Both techniques were performed under general anesthesia. Cottonoid packs with (1: 100,000) adrenaline in saline solution were used at the beginning of the operation.

Infiltration :

Subperchondrial infiltration of the nasal septum was done bilaterally using 1: 100,000 adrenaline in saline solution. In cases of septal spur, infiltration was done under endoscopic visual control at the spur itself (Fig. 2).

Incision:

Standard Killian incision from down upword on the left side of the septum was used except in cases of isolated septal spur, incision was done on the spur itself using a sickle knife or using No. 15 knife blade (Fig. 3).

Flap elevation and dissection:

After mucosal incision, mucoperichondrial flap elevation was performed with a Cottle elevator. A suction elevator was used as a useful alternative dissecting instrument to simultaneously clear any blood from the field of view during flap elevation. Further elevation was done using 0° Karl Storz nasal endoscope (4 mm), held in left hand, keeping the tip of the endoscope between mucoperichondrial flap and the septal cartilage. The right hand was used for instrumentation. Exposure was limited to the target area (Fig. 4).

The septal cartilage was then incised just posterior to the mucosal incision. The contralateral mucoperichondrial flap elevation was then performed under endoscopic visual control. Flap elevation was continued bilaterally until complete extent of septal deformity had been dissected (Fig. 5).

At this time, an angled scissors was introduced and used to cut through septal cartilage on a direction, which is parallel to, and at least 1cm posterior to the nasal

dorsum. We removed only the obstructing cartilage, leaving at least caudal and dorsal struts to maintain support of nasal dorsum and columella.

The cartilage was removed with Luc's forceps or a Ballenger's swivel knife under endoscopic visual control (Fig. 6).

Punches or forceps could also be used. Any deviated bone only in the perpendicular plate of ethmoid was then removed under endoscopic visual control with punches or forceps. Large pieces of excised cartilage and bone were saved in saline solution in order to re-insert one of them between the two flaps, if needed at the end of the surgery.

If the maxillary crest is deviated, elevation of the flaps of the maxillary crest and vomer was done under endoscopic visual control. Then deviated crest was removed with gouge held in surgeon right hand and hammer used by the assistant.

In cases of subluxated cartilage from the crest, the excess cartilage inferiorly was shaved using No. 15 knife blade. Then it was repositioned over the crest to prevent a supratip deformity. If there was associated vomerine spur or crest, it was resected.

In cases of septal spur only, incision was done directly over the spur under endoscopic visual control especially for posterior spurs. Exposure was done by elevating the flaps with the sharp end of Cottle elevator superiorly and inferiorly. It was removed either by straight biting forceps or osteotome.

At the end of the operation, suturing of the two flaps with 4/0 catgut suture was done. Splinting of half of the patients was done using readymade Reuter nasal splints (Fig. 7).

Both nasal cavities were packed with small sofratulle packs.

In traditional group, Cottle technique or mobilization of quadrilateral cartilage and re-position it into the columella were used without use of endoscope.

Postoperatively, all patients were viewed in the outpatient clinic. Once weekly for the 1st month then every 2 weeks for 3 months then once every month till the end of follow up period after 6 months. They were subjected to an assessment protocol similar to the pre-operative.

Results

The age in the traditional group varied from 16 to 38 years with a mean of 26.8 years. The age of the endoscopic group varied from 17 to 36 years with a mean of 25.13 years. In our study, we excluded old age patients to avoid calcification of septal cartilage. We didn't find any significant difference in pre-operative results between both groups as regards age, sex, duration, side of nasal obstruction, number of patients having associated symptoms and types of septal deformities. So, the pre-operative circumstances were similar between both groups; consequently any expected difference in the post-operative results would depend mainly on the surgical technique used in each group. By rhinomanometry, the mean pre-operative total nasal resistance was 0.4815 in group A and 0.5069 in group B. There was no significant difference in all pre-operative results between both groups.

As regard the duration of each procedure in our study, endoscopic septoplasty consumed 15 minutes in isolated septal spur up to 27 minutes in broadly based deflections or more than one septal deformity. But in traditional septoplasty, it is ranged from 22 to 34 minutes which means that, in cases of isolated septal spur the duration of operation is less by endoscopy.

Concerning early post-operative complications; in group A, we found that 2 cases (10%) had mild nasal bleeding upon removal of the packs that was controlled by insertion of ephedrine packs for few minutes. Neither pain nor discomfort was experienced in this group.

No smell of bad odour. Neither septal hematoma nor septal perforation was diagnosed. In Group B, 3 patients (15%) had mild nasal bleeding. Mild pain and discomfort were experienced in 2 patients

(10%). 3 patients (15%) had a smell of bad odour due to infection and crustations. One case (5%) of septal hematoma was diagnosed but incision and drainage was done and the patient was improved. No septal perforation was diagnosed. The post-operative results (after 6 months) in both groups subjectively, 18 patients (90%) were benefited (resolved or improved) in group A and 15 patients (75%) were benefited in group B (Table 1).

Objectively, the mean post-operative total nasal resistance was 0.1837 in group A and 0. 2279 in group B. our results show high significant difference between pre- and post-operative values in each group indicating improvement of the patients in both groups as regard nasal obstruction. Results of endoscopic findings on last available follow up is given in (Table 2).

We did not find any importance in use of septal splints at the end of the operation as they not only offer no additional help in stabilizing the septum post-operatively but also cause pain to the patients especially that we didn't perform any surgery at the turbinates, so risk of adhesion formation is minimal (Table 3).

Table (1): Comparison of the subjective results between both groups (6 months post-operative) based on the pre- and post-operative visual analogue scores

Symptoms (No. of cases E/T)		Endoscopic				Traditional				P	Significance
		Benefited		Not Benefited		Benefited		Not Benefited			
		R	I	S	W	R	I	S	W		
Obstruction (n=20/20)	No.	13	5	1	1	8	7	2	3	0.0464	Sig.
	%	65%	25%	5%	5%	40%	35%	10%	15%		
Snoring (n=9/7)	No.	4	3	2	0	1	2	3	1	0.276	N.S
	%	44.4%	33.3%	22.2%	0%	14.29%	28.57%	42.86%	14.29%		
Headache (n=14/12)	No.	6	4	3	1	4	3	5	0	0.423	N.S
	%	42.86%	28.57%	21.43%	7.14%	33.33%	25%	41.67%	0%		
PND (n=6/8)	No.	1	2	3	0	3	2	2	1	0.598	N.S
	%	16.67%	33.33%	50%	0%	37.5%	25%	25%	12.5%		
Hyposmia (n= 6/5)	No.	0	3	3	0	0	2	3	0	0.659	N.S
	%	0%	50%	50%	0%	0%	40%	60%	0%		
Sneezing (n=4/5)	No.	0	2	2	0	0	2	3	0	0.709	N.S
	%	0%	50%	50%	0%	0%	40%	60%	0%		

NB: E= endoscopic, T= traditional, R= resolved, I= improved, S= same and W= worsened.

Table (2): Nasal endoscopic findings on last available follow-up

Nasal endoscopic findings on last available follow-up	Endoscopic		Traditional		P	Significance
	No.	Percentage	No.	Percentage		
Persistent deformity						
a) Anterior deviations	0	0%	0	0%		N.S
b) Posterior deviations	1	5%	7	35%	0.031	Sig.
c) Spurs	0	0%	5	25%	0.0317	Sig.
Persistent contact with turbinates	0	0%	7	35%	0.0143	H.S
Nasal crustations	2	10%	3	15%	0.543	N.S
Nasal synechiae	0	0%	3	15%	0.0678	N.S
Septal hematoma	0	0%	0%	0%		N.S
Septal perforation	0	0%	0%	0%		N.S

Table (3): Study of effect of Splinting

	Splint		No splint		P	Significance
	No.	Percentage	No.	Percentage		
Discomfort after pack removal	5	25%	1	5%	0.0273	Sig.
Splint extrusion	1	5%	0	0	0.487	N.S
Septal hematoma	0	0	1	5%	0.487	N.S
Septal perforation	0	0	0	0		
Nasal synechiae	0	0%	1	5%	0.558	N.S

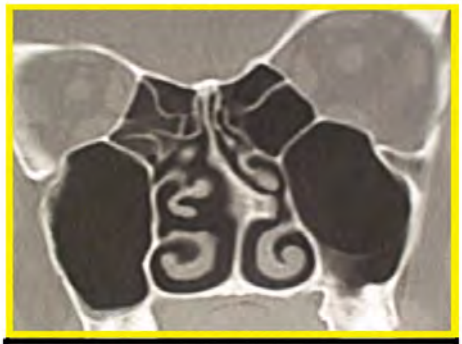

Fig. (1): CT scan on paranasal sinuses of a case of left posterior septal deviation.

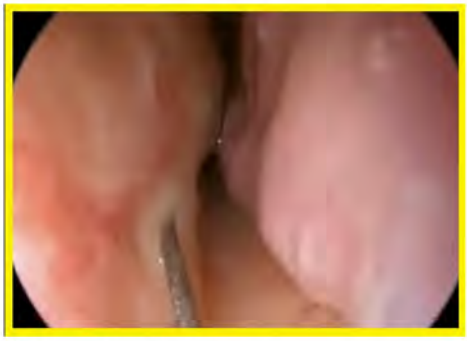

Fig. (2): Infiltration of the nasal septum using 1: 100,000 adrenaline in saline solution.

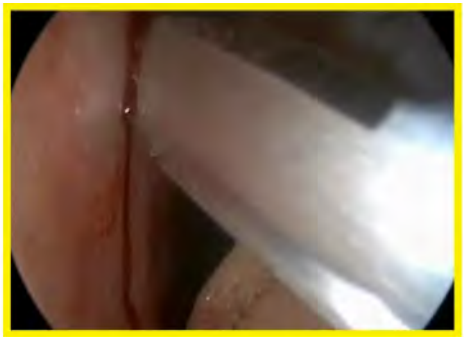

Fig. (3): A longitudinal incision is made by a number 15 knife blade.

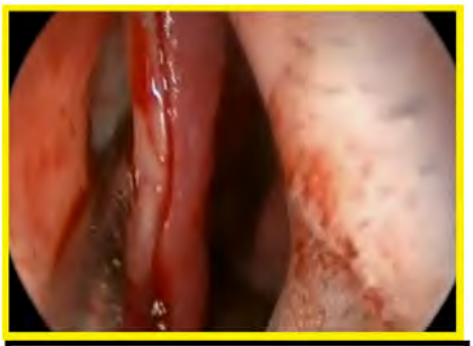

Fig. (5): Suction elevator is used for flap elevation on the contralateral side of the cartilage.

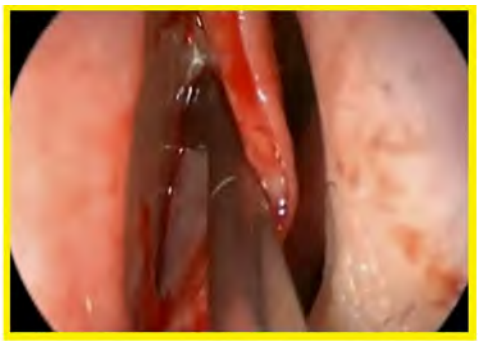

Fig. (4): Suction elevator is used for flap elevation on the left side.

Fig. (6): Deviated cartilage is removed with Ballenger's swivel knife under endoscopic visual control.

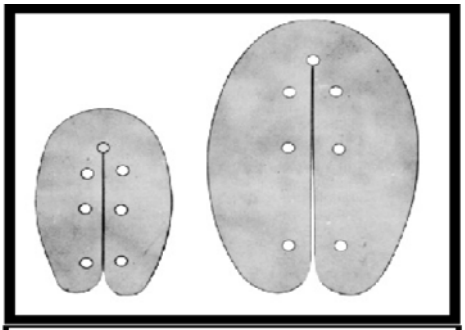

Fig. (7): Reuter nasal splints.

Discussion

Various techniques have been described for the correction of a deviated septum (Freer, 1902; Metzenbaum, 1929; Galloway, 1946; Cottle et al,1958; Maran, 1974). The concept of submucus resection was popularized and refined by Killian.

The application of endoscopic technique for the correction of septal deformities was initially described in 1991 by both Lanza et al. and by Stammberger. This had led to search in the indications, technique and effectiveness of the use of an endoscope in correction of septal deformities by many authors (Lanza et al., 1993; Giles et al., 1994; Yanagisawa & Joe, 1997; Nayak et al., 1998; Vanclooster & Jorissen, 1998; Hwang et al., 1999; Christmas & Yanagisawa, 1999; Durr et al., 2003; Gupta et al., 2005; Getz and Hwang, 2008 and Bothra and Mathur, 2009).

We found that endoscopic septoplasty is superior to traditional septoplasty because of the following:

- No limitation of age.
- It is more effective in treating nasal obstruction.
- It is more effective in preventing contact between septum and turbinate.
- It facilitates accurate identification of the pathology.
- It allows better visualization and accessibility to the posterior deviations and spurs.
- Endoscopic septoplasty could be apart of Rhinoplasty.
- It is valuable in patients who have undergone prior septal cartilage resection.
- It is very useful as an effective teaching tool.
- It allows better understanding of the lateral nasal wall pathology associated with the deformity.
- Instruments used in endoscopic septoplasty are similar to those used for FESS.
- It allows correct identification of the cleavage planes of flap elevation especially in revision and post-traumatic cases. This minimizes chances of tears and prevents perforations.
- Also, elevation of the flap in the correct plane minimizes intra-operative bleeding.
- It helps in documentation of cases.

But there is some limitation of use of nasal endoscopy which include:

- Loss of binocular vision.
- The proximity of nasal endoscope to the surgical field results in frequent tip soiling and the need for intermittent cleaning of the endoscope tip. But, there is irrigation systems that are available for selected endoscope application resolve tip soiling without withdrawing the endoscope.
- The surgeon cannot operate bimanually.

Conclusion

We conclude from our study that deviated septum is one of the important causes for nasal obstruction. It can be corrected by different techniques. Endoscopic septoplasty can be used to correct different types of septal deformities. In cases of isolated septal spur, incision can be done over the spur itself under endoscopic vision, so that less time consuming during surgery and less morbidity. Endoscopic septoplasty is superior to traditional septoplasty in:

- Correction of posterior deviations and septal spurs.
- Treating nasal obstruction.
- Prevention of contact between septum and turbinates.

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ZINC, MAGNESIUM AND CUPPER BLOOD LEVELS IN PRETERM INFANTS

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Abstract

Background: *Increasing survival of extremely low birth weight (ELBW) infants heightens importance of nutritional needs especially trace elements which are not routinely monitored inspite of their important biological functions.*

Aim of the study: *We aimed to determine the serum cupper, magnesium and zinc in preterm newborn with different gestational ages.*

Subject and method: *This study was carried out on 36 preterm infants; 24 female and12 male with mean gestational age (33.1-/+1.8wks) and 20 full term newborns with mean gestational age (38.3+/-1.3). Blood samples were drown for measuring serum copper, zinc and magnesium in both groups.*

Results: *We found in this study a significant lower level of weight, serum copper and zinc in preterm (1.97+/-1.78kg ,21.67+/-10.23ug/dl,116+/-31.53ug/dl respectively) compared to full term infants(3.5+/-.6kg, 30.66+/-11.66ug/dl ,147.62+/-24.89ug/dl respectively) p value was 0.001, 0.022 and 0.006 respectively. Serum magnesium was not significantly different between both full term and preterm (1.94+/-.064mg/dl vs 1.98+/-0.44mg/dl),p was 0.83. No relation to gender, gestational age and duration of admission was found regarding copper, magnesium and zinc. However positive correlation was found between serum zinc and birth weight (r = 0.375 and p = 0.029). No significant statistical differences between simple and sick preterm regarding these elements. Further investigation is required regarding these trace elements in sick preterm with more wide study.*

Determination of serum copper and zinc may be required in unex-

plained and resistant cases of sick preterm.

Keywords: *preterm zinc copper magnesium.*

Conclusion: *From this study we conclude that there is a significant lower level in serum cupper and zinc in preterm infants compared to full term. Also we found no significant difference in both preterm and full term infants as regard to serum magnesium level.*

Introduction

Trace elements are group of chemical elements that are needed in minute quantities for th proper growth, development, and physiology of an organism[1]. They are essential nutrients for metabolism growth, and neurological and immunological function[2]. Their function for preterm seems to be very critical in health and disease, because of their limited stores[3].

The increasing survival of extremely low birth weight (ELBW≤1,000 g) and very preterm (less than 28 weeks' gestation) infants heightens the importance of understanding their unique nutritional needs, including trace elements such as copper. Most fetal accretion of copper, like zinc, occurs in the third trimester, placing babies born before the third trimester (28 wk) at increased risk of copper deficiency(4). Requirement of preterm fore trace element may increase in comparison to full term because of rapid growth stressful events(5). Variation in level in trace element in different reports in preterm infants have been observed among different localities and times because difference in maternal nutrition and methods of study(6,7).

Zinc and copper are important constituents of the diet of preterm infants(1,4); and deficiencies in these trace elements interfere with body development during the accelerated growth period.

Zinc is essential for normal growth and protection against preterm complication(8,9). Because fetal zinc accretion occurs in the third trimester(10), such babies are extremely vulnerable to zinc deficiency. Zinc contributes to many vital metabolic functions (11). Zinc is very stable in its single oxidation state (ZnO2), ena-

bling it to be involved in more than 200 zinc metalloenzymes. It is important in gene expression, neurotransmission, and apoptosis (possibly related to zinc deficiency in mothers and to teratogenicity [12] and has a role in the inflammatory response[13]. Zinc also is vital for optimal metabolism of protein, carbohydrate, and lipids, serving an important secondary role in nutrition[12]. Zinc is necessary for the function of T-helper lymphocytes, natural killer cell activity, and normal structure and function of lymphatic tissues[5]. Although clinical deficiency is uncommon, increasing evidence suggests that there may be problems associated with subclinical zinc deficiency and all the data on zinc requirements come from small studies on term or, at best, very low-birthweight (VLBW) (less than 1,500g) infants, and the results are somewhat conflicting. Recommendations for ELBW/very preterm infants are extrapolated from these data[14].

Copper is a cofactor in several metalloproteins, essential for oxidative metabolism, myelination and the metabolism of several steroid hormones. Clinical copper deficiency is a recognized hazard among preterm infants[15]. In addition to being an essential component of several enzymes, copper is required for bone growth and maturation of red and white blood cells. It also takes part in iron transport, cholesterol metabolism, myocardial contractility, and cerebral development[16]. Also, some complication of preterm was associated with decreased its serum level[17]. Because the importance of copper and its physiology is not fully understood, it is difficult to be certain of the exact requirements. However, it is clear that some babies suffer significant consequences from copper deficiency, so copper must be considered in caring for the highest risk groups. It is currently only possible to estimate the copper requirements of ELBW very preterm babies from some small studies[18].

Magnesium is the second intracellular anion. It is essential for the synthesis of nucleic acids and proteins, for intermediary metabolism and for specific actions in different organs such as the neuro-

muscular and cardiovascular systems. Hypo or hypermagnesaemia occur in preterm with different pathological conditions, however no recent reports described serum magnesium in preterm in comparison to full term[19].

Because of importance of trace elements for preterm in various conditions their level needs to be revised.

Subjects & Methods

36 preterm infants of different gestational ages was selected from Banha Teaching Hospital NICU. Cases was selected from mothers with near socioeconomic classes. Extreme very low birth weight [EVLBW <1000gm], critically ill, twins, babies whose mothers had evidence of placental insufficiency e.g. pre-eclampsia, severe malnutrition ,small for gestational age was excluded from the study. Those with major congenital malformation(cardiac, renal, and pulmonary)were also excluded.

Sample was taken on day 1 just before starting oral feeding started.

Detailed antenatal history including antenatal infection, pregnancy complication, medication intake, exposure to teratogen, antenatal care was taken .Natal history include mode of delivery, premature rupture of membrane (PROM), neonatal asphyxia was recorded. Postnatal course and complication was recorded.

Twenty control cases were selected from healthy full term newborns just before starting oral feeding, those who developed complicated course were excluded.

Routine investigation for simple preterm cases including complete blood count, bleeding time, coagulation time and blood typing was done. Other investigations as radiological studies was done when needed in complicated preterm.

Blood sample (2-3ml) was taken by venipuncture after application of local anesthetic from each patient and control subject, allowed to clot at room temperature. Then serum was separated and stored at -20^{o}C until analysis was performed .

Copper was measured by a colorimetric method by a kit from Centronic GmbH: Copper forms with (3,2 Dibromo -2- pyridyiazo) Methyl-N-sulfopropylaniline a chelate complex.

The excess of absorbance of the complex can be measured and is proportional to the total copper in the sample.

Zinc was measured by a colorimetric method by a kit from QUIM1CA CLINICA APLICADA, S.A: In an alkaline solution, the zinc ions of the sample will produce a red color with 2- (5- Brom -2- pyridyazo) -5-[-N-Propyi-N-(3-Sulfo the zinc ions concentration present in the sample.

Magnesium was measured by a colorimetric end point method by a kit from Centronic GmbH: In alkaline solution magnesium forms a colored complex with xylidyl blue, which is determined photometerically. The color is proportional to the magnesium concentration[20].

Written consent have been taken from parents of all children in this study.The study also was approved from research ethics committee in general organization for teaching hospital and institutes in Cairo.

Statistical Analysis

Data were entered and analyzed by using spss 20.Data were expressed as mean±SD for categorized variables. Chi-square, student -T test and correlation when appropriate $P<0.05$ was statistically significant.

Results

We selected 36 preterm infants from Banha Teaching Hospital, 12 was male and 24 was female, 22 delivered by normal vaginal delivery while 14 delivered by cescarian section (C.S.). Their mean weight was 1.84±0.4 kg. Mean gestational age was 32.61±3.87 weeks, no significant difference was recorded regarding sex and the modes of delivery (P was 0.85&0.6 (respectively). But between patients and control newborn cases (table 1), however there is a significant difference regarding weight and gestational age (P was 0.001&0.0001 respectively).

Among our cases, 16 cases was simple preterm did not developed any complication, 8 developed RDS, 6 had sepsis and 6 had transient tacypnia of newborn TTN (table 2).

In this study we founded significant decrease in cupper in preterm compared to full term 21.67±10.23 vs 30.66±11.66 p was 0.022. Also zinc was significantly lower in preterm compared to full term (116±31.53 vs 147.62±24.89 (p was 0.006). However, no significant difference was found regarding magnesium between both both groups(p was 0.83) table 3.

No significant difference in copper, zinc, magnesium in relation to sex, gestational age, or mode of delivery. Also no relation between copper, magnesium and weight, however, a positive correlation was found between zinc and weight (p was 0.029) table 4,5.

We found positive a correlation between birth weight and gestational age (r=0.506 p=0.002),no correlation was found between serum copper and zinc r=0.065 p= 0.71 table 6.

No significant differences was founded between simple preterm and those with medical complications regarding copper, magnesium and zinc table 7.

Table 1: Trace Elements Of Preterm Infants Demographic Data

		Preterm N=36	**Control(full term) N=20**	**P**
Sex	♀	12	6	0.85
	♂	24	14	
Wt (kg)	X̄ ±SD	1.84±0.4	3.12±0.47	0.001
	Range	1- 2.6	2.5- 3.8	
Modes of delivery	NVD	22	12	0.6
	C.S.	14	8	
Gestational age	X ±SD	33.1±1.8	38.3±1.3	0.001
	Range	28-36	37-40	

Table 2: Course of preterm babies.

Course	number	Percentage
Healthy preterm	16	19.4%
RDS	8	22.2%
Sepsis	6	16.7%
TTN	6	16.7%

Table 3: Serum level of trace elements in preterm versus full term newborn

		Preterm cases (36)	Control cases (full term 20)	p
Copper(ug/dL)	X̄ ±SD	21.67±10.23	30.66±11.33.	0.004
	Range	6.55-42.8	10.02-43.97	
magnesium(mg/dL)	X ±SD	1.94± 0.64	1.98± 0.44	0. 778
	Range	1.03-4.62	1.32-2.77	
zinc(ug/dL)	X̄ ±SD	116±31.53	147.62±24.89	0.001
	Range	62-177.6	109-184	

Table 4: Trace elements serum level regarding gender.

		Male(12)	Female(24)	P
Cupper(ug/dL)	X̄ ±SD	24.6±10.7	20.18±9.85.	0. 306
	Range	6.9-41	6.55-42.8	
Magnesium (mg/dL)	X ±SD	1.78±0.43	2.1±0.7	0.234
	Range	1.39-4.44	1.67-2.83	
Zinc (ug/dL)	X ±SD	120.0958±31.62	109.03 ±31.34	0.328
	Range	36.85-158.24	36.5-150.51	

Table 5: Trace elements serum level regarding weight, duration of admission, gestational age

	Cupper		magnesium		zinc	
	R	P	R	P	R	P
Gestational age	0.294	0.086	0.245	0.151	0.188	0.221
Duration of admission	0.101	0.564	0.023	0.893	0.269	0.112
Weight	0.147	0.414	0.002	0.99	0.375	0.029

Table 6: Gestational age versus weight & Zinc versus cupper(6)

	Zinc		Gestational age	
	R	P	R	P
Weight	0.375	0.029	0.506	0.002
Cupper	0.065	0.71	0.294	0.086

Table 7: Trace elements serum level in simple versus sick preterm

		Simple Preterm cases (16)	Sick preterm cases(20)	p
Copper(ug/dL)	X ±SD	21.1±10.463	20.54±9.74.	0.548
magnesium(mg/dL)	X ±SD	1.95± 0.92	1.92± 0.46	0. 883
zinc(ug/dL)	X ±SD	120±27.84	113.53±34.63	0.673

Discussion

In this study we investigated serum copper, zinc and magnesium in preterm newborn. Our preterm cases was matched with control regarding sex distribution, modes of delivery, natal, antenatal history socioeconomic classes and maternal nutritional states. 16 of our cases was simple preterm,8 had RDS, 6 had sepsis and 6 had TTN.

We found significant decreased level of copper in preterm infants (21.67±10.23) compared to full term control (30.66±11.66) p was 0.022.Similar to our results Kleopatra et al., 2004[21] who found significant lower serum cupper in preterm with hemolytic and non-hemolytic jaundice compared to full term newborn whether have hemolytic or non-hemolytic jaundice. Also similar to our results Sharda et al., 1999[22] found lower serum copper in preterm compared to full term newborn They found also lower copper level in both serum, breast milk of mother of preterm newborn compared with those of full term newborn . Also they founded high urinary copper excresion in preterm compared to full term newborn. Similar to our results Nasser et al.,1998[23] found significant higher serum level of copper in full term newborn (44.42±1.26 ug/dL) relative to that of preterm neonates (30.30±1.14 ug/d) $p < 0.001$.

Contradictory to our results Iqbal ASM 2001[24], found that copper concentration in preterm infants was significantly higher than in the full term infants. They explained their results on basis of higher blood volume in full term relative to preterm .The difference from our results may be explained difference in sample selected. Their cases has higher weight and gestational age relative to our cases.

Comparable to published data

about copper in preterm wide variation with available reports Our data are lower compared with Kleopatra et al., 2004 (21.67±10.23 ug/dL vs 40±8-121±12 ug/dL). Higher values in latest study from our study may be attributed to type of preterm cases were different from our study. Those preterm cases were jaundiced both hemolytic and non-hemolytic with possible higher copper serum level. Higher serum copper may be a cause of non-hemolytic jaundice due to displacement of bilirubin from albumin in the plasma[21]. In cases of hemolytic jaundice copper is released from the RBCS and displace bilirubin from albumin and toxic effect on the liver and other organs[25].

Compared with Marriott et al., 2007[7] our results are lower (21.67±10.23 vs 64.56±16.38 ug/dL). This differences is explained on bases that Marriott cases received breast feeding while our cases did not receive feeding before taking blood sample.

Compared to Sharda et al., 1999[22] our results are lower (21.67±10.23 ug/dL vs 46.62± 13.23 ug/dL). This can be explained on basis of environmental factors and differences in feeding pattern of mother and environmental factors. Low level of copper in preterm has different explanation, fetal serum copper concentrations reach a maximum at the end of the last trimester of pregnancy, whereas the livers of premature infants are immature and cannot accumulate the metal [27]. Another explanations that excessive urinary excursion of copper,low maternal serum copper, breast milk cupper may associate preterm labor[22].

No significant differences in serum copper was found between simple preterm and preterm cases who developed complicated course as infection, TTN, RDS, table (7) Many reports recorded that copper deficiency is associated with complication in preterm. Airede 1998 [28] founded significant decreases in serum cupper in sick preterm compared to stable group which reach level of stable group by 24 weeks with improved clinical manifestation. The difference from our results is attributed to difference in sample size and difference in

type of complicated course from our cases. These cases were had pedal edema, anemia, decreased rate of growth and chronic lung diseases. Cupper is known to have anti infectious function in some reports because of its anti-oxidant character[29] in infants and children, but this role in preterm needs further investigation. Hong Y et al., 2007 found decrease copper in preterm is associated with retinopathy in preterm[30]. Sutton AM et al., 1985[31] recorded 4 preterm cases with cupper deficiency who developed RDS, neutropenia, anemia, edema, PDA and bone changes similar to rickets. Edema, neutropenia, bone changes and anemia improved with treatment with copper. Further investigation may be needed in large studies with bigger sample size for each individual disease.

Cupper deficiency in preterm has clinical significance of possible copper replacements and determination in certain clinical problems and total paretral nutrition (TPN). This needs further study to characterize clinical indications and level in preterm babies.

In this study we found weak and non significant correlation between serum copper with birth weight. This may be due to small sample sized study. No relation to gestational age, sex and duration of admission was found.

We did not found significant differences between preterm and full term newborn regarding serum magnesium (p was 0.83) .Little reports available about serum magnesium in fullterm compared to preterm newborn. Contradictory reports about serum magnesium in preterm newborn. Low level of magnesium was reported in preterm compared to full-term newborn[32] in many reports. This explained on basis that accumulation of magnesium in last trimester of pregnancy, associated hypocalcaemia due to transient hypoparathyrodism, and increased tissue breakdown in preterm, however, higher values for total serum magnesium was found by Tsang RC 1970 and Ariceta G 1995[33&34] in preterm. The latest study proven that Fractional excretion of Mg and the ratio of urine Mg to urine creatinine did not vary as a function of postcon-

ceptional age. Furthermore, no functional immaturity is present for renal tubular reabsorption of Mg, even in very low birth weight infants. Noone D et al 2012[35] found that Mg tends to rise initially in 1st week in ELBW then stabilize and remain normal thereafter. The difference from our study may be attributed to difference in gestational age, type of preterm cases. Magnesium is predominantly intracellular cation participating in many membrane-enzymatic functions, less dependent on maternal transfer as copper and zinc. Factors contributing to hypo or hypermagnesaemia are not significantly different between preterm and full term newborns[36].

We found significant lower level of serum zinc in preterm infants compared to full-term new borns (p was 0.006). Also, positive correlation between birth weight and serum zinc was found (r was 0.375 p was 0.029). In agreement with our results McMaster D et al., 1983[37] found that serum level of zinc in preterm infants 103 +/-16 micrograms/dl at birth, which decreased to 71 +/-11 micrograms/dl at 24 weeks age and start to increased to 119 +/-34 micrograms/dl at 52 weeks age.Similar results was found by Islam MN et al., 2008[36] in Bangladesh in both preterm AGA and preterm SGA babies. However Iqbal ASM 2001[24] did not found significant differences between full term and preterm new born regarding serum zinc the difference from our results may be due to higher gestational age and birth weight.

Zinc deficiency in preterm may be due to most accumulation of zinc (about 60%) occur in last trimester[39] also maternal zinc deficiency has been associated with decrease birth weight[40].

Compared with published data about serum zinc in preterm before, our results are comparable to those of Vileisis RA et al., 1981[41] (121±15 n=9 vs116±31.53 µgm no=36 and higher compared to Lynne D Marriott DL et al.,[6], 2007 and Iqbal ASM[24] (78.46± 17 µgm/dl no=67&90±0.470 µgm/dl respectively. The differences of our results from last two reports may be due to most of cases in 1st

report was sick and lower maternal serum zinc in the second or due to environmental and feeding habits of their mothers.

No correlation was found between serum copper and zinc (r= 0.065 p=0.71).Some reports described negative correlation between serum copper and zinc [26&49&52]. The negative correlation of copper and zinc may be explained by their competition either for the same absorptive binding sites on the intestinal mucosal cells or for similar functional protein systems. Both elements form prosthetic group for superoxide dismutase enzyme[50]. However in agreement with our results low serum copper was associated with clinical zinc deficiency in preterm which was corrected with zinc supplement and improve of clinical manifestation of zinc deficiency[51&52]. This contradictory relation in serum copper and zinc in literatures, together with possible synergistic clinical effect may need further study.

No significant statistical differences was found in serum zinc between sick and simple preterm newborn. Contradictory to our results several case reports described zinc deficiency in preterm associated with serious complications. Clinical zinc deficiency similar to acrodermatitis enteropathica was reported in breast fed preterm newborn at different postnatal ages which improved on oral treatment with oral zinc[41,42,43]. Also case reports about association of zinc deficiency with retinopathy, impaired growth and necrotizing enterocolitis in preterm new born was recorded. The differences of these reports from our report may be due to our small sample size of sepsis and respiratory distress. Further investigations is required with adequate number of cases of isolated sick preterm. Also it is recommended to check serum zinc, copper in unexplained sick preterm especially with cutaneous manifestations, bony changes, RDS, receiving and in those treating with TPN for possible zinc and copper deficiencies.

Conclusion

From this study we conclude that there is a significant lower level in serum cupper and zinc in preterm infants compared to full

term. Also we found no significant difference in both preterm and fullterm infants as regard to serum magnesium level.

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ZINC, MAGNESIUM AND CUPPER BLOOD LEVELS IN PRETERM INFANTS

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TRENDS IN HEPATITIS B, C AND HIV VIRUSES AMONG BLOOD DONORS IN TRIPOLI REGION OVER 4 YEARS

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Abstract

Background: *Hepatitis B (HBV) and C (HCV) and HIV infections are a serious global and national public health problem. Libya has no active surveillance program to monitor trends of these virus infections.*

Object of the study: *The objective of this study was to verify the trend rates of occurrence of HBs-Ag, Anti-HCV, and anti-HIV-antibody in blood donors at Tripoli Central Hospital.*

Methods: *The study analysed the data of blood donors who donated blood at Central Hospital between January 1, 2005 to December 30, 2008. HBsAg, anti-HCV antibody and anti-HIV antibody status was determined immunoassay method (Vitros EciQ, Orthodiagnostic).*

Results: *The overall sero-positive HBs-Ag prevalence was of 12.8, anti-HVC antibody positive was 6.9, and anti-HIV-antibody was 0.9 per 10.000 per 4 years. The incidence per year increased into the donors for the three viruses, HBs-Ag was ranging between 22-58, Anti-HCV antibody was between 15-45 and anti-HIV antibody was 0.7 per 10.000 per year. Prevalence and incidence in this study increased, although statistically was not significant (P value ≥ 0.05).*

Conclusion: *We have concluded that the trend rate of occurrence increases for the three viruses, therefore strict rules to screen every blood donor, and further studies to assess the actual prevalence of these viruses in common blood donor age-group.*

Key words: *Trends, Seroprevalence, HBV, HCV, HIV.*

Introduction

HBV, HCV, and HIV infections represent a global public health problem. Transmission of these viruses occurs mainly via blood and blood products transfusion, and also by sexual contacts[1]. The infections caused by the three viruses are not curable even with the latest available treatments. Effective vaccine is available only for HBV infection prevention. Infection by these viruses can lead to chronic liver and other organ complications as cirrhosis, hepatocellular carcinoma and renal failure, causing financial impact on the families' and death of suffers. All the three viruses have same transmission mode. Health education, safe legal sex, and precaution in blood transfusion are strictly recommended to prevent infection transmission.

Offergeld et al[2] reported, the prevalence rates of the HIV, HCV, and HBV infections were 8.2, 99.3, and 158.9 for HBV (per 100,000 donations). In 2004, the prevalence rates were 4.8 for HIV, 85.3 for HCV and 156.3 for HBV per 100,000 donations. The analysis showed a very low incidence of infections with a decreasing trend for HCV infections and an increase in HIV. In Japan, the incidence of HBV and HCV infections among blood donors, during 10 years, was 2.78 and 1.86 per 100,000 person-years for HBV and HCV respectively[3].

The prevalence of hepatitis B and C has decreased markedly between 1989 and 2004 in Turkey, and that decrease was related to the significant increase in the number of volunteer blood donors [4]. In Turkey, other study by Akcam et al[5] showed that 2.5% were HBV, and 1% were HCV.

Bajubair et al[6] reported that the prevalence of HBV in healthy volunteers was 8% and 10.8% in blood donors, while HCV prevalence was 1.7% in healthy volunteers, 2.7% in blood donors. In another study carried out in Yemen by Haidar[7] reported that the prevalence was 9.8% for HBV and 1.1% for HCV in screened blood donors.

Ali et al[8] reported, the prevalence among healthy adults (blood donors and non-donors) was 2.4%

for HBV, and 3.0% for HCV.

El Beltagy et al[9] reported that the prevalence of HBs-Ag was 3.0% in the northwest region of Saudi Arabia blood donors. Furthermore, it has been concluded that the prevalence of HBV infection among male Saudi blood donors in the northwest region of Saudi Arabia is higher compared to that reported from the central capital area (Riyadh).

In Libya, Zaid et al (2010), reported that the prevalence rates were (0.3% and 0.4%), (2.6% and 2,6%) , (3% and 3%) for anti- HIV antibodies, HBsAg and anti-HCV antibodies respectively[10], that also reported by Habas et al (2009), and Aboudaher et al [11,12].

The prevalence and the incidence was not studied in blood donors in Libya, therefore this study was to determine changes (trends) in infection incidence and the prevalence rates of HBs-Ag, Anti-HVC antibody , and anti-HIV-antibody detection in Tripoli Central Hospital blood donor population.

Materials and Methods

The data were collected retrospectively from the statistical department registry at Tripoli Central Hospital. The data included blood donors who were screened for HBV, HCV, and HIV during 2005-2008. For detection of HBs-Ag, anti-HCV and HIV antibodies, antibodies, immunoassay method (Vitros EciQ, Orthodiagnostic) were used. The data were arranged in Excel Microsoft Program, and the statistic was done by Excel Microsoft version, Minitab version 15 and SPSS version 16 window programs were used. Descriptive statistics were used to describe the data, and prevalence was calculated.

Chi- square test for trend was used to measure the statistical significance.

Results

The screened donors were 14.105 subjects. Out of those subjects 181, 98 and 12 subjects were positive for HBs Ag, anti-HCV and anti-HIV antibodies, with a prevalence of 128,69, and 8.5 were HBV, HCV, and HIV respectively. Furthermore, the incidence was 124,

58, 4.8 subjects per 10.000 during 2005 for HBV, HCV, and HIV. A 4169 subjects were screened during 2006 revealed that the incidence was 122, 47, and 4.8 subjects per 10.000 for HBV, HCV, and HIV. During 2007, 5802 persons screened and showed a 86, 31, and 5 persons per 10.000 were HBV, HCV, and HIV respectively. Lastly, the result revealed that in 2372 blood donors screened in 2008, a 244, 189, and 29 subjects per 10.000 during 2008. Table 1,2,3 and Figure 1,2,3,4,5 demonstrate the results.

Table (1): Demonstrate the total screen subjects and the positive test subjects each year For 4 years.

Year	total donors	B	C	HIV
2005	1762	124	85	0
2006	4169	122	47	4.8
2007	5802	86	31	5.1
2008	2372	244	189	29
total	14105	128	69	8.5

Table (2): Demonstrate the incidence per 10.000 of the screened donors.

Year	total donors	B	C	HIV
2005	1762	22	15	0
2006	4169	51	20	2
2007	5802	50	18	3
2008	2372	58	45	7

Table (3): Number of positive donors and prevalence per 10.000

Time	Nuumber of +ve subjects			Prevalence			
Year	HBV	HCV	HIV	Total screened	HBV	HCV	HIV
2005-06	73	35	2	5931	12.3	5.9	0.3
2005-07	123	53	5	11733	10.5	4.5	0.4
2005-08	181	98	12	14105	12.8	6.9	0.9

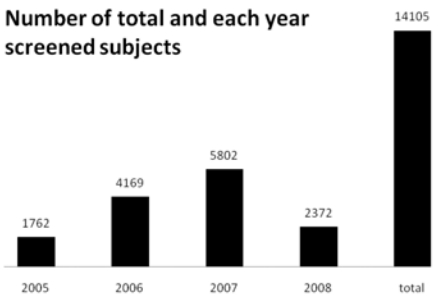

Fig. (1): Demonstrate the total and each year screened subjects.

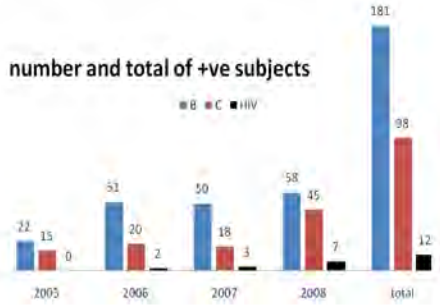

Fig. (2): Demonstrates the number of subjects screened.

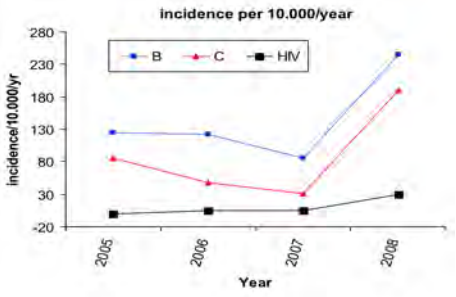

Fig. (3): Demonstrate incidence of the positive subjects.

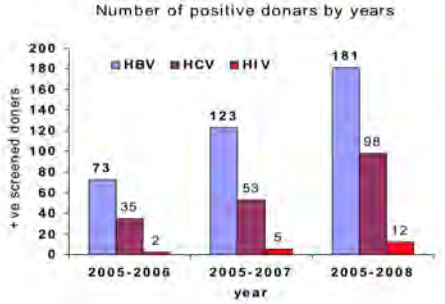

Fig. (4): Number of positive donors per years.

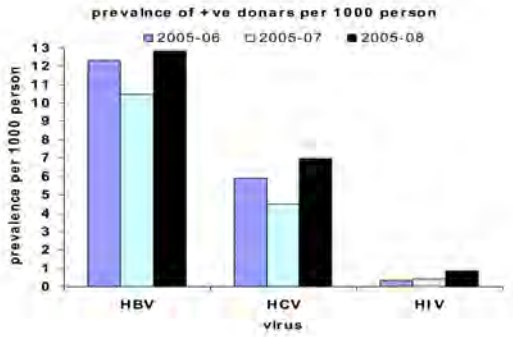

Fig. (5): Prevalence per 10.000/year.

Discussion

HBV, HCV, and HIV infections represent a major worldwide health problem, and it has attracted the attention of the health authorities during the last decades, as these infections are associated with a great morbidity, and mortality. All the three diseases may run a chronic course causing financial drain of the family and can result in death. Hepatitis B and C chronicity results in an increased risk of chronic carrier state, cirrhosis, and hepatocellular carcinoma. Hepatitis B and C are endemic in most parts of the developing countries while HIV is spreading as an epidemic. Since these infections are not curable, preventive measures as health education, safe sex, precautions in blood transfusions and the use of disposable syringes and razors etc [13]. There are few reports dealing with the frequency of HBV, HCV, and HIV infection in our community, therefore, this study was per-

formed to assess the prevalence and the incidence of the viral markers in healthy blood donors within 4 years screened at Tripoli Central Hospital. Our results showed that the incidence of the HBs-Ag positive tends to increase, although the trend was not significantly different per years. anti-HCV and anti-HIV antibodies positive donors incidence tends to rise, but again was not statistically significant (Chi-square). The same increments can be seen in partial and cumulative prevalence in spite of the difference of subjects screened count. In this study, HBs-Ag positive incidence per year per 10.000 increased every year as well as the anti-HVC and anti-HIV-antibody. The trend of the incidence increase was consistent for the three viruses' indices, although the incidence of indices positively was higher in HBs-Ag than anti-HVC and anti-HIV antibody. In spite of the health authorities' legalisation in Libya for the vaccination programmes and the compulsory vaccination for Libyan-nationality subjects, hepatitis B carrier subjects still occur. A Local study conducted in Tripoli in randomly recruited subjects using multi-stage sampling technique reported that HBV and HCV infections were detected in (2.2%) and (1.2%) respectively[12]. Other study conducted at 2007 in Benghazi by Kutrani et al (2007) revealed that 51.2% were infected with hepatitis B virus, 46.9% with hepatitis C virus and 1.9% with hepatitis B and C[13].

The increase of the viruses indices among healthy blood donors during the 4 years showed in this study is not different from Germany, Turkey, Yemen, Pakistan and Japan[2,3,7,8] as well as in at northwest region of Saudi Arabia, but was higher than reported at Riyadh region[9]. On the other hand Bajubair et al[7] concluded that this difference might be due to the cultural, age, early marriage age, education and vaccination programme. On the contrary, Akhtar et al[15] and Syed et al[16] reported a decreasing trend of HBs-antigen and aAnti-HVC antibodies into two different areas in Pakisoan.

In Libya, most of the blood donors are male and younger sub-

jects (20-40 years of age). As it is known that this age group is usually high-risk group for drug abuse, illegal sex and other insecure habits for the virus's transmission.

From this study, it can be concluded that HBV & HCV and HIV virus infection is a problem and every donor must be screened. National programmes should be initialised to screen the whole population in the community. Public health education programs on HBV & HCV and HIV infection, adult hepatitis B immunization programs, and raising socioeconomic standards should be initiated in order to target the high-risk groups. Furthermore, there is an urgent need to assess the actual prevalence of these infections. Therefore, further community-based studies should be planned targeting risky and non-risky subjects to investigate this problem severity and its impaction on the community to adopt preventive strategies.

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TRENDS IN HEPATITIS B, C AND HIV VIRUSES AMONG BLOOD DONORS IN TRIPOLI REGION OVER 4 YEARS

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THE EFFECT OF MATERNAL ANEMIA (IRON DEFICIENCY ANEMIA) ON PREGNANCY OUTCOME

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Abstract

Background: *Iron deficiency anemia (IDA) is relatively common in the third trimester of pregnancy, but causal associations with low birth weight, premature delivery, are still under study.*

Objective: *The aim of the study was to assess the relationship between maternal hemoglobin (IDA) and Perinatal outcome (birth weight, gestational age) in a cohort of 200 pregnant women in the third trimester.*

Methods: *This study was performed on a cohort of 200 pregnant women in their third trimester that referred to Elzahra Hospital in Eljfara area for antenatal care and delivery during November 2008 till May 2009. The data collected were based on questionnaires, clinical examination and laboratory investigations.*

Hematologic and iron-status measures, pregnancy outcomes, and fetal and neonatal evaluations were compared between iron deficiency anemia pregnant women (n=66) and controls (n=132, pregnant with no anemia).

Results: *Complete data were available for the 200 women. Anemia (hemoglobin <11 g/dL) was present in 68 (34%) pregnant women. IDA (Hb <11g/dl & ferritin ≤ 9 ng/ml was present in 66 (97.1%) pregnant women out of the 68 anemic women. The pregnancy outcome (prematurity, birth weight) has not been affected by the maternal IDA in the third trimester of pregnancy.*

Conclusion: *Maternal anemia detected during the later stages of pregnancy, especially the third trimester, often reflects the expected*

(and necessary) expansion of maternal plasma volume. This might explain our findings in this study which showed no significant association between maternal iron deficiency anemia in the third trimester and the health of the infant at birth in terms of premature delivery (P-Value 0.134), low body weight (P-Value 0.082).

Key words: *IDA, Pregnancy, Prematurity, Birth weight*

Introduction

Iron deficiency is the commonest cause of anemia worldwide and is frequently seen in general practice[6]. It is estimated that about 2,150 million people are iron deficient, and the iron deficiency is severe enough to cause IDA in 1,200 million people globally[47]. In the developing world nearly 1/2 of the population are iron deficient, while in Europe about 11 % of population has iron deficiency.

Anemia detected in about 47% of non-pregnant women and 60% of pregnant women worldwide. In the industrial world as a whole, anemia prevalence during pregnancy averages 18%, and over 30% of these populations have IDA[24].

Several studies, performed on pregnant females with IDA revealed an association with both LBW and preterm delivery. An assessment of pregnancy outcome in women with anemia during pregnancy was performed in 2003 in Finland. The frequency of anemia was 2.6%, with 0.3% occurring in the first trimester, and anemia detected in the first trimester was associated with low-birth-weight infants, whereas the mid- and third-trimester anemia groups showed no significantly different outcomes when compared with the non-anemic women. A Sudanese study, revealed that the prevalence of anemia (73.2%) was significantly high in grand multigravidae[14]. In Saudi Arabia the incidence of nulliparous teenage pregnancies was 0.8% and teenagers were at higher risk of preterm birth and low birth weight compared with older mothers[1]. In Nigeria, women younger than 15 years were at higher risk of anemia, premature labor, low birth weight, and operative deliveries[4]. In Ghana in 2007, the prevalence and risk factors for

iron deficiency and anemia in pregnant women from urban areas is less than previously reported[5]. Bakhtiar et al reported that the risk of preterm and Low birth weight among anemic women was 3.4 and 1.8 times more than non anemic women respectively, while others in Pakistan, reported that the Frequency of anemia was 69.9% and that of severe anemia was 4.8%, and the Preterm birth was seen in 23.5% cases and 10.2% in controls and the difference was statistically significant. Of the severely anemic mothers, 29.6% babies were low birth weight (p=0.022) and 27.8% were small for gestational age (p=0.001), as compared to 14.5% and 8.2% of controls, respectively. In France, retrospective study comparing two groups of pregnant women: 111 (pregnant women) with anemia (Hb < 8 g/dl), 111 non-anemic pregnant women (Hb > 10 g/dl) was preformed. Clinical and biological characteristics for both groups were compared. Data on the new born babies were collected. In the anemic group: iron deficiency was the most common cause of anemia (92.7%). There was no significance difference between the two groups with respect to age and parity. Maternal anemia was found to be more significantly associated with more frequent preterm birth (29.2% vs. 9.2%) and increased low birth weight (2933 g vs. 3159 g)[2]. The risk of preterm delivery and LBW among exposed group was 4 and 1.9 times higher among anemic women, respectively. The study concluded that low maternal hemoglobin levels are associated with increased risk of preterm delivery, LBW babies[21].

Lu et al reported that, hematocrit (<37%) was weakly associated with an increased risk of preterm delivery and hematocrit 40% before 20 wk, and between 31 and 34 wk gestation were significantly associated with an increased risk for preterm delivery. Preterm delivery was significantly associated with hematocrits 43% at 31-34 wk gestation[22]. Scholl et al. reported that in 755 pregnant women with IDA receiving initial antenatal care at 16.7±5.4 wk gestation, had more than a twofold risk for preterm delivery whereas, anemia's from other causes were not associated with any increased risk for

preterm delivery, also as including those women in later pregnancies[38,39].

These findings were supported by Zhou et al. (1998) who described the relationship of maternal hemoglobin concentrations during the first trimester and poor pregnancy outcome in 829 Shanghai women, the risk of preterm delivery was increased 1.6 times for women with hemoglobin concentrations between 100 and 109 g/L. A 2.6-fold increase in risk was noted for hemoglobin concentrations ranging from 90 to 99 g/L. The risk for preterm delivery increased 3.7-fold for hemoglobin concentrations between 60 and 89 g/L[46].

Garn et al. (1981) reported that there is increased risk of preterm delivery with low hematocrit (<29% at any gestation) was modest, and the risk of preterm delivery was doubled for white, but not African-American women[11].

Klebanoff et al.[18] concluded that the relationship between maternal anemia at the time of delivery and preterm delivery was an artifact of blood sample collection time. During pregnancy, the normal physiologic changes in plasma volume and red cell mass occur at different periods during gestation. This report did demonstrate a weak association between anemia early in the third trimester and preterm delivery. After 30 wk, anemia was not associated with an increased risk of preterm delivery.

Objectives:

To study the relationship between maternal hemoglobin (iron deficiency anemia) and prenatal outcome in terms of birth weight, gestational age, in a cohort of 200 pregnant women in the third trimester and to highlight the importance of antenatal care regarding maternal health and fetal outcome.

Materials & Methods

This study was performed on a cohort of 200 selected pregnant women in their third trimester that referred to Elzahra Hospital in Eljfara area for antenatal care and delivery from November 2008 to May 2009. The data collected were based on questionnaires, clinical examination and laborato-

ry investigations.

Some of the pregnant women attended Elzahra Hospital for antenatal care routinely received iron and folate supplements where others did not. Women with diabetes mellitus, cardiovascular disease, chronic hypertension and diseases other than anemia, multiple pregnancies, and those who delivered infant with congenital malformations were excluded from the study.

The laboratory investigations included CBC, S.iron, TIBC, and S.feritten, The two hundred pregnant women was classified into anemic (iron deficiency anemia Hb < 11 g/dl & s.feritten < 9.3 ng/ml) and non-anemic pregnant women (Hb > 11 g/dl). The non-anemic group would be used as a control, where the anemic group (IDA) outcome would be checked for birth weight, and gestational age at delivery. LBW was defined as a birth weight below 2500 g, preterm delivery as a gestational age of less than 37 weeks.

Statistical Analysis

Hemoglobin levels were categorized as "anemic" and "non-anemic," according to The World Health Organization's accepted values to define anemia. Averages of collected data of pregnant women of age, Hb, s.feritten, gestational age, birth weight, were calculated for both "anemic" and "non-anemic" patients. These averages were analyzed for statistically significant relationships with s.feritten levels using the Anova in Microsoft Excel Data Analysis Tool pack. The Statistical Package for Social Science version 10 (SPSS), software for biostatic analysis was used to achieve valid and reliable results obtained in this study. P value (<0.05 was considered statistically significant). Data were then presented in tables and figures.

Results

A total of 200 pregnant women were enrolled in the study, the mean age of these women was 29.95±4.9 years with a range of 18 to 43 years.The difference in the age groups between groups was found to be statistically insignificant (P-value 0.531). The age interval (20-35) years contained the highest frequency of age (164)

(82.4%) pregnant women where the age interval (<20) years showed the lowest frequency of age (2.0) (1.0%) pregnant women. The mean maternal age in the control group was 30.2±5.0 years (range, 18-43). The frequency of age of the control group among the three different age intervals was found to be similar to that of the study group where the age interval (20-30) years contained the highest frequency (107) (81.1%) of age of non-anemic pregnant women, and where the age interval (< 20) years contained the lowest frequency of age (2) (1.5%) of non-anemic pregnant women. The mean maternal age of the IDA group in the third trimester (Hb < 11 g/dl & S.Feritten < 9.3 ng/ml) was 29.5±4.9 years (range 20-40).The age interval (<20) years did not contain any IDA pregnant women, where the age interval of (20-30) years contained the highest frequency of age (56) (84.8%) of IDA pregnant women. The mean hemoglobin (Hb) for the entire cohort was 11.7±1.4 g/dl (range, 8.0-15.3). Anemia (Hb <11 g/dl) was present in 68 pregnant women out of 200 (34%) women as illustrated in (figure 1). Of 68 anemic women, 66 (97.1%) had feritten < 9.3 ng/ml which represented IDA. The Average hemoglobin of the control group Hb ≥ 11 g/dl was 12.4±1.0 g/dl (range 11-15.3), where the median was found to be 12.3 g/dl. The mean hemoglobin of the iron deficiency anemic group Hb < 11 was 10.2±0.75 g/dl (range, 8-10.9). The 66 iron deficiency anemic pregnant women (Hb < 11 g/dl & s.feritten < 9.3 ng/ml) represented (33%) of the 200 studied women. and represented (97.1%) of the 68 anemic pregnant women (Hb < 11 g/dl & s.feritten ≥ 9.0 ng/ml). The mean serum ferritin level for the entire cohort was 16.9 ng/ml ± 12.9 (range, 1.5-71 ng/ml). The average of s.feritten among the control group was found to be 23±11.2 ng/ml (range, 10.1-71.0). The mean of the s.feritten level among the IDA cases turned to be 4.54±3.9 ng/ml (range, 1.5-9). In our study, s.feritten was used as the decisive hematological parameter (s.feritten < 9.0 ng/ml) for the determination of iron deficiency anemia. The number of pregnant women who had s.feritten < 9.0 ng/ml among the 88 anemic women turned to be 66 (97.1%) which

represented IDA. The two anemic cases that had s.feritten ≥ 9.0 ng/ml & Hb < 11 g/dl represented other types of anemia. The average of the gestational age among the study group was found to be 39.3±1.6 weeks (range, 35-43) and the median was found to be 40 weeks. The gestational age interval (37-42) weeks showed the highest frequency of gestational age (192 women) (96.0%) among the study group where the age interval < 37 weeks which represents premature delivery and the age interval > 42 weeks showed equal frequencies (4.0 pregnant women) (2.0%) each among the study group. The control group was found to have an average of 39.2±1.6 weeks (range, 35-43), where the median was found to be 39.5 weeks. As in the study group, the gestational age interval (37-42) weeks also showed the highest frequency (126 non-pregnant women) (95.5%) among the control group. The iron deficiency group (66 pregnant women) was found to have an average of gestational age 0f 39.5±1.5 weeks (range, 37-42). The gestational age interval <37 weeks which represents premature delivery did not contain any frequency (0) (0%) of gestational age of IDA of pregnant women, where the interval of 37-42 weeks showed the highest percentage of gestational age (98.5%). As demonstrated in figure 1.

The mean of the newly born infants of the study group was found to be 3.37±0.44 Kg (range, 2.10-4.50), where the median was found to be 3.4 Kg. Among the studied group, six (3.0%) newly born infants weighed < 2.5 Kg which is considered to be low body weight, where 194 (97.0%) newly born babies weighed ≥ 2.5 Kg. The 132 non-anemic pregnant women were found to have an average of newly bor n baby of 3.39±0.446 Kg (range, 2.1-4.5), where the median was found to be 3.4 Kg . The control group was found to have 96.2% of the newly born babies weighing ≥2.5 Kg, where 3.8% of the infants weighed <2.5 Kg which is considered to be low birth weight. The iron deficiency anemia pregnant women had an average of baby weight of 3.32±0.43 (range, 2.50-4.12) and a median of 3.39 Kg. The entire 66 iron deficiency anemia pregnant woman

had a baby weight ≥2.5 Kg as illustrated in figure 2.

There was no association of iron deficiency anemia with age, (P-0.74).Hb was significantly associated with the distributions of s.feritten (P-0.000). Medians of s.feritten increased from low to high Hb. Similarly, the prevalence of low s.feritten also showed a significant decrease with increasing Hb. The proportion of women with indication of low s.feritten, seemed to decrease with increasing Hb. The mean gestational age was found to be higher in patients with lower hemoglobin (IDA Group) (39.5±1.5 weeks). No statistically significant difference was found between the anemic (IDA) and the non-anemic groups in gestational age (P-value =0.134). The average birth weight of infants of anemic patients was 70 g less than the average birth weight of those of non-anemic patients, however, insignificant statistical significance was also observed in this analysis (P=0.082).

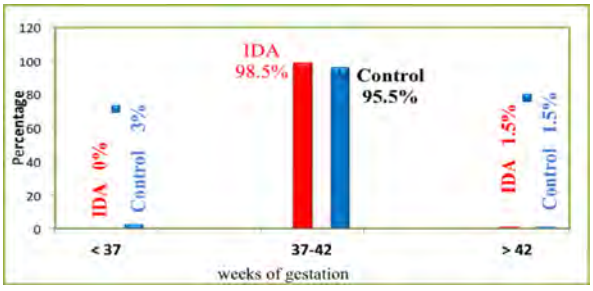

Fig. (1): Representing weeks of gestation and percentage among patients & control group.

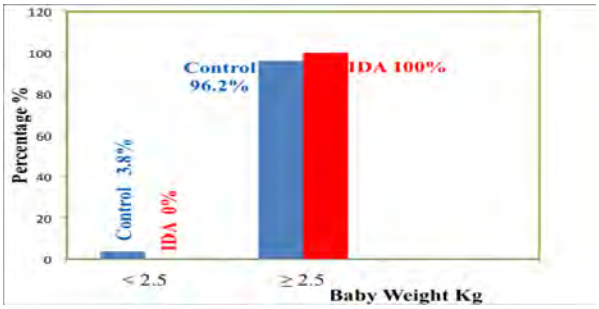

Fig. (2): Showing the baby weight intervals and its corresponding percentages among IDA & control group.

Discussion

This is the first study that has been carried out that is concerned with maternal effect of IDA on pregnancy outcome in the third trimester in Eljafara area, Tripoli-Libya. Most of the previous studies in Libya have concentrated on the prevalence of anemia in a certain population.

In our study, 200 pregnant women underwent hematological investigation and were classified as: 68 pregnant women (34% of the total study group) with anemia (Hb <11 g/dl), and 132 non-anemic pregnant women (66% of the total study group) (Hb ≥11 g/dl). Clinical and biological characteristics for both groups were compared. Data on the new born babies were collected. In the anemic group: iron deficiency was the most common cause of anemia (97.1%).

These findings appear to be in accordance with the prevalence rates of anemia and iron deficiency anemia which reported by most of the previous studies e.g. Kelly et al[16], found that the prevalence of anemia among women participating in public health nutrition programs is approximately 29% in the third trimester. Our results were also in accordance with the prevalence rates of anemia and iron deficiency anemia which reported by Cyril et al[5], Bakhtiar et al (3), Riffat et al[33], Klebanoff et al[19], Lu Z et al[22], Murphy et al[26]. Retrospective cohort analysis of hemoglobin and birth outcome among 173,031 pregnant women who attended publicly funded health programs in ten states and delivered a live born infant at 26-42 weeks' gestation was performed in the United States of America. They defined preterm as less than 37 weeks of gestation, where they concluded that the risk of preterm birth was increased in women with low hemoglobin level in the first and second trimester.

The study highlighted the importance of considering anemia as indicator for adverse as pregnancy outcome[28,36,39,46].

We have evaluated multiple factors of pregnancy outcome in relation to maternal Hb (s.feritten < 9 ng/ml) in the third trimester.

Such risk factors included premature delivery (<37 weeks of gestation), low birth weight (<2.5 Kg). Our findings showed that there was no significance difference between the two groups (cases and controls) with respect to age (P-Value=0.74). In the other hand, the statistical analysis showed that Hb was significantly associated with the distributions of s.feritten (P-Value=0.000). Medians of s.feritten increased from low to high Hb. The proportion of women with indication of low s.feritten seemed to decrease with increasing Hb.

The comparison of means of gestational age between the iron deficient group and the non-anemic group showed that the mean gestational age of the anemic group was found to be higher than the non-anemic (39.5±1.5 weeks) vs. (39.2±1.6 weeks). Also our study reported four premature deliveries among the control group where there were no cases of premature delivery were reported among the anemic group. Regardless of these findings, there was no statistically significant difference found between the anemic (IDA) and the non-anemic groups in gestational age (P-value=0.134). The average birth weight of infants of anemic patients was 70 g less than the average birth weight of those of non-anemic patients. Although there was five cases of low body weight among the control group and there was no cases of low body weight among the anemic group. There was no statistical significance between the two groups (P=0.082).

Previous studies have demonstrated a relationship between maternal Hb in early pregnancies and birth weight of the infant[29], as well as gestation age (1) The findings of our study found no significant association between maternal IDA in 3rd trimester and the health of the infant at birth in terms of premature delivery (P-Value 0.134), low body weight (P-Value 0.082).

The above mentioned findings appear to be in accordance with previous studies performed in 1994 in a study in America which showed that anemia diagnosed early in pregnancy is associated with increased risks of low birth

weight and preterm delivery, where the association between anemia and outcomes reversed direction during the third trimester; maternal anemia was no longer a risk factor for poor pregnancy outcomes[36].

Our results were also in accordance with a study performed by Klebanoff et al. Used a case-control design to examine the relationship between second and third trimester hematocrit and risk of preterm birth in >1700 gravidas from the Kaiser Permanente Birth Defects Study. For bi-weekly intervals between 13 and 26 wk gestation, odds for preterm delivery with anemia were almost doubled (adjusted odds ratio=1.9), when controlling for age, education, ethnicity, marital status, smoking and gestational stage at study entry and consistent for all ethnic groups. However, during the third trimester, anemia was no longer a risk factor for preterm delivery[19].

In the other hand, our results are not consistent with low prevalence rate was found in some previous studies e.g.Fatemeh et al[4], Khyrunnisa et al[7], in India which reported a prevalence percentage of 16% in the third trimester of 500 studied pregnant women.

Our results also agreed with a follow-up study of a population at 28 wk gestation, Scholl and Hedgier (1994) demonstrated that the risk was no longer increased for women who had iron deficiency anemia (15.6%) at this time or anemia from other causes. Although risk for preterm delivery was increased when IDA occurs early in gestation, iron deficiency later in pregnancy probably reflects mainly normal physiological expansion of maternal plasma volume[38].

3rd-trimester anemia usually is not associated with increased risk of preterm delivery; however, all have been associated with increased preterm delivery. This might be a reason for the presence of four cases of premature delivery among the control group. This increased risk may reflect in part the failure to expand maternal plasma volume adequately, thus diminishing appropriate placental perfusion[18,42].

Conclusion

Maternal anemia detected during the later stages of pregnancy, especially the third trimester, often reflects the expected (and necessary) expansion of maternal plasma volume. This might explain our findings in this study which showed no significant association between maternal iron deficiency anemia in the third trimester and the health of the infant at birth in terms of premature delivery (P-Value 0.134), low body weight (P-Value 0.082).

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THE EFFECT OF MATERNAL ANEMIA (IRON DEFICIENCY ANEMIA) ON PREGNANCY OUTCOME

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EPIDEMILOGY OF NON-HODGKIN LYMPHOMA IN LIBYAN PATIENTS

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Abstract

Background: *Non-Hodgkin lymphoma (NHL) is a heterogeneous group of hematologic malignancies with varied aggressiveness and many therapeutic options.*

As there is a lack of data base in cancer patients in Libya and no epidemiologic studies were performed and we think studding the epidemiology of NHL is an important issue that may help hematologists and oncologists to orient about the subject and to have a comparative view with other studies.

Objective: *The study objective was to analyze some important epidemiological parameters that have an impact on the disease course and management in the Libyan patients with NHLs.*

Materials and method: *We have reviewed retrospectively data from the archive at medical department Tripoli central hospital and hemato-onclogy department at Tripoli medical center for patients diagnosed as NHL during 2006-2010.*

Results: *Our study revealed some epidemiological data of Libyan patients with NHL and the results were shown in figures.*

Key words: *NHL, Epidemiology, Libyan patients.*

Introduction

Cancer in all forms causes 9% of deaths throughout the world. In the developed countries it is the second cause of death and in the developing countries it is ranked as the fourth cause of death. Malignancies of lymphoid cells can be divided into Hodgkin and non-Hodgkin lymphomas (NHL) on the basis of pathologic features, clinical manifestations and treatment.

B-cell non-Hodgkin lymphoma (B-cell NHL) is composed of a heterogeneous group of clonal lym-

phoproliferative disorders. According to the 2008 World Health Organization classification of lymphomas, this group includes more than 30 different disease subtypes [1]. Approximately 70,000 patients with NHL are diagnosed annually in the United States, with approximately 85% of these originating from B cells[2]. The peak incidence for diffuse large B-cell lymphoma (DLBCL) occurs in the seventh decade of life[3]. Extranodal involvement or associated constitutional symptoms are uncommon. Bone marrow involvement is not commonly seen at diagnosis, with only 20% to 30% of patients having evidence of DLBCL in the marrow[4].

Objectives

Materials and Methods

This study retrospectively analyzed the characteristics of clinical and pathological data for the 89 NHL Libyan patients between 2006 and 2010. Then data were obtained from the files of patients at Tripoli central hospital and Tripoli medical center. All cases were diagnosed based on the clinical characteristics, the pathological morphology of the tissue samples, and the immunohistochemical results. The clinical data of patients included gender, age, sex, pathological type and immunohistochemistry, stage, lesion location, date of diagnosis.

Statistical Analysis

Clinical data of all patients including gender, age, lesion location, pathological type, and clinical stage were counted or measured. The proportions or the averages were used for processing and analysis. Significant differences between data were analyzed using chi-square test or t test. The statistical software SPSS 13.0 was used for all statistical analyses.

Results

The study revealed that in 89 patients with NHL 52 was males and 47 females and the age of the patients was between 22-90 years (mean 54.6). Fig 1 demonstrate that the majority of the patients (56.18%) were men and 41.57% were females and fig 2 demonstrate that 47.7 were at the age of more than 60 years, 28% were between the age of 40-59y and only 23.6% at the age between 20-39y.

The distribution of the patients according to the stage of the disease demonstrated in fig 3 and in 11.24% were in stage I (A and B), 19.10% in stage II (a, b), 35.95% in stage III(a,b) and 11.23% in stage VI.

Fig 4 demonstrate that the majority of the patient were diffuse large B cell lymphoma (DLBCL, about 42.7%), Mucosa associated lymphoma (MALT, 10.11%), small cell lymphocytic lymphoma (SCLL, 2.24%), Follicular lymphoma (FL, 1.12) and T-cell lymphoma in only 3.37%. About 47% of patients were CD 20+;35.96% were CD 20- as shown in fig 5.

Also, in our study the majority of the patients presented with nodal type (about 73%) and only about 22% of patients were with extranodal presentation.

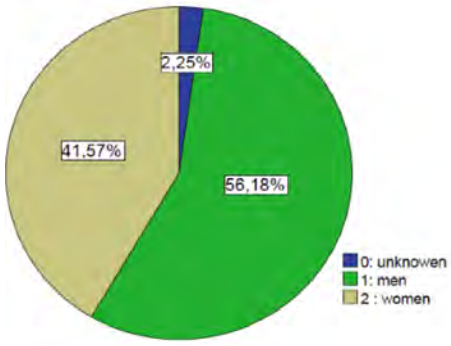

Fig. (1): Distribution of patients according to gender.

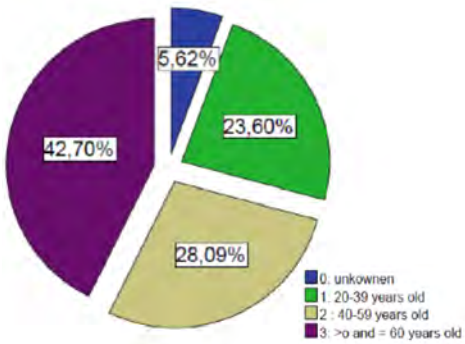

Fig. (2): Distribution of patients according to the age.

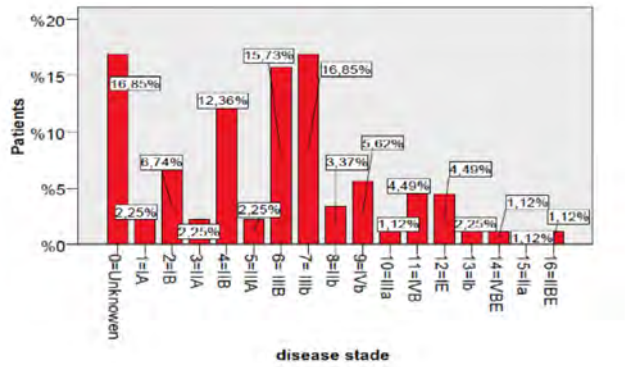

Fig. (3): Distribution of patients according to the stage of the disease.

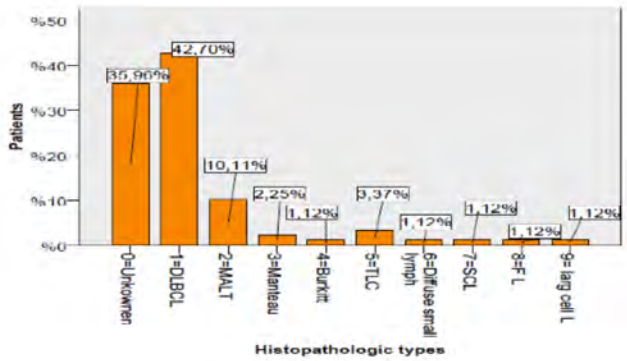

Fig. (4): Distribution of patients according to histopathologic types of NHL.

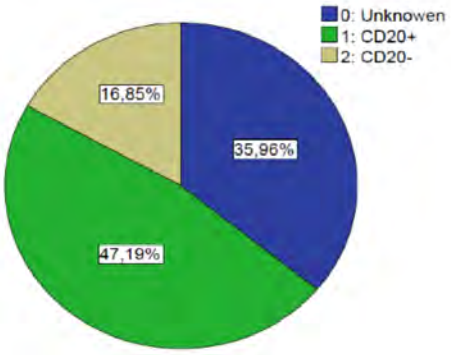

Fig. (5): Distribution of patients according to the immunohistopathologic types of NHL.

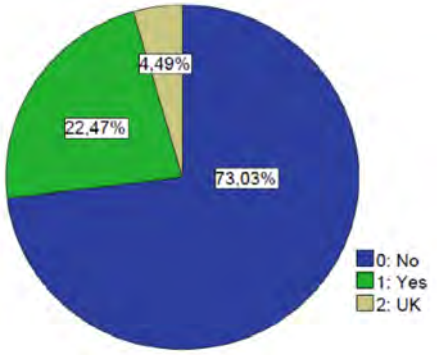

Fig. (6): Distribution of patients according to extranodal localization of NHL.

Discussion

The study of NHL in Libya is useful as no previous data especially on epidemiology was published in the literature and it will provide the clinicians with important information about the disease incidence in Libya.

There several factors which have a great impact on the course and prognosis of NHL. One of the most important clinical predictors of survival in DLBCL patients is the IPI, which uses patient age, Ann Arbor tumor stage, serum lactate dehydrogenase, performance status, and number of involved extranodal sites to identify patients as low risk, low-intermediate risk, high-intermediate risk, and high risk[5]. Jie Liu et al[6] showed that the average age of onset was 47.7±16.3 (18-85), and the male

to female ratio was 1.57:1.

We have found in the study that the majority of the patients were at 60y and mainly males were affected. Age influenced survival strongly as older persons typically experienced poorer survival, and even within stage, older persons had lower survival rates. Although, NHL incidence rates were higher in males than females across the age spectrum and females had slightly higher survival rates. As with most cancers, stage at diagnosis exerted considerable impact on survival. The We have found that the majority of the patients presented with stage III (a, b) about 35.95% and 69.65% were presented with B symptoms at diagnosis.

Presence of B-symptoms (B-symptoms mean the constitutional symptoms like fever, chills and weight loss) dramatically lowered survival within all stage and age groups[6,7].

Jie Liu et al[6] showed that diffuse large B cell lymphoma (DLBCL) (40.9%), extranodal NK/T-cell lymphoma, nasal type (NK/T) (10.0%); peripheral T cell lymphoma, unspecified (PTL) (9.2%); follicular lymphoma (FL) (6.4%); extranodal marginal zone B cell lymphoma (MALT) (5.4%); precursor T lymphoblastic leukemia/lymphoma (T-LBL) (4.5%); and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) (3.2%).

Our results revealed that the majority of the patients were DLBCL (42.7%) and this result is slightly more than that in the developed countries where DLBCL is the most common type of lymphoma seen, accounting for 30% of all newly diagnosed NHL[8]. The second common type is the MALT type (about 10%) and FL is only about 1% which is not compatible with other studies where they found that the FL is considered an indolent type of NHL. It comprises about 20% of all NHLs and is the most common subtype of indolent NHL[9].

The study of CD20 expression (cluster of differentiation antigens) in lymphoma cells is vital not only to establish an accurate diagnosis but also to prepare an appropriate

plan of treatment with biological drugs[10,11].

It has been confirmed that, in the majority of B-cell lymphomas, the CD20 antigen. Is expressed on the surface of neoplastic cells; however, the intensity of CD20 expression varies by the type of lymphoma and by the differentiation of lymphoma B-cells. It is therefore assumed that the CD20 expression is low in the cells of B-cell chronic lymphocytic leukemia (CLL), while it is most intense in the cells of diffuse large B-cell lymphoma (DLBCL) and of hairy cell leukemia[12,13,14].

Jie Liu et al[6] Our study revealed that Among the total, 64.7% were B-cell NHL, 30.3% were T-cell NHL, where as in our study we found that more than 97% of the studied were B-cell type and the rest is T-cell. About 47% of them express CD 20, 16,85% were negative while 35.96 unknown results which could be explained on the bases that the CD20 test was not available during histopathological diagnosis.

The extranodal presentation of NHL may represent a poor prognostic criteria particularly the involved primary site is extranodal and also the extranodal dissemination in nodal disease and our study showed that the majority of the patients were with nodal presentation (about 73%) and only in 22.47% were extranodal.

Conclusion

We conclude that Libyan patients were not totally different from other areas in the world concerning with some epidemiological parameters and the study of these factors might help hematologists and oncologist about course and progress of NHL in our region.

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A RETROSPECTIVE STUDY ABOUT THE SERUM FERRITIN LEVEL BETWEEN RESPONDER AND NON RESPONDER PATIENTS WITH CHRONIC HCV TO TREATMENT BY [PEG INTERFERON & RIBAVIRIN THERAPY]

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Abstract

The goal of treatment in patients with chronic hepatitis C virus (HCV) infection is to eradicate hepatitis C virus (HCV) RNA, the predictors for the patients that underwent interferon therapy is crucial. This is retrospective study carried on 50 patients (40 males, 10 non pregnant females). The patients divided into 2 groups, (1) responder (29 males, 8 females). Mean age (50.5±4.53 years old) and group (2) non responder (11 males, 2 females). Mean age (48.6±6.37 years old). The results of the investigations between responders and non responders was of no statistical significant, P value >0.05 for all including iron indices (serum ferritin, serum iron and total iron binding capacity). There was statistical significant difference for T4, PCR viral load and INR, p value <0.05. Serum ferritin cut-off level 19.25um/l, with sensitivity 75.7% and specificity 38.5% and Coefficient interval (CI 0.42 - 0.8) by ROC Curve, p value >0.05 and that was not statistically significant. Conclusion, patients with chronic HCV need more care for the prediction to the therapy to avoid the side effects as well as the economic cost. Serum ferritin level has no role to the prediction of therapy in this study. Recommendation for further study to proove or disprove this issue .

Keywords: *Chronic HCV, Responders, non Responders.*

Introduction

The factors that contribute to histological progression of liver disease in HCV infection are still under investigations, it is known that many factors associated with response to treatment by dual therapy (Peg INF + Ribavirin) was classified into viral e.g, the base line viral load, the genotype[1,2] and host factors which can be further divided into general parameters like age, sex, liver fibrosis, iron load and cellular factors including genetic polymorphism[3,4]. Steatosis may be either virus related or host related, this is supported by the higher prevalence of liver steatosis in patients with chronic hepatitis C than in the general population[5], however nearly half patients with chronic viral hepatitis (B & C) have an abnormal transferrin saturation and or serum ferritin[6]. A higher iron level and serum ferritin level reduces the response rate to interferon in chronic HCV. Removal of iron by venesection increases the end of treatment virological and histological response to short term interferon therapy, but there is no significant benefit to the sustained response[6,7]. Long term benefits are the reduction of HCV - related morbidity and mortality[8]. This study aimed to evaluate serum ferritin level in chronic HCV patients treated by pegylated interferon and ribavirin and its impact on treatment response [end treatment (ETR) and sustained virological responses (SVR)].

Patients and Methods

This is retrospective study included 50 patients from Gastroenterology, Hepatology and Infectious diseases department, Benha University Hospital and Internal Medicine Department, Police Hospital, El–Agoza. All the patients are known chronic HCV (40 males & 10 females), they were divided into 2 groups (responders and non responders). Mean age was (50.5± 4.5 years old), (48.6±6.8 years old) respectively. All the patients have compensated chronic HCV diagnosed by laboratory investigations that include (HCV antibodies, PCR for HCV RNA and liver biopsy with assessment of degree of inflammation and stage of fibrosis by METAVIR score), and all patients were also investigated to assess candidacy for interferon therapy and to exclude other etiologies of chronic

liver disease (CBC, ESR, liver function tests, kidney function tests, FBS, lipid profile, autoimmune markers (ANA, AMA, SMA), alpha-fetoprotein (AFP) and serum iron indices, that include (serum ferritin, total iron binding capacity (TIBC), serum iron.

PCR for HCV RNA was done at baseline then 3, 6, 12 months of treatment and 6 months after end of treatment (SVR).

Abdominal ultrasound to evaluate liver size, echogencity focal lesion, portal vein diameter, blood flow, splenic size...etc.

Ferritin test by Micro-Elisa :

Principle of the procedure:

This test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes two unique antibodies (mouse monoclonal and a goat polyclonal) directed against distinct antigenic determinants on the ferritin molecule.

Statistical methods:

Data was statistically analyzed using SPSS (statistical pakage for social science) program version 13. Data are shown as mean, range or value and 95% confidence interval (95%CI) and frequency and percent.

Chi square test was done for qualitative variable analysis and P value <0.05 was considered significant.

Student t-test was done for normally distributed quantitative variable to measure mean and standard deviation and P value <0.05 was considered significant.

ROC curve (Receiver operating characteristic curve) was done to detect cut level of any tested variable where at this level the best sensitivity and specificity cut-off values of the variables for the presence of the disease. The validity of the model was measured by means of the concordance statistic (equivalent to the area under the Roc curve). A model with value above 0.7 is considered useful while value between 0.8-0.9 indicated excellent diagnostic accuracy.

Multiple logistic regression analysis was performed on factors which were significantly different in a uni-variance analysis between

patients of both groups in order to determine adjusted Odd ratio (OR) and 95% confidence (CI) to detect the factors that are independtly associated with the presence of the factors under the study.

Sensitivity: True positive cases divided by all positive cases.

Specificity: True negative cases divided by all negative cases.

Accuracy: All true positive plus true negative cases divided by all cases (either true positive or true negative or false positive or false negative).

Results

There was no statistical significant difference for the investigations before and after interferon therapy especially the ferritin level, except for free T4, HCV load with p value <0.05 (Table 1).

Table (2) shows that the multivariable logistic regression analysis of different studied variables (Enter model). All variables are dependent predictors of response (P >0.05) except INR is independent predictor of response (P<0.01).

Cut-off level of serum ferritin 19.25 ng/ml, that of no statistical significant to be used as predictor for the response to peginterferon (as shown in Table 3).

The sensitivity and specificity was 75.7%, 38.5% respecitively (Table 3).

Table (1): Show the results of responder and non responder patients to the investigations.

Studied variables	Response				t -test	P – vale
	Responder (N = 37) Mean ±SD		Non responder (N = 13) Mean ±SD			
HB	14.6 ± 1.71		15.4 ± 1.49		1.4	> 0.05
WBCs	7.05 ± 1.83		5.93 ± 1.46		2	> 0.05
Platelets	179.9 ± 52.7		199.3 ± 49.15		1.08	> 0.05
RBS	123.6 ± 32.3		128.8±43.6		0.39	>0.05
Serum creatinine	0.95 ± 0.15		1.02±0.16		1.5	>0.05
Serum Urea	113.6±40.16		94±45.7		0.76	>0.05
Serum cholesterol	160.09±24.37		168±36.6		0.45	>0.05
Serum TGS	159.08±56.9		162.3±75.96		0.08	>0.05
Serum ferritin	331.9±174.9		276.15±149.9		1.22	>0.05
TIBC	346±62.2		407±103.4		0.92	>0.05
Serum iron	131.92±35.55		143.25±11.67		0.62	>0.05
Studied variables	Responder(N = 37) Mean ±SD		Non responder(N = 13) Mean ±SD		Mann Whitney test	P – vale
TSH	4.88± 1.86		9.07 ± 3.12		1.64	> 0.05
Free T3	3.48 ± 1.78		2.61 ± 1.04		1.51	> 0.05
Free T4	2.42±2.1		5.93± 4.68		2.37	< 0.05*
AFP(ng/ml)	7.35±3.49		6.75±4.71		0.64	>0.05
PCR	190798.3±26326.54		952021.9±20959.5		1.98	< 0.05*
Gender	Responder(N = 37) Mean ±SD		Non responder (N = 13) Mean ±SD		X^2 test	P – vale
	No.	**%**	**No.**	**%**		
Male	29	78.4	11	84.6	0.23	>0.05
Female	8	21.6	2	15.4		
Diabetic patient	7	18.9	3	23.1	0.1	>0.05
Non diabetic patient	30	81.1	10	76.9		
Fibrosis	**Mean ± SD(n=37)**		**Mean ± SD(n=13)**		1.77	>0.05
F0 – F1	10	28.6	3	27.3		
F2 – F3	9	25.7	5	45.4		
F4 – F5	16	45.7	3	27.3		
A1	4	11.4	1	9.1	0.11	>0.05
A2	27	77.1	9	81.8		
A3	4	11.4	1	9.1		

Table (2): Multivariable logistic regression analysis of different studied variables (Enter model)

Studied variables	Response				
	β0 (Constant)	β	SE	Resquare	P-value
Age	5.08	0.08	0.08	0.11	>0.05
Sex		0.69	1.22		>0.05
BMI		0.01	0.15		>0.05
DM		0.98	0.97		>0.05
ALT	8.54	0.003	0.03	0.38	>0.05
AST		0.07	0.045		>0.05
ALP		0.05	0.043		>0.05
Serum Albumin		2.27	1.69		>0.05
Total bilirubin		2.02	1.77		>0.05
INR		14.8	12.31		<0.01**
Serum creatinine	23.16	31.6	3.15	0.65	>0.05
Serum urea		2.08	5.13		>0.05
Serum cholesterol		0.97	4.8		>0.05
Serum TGs		1.42	3.8		>0.05
HB	8.92	0.66	0.47	0.21	>0.05
WBCs		0.5	0.38		>0.05
Platelets		0.005	0.01		>0.05
TSH		0.004	0.07		>0.05
Free T3		0.23	0.35		>0.05
Free T4		0.13	0.15		>0.05
PCR	1.48	0.0001	0.00	0.14	>0.05
AFR	0.65	0.04	0.1	0.01	>0.05
HAI	1.18	0.24	1.01	0.02	>0.05
Fibrosis		0.39	0.58		>0.05
Steatosis		0.003	0.02		>0.05
Serum Ferittin	0.35	0.002	0.002	0.03	>0.05
TIBC	9.25	0.02	0.02	0.22	>0.05
Serum iron	3.56	0.01	0.02	0.03	>0.05

Table (3): Show specificity and sensitivity of serum ferritin level.

Serum ferritin	Sensitivity	Specificity	AUC	Accuracy	P-value	CI
Cut level 19.25	75.7%	38.5%	0.61	62.9%	> 0.05	0.42-0.8

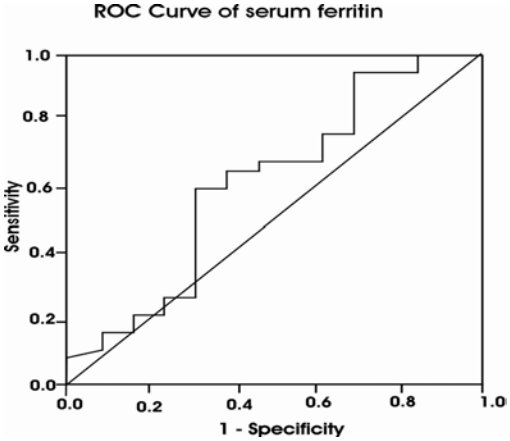

Fig. 1: ROC curve to detect sensitivity, specificity and accuracy of serum ferritin in HCV cases for prediction of response to peg-interferon treatment with accuracy 62.9%, and area under the curve 0.61, P value >0.05.

Discussion

It is estimated that about 170 million peoples worldwide are infected with HCV[1]. Cohort studies in Egypt found that incidence rate of 0.8/1000 person per year. Despite the implementation of blood -donor screening in the early 90s, there will be an anticipated increase of HCV related cirrhotic complications[9]. So the eradication and cure from HCV is crucial, beside search for the associated factors related to the host or virus. This is the aim of the present study, that includes 50 patients with chronic HCV (responders 37 patients both males and females & non-responders 13 patients both males and females). The evaluation of studied variables between the responders and non-responders was of statistically significant difference for (free T_4 & Viral load) variables, P value < 0.05 (table 1) that agree with (Hadziyannis et al., 2004), the explanation that in non responders there was high virus load and hyperthyroidism as side effects of interferon therapy[10]. Most of the other variables was of no statistical significant, P value >0.05 (table 1). As regard BMI (table 2) the result of the present study was of no statistical significant, P value 0 >0.05 that in agree with (Zeuzem et al., 2004 & Jacobson et al., 2006)[5,11], disagree with (Fried et al., 2003 & Manns et al., 2001) [12,13]. Spontaneous clearance of HCV infection appears to be more frequent among women after acute infection.[14] In the present

study, there was no statistical significant difference as regard age and gender, that agree with some Western studies[13,15,16] carried out among patients older than 50 years. Also in Asian studies[17] confirm that, the explanation for this results, in the menopause women appears to be associated with an accelerated rate of fibrotic progression[18,19]. However, there is an idea that estrogen has a protective role was also suggested by a study on chronic HCV patients younger than 50 years old[17]. Also Reddy et al., 2009 demonstrated that SVR rate among patients less than 50 years old was greater than the rate among patients older than 50 years old.[14] The association of co-morbid diseases like DM between the responders and non responders was of no statistical significant, P value >0.05 (table 1), the explanation for that may be most probably due to the good control of DM among the studied group. As regard the host factors in the evaluation of SVR, Iron load, liver fibrosis and cellular factors including genetic polymorphism were evaluated[3,20,21]. Nearly half of patients with chronic hepatitis (B & C) have an abnormal transferrin saturation and or serum ferritin[6]. Ferritin is a good indicator of largely increased iron stores in the liver and reliably indicate iron deficiency as well as an indication for timing of therapy, check the effect of iron chelation[7]. In the present study the cut-off level of serum ferritin 19.25ng/dl, it was of no statistical significant with sensitivity 75.7% and specificity 38.5%, CI 0.42-0.8 (table 3, Fig 1). However Bonkvesky et al., 2007 reported that decrease the basal serum ferritin level to less than 100ng/dl is associated SVR, While Tung et al., 2003 reported that removal of iron by venesection increases the end of treatment virological and histological response to short term interferon therapy, but there is no significant benefit to SVR[6,7].

Conclusion

Still HCV infection and its complications is a major health problem worldwide, as well as Egypt. There was no statistical significant difference between the responders and non responders as regard iron indices especially serum ferritin level to be used as predictor for response.

Recommendations

It is not needed to do iron indices in patients with chronic HCV scheduled to interferon -ribavirin therapy.

Prevention of excess iron accumulation in patients with chronic HCV, and the populations in general is advisable by counseling.

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A RETROSPECTIVE STUDY ABOUT THE SERUM FERRITIN LEVEL BETWEEN RESPONDER AND NON RESPONDER PATIENTS WITH CHRONIC HCV TO TREATMENT BY [PEG INTERFERON & RIBAVIRIN THERAPY]

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MOLECULAR DIAGNOSIS AND GENOTYPING OF CRYPTOSPORIDIUM

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Abstract

Background: *Cryptosporidiosis is an infection caused by Cryptosporidium; a protozoan parasite that infects the gastrointestinal tract. The infection is of major public health concern in both developed and developing countries.*

Aim: *The current study aims to evaluate PCR technique as a diagnostic tool for Cryptosporidium infection comparable to conventional methods and to identify Cryptosporidium genotypes among patients receiving chemotherapy in oncology center of Mansoura university , Egypt.*

Methodology: *This study was carried out from May 2010 to June 2013 on 164 immuno-compromised cases were complaining of different types of malignant diseases and all were receiving chemotherapy in Oncology Center of Mansoura University. All were subjected to history taking , clinical examination and laboratory investigations in form of microscopic examination of samples by using modified zeihl neelsen and auramine phenol stains followed by coproantigen detection using ELISA technique and finally PCR and genotyping of study cases.*

Results: *Statistical difference was detected between the four diagnostic techniques ($\chi 2 = 50.953$, $P<0.001$). The most frequent GIT symptom among symptomatic cases was abdomenal pain (68.1%). C.parvum*

showed the highest percentage (50%) among PCR positive cases.

Conclusion: *Cryptosporidiosis is a potentially life-threatening disease in immune-compromised persons and it should be taken in mind as a routine examination in all diarrheal samples.*

Introduction

Cryptosporidiosis is a disease caused by cryptosporidium species which infect both human and animals[1]. Diarrhea caused by parasitic infection account for more than 3.1 million death annually especially among children less than 15 years of age and mostly in developing countries[2]. Infection with the parasite accounts for 6% of all diarrheal diseases in immune-competent persons and presents up to 24% in immune-compromised cases[3]. Transmission occurs via feco-oral route, direct person to person and indirect zoonotic food borne, water borne and air borne route of infection[4]. Methods to detect cryptosporidium spp. in feces usually involve microscopic examination of stained fecal smears (modified zeihl neelsen and auramine phenol), antigen detection (ELISA) and genome detection(PCR). Each varies in sensitivity and specificity and there is no accepted gold standard[5]. Big differences in the prevalence of the disease; 19 studies were carried out on patients with diarrheal illness and the prevalence varied between 0% and 47%[6].

Subjects, Materials and Methods

This study was carried out from May 2010 to June 2013 on 164 immuno-compromised cases of both sexes with a written consent obtained from them. They were complaining of different types of malignant diseases and all were receiving chemotherapy in Oncology Center of Mansoura University. All were subjected to history taking stressing on name, age, sex, abdominal pain, vomiting and diarrhea then clinical examination searching for tender abdomen, heatomegaly, ascitis and signs of dehydration followed by laboratory investigations:

I- stool concentration using sheather's floatation technique followed by microscopic examination of stained modified zeihl neelsen and auramine phenol smears done for all samples[7].

II- Coproantigen detection of cryptosporidium by Ridascreen Cryptosporidium, product number C1201 R-Biopharm Ag, Darmstadt, Germany, Email: www.r-biopharm.com.

Evaluation:

1) Calculating the cut-off:

Cut-off = Extinction for the negative control+ 0.15.

2) Test result:

Samples were considered positive if their extinction was more than 10% above the calculated cut-off. Samples were considered equivocal and were repeated if their extinction within the range 10% above to 10% below the cut-off, if the repeat yielded a value within the cut-off range, the sample was considered negative. Samples were considered negative if their extinction was more than 10% below the calculated cut-off.

III- PCR for detection of cryptosporidium:

DNA extraction:

A commercial extraction kit i-genomic stool DNA extraction mini kit of iNtRON Biotechnology , Inc. Korea was used.

DNA amplification:[8]

* PTC-200 Peltier Thermal cycler (MJ research, Watertown, USA).

*** Primers (Sigma):**

Primers targeting 18S rRNA gene were used.[9]

CPB-DIAGF(forward;50AAGCT CGTAGTTGGATTTCTG30)

CPBDIAGR(reverse;50TAAGGT GCTGAAGGAGTAAGG30)

*** For each PCR reaction the following reagents were added:** 13 ul sterile bi-distilled water, 25 ul of Ready Mix RED Taq PCR reaction mix, 1 ul of each primer set and 10 ul DNA sample to make the sum 5o ul.

* Reaction cycles of the polymerase chain reaction were 40 cycles in which temperature time (hold 1) 94 c for 5 min then Denaturation 94 c for 30 sec then annealing 60 c for 1 min then extension 72 c for 30 sec and temperature time (hold 2) 72 c for 10 min.

Reporting results of PCR-RFLP/sequencing examination

* Negative specimens were reported as 'NO Cryptosporidium DNA detected'.

* Positive specimens were reported as 'Cryptosporidium DNA detected' inserting the species and genotypes.

VI- Restriction Fragment Length Polymorphism (RFLP) analysis for identification of Cryptosporidium genotypes:

Two microliters of amplified PCR products was restricted with either SspI or VspI. Digestion were carried out under standard conditions[10]. The amplified and digested products were detected by agarose gel electrophoresis.

Statistical Analysis

Statistical analysis was performed by using Statistical Package of Social Sciences (SPSS) version 13.

Results

Table (1): Laboratory diagnosis of *cryptosporidium* cases in 164 patients : modified ziehl neelsen , auramine phenol , coproantigen (ELISA) and PCR.

Methods	Positive cases (n= 164)	%	χ^2	P
Modified ziehl neelsen	30	18.3	50.593	< 0.001
Auramine phenol	34	20.7		
Coproantigen (ELISA)	36	22		
PCR	80	48.8		

Table (2): Sex distribution among *cryptosporidium* infected cases:

Sex	Methods							
	Modified ziehl neelsen		Auramine phenol		Coproantigen		PCR	
	No	%	No	%	No	%	No	%
Male (80)	14	46.7	12	35.3	16	44.4	38	47.5
Female (84)	16	53.3	22	64.7	20	55.6	42	52.5
Total (164)	30		34		36		80	
χ^2	0.066		3.122		0.347		0.103	
P	0.798		0.077		0.556		0.749	

Table (3): Age distribution among *cryptosporidium* infected cases:

Age	Methods							
	Modified ziehl neelsen		Auramine phenol		Coproantigen		PCR	
	No	%	No	%	No	%	No	%
Up to 5 years (30)	8	26.7	8	23.5	6	16.7	9	11.3
5-20 years (32)	2	6.7	4	11.8	6	16.7	13	16.3
20-40 years (50)	8	26.7	8	23.5	8	22.2	17	21.3
More than 40 years (52)	12	40	14	41.2	16	44.4	41	51.3
Total (164)	30		34		36		80	
$\chi 2$	5.290		3.556		3.171		25.380	
P	0.152		0.314		0.366		< 0.001	

Table (4): Clinical presentations among *cryptosporidium* infected cases:

Clinical presentations	Numbers	(%) N=164
Asymptomatic	20	12.2
Symptomatic	144	87.8
Abdominal pain	98	68.1
Loss of weight	68	47.2
Vomiting	77	53.5
Diarrhea	95	66

Table (5): Signs among *cryptosporidium* infected symptomatic cases:

Signs	Numbers	(%) n= 144
Tender abdomen	98	65.3
Hepatomegaly	73	50.7
Dehydration	90	62.5
Ascities	41	28.5

Table (6): Genotypes distribution among PCR positive *cryptosporidium* infected cases.

Genotypes	Numbers	(%) n= 80
C.parvum	40	50%
C.hominis	24	30%
C.meleagridis	12	15%
C.muris	4	5%

Discussion

Cryptosporidiosis is a common gastrointestinal disease, and it has been recognized worldwide as a common cause of diarrhea in otherwise healthy individuals. The disease is widespread in many developed and developing countries [11]. According to the World Health Organization, more than 33% of global deaths are due to parasitic diseases[12]. parasitic infections are among the most common infections in the world responsible for mortality and morbidity[12]. In terms of pathogenic importance, G. lamblia, Cryptosporidium parvum and E. histolytica have been shown to be responsible for severe diarrhoeal episodes especially in immune-compromised individuals. In humans, Cryptosporidium infection can result in severe diarrhoea, which is usually self-limiting in immunocompetent individuals, but may be chronic and life-threatening to those that are immunocompromised[1]. Cryptosporidium is prevalent in Egypt[6]. But to date limited genotyping studies have been conducted on Cryptosporidium isolates from humans. The aim of the present study, therefore was to examine samples from immune-compromised cases of cryptosporidiosis receiving chemotherapy in Oncology center of Mansoura University over May 2010 to June 2013 to identify the species and genotypes of Cryptosporidium involved to better understand the transmission, dynamics and distribution of the parasite in Egypt. In our study, (18.3%) of the 164 studied cases were positive for cryptosporidium using modified ziehl neelsen stain, by using auramine phenol stain (20.7%) were positive, by using ELISA (22%) were positive while by using PCR (48.8%) were positive with statistically significance ($\chi 2=$ 50.953, $P<0.001$). (table 1). It could be concluded that PCR was the most specific and sensitive method in diagnosis of infection with cryptosporidiun among study cases.

These results were in accordance with previous results by Ab-

del messeh et al., 2005[13] who recorded 17% for modified Ziehl neelsen stain and 20% for ELISA. On the other hand our results were lower than Al-Shamiri et al., 2010[14] in Yemen who recorded 34.7% were positive by microscopy and 26.1% were positive by ELISA. In another study in Jordon, molecular analysis of 104 cases 40.3% were positive[15]. Regarding gender variation in this study, cryptosporidiosis was found to be relatively higher among females than males as 51% were female and 49% were male with statistically insignificant difference (p=0.749) (table 2). These results agreed with Park et al., 2006[16] who recorded 1.2% in males and 1.9% in females with no statistical difference. As regard the age of the studied groups (table 3), the highest frequency was among adult older than 40 years (31.7%) followed by adolescents 20-40 years (30.5%) then children 5-20 years (19.5%) and the least was among infants up to 5 years (18.3%). Prevalence of cryptosporidiosis was significantly higher among age group older than 40 years in PCR technique and was statistically significant ($\chi 2=$ 25.380, $P<0.001$). These results agreed with Casemore., 1995[17] who recorded higher prevelance among age 20-40 years and age more than 40 years. In the present study, Among 164 study cases, (87.8%) were symptomatic (suffered from GIT manifestations as Abdominal pain, loss of weight, vomiting, diarrhea) and (12.2%) were asymptomatic (table 4). The most frequent GIT symptom among symptomatic cases was abdominal pain (68.1%) followed by diarrhea (66%) then vomiting (53.5%) and the least one was loss of weight (47.2%). These results were in agreement with Al-Shamiri et al., 2010[14] who recorded that 38.45% of infected cases by cryptosporidium spp. had diarrhea, also Mirzaei, 2007[18] recorded 25.6% of cryptosporidiosis cases had diarrhea and 28% had abdominal pain. On clinical examination of the symptomatic cases in our study, among 144 symptomatic cases, tender abdomen was

detected in 98 cases (65.3%), dehydration was detected in 90 cases (62.5%), Hepatomegaly was detected in 73 cases (50.7%), in addition, signs of ascities were detected in 41 cases (28.5%) (table 5). In the present study, four different Cryptosporidium genotypes were detected; C. parvum, C. hominis, C. meleagridis and C. muris, which were distributed among PCR positive cases as C.parvum showed the highest percentage (50%) followed by C.hominis (30%) then C. meleagridis was (15%) and the least was C.muris (5%) (table 6). These results were agreed with study in Jordon in which 44 isolates that were typed, 50% were C. parvum and _45% were C. hominis[6]. Previous studies in humans in the Middle East have also reported that C. parvum is the predominant Cryptosporidium species. For example, in Iran, 15 microscopy positive human fecal samples analysed using molecular tools identified 11/15 as C. parvum and 4/15 as C. hominis[19]. In Kuwait, 94% (58/62) of children screened had C. parvum, 5% (3/62) had C. hominis, and 1% (1/62) had both C. parvum and C. hominis[20]. Another study in Shahriar (a suburb of Tehran, Iran), identified 18/24 isolates as C. parvum and the remaining 6 isolates as C. hominis from patients with diarrhea[21].

Conclusion

In this study, infection with cryptosporidium was of high prevalence among studied group and it should be considered as a routine examination in all cases with diarrhea.

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MOLECULAR DIAGNOSIS AND GENOTYPING OF CRYPTOSPORIDIUM

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