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ULTRASONOGRAPHIC DIAGNOSIS OF THICKENED UTEROSACRAL LIGAMENT: COULD IT BE A SIGN OF MINIMAL ENDOMETRIOSIS?

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Abstract

Objectives: *To assess the value of thickened uterosacral ligament detected at transvaginal ultrasonography as a marker of minimal endometriosis..*

Design: *Observational study.*

Setting: *Mansoura University Hospital, Dakahlia, Egypt.*

Patient: *Patients with infertility and/or chronic pelvic pain with suspected endometriosis and were booked for laparoscopy.*

Intervention: *Transvaginal ultrasonography was done for all patients before laparoscopy. When uterosacral ligament is visualized at transvaginal ultrasonography its thickness is measured in millimeters. At laparoscopy, all endometriotic patients were classified according to the revised American Fertility Society classification 1985.*

Main outcome measures: *sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated with 95% confidence Intervals (CIs).*

Results: *The accuracy of thickened uterosacral ligament detected at transvaginal ultrasonography as a marker for minimal endometriosis was (81%, 95% CI 73.9-86.7). The sensitivity, specificity, positive predictive value and negative predictive value were (64%, 95% CI 52.8-72.9), (88.8%, 95% CI 83.7-93), (72.7, 95% CI 60-82.9), and (84.2%, 95% CI 79.3-88.1) respectively.*

Conclusion: *Thickened uterosacral ligament detected at transvaginal ultrasonography in patients with suspected endometriosis might be a marker of minimal endometriosis.*

Keywords: *Endometriosis, uterosacral ligament, ultrasonography, laparoscopy.*

Introduction

Endometriosis is an estrogen-dependent disorder defined as the presence of endometrial tissue outside the uterine cavity.[1] It affects 5% to 10% of women in reproductive age.[2] Ovarian endometriotic cysts, superficial peritoneal lesionsand deeply infiltrating endometriotic lesions of rectovaginal septum were identified as three different entities.[3] The most prominent features of the disease is infertility and chronic pelvic pain.[4]

The diagnosis of endometriosis has traditionally been established by visual inspection of the pelvis, at the time of laparoscopy or laparotomy.[5] The procedure is not without complications. It may be associated with vascular injury, gastrointestinal injury, extra peritoneal gas insufflation, wound infection, wound dehiscence and hernia.[6] Given these potential hazards, there has been considerable interest in using non-invasive techniques, such as ultrasound, to detect endometriosis.[5]

Transvaginal ultrasonography is used to diagnose endometrioma with a high degree of accuracy.[7,8] Also this diagnostic tool is of high value in diagnosing ovarian adhesions[9,10] and Douglas pouch obliteration,[11] however its role in detecting peritoneal endometriosis is limited.[12] This may represent a limiting step in ultrasonographic staging of endometriosis and may delay the diagnosis and management.

Patient and Methods

This prospective observational study was carried out on 158 women admitted to our department during the period between August 2010 and June 2013. All patients were complaining of infertility and/or chronic pelvic pain and were booked for laparoscopy. Transvaginal ultrasonography was done for all patients using Medison digital sonace 5500, with 5 Mhz transducer or Philips clear vue 350 with 4-9 MHz transducer.

For assessment of the thickness of the uterosacral ligament (USL), the probe was introduced in the vagina and advanced until the midline image of the cervix was visualized using the longitudinal view. The probe is placed in

the posterior fornix and then rotated 90º and pressed gently against the site of the USL to obtain a cross section and its thickness is measured in millimeters.

Laparoscopy was done for all patients and they were classified according to the revised American Fertility Society (r AFS 1985) scoring system for pelvic endometriosis.

Results

Patient characteristics are displayed in table 1. The mean age was 26.4±3.3. The mean body mass index was 24.2±2.3. The frequency of symptoms is shown in table 2. Infertility was a universal symptom, dysmenorrhea in (60/158-37.9%), dyspareunia in (36/158-22.8%) and chronic pelvic pain in (31/158-19.6%). Symptoms were evaluated on the basis of whether they were present or not rather than their degree of severity.Many patient presented with more than one symptom.

Forty four cases with thickened uterosacral ligament (USL) were identified at TVS. The relation between thickened USL detected by TVS and the different stages of pelvic endometriosis according to the r AFS scoring system was evaluated. Thickened USL by TVS was higher in stage I compared to stages II,III and IV. P value <0.001. Table 3.The accuracy of thickened USL detected by TVS in diagnosing minimal endometriosis was evaluated. (Table 4). The sensitivity was (64%, 95% CI 52.8-72.9), specificity was (88.8%, 95% CI 83.7-93), positive predictive value was (72.7, 95% CI 60-82.9), negative predictive value was (84.2%, 95% CI 79.3-88.1) and accuracy was (81%, 95% CI 73.9-86.7).

The USL thickness in our study range between (10-18) mm with different variability in sensitivity and specificity in diagnosing minimal endometriosis. A thickness of 14 mm was chosen as a cut off value that achieved the best sensitivity and specificity.(table 5 and figure 1).

Table (1): Patient characteristics (n=158).

Parameter	Mean ± SD
Age (years)	26.4 ± 3.3
Infertility duration (years)	4.59 ± 2.35
Age of menarche (years)	12.14 ± 0.86
Duration of menstrual flow (days)	4 ± 0.93
BMI(kg/m^2)	24.2 ± 2.3

Table (2): frequency of symptoms in the population of the study (n=158).

Symptom	Number	Percentage
Dysmenorrhea	60	37.9%
Dyspareunia	36	22.8%
Chronic pelvic pain	31	19.6%
Infertility	158	100%

Table (3): TVS detection of thickened USL in relation to endometriosis stage (rAFS).

USL by TVS / r AFS stage	USL +ve n= 44	USL –ve n=114	P value
0(30 case)	0	30	<0.001
I(50 case)	32	18	<0.001
II(7 case/s)	3	4	0.37
III(35 case)	3	32	0.008
IV(36 case)	6	30	0.09

Table (4): The accuracy of thickened USL detected by TVS in diagnosing stage I (minimal) endometriosis according to the r AFS system.

rAFS stage I / Thick USL by TVS	Yes	No
Yes	32	12
No	18	96

Table (5): Different values of the (USL thickness) in relation to sensitivity and specificity in diagnosing stage I pelvic endometriosis.

USL thickness	Sensitivity	95% CI	Specificity	95% CI
>14 mm	62.00	47.2 - 75.3	92.59	85.9 - 96.7
>15 mm	46.00	31.8 - 60.7	94.44	88.3 - 97.9
>16 mm	20.00	10.0 - 33.7	98.15	93.5 - 99.8
>17 mm	12.00	4.5 - 24.3	99.07	94.9 - 100.0
>18 mm	0.00	0.0 - 7.1	100.00	96.6 - 100.0

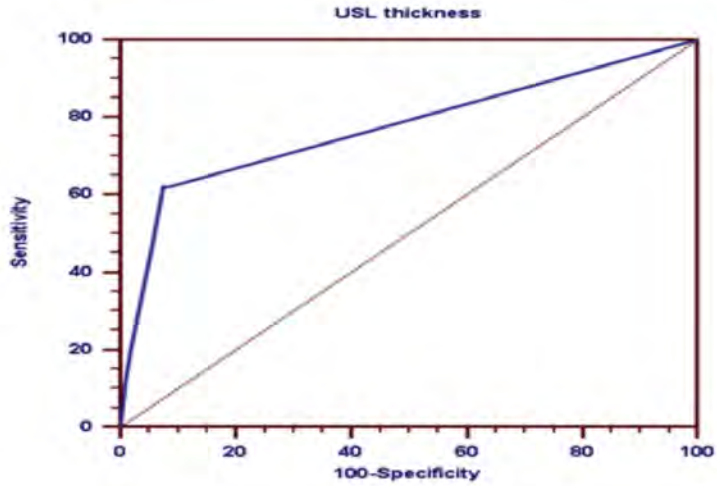

Fig. (1): Receiver Operating characteristic (ROC) for USL thickness by TVS in diagnosing stage I pelvic endometriosis.

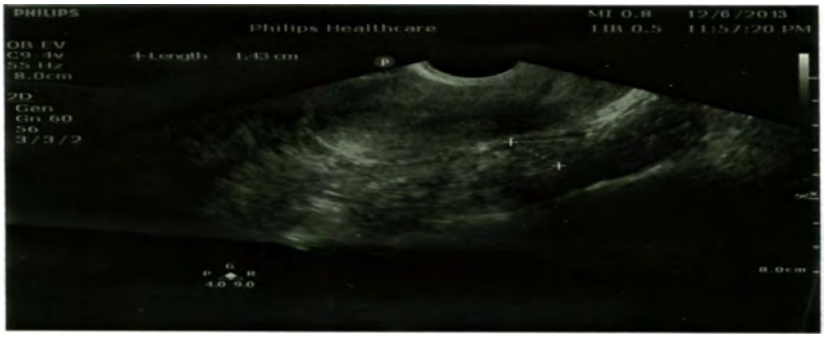

Fig. (2): Thickened USL at TVS.

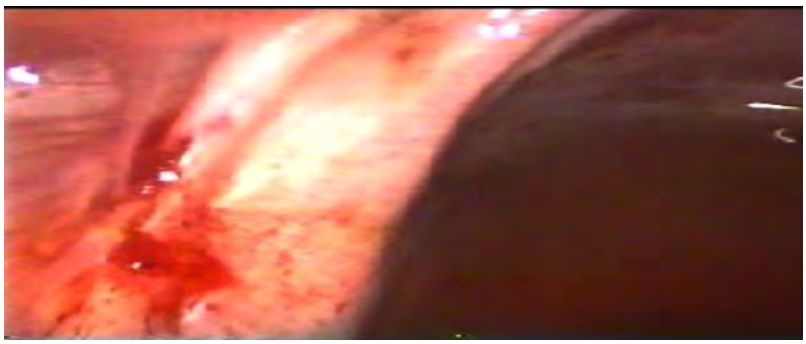

Fig. (3): Laparoscopy confirmed thick USL with peritoneal endometriosis.

Discussion

Transvaginal ultrasonography has been used for diagnosing ovarian endometriotic cysts[7,8], ovarian adhesions[9,10] and Douglas pouch obliteration[11] with good accuracy, but it has a limited value in the detection of minimal peritoneal lesions.[12] This limitation may delay the diagnosis and treatment of the disease. However, in our research, we found an association between the thickened uterosacral ligament (USL) at TVS and presence of minimal endometriosis at laparoscopy and this sign could be proposed as an indirect marker for stage I endometriosis. Bazot et al[13] found that normal USL is barely detectable on TVS, but, when harboring DIE, it thickens and becomes visible. Somigliana et al[14] documented a strong association between DIE and other forms of the disease. They observed superficial peritoneal implants and / or endometriotic ovarian cysts in association with DIE in 93.5% of population participated in their study. This support the common pathogenic mechanism for different forms of endometriosis.

In our study, The USL thickness range (10-18) mm with different variability in sensitivity and specificity in detecting minimal endometriosis. A thickness of 14 mm was chosen as a cut off value that achieve the best sensitivity and specificity.

To our knowledge this is the first study to evaluate the relation between thick USL detected at transvaginal sonography and the AFS staging for pelvic endometriosis. We achieved sensitivity, specificity, PPV, NPV and diagnostic accuracy of 64% (95% CI 52.8-72.9), 88.8%(95% CI 83.7-93), 72%(95% CI 60-82.9), (84.2%, 95% CI 79.3-88.1) and 81% (95% CI 73.9-86.7) respectively for diagnosing minimal stage pelvic endometriosis based on the sonographic detection of thickened USL.

The higher prevalence of thickened USL detected by TVS in stage I compared to stages II, III and IV pelvic endometriosis poses a question weather thick USL represent an early stage in the evolution of the disease or it might be masked by dense adhesions and/or fixed RVF uterus in advanced stages. More studies are required to as-

sess the value of thickened USL detected at TVS as a marker for minimal endometriosis.

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NUTRITIONAL MARKERS AFTER LOOP DUODENAL SWITCH (SADI-S) FOR MORBID OBESITY: ANEW TECHNIQUE WITH FAVORABLE NUTRITIONAL OUTCOME

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Abstract

Background: *A reduction of body weight can be achieved after Biliopancreatic diversion, but there is a risk of malnutrition and diarrhea. This risk may be reduced by pyloric preservation with duodenal switch. Loop duodenal switch (Loop DS) or (Single anastomosis duodeno-ileal bypass with sleeve gastrectomy= SADI-S) is hybrid operation combining moderate intake restriction with moderate malabsorption for treatment of morbid obesity. It is considered a modified version of the original duodenal switch operation in which after the sleeve gastrectomy, the duodenum is anastomosed in end to side, ante colic and isoperistaltic manner to the selected ileal loop with a length of 2 meters from ileocacal valve.*

Objective: *To evaluate the nutritional outcomes well as to determine weight loss success of Loop duodenal switch Procedure as surgical treatment for morbid obesity on a series of 37 consecutively operated patients in Endocrine surgery Unit, Mansoura University hospital, Mansoura University, Mansoura, Egypt*

Patients and methods: *A prospective study conducted during the period from July 2010 to January 2013. The mean age was 35.37±7.78years. The mean BMI was 56.25±8.43 kg/m2 .All patients were subjected to Loop Duodenal Switch after preoperative preparation and laboratory investigations including: Haemoglobin, serum iron, serum ferritin, serum vitamin B12, serum folic acid, serum calcium, serum magnesium, serum phosphorus, serum alkaline phosphatase, serum*

cupper, serum zinc, serum sodium, serum potassium, serum albumen, aspartate aminotransferase (AST), alanine transaminase (ALT) and serum bilirubin were followed up over 1 year.

Results: *Most of the patients had smooth postoperative course with no major morbidity and only 1 mortality. The BMI decreased significantly, from: 56.52±8.47, to 33.21±3.91, with decrease of the amount of food ingested. Both hemoglobin and calcium in Loop DS readily returned to the normal reference range following supplementation with iron and calcium respectively. The mean serum iron, serum ferritin, serum vitamin B12, serum folic acid, serum magnesium, serum phosphorus, serum Alkaline phosphatase, serum cupper, serum zinc, serum sodium, serum potassium, serum albumen, aspartate aminotransferase (AST), alanine transaminase (ALT) and serum bilirubin remained within the normal range with no significant nutritional deficiency.*

Conclusion: *Loop DS is not associated with broad nutritional deficiencies and does not appear to pose a threat to nutritional status. It provides excellent weight loss with preservation of good alimentation, even in the super obese. Postoperative supplementation with iron, multivitamins, calcium and vitamin D may be required continuously to prevent nutritional deficiency especially for adults and females in the Child bearing period.*

Keywords: *Nutritional markers, Morbid Obesity, Weight loss, Loop Duodenal Switch, single anastomosis Duodeno-ileal bypass, SADI-S., Bariatric surgery.*

Introduction

Morbid obesity is a chronic insidious disease which leads to progressing co-morbidities, socio-economic problems, undesirable quality of life, and earlier death[1].

The etiology of morbid obesity is multifactorial and is related to inheritance, physiological, metabolic, sociocultural, behavioral, and psychological factors[2].

People are considered to have morbid obesity if they have a body mass index (BMI) of 40 kg/m2 or

more or they have a BMI of between 35 kg/m2 and 40 kg/m2 and other significant disease (for example, diabetes, high blood pressure) that may be improved if they lose weight[3].

Overweight and obesity are estimated to be present in1.7 billion people worldwide[4]. In the Eastern Mediterranean region, the highest levels of overweight persons (BMI ≥ 25) were in Kuwait, Egypt, United Arab Emirates, Saudi Arabia, Jordan and Bahrain, where the incidence of overweight/obesity for those aged ≥ 25 years was between 74%-86% (women) and 69%-77% (men)[5].

Significant comorbidities, defined as medical problems associated with or caused by obesity, are numerous. The most prevalent and acknowledged of these include degenerative joint disease, low back pain, hypertension, obstructive sleep apnea, gastroesophageal reflux disease (GERD), cholelithiasis, type 2 diabetes, hyperlipidemia, hypercholesterolemia, asthma, hypoventilation syndrome of obesity, right-sided heart failure, migraine headaches, pseudotumorcerebri, venous stasis ulcers, deep vein thrombosis, fungal skin rashes, skin abscesses, stress urinary incontinence, infertility, dysmenorrhea, depression, abdominal wall hernias, and an increased incidence of various cancers such as those of the uterus, breast, colon, and prostate[6].

According to the 1991 National Institutes of Health (NIH) consensus conference on gastrointestinal surgery for severe obesity, patients are candidates for bariatric surgery if they are morbidly obese (BMI >40 kg/m2 or ≥35 kg/m2 with comorbidities), have failed attempts at diet and exercise, are motivated and well informed, and are free of significant psychological disease[7].

None of the medical methods of weight reduction provide a lasting weight reduction. Surgery offers the only achievable long-term solution[8]. Bariatric surgery has demonstrated its efficacy in weight loss and in reducing the comorbidities in the morbid obesity patient[9].

Bariatric surgical techniques can be divided into restrictive, malabsorptive, and combined (restrictive

and malabsorptive) procedures. Commonly performed procedures include: Laparoscopic Adjustable Gastric Banding (LAGB), Sleeve Gastrectomy (SG), Vertical Banded Gastroplasty (VBG), Roux-en-Y Gastric Bypass (RYGB), Biliopancreatic Diversion (BPD), and BPD with Duodenal Switch (DS)[10].

The biliopancreatic diversion with duodenal switch (BPD-DS) is often referred to as the duodenal switch operation and is a modification of the original biliopancreatic diversion described by Scopinaro in 1979[11].

The essential difference between these two operations is that in the BPD-DS version, a sleeve gastrectomy is performed and the pylorus is preserved, whereas in the original Scopinaro operation, a distal gastrectomy sacrifices the pylorus. In both operations, the stomach pouch has a capacity of 250 mL and malabsorption results from a distal Roux-en-Y reconstruction of the bowel, with a common channel of 50 to 100 cm and an alimentary limb of 250 cm.[12,13].

Proximal duodenal-ileal end-to-side bypass, or loop duodenal switch, is a modification of the original biliopancreatic diversion with duodenal switch[14] in which after the sleeve gastrectomy, the duodenum is anastomosed to the selected ileal loop in a Billroth-II fashion, with no Roux-en-Y reconstruction[15].

SADI-S which is simplified 1-loop duodenal switch with a 200-250 common channel that was introduced by Sánchez-Pernaute A et al.[15]. It has theoretical benefits over RYGP which is considered by many authors to be the gold standard bariatric surgery[16]: half number of performed anastomoses, retained pylorus that controls gastric emptying, no dumping syndrome, reduction of operative time and possible lower rate of postoperative complications[15]; But due to the novelty of this procedure the effects on nutritional status have yet to be clearly defined.

The aim of this study was to establish baseline nutritional status, record prevalence of nutritional deficiency, as well as determine weight loss success in the Loop Duodenal Switch patient during the first 12 month post-surgery.

Patients and Methods

A total numbers of 37 consecutive morbidly obese patients were included in the study between July 2010 and January 2013, Males and females were considered for inclusion. All patients were treated by Endocrine Surgery Team in the Endocrine Surgery Unit (ESU), Mansoura University Hospitals. The Inclusion criteria were (1) MO patients with BMI greater than 40 kg/m2 or 35 kg/ m2 with comorbidities. (2) Previous failed attempts at losing weight by diet and exercise (3) Cooperative and motivated patient. The Exclusion criteria were (1) Lack of motivation, (2) History of previous Bariatric surgery and (3) Patients unfit for general anesthesia. All patients provided informed consent for inclusion in the study after explanation of the nature of the procedure and possible complications. The study was approved by Mansoura Faculty of Medicine ethical committee.

The preoperative evaluation included careful history taking regarding age, sex, obesity associated comorbidities, clinical examination including weight by kg, height by meter and body mass index (BMI), body circumferences, blood pressure and laboratory investigations included: Haemoglobin, serum iron, serum ferritin, serum vitamin B12, serum folic acid, serum magnesium, serum phosphorus, serum alkaline phosphatase, serum cupper, serum zinc, serum sodium, serum potassium, serum albumen, aspartate aminotransferase (AST), alanine transaminase (ALT) and serum bilirubin. In addition to the routine preoperative assessment as for any other major abdominal surgery, the patient may undergo further assessment for pulmonary functions, endocrine disorders or gastro-esophageal disorders.

Patients were advised to lose weight prior to surgery to help to facilitate the operative procedure by placing them on protein diet for 2 weeks prior to surgery. This helps to shrink the visceral fat and particularly the fatty deposits within the liver. Patients were admitted to the hospital one day before surgery, at which time they underwent most of their preoperative tests. Upon admission, they were seen by an anaesthesiologist and an internist, and received bowel preparation. Patients were

given Enoxaparin (Clexan) 60 mg on the evening of admission, and daily thereafter during the hospital stay. An epidural catheter was placed for postoperative pain management. Sequential compression stockings were used.

The sleeve gastorectomy was performed by mobilizing the greater curvature of the stomach distally to about 4cm past the pylorus and proximally to the angle of His; using ultrasonic shears .The stomach and fundus were fully mobilized during the dissection. The filmy posterior attachments were divided so the entire posterior surface of the stomach can be seen. Once this dissection was complete, the first stapler was placed tangentially across the antrum. The authors used green loads for the first two staple firings because of the increased thickness of the stomach in this area. The assistant flattened the stomach with lateral retraction and the anesthesiologist removed the orogastric tube before the first staple firing. The angle of the first firing was determined by the patient's anatomy, but care should be taken to not use an angle that will narrow the lumen at the incisura. Next 36 Fr. Bougie was passed into the stomach along the lesser curve to the pylorus. If any concern existed that the lumen is too narrow at the incisura, the stapler was moved laterally before firing. Once the surgeon was satisfied with the lumen size, the stapler was fired. Care was taken to create a straight staple line and avoid anterior or posterior "spiraling" of the staple line. The position of the final staple firing was critical to avoid a leak by avoiding the gastro esophageal junction and esophagus during firing. Approximately 1cm of gastric serosa should be seen to the left of the stapler cartridge before the stapler is fired. The entire staple line is then oversewn with continuous, imbricating Vicryl 2/0 sutures (Fig. 1).

The duodenum was sectioned at the level of the gastroduodenal artery, warranting a 2- to4-cm proximal duodenal stump and avoiding damage to the common bile duct (Fig. 2). The distal duodenal stump was over sutured in 2 layers; through and through layer using Vicryl 2/0 sutures, seromuscular layer using silk 2/0 su-

tures. (Fig.3). We observed that starting the Loop D.S technique with duodenal transection facilitates the sleeve gastric resection.

The ileo-cecal junction was identified, and 250 cm was measured upwards. The selected loop was ascended up and anastomosed to the proximal duodenal stump in an isoperistaltic way end-to-side hand-sewn anastomosis (Fig.4).

Postoperative course included: Proton pump inhibitor was administrated routinely in all patients. Ryle tube was removed at the 2nd postoperative day. Gastrografin meal was performed at the 5th postoperative day (Fig.5) and oral fluids intake was started if there was no leak. Early ambulation was advised from the first postoperative day. Drain was removed once oral intake was started after assurance that was no leak. The patients were discharged 6-9 days postoperatively according to postoperative course.

After discharge patients continued on clear fluids for one week (sugar free) then full fluids during the second week and soft food was allowed on the third week followed by regular food. Supplementation with Calcium, Vitamin D, Vitamin B12, Iron and multivitamins was done.

Follow up visits were scheduled postoperatively at three weeks, three months and then every 3 months. Follow up included clinical examination to detect any postoperative complications, body weight and BMI, laboratory investigations included: CBC, Iron, ferritin, vitamin B12, folic acid, serum sodium & potassium, calcium, phosphorus, magnesium, alkaline phosphatase, serum albumen and liver function tests were performed at each visit.

Results

The study included 37 morbidly obese patients who underwent open SADI-S. Females, 31 (83.8%) patients, comprised the majority of cases while 6 (16.2%) were males. Thirty two (86.5%) patients were sweet eaters, and 35 (94.6%) had family history of obesity. Demographic data of all patients is shown in table (1).

There is a highly statistical significant difference between mean

scores of weight and BMI before surgery and after 3, 6 and 12 months after surgery (Table 2, Fig. 2, 3). The mean weight of our patients decreased significantly from 150.75±20.15 kg preoperatively to 89.13±10.09kg one year after surgery ($p<0.001$) with a mean reduction in weight of 61.62 kg. The mean BMI decreased significantly from 56.25±8.43kg/m^2 preoperatively to 33.21±3.91 kg/m^2 one year after surgery ($p=0.001$) At 3 weeks, 3 months, 6 months and one-year follow up, the mean reduction in BMI was 4.49 kg/m^2, 10.67 kg/m^2, 16.03 kg/m^2 and 23.04 kg/m^2 respectively.

As regard Postoperative Nutritional and laboratory follow up: All patients receive follow-up nutritional counselling for a protein-enriched diet (80 to 100 g/day), and multivitamins, iron, and fat-soluble vitamins (D, E, A, and K) are given on a daily basis. The mean level of haemoglobin shows an increase from 10.85±0.68 mg/dl preoperative to 11.47±0.59 mg/dl at the end of the first year (Not significant); also the mean haematocrit value shows an increase from 34.45±2.83 mg/dl preoperative to 35.31±1.85 mg/dl at the end of the first year (Not significant). The mean serum Iron increased from 87.19±28.65 mg/dl preoperative to 97.69±16.67 mg/dl at the end of the first year. Averages for each time-point fell within normal reference ranges (Not significant). The mean serum Ferritin increased from 105.89±47.10 mg/dl preoperative to 123.26±36.21 mg/dl at the end of the first year. Averages for each time-point fell within normal reference ranges (Not significant). The mean serum Magnesium decreased from 1.94±0.24 mg/dl preoperative to 1.86±0.27 mg/dl at the end of the first year. Averages for each time-point fell within normal reference ranges (Not significant). The mean serum Zinc increased from 80.32±11.48 mg/dl preoperative to 83.58±11.59 mg/dl at the end of the first year. Averages for each time-pointfell within normal reference ranges. (Not significant). The mean serum Cupper increased from99.72±19.33 mg/dl preoperative to 102.60±19.66 mg/dl at the end of the first year. Averages for each time-point fell within normal reference ranges. (Not significant). The mean serum Vitamin B12 increased from 479.08±153.70

mg/dl preoperative to 520.82±169.60 mg/dl at the end of the first year. Averages for each time-point fell within normal reference ranges. (Not significant). The mean serum Folic acid increased from 10.48±4.07 mg/dl preoperative to 12.01±2.44 mg/dl at the end of the first year. Averages for each time-point fell within normal reference ranges. (Not significant). The average values of serum electrolytes for each time-point fell within normal reference ranges. (Not significant).

After surgery, all patients received oral supplementation of 1000 mg calcium and 800 IU vitamin D per day. From this table, it is shown that the mean serum calcium decreased from 8.82±0.29 mg/dl preoperative to 8.80±0.29 mg/dl at the end of the first year (Not significant); the mean serum Phosphorus decreased from 3.81±0.56 mg/dl preoperative to 3.81±0.28 mg/dl at the end of the first year. Averages for each time -points fell within normal reference ranges (Not significant); also the mean serum.

Parathyroid hormone (PTH) levels increased significantly from a preoperative level of 21.79±4.69 pg/ml to 70.39±21.12 pg/ml at the end of the first year but averages for each time-point fell within normal reference ranges. Lastly, the mean value of Bone specific alkaline phosphatase (BSAP) levels increased significantly from a preoperative level of 59.72±15.19 ng/ml to 74.65±15.44 ng/ml at the end of the first year. But averages for each time-point fell within normal reference ranges.

The mean serum Albumen shows a significant decrease from 4.00±0.19 mg/dl preoperatively to 3.70±0.22 mg/dl at the end of the first year but still within the normal reference range. The mean serum SGOT, SGPT and sr. bilirubin show no significant changes from the preoperative values.

Table (1): Demographic features of included patients (N:37):

Variables	Mean	SD	Range
Age (Years)	35.73	7.78	17.00 -53.00
Weight (Kg)	151.14	20.01	119.00-200.00
Height (m)	1.63	6.54	1.55.00-1.83.00
BMI (Kg/m²)	56.52	8.47	42.56-79.92

Table (2): Weight and BMI changes during follow up (N:37):

	Preoperative (Mean ±SD)	3weeks (Mean ± SD)	3 months (Mean ± SD)	6months (Mean ± SD)	1 year (Mean ± SD)	P value
Weight (kg)	150.75±20.15	145.67±22.56	141.83±25.81	107.83±13.72	89.13±10.09	P1 < 0.001 P2 < 0.001 P3 < 0.001 P4 < 0.001
BMI (kg/m^2)	56.25±8.43	51.76±8.32	45.58±8.17	40.22±5.51	33.21±3.91	P1 < 0.001 P2 < 0.001 P3 < 0.001 P4 < 0.001

P1: preoperative versus 3weeks postoperatively; P2: preoperative versus 3months postoperatively;
P3: preoperative versus 6 months postoperatively; P4: preoperative versus 1 year postoperatively.

Table (3): Postoperative Nutritional and laboratory follow up changes (N: 37):

	Preoperative (Mean ± SD)	Postoperative (Mean ± SD) 3weeks	Postoperative (Mean ± SD) 3months	Postoperative (Mean ± SD) 6months	Postoperative (Mean ± SD) 1 year	P value
HB(g/dL)	10.85±0.68	10.10±0.42	11.10±0.67	11.08±0.65	11.47±0.59	P1 = 0.445 P2 = 0.129 P3 = 0.160 P4 = 0.088
HCT	34.45±2.83	33.10±3.25	34.41±2.85	35.01±2.06	35.31±1.85	P1 = 0.063 P2 = 0.953 P3 = 0.354 P4 = 0.141
Sr. iron (µm/dL)	87.19±28.65	80.93±21.17	95.58±19.99	96.96±20.70	97.69±16.67	P1 = 0.267 P2 = 0.142 P3 = 0.111 P4 = 0.065
Sr. Ferritin (ng/mL)	105.89±47.10	113.73±41.34	117.81±43.62	120.39±46.92	123.26±36.21	P1 = 0.361 P2 = 0.360 P3 = 0.215 P4 = 0.092
Sr. Magnesium (mg/dL)	1.94±0.24	1.21±0.91	1.88±0.22	1.91±0.22	1.86±0.27	P1 = 0.192 P2 = 0.286 P3 = 0.596 P4 = 0.500
Sr.Zinc (µg/dl)	80.32±11.48	75.84±13.25	84.49±12.95	85.65±14.32	83.58±11.59	P1 = 0.062 P2 = 0.148 P3 = 0.099 P4 = 0.242
Sr.Cupper	99.72±19.33	91.93±19.31	96.37±15.33	100.14±17.38	102.60±19.66	P1 = 0.089 P2 = 0.451 P3 = 0.363 P4 = 0.426
Sr. Vitamin B12 (ng/L)	479.08±153.70	452.90±180.16	451.09±185.50	483.93±197.96	520.82±169.60	P1 = 0.317 P2 = 0.415 P3 = 0.452 P4 = 0.298
Sr.Folic acid	10.48±4.07	9.54±3.65	11.39±4.52	11.58±2.99	12.01±2.44	P1 = 0.304 P2 = 0.386 P3 = 0.099 P4 = 0.062
Sr Na+	138.94±4.15	137.94±4.02	140.42±3.75	139.11±4.23	140.42±3.01	P1 = 0.347 P2 = 0.187 P3 = 0.869 P4 = 0.083
Sr.K+	3.83±0.41	3.80±0.22	3.82±0.27	3.83±0.81	3.84±0.26	P1 = 0.657 P2 = 0.887 P3 = 0.947 P4 = 0.898

Table (4): Postoperative skeletal health follow up changes (N: 37).

	Preoperative (Mean ±SD)	3weeks (Mean ± SD)	3 months (Mean ± SD)	6months (Mean ± SD)	1 year (Mean ± SD)	
Serum calcium (mg/dL)	8.82±0.29	8.75±0.32	8.77±0.33	8.74±0.23	8.80±0.29	P1 = 0.331 P2 = 0.510 P3 = 0.505 P4 = 0.780
Serum phosphorus (mg/dL)	3.81±0.56	3.66±0.40	3.59±0.38	3.72±0.37	3.81±0.28	P1 = 0.121 P2 = 0.057 P3 = 0.389 P4 = 0.934
PTH (pg/mL)	21.79±4.69	32.47±7.07	65.65±12.80	67.10±16.76	70.39±21.12	P1 = < 0.001 P2 = < 0.001 P3 = < 0.001 P4 = < 0.001
AP (ng/mL)	59.72±15.19	69.64±10.93	77.43±9.61	75.50±11.72	74.65±15.44	P1 = < 0.001 P2 = < 0.001 P3 = < 0.001 P4 = < 0.001

Table (5): Liver function tests changes during follow up (N:37):

Liver function tests	Preoperative (Mean ±SD)	3weeks (Mean ± SD)	3 months (Mean ± SD)	6months (Mean ± SD)	1 year (Mean ± SD)	P
Sr. Albumen(g/dl)	4.00±0.19	3.98±0.17	3.82±0.19	3.74±0.20	3.70±0.22	P1 = 0.726 P2 = 0.005 P3 < 0.001 P4 < 0.001
Sr. Bilirubin(mg/dl)	0.70±0.09	0.69±0.09	0.68±0.08	0.70±0.09	0.69±0.12	P1 = 0.727 P2 = 0.459 P3 = 1.0 P4 = 0.765
SGOT(U/L)	24.89±6.70	25.78±5.17	25.81±4.70	24.61±4.45	24.43±5.17	P1 = 0.640 P2 = 0.619 P3 = 0.877 P4 = 0.809
SGPT(U/L)	22.44±5.87	24.00±3.90	23.74±9.87	23.64±2.84	22.84±1.84	P1 = 0.330 P2 = 0.616 P3 = 0.413 P4 = 0.777

P1: preoperative versus 3weeks postoperatively; P2: preoperative versus 3 months postoperatively;
P3: preoperative versus 6 months postoperatively; P4: preoperative versus 1 year postoperatively.

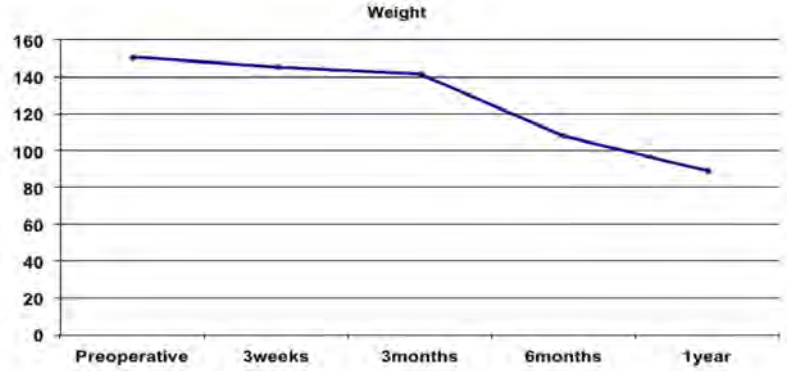


Diagram (1): representing the weight follow up changes.

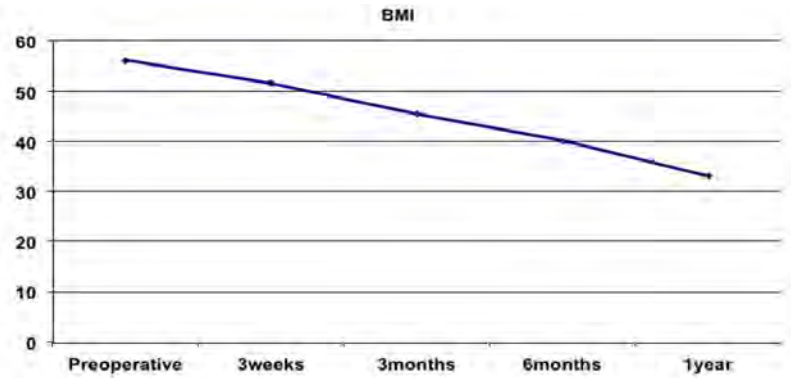
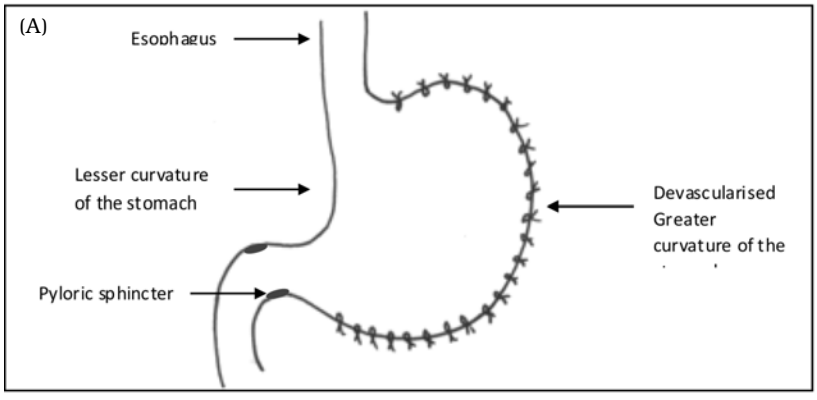


Diagram (2): representing the BMI follow up changes.

Fig. (1a): Steps of sleeve gastorectomy:

A. Devascularization of the greater curvature of the stomach.



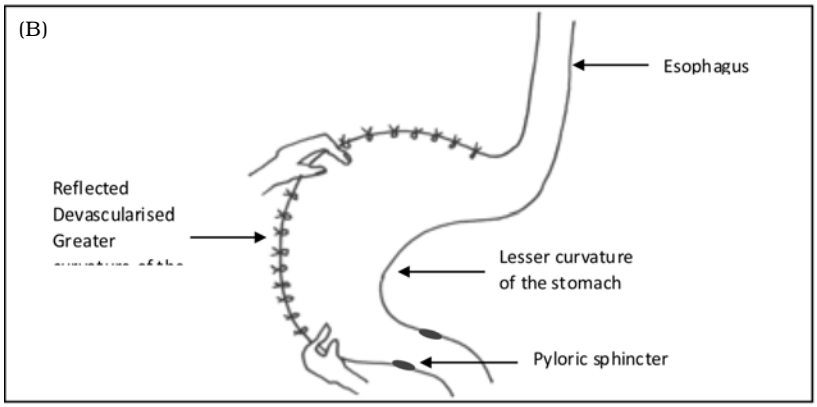


Diagram (3): A- Devascularization, **B-** reflection of the greater curvature of the stomach.

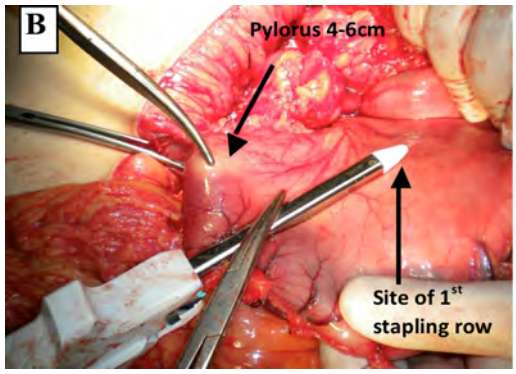


Fig. (1b): Steps of sleeve gastorectomy:
B. Application of the linear cutter 75mm, 4-6cm from the pylorus.

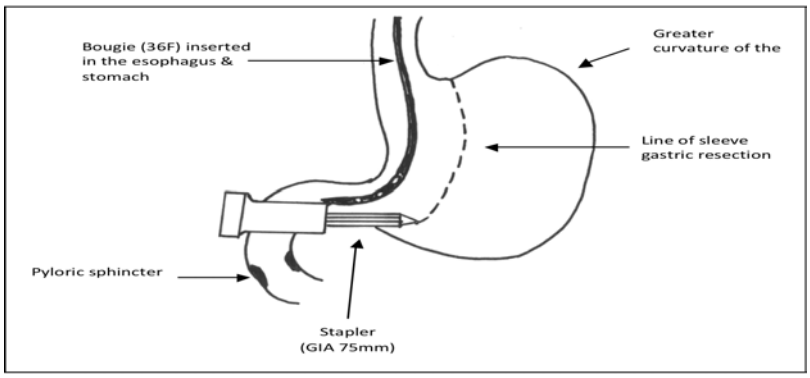


Diagram (4): Application of the linear cutter 75mm, 4-6cm from the pylorus.

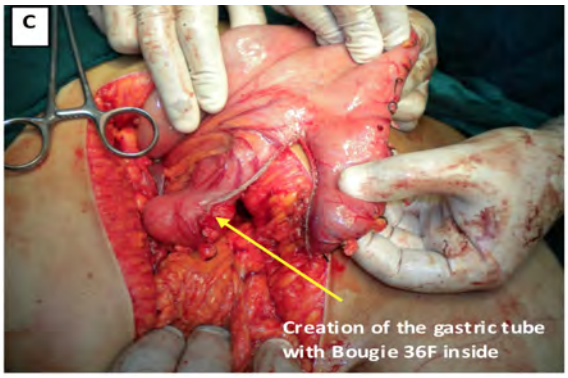


Fig. (1c): Steps of sleeve gastorectomy:
C. Creation of the gastric tube with Bougie 36F inside.

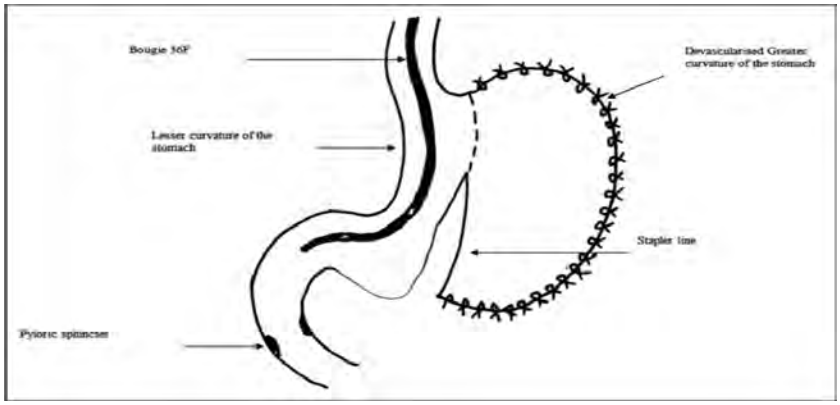


Diagram (5): Creation of the gastric tube with Bougie 36F inside.

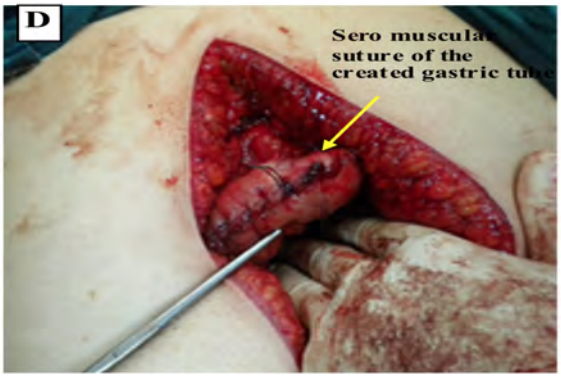


Fig. (1d): Steps of sleeve gastorectomy:
D. Taking sero muscular layer with vicrle 2/0.

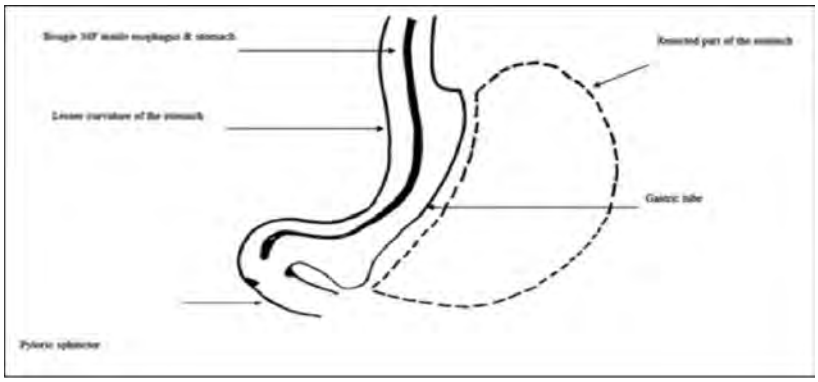


Diagram (6): Taking sero muscular layer with vicrle 2/0.

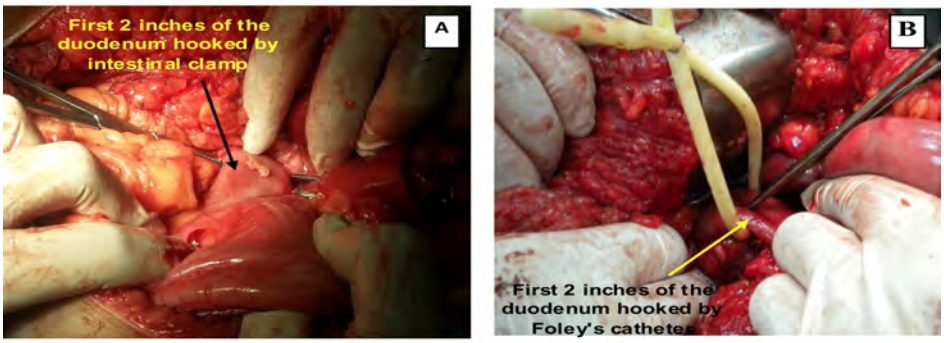


Fig. (2): Landmark oftransection of the duodenum at the level of the gastroduodenal artery warranting a 2 to 4cm proximal duodenal stump.

A. Using intestinal clamp. B. using Foley's catheter.

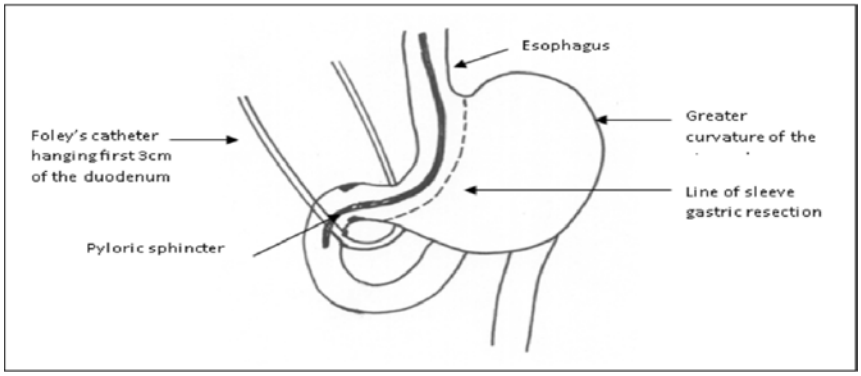


Diagram (7): Landmark of transection of the duodenum at the level of the gastroduodenal artery warranting a 2 to 4cm proximal duodenal stump.

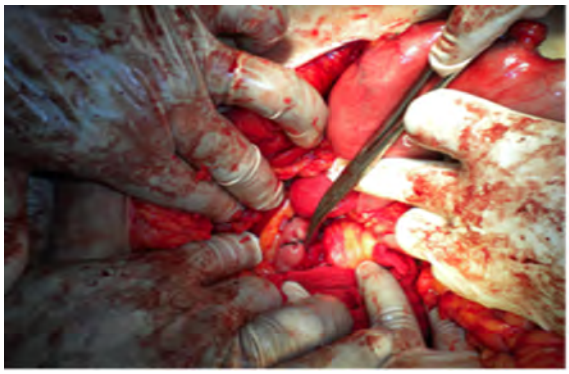


Fig. (3): Closed distal duodenal stump (Arrow) after transection of the proximal duodenum.

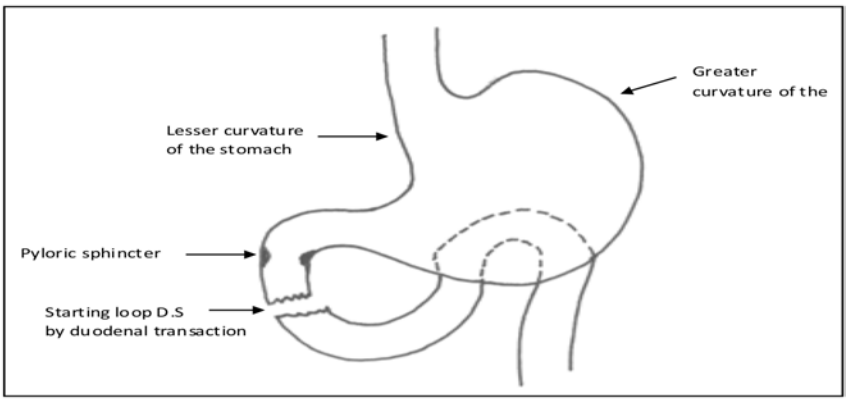


Diagram (8): Closed distal duodenal stump (Arrow) after transection of the proximal duodenum

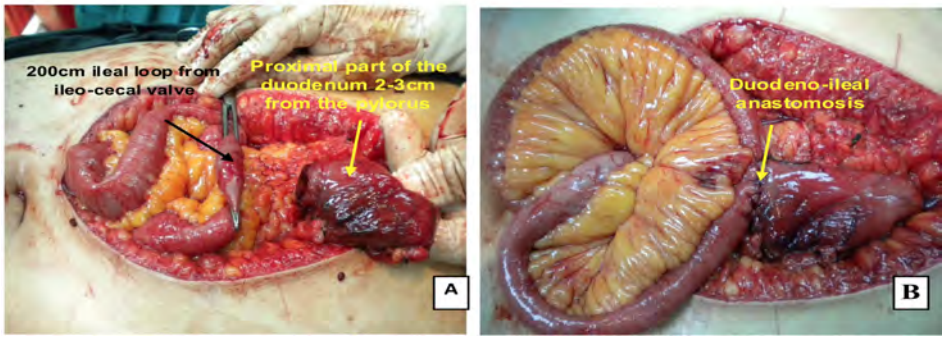


Fig. (4): The ileo–cecal junction is identified, and 200 cm is measured upwards. The selected loop is ascended up to the proximal duodenal stump and anastomosed in an isoperistaltic way end-to-side hand-sewn anastomosis performed manually.

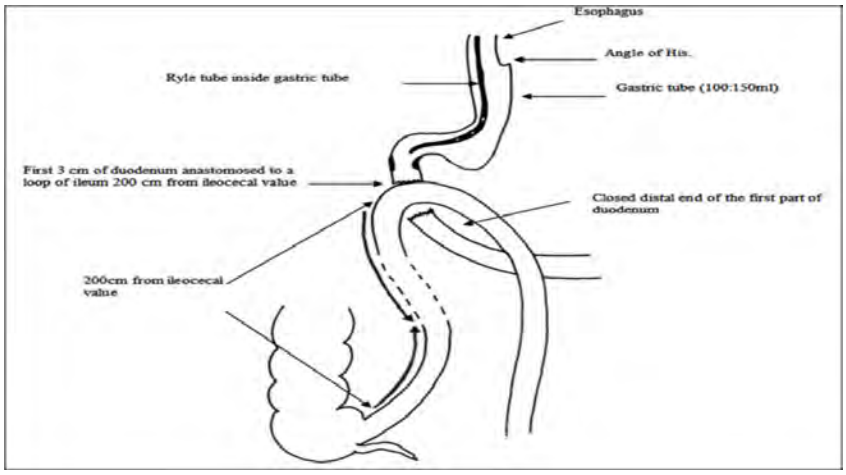


Diagram (9): The ileo–cecal junction is identified, and 200 cm is measured upwards. The selected loop is ascended up to the proximal duodenal stump and anastomosed in an isoperistaltic way end-to-side hand-sewn anastomosis performed manually.

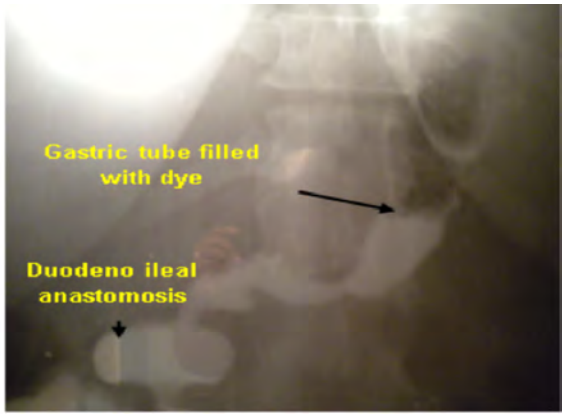


Fig. (5): Gastrografin meal and follow through showing the gastric tube (arrow) and filling of the first part of the duodenum and passage of the dye to the ileal loop (arrow head).

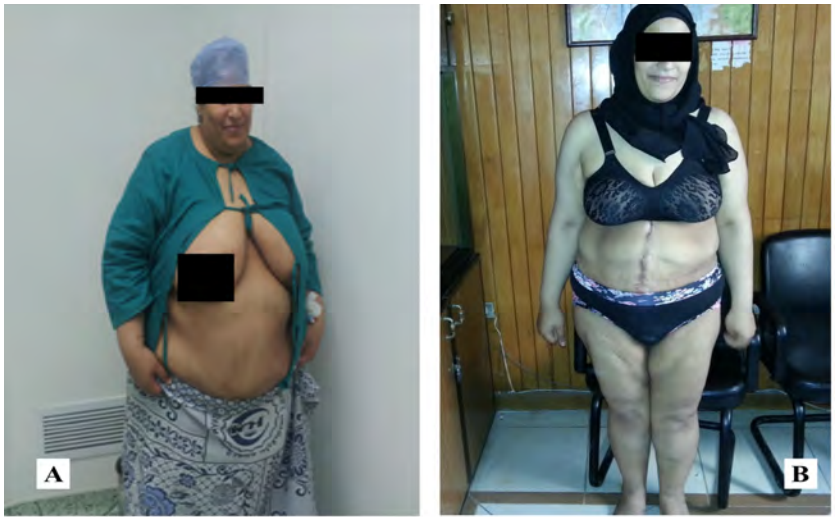


Fig. (6): Case (1) Female patient
(A) Before surgery BW (172kg), BMI (64kg/m2).
(B) One year after surgery BW (92kg), BMI (34.7kg/m2).

Fig. (7): Case (2) Female patient.
(A) Before surgery BW (165 kg), BMI (63kg/m2).
(B) One year after surgery BW (90kg), BMI (33kg/m2).

Discussion

Our results consistently demonstrated the presence of a link between morbid obesity and sweet eating and which is defined by the Dutch Sweet Eating Questionnaire as an eating behavior in which at least 50% of daily consumed carbohydrates consist of simple carbohydrates and which can be triggered by emotional factors (i.e., stress)[17].

In the previous literature by Sánchez-Pernaute A et al.[15] sleeve gastrectomy was done before duodenal switch transection. In our study duodenal transection was done primarily in most of the cases (Abdlatif Modification). We observed that starting with duodenal transection facilitates the later sleeve gastric resection.

In our study weight loss was assessed by decrease in BMI one year after surgery. We achieved a decrease in BMI from 56.25±8.43 kg/m2 to 33.21±3.91 kg/m2. This reduction in BMI was higher than that reported after BPD[18]; as they reported that the decrease in BMI was from 47.4 6±9.5 to 30.8 6±5.6. It was also pointed that DS patients decreased their BMI from a mean of 55.2 kg/m2 to 32.5 kg/m2[19]; whereas the RYGB patients decreased their BMI from 54.8 kg/m2 to 38.5 kg/m2. These results are comparable to our results.

Many obese persons undergoing WLS have preoperative nutritionaldeficiencies[20] that can be exacerbated by malabsorptive procedures. Evenpatients undergoing purely restrictive procedures are at risk for nutritionaldeficiencies due to poor eating habits as well as food intolerances andeating restrictions[21,22].

We agree that all Malabsorptive procedures as duodenal switch usually require strict nutritional supplementation especially postoperatively as most of the Intestinal tract is bypassed. This heightened risk underscores the importance of lifelong follow-up of WLS patients, and the need for clinicians to have a high index of suspicion for nutritional-related abnormalities. Patients may suffer malnutrition due to incompliance and/or operation complications[23].

Although multivitamins typical-

ly contain the U.S. Recommended Daily Allowance for most vitamins and minerals such as iron and calcium, the available data show that multivitamins alone do not consistently protect patients from metabolic deficiencies after either RYGB or BPD. The one exception is folate, which can be maintained at normal levels in patients who regularly take multivitamins after RYGB[24,25].

In our series, after loopduodenal switch procedure, the degree of anemia usually was mild; we reported average mean values of both iron and ferritin and gradual improved values of both hemoglobin and hematocrit towards the end of the first year with patient compliance and regular follow up. On the other hand, a certain percentage of patients usually develop iron and ferritin deficiency after any operation which bypasses the duodenum, as RYGB whileiron deficiency did not differ in BPD with or without duodenal switch, and proved to occur in some studies[26,27] while others not[28]. This is because iron is absorbed preferentially in the distal duodenum and proximal jejunum. The frequency of anemia after duodenal switch (about 50%) is comparable to that afterRYGB, where it varied from 41% after a short limb to 74% after a long Roux lim[29].

Vitamin B12 and folate deficiencies are often evaluated together.Studies indicate that these deficiencies are fairly prevalent after bariatric operations despite the patients being advised to follow a multivitamin regimen[26,29,30]. After 1year of RYGBfollow up Vitamin B12 and folic acid deficiency were reported to be 33%, 63% respectively[31] and that value washigher thanthat afterBPD (22%) after 4 years of follow up[26]. We reported only 5.4% and 2.7% of Vitamin B12 and folic acid deficienciesduring our follow up period especially with good supplementation and patient compliance; although that, the meanvalue did not alter significantly over 1 year. We agree that vitamin B12 and folic acid deficiency does not usually occur in patients after the duodenal switch in contrast to that was reported after Roux-en-Y gastric by passdue to the preservation of more gastric mucosa that secreted inadequate intrinsicfactor

maynot lead to a vitamin B-12 deficiency with the duodenal switch-procedure[26,32].

We also did not report Zinc deficiency. Although it is seen mainly after BPD,[33] but can also occur after purely restrictive procedures due to poor dietary intake[34].

Parathyroid hormone (PTH) level showed slightly elevated levels but fluctuating within normal reference range, it goes ahead with other studies conducted[35]. Serum calcium and phosphorus in our study showed levels move within normal reference range, but this with continues supplementation it goes ahead with other studies conducted[35]. In comparison, Dolan et al [27] compared the nutritional side-effects of BPD alone vs BPD-DS, by performing a nutritional screen at a median follow-up of 28 months in both groups of patients. One-quarter of the patients were hypocalcaemia despite more than 80% taking vitamin supplements. There were no significant differences between the BPD and BPD-DS, suggesting that duodenal switch does not lessen the nutritional side-effects of BPD[27].

Bone specific alkaline phosphatase (BSAP) (marker of bone formation) showed levels fluctuating within normal reference range in most patients of our study.

Although there is no reported evidence of clinical complications of magnesium deficiency following bariatric surgery[36], we did not report magnesium deficiency in our series. Marceau et al[37] found also no significant abnormalities in magnesium levels before BPD, after 4 years and after 10 years.

According to Scopinaro N et al. [38] in all BPD patients, protein malnutrition is a major concern. It is manifested by clinical hypoalbuminemia, edema, asthenia, and alopecia. In our study only 1 (2.7%) patient developed protein malnutrition. We treated him conservatively by continuous 3 weeks of parenteral feeding and he showed good improvement then he continued on high protein diet and protein rich formulas. After loop DS also reported 4(8%) cases of significant protein malnutrition, 2 of whom underwent revision to RYGB. This rate considered to be high that made him later change

the common limb length to from 200 to 250 cm[35]. After open BPD it wasreportedthat 91(6.7%) of cases developed protein malnutrition[38].

Our data was consistent with that of Sánchez-Pernaute A et al.[35] that there was no evidence of hepatic dysfunction or liver failure manifested by either elevated serum transaminases or bilirubin levels.

In our series, the average number of bowel movements was 2.5/ day, which was similar to that reported after loop DS[35]. We did not record any cases of excessive diarrhea which is frequent episodes of diarrhea not adequately controlled. After open classical DS, 2 (0.45%) cases of excessive diarrhea that required revision surgery to lengthen both the common and alimentary channels[39].

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NUTRITIONAL MARKERS AFTER LOOP DUODENAL SWITCH (SADI-S) FOR MORBID OBESITY: ANEW TECHNIQUE WITH FAVORABLE NUTRITIONAL OUTCOME

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MINIMAL INVASIVE VIDEO ASSISTED ENDOSCOPIC THYROIDECTOMY (MIVAET) STUDY OF 20 PATIENTS

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Abstract

Minimal invasive video assisted endoscopic thyroidectomy remains rare. Here in we present this method for more experience and to show how it became more faceable and easily applied. It is called video-assisted endoscopic thyroidectomy, where it was first discovered in1997 (VAET), for the management of various thyroid diseases.

Methods: *20 consecutive patients who underwent MIVAET for simple thyroid diseases were solitary or multi-nodular goiters. The study group included colloidal nodules in 9 patients, follicular adenoma in two, and simple cyst in three cases, and simple multinodular goiter (colloid) in six patients. 2 to 3.5 cm transverse incision was made on the suprasternal notch. The wound was deepened to expose the underlying trachea from which the plane of the thyroid fascia was accessed directly, and the working space was established with lifting method using conventional instruments. All surgical procedures could be manipulated and monitored under laparoscopy without gas insufflation. The ultrasonically activated scalpel was the principal instrument used for MIVAET.*

Results: *All 20 patients underwent MIVAET successfully with one case converted to open thyroidectomy. The operation time ranged from 30 minutes to 90 minutes (average 45 minutes). The swelling size was up to 3.5 cm and may reach 8.0 cm in case of cystic swelling.posoperatively there was only one case of transient hyporparathyroidism, and another case of transient recurrent laryngeal nerve concussion. All patients were discharged one day postoperatively. During follow-up, all patients showed within normal thyroid function testes and*

satisfactory cosmetic results.

Conclusions: *MIVAET emerges as a promising minimally invasive surgical technique replacing conventional thyroidectomy for thyroid diseases in selected cases, with the advantage of satisfactory cosmetic results.*

Introduction

Thyroidectomy is one of the most common operations performed throughout the world and it has a low morbidity if performed by skilled surgical teams[1].

The conventional thyroidectomy normally requires a long collar incision, usually 6-10-cm in length, wide skin flaps on the anterior neck, and a long midline opening of the strap muscles to provide exposure of the thyroid gland, regardless of the extent of thyroidectomy planned. This conventional approach may cause a wide neck scar and a variety of potential complications related to raising skin flaps and vertically opening the strap muscles[2].

The concept of minimally invasive surgery has recently found many supporters in the management of thyroid disorders. The thyroid gland is frequently affected by benign or low-grade malignant diseases and the majority of patients are young women who consider the aesthetic appearance to be very important. For this reason many surgeons have been prompted to try a minimally invasive approach to thyroid gland. The cosmetic result is not the only factor that has prompted the use of the minimally invasive approach to the thyroid gland. In fact, the concept of minimally invasive surgery is also strictly connected to the attempt to reduce surgical morbidity and consequently the social costs of hospital admission by reducing postoperative hospital stay[3].

Since the 1980s, minimally invasive surgical techniques have attracted interest in all surgical specialties. During the last 10 years, laparoscopic surgery has begun to replace conventional open surgical techniques for abdominal and thoracic surgery. In neck surgery, Ganger[4] described

the 1st endoscopic subtotal parathyroidectomy using constant gas insufflation for hyperparathyroidism obtaining good clinical and cosmetic results. Subsequently, alternative techniques were devised to further improve the results of endoscopic surgery for neck lesions. Several techniques for minimally invasive thyroidectomy have been described with the primary aim of obtaining better cosmetic results. Minimal invasive thyroidectomy may be done by endoscopic and non-endoscopic methods. The endoscopic method may be done through direct and indirect approaches. In the direct approach through placing the access ports within the cervical region and was considered the least invasive[5], this approach include: lateral cervical, submandibular and suprasternal approach of minimal invasive video assisted thyroidectomy (MIVAET). Indirect approaches include minimally invasive total endoscopic approaches with constant gas insufflation[6] which include the anterior chest wall approach[7], the breast approach[8], and the Trans axillary approach[6]. Although the indirect approaches provide superior cosmoses, they are the most invasive, requiring a relatively large working space to access the neck and increases the operation time.

Suprasternal approach in surgical treatment of simple nodular goiters is characterized by being a direct approach, less invasive and each lobe of the thyroid gland can be dealt from the same small incision while all other methods whether direct or indirect deal only with ipsilateral lobe of the thyroid gland.so resection of the two thyroid lobes necessitate two approaches.

The aim of this study was to evaluate the suprasternal approach (Minimal invasive video assisted endoscopic thyroidectomy MIVAET) in treatment of simple nodular goiter, regarding to Operative time, cosmetic aim, and anatomical identification of recurrent laryngeal nerves and parathyroid glands which cause the majority of complications in thyroidectomy.

Patients and Methods

This study had been conducted on 20 patients selected from the outpatient clinic of Mansoura Uni-

versity Hospital from October 2010 to August 2013, suffering from (simple solitary nodule or simple multi nodular goiter (MNG). All patients were subjected to complete history taking with special emphasis on the thyroid complaints such as swelling in the neck, pressure symptoms, toxic symptoms and neoplastic symptoms, complete clinical examination both general and local including inspection, palpation, percussion of upper chest wall and auscultation, Routine laboratory investigations: Complete blood picture, liver function tests, renal function tests, blood glucose level and INR.

Specific investigations in the form of pre and post- operative measurement of (T3, T4, and TSH) and (serum calcium and PTH) to evaluate the thyroid and parathyroid function respectively. Ultrasonography, fine needle aspiration biopsy and vocal cord mobility assessment by ENT specialist .

Exclusion criteria are (1) lobe size more than 4cm except in case of cystic nodule where the size can reach 8 cm because it can be evacuated by using a syringe.(2) Thyroiditis.(3) previous neck surgery.(4) Previous neck irradiation.(5) Thyroid malignancy. (6) Hyperthyroidism .

All patients provided informed consent for inclusion in the study after explanation of the nature of the procedure and possible complications.The study was approved by Mansoura Faculty of Medicine ethical committee.

(1) Surgical Procedures

All patients were subjected to Minimally Invasive Video-assisted Endoscopic Thyroidectomy (MIVAET) through a suprasternal transverse incision about 2 to 4cm (suprasternal approach). The operative procedure included localizing the incision, division of the main thyroid vessels, visualization and localization of the RLN and parathyroid glands, delivery of the lobe in case of solitary one and doing the same to the other lobe in case of multinodular swelling, and lastly closure of the incision.

Localizing the Incision: The operation was performed under general anesthesia. The patient

was placed in the supine position with no hyperextension of the neck. It is important to avoid hyperextension of the neck as this makes it more difficult to open surgical planes with retractors. The primary surgeon was to the right of the patient and this was not dependent on the side of the thyroid pathology. Two assistants were required with the 1st assistant opposite the main surgeon and the 2nd assistant at the head of the patient. The operating room nurse and instruments were to the right of the primary surgeon .The process of incision design involves the 3 steps described in the following figure (Fig.2).

Determine the Midline of the Neck: The midpoints of sternal notch, thyroid notch, and mental region were identified and marked respectively as points 1, 2, and 3. The midline of the neck is identified by stretching a 2-0 silk tie between points 1 and 2. For patients with no prominent thyroid notch, the midline is determined by points 1 and 3.

Determine the Height of the Incision: Point 4 was marked 2-cm above point 1 in the midline. The distance between points 1 and 4 determines the height of the incision. A 2-0 silk tie running perpendicular to the midline was pressed against the neck through point 4.

Determine the Length and Shape of the Incision: Points 5 and 6, equally distant from point 4, were marked symmetrically in the perpendicular line. The distance between points 5 and 6 was the planned incision length (1.5-3.0-cm). The skin incision was achieved by connecting points 5 and 6 along Langer's lines. It was preferable to place the incision in the natural skin crease if possible.

After determining the site of the incision, the incision was made through the skin, subcutaneous fat, and platysma which were carefully dissected so to avoid any minimum bleeding. The skin flaps were raised by blunt and sharp dissection staying in the midline exposing the strap muscles superiorly and inferiorly for approximately 3-4-cm.we did not dissect laterally as this was not required and leads to unwanted bleeding.

As with normal thyroidectomy, the primary surgeon found the midline (linea Alba), a vertical white line lying between strap muscles since this is a relatively avascular plane. If this proves to be difficult, this is best found superiorly in relation to the laryngeal cartilages. This was opened from superior to inferior for at least 3-4-cm being careful to stay in the midline and cauterize any vascular tributaries of the anterior jugular veins. This was a critical point because it is necessary to incise the midline on an absolutely bloodless plane; any minimal bleeding at this point prevents the surgeon from carrying out the procedure.

The strap muscles on the affected side were then gently retracted with a small conventional retractor; with long blade shown in the fig. (6). A 2nd retractor was placed directly on the thyroid lobe which is retracted medially and lifted up. The dissection of the lobe from the strap muscles is completely carried out through the skin incision by gentle retraction and using conventional instruments. The 2 small retractors maintain the operative space. A 30º 10-mm endoscope was inserted through the skin incision to help continued dissection. The tip of the endoscope should be kept free of blood and avoid rotation of the endoscope to prevent disorientation of the surgical field.

Dissection of the thyrotracheal groove was completed under endoscopic vision by using small conventional instruments.

Washing and cleaning the operative field is very simple because there were not trocars in this procedure and sterile saline can be injected directly with the syringe, its aspiration can be greatly facilitated by the use of the fine tip aspirator. It allowed one to keep on operating through aspirating smoke and liquids without introducing extra-instruments into the incision.

Division of the Main Thyroid Vessels: Avoiding the electrocautery (either bipolar or monopolar) was particularly important when laryngeal nerves are not yet exposed. The 1st vessel to be divided is the middle thyroid vein, if present, or the small veins between IJV and thyroid capsule. Di-

vision and hemostasis was achieved by means of ultrasonic scissors (Harmonic Scalpel) (Fig. 4). This step allows a better preparation of the thyrotracheal groove where the RLN will be later searched for.

Usage of ultrasonic scalpel (harmonic scalpel) (Ethicon 10 mm) made the process of dissection, cutting and hemostasis so easy and not time consuming.

Further thyroid gland mobilization was started 1st by dissection of the superior or inferior pedicle according to accessibility. Partial division of the sternothyroid muscle insertion into the laryngeal cartilage may allow better exposure of the superior pedicle. The lateral aspect of the superior pedicle was 1st exposed, followed by opening of the potential space medially between the superior thyroid pedicle and the cricothyroid muscle after dissection the superior pedicle which was divided using the harmonic scalpel (Fig. 5). The thyroid lobe is retracted downward by the retractor. The spatula is used to separate the larynx from the vessels and to retract them laterally. The vessels are then selectively divided by Harmonic Scalpel (Ultracision) and they are cut (Fig. 6). The tip of the Harmonic Scalpel must be carefully checked while the upper pedicle was divided; it reaches high temperatures that could damage the larynx and even the pharynx if the tip is kept too long on their walls. The EBSLN (external branch of the superior laryngeal nerve) can be easily identified during most procedures once the different components of the upper pedicle have been prepared. The inferior vessels were similarly divided, exposing the anterolateral side of the trachea.

Visualization and Dissection of the RLN and Parathyroid Glands: After retracting medially and lifting up the thyroid lobe, the fascia can be opened by a gentle spatula retraction. The RLN generally lies in the thyrotracheal groove behind the Zuckerkandl tuberculum (posterior lobe) which is an important landmark in this step (Fig.7). Both parathyroid glands can be easily visualized thanks to endoscopic magnification (Fig. 8). Their vascular supply was preserved by selective ligature of the branches of the ITA (inferior thyroid artery). Dur-

ing dissection, hemostasis was achieved by Harmonic Scalpel when dealing with large or small vessels close to the nerve. In this way the RLN and the parathyroid glands were dissected and freed from the thyroid gland.

Blunt dissection and division of the isthmus was performed using the harmonic scalpel. Extraction and Resection of the Lobe: After removing the endoscope and the retractors, the surgeon carefully pulled out the upper portion of the gland using conventional forceps. Gentle traction over the lobe allows the complete exteriorization of the gland (Fig.9). Thyroid cysts were also a good indication for this procedure but they were often larger than 3.5-cm. Nonetheless, even cysts as large as 4- or larger can be operated on this way as long as they are aspirated before pulling out the thyroid lobe from the skin incision. Emptying these cysts in fact results in a sharp decrease in their size so as to allow the lobe to be easily extracted.

It was very important to check once again the RLN to avoid its injury. The isthmus was then dissected from the trachea and divided using the Harmonic Scalpel (Ultracision) (Fig.10). After completely exposing the trachea, the lobe is finally removed in case of solitary nodule or complete the other lobe as previous in case of multi-nodular goiter.

(2) In cases of multi nodular goiter: exposure the isthmus was carried out first and by using harmonic scalpel dissection of both anterior and posterior planes (between isthmus and trachea).each lobe was individually dealt with in the same step described previously.

(3) The following data were recorded: (a) The time of operation from the start of incision till closure in each case, (b) Exposure of important structures like recurrent laryngeal nerve, blood supply and parathyroid glands, (c) Incidence of intraoperative complications like bleeding, recurrent laryngeal nerve injury and parathyroid gland injury.

(4) Closure: Drainage was not necessary. The linea Alba and platysma were sutured with absorbable suture and the wound was

closed with subcuticular suture. By using 3-0 polygalatine.

(5) All removed specimens were send for pathological examination and compared to the pre-operative (FNAC).

(6) Post-operative care and follow up: from the day zero post operatively till six months we observed the following:

In the first 24 hours, patients were observed for vital signs, dyspnea, voice changes or hematoma the patients started oral fluid after 6 hours of the operation and then started regular diet if there was no GIT upset. Measurement of post-operative pain was carried out VAS.

The visual analogue scale (vas) was conducted in our study according to[9,10]. It consisted of a 100-mm line with the words no pain on the left side and worst pain imaginable on the right side. All patients were asked to evaluate their pain 6 and 24 hours after operation by indicating its level on the line. Pain severity was defined. Patients with scores of 3 or less were defined as having mild pain, those with score of 7 or more were considered to have severe pain and those from 3.1 to 6.9 moderate pain. They were also asked to describe the location of the pain, in particular, incision pain versus back or neck pain.

Mild pain may needs small dose of analgesic. The dose of analgesic increased with severity of pain.

The analgesic used was (NSAID) to relieve patient complaint.

The patients were discharged from hospital at the next day of operation, the patients were followed up in outpatient clinic as regard to the post-operative serum calcium 42 hours postoperatively, and parathyroid hormone and thyroid function testes within two weeks of operation as usual in the follow up of conventional cases and all patients were observed for seroma or wound infection. Observation of the post-operative scar within the follow up period which was one month through verbal response scale (V.R.S) was conduct in our study according to[11] Which had 4 options: 1= poor, 2 = acceptable, 3 = good and 4 = excellent. The patients were asked to

grade the cosmetic appearance of their wound 1 month after surgery, and determining the time needed by the patient to return to activity and to return to work in free pain condition. Follow up for recurrence within six months.

Results

Twenty patients with thyroid swellings underwent MIVAET (minimal invasive video assisted thyroidectomy), 12 (60%) were females and 8(40%) were males with mean age 36.7±8.7 tab. (1).

Fourteen (70%) cases were solitary thyroid nodule, where the pathological examination revealed: 9 (45%) cases were colloidal nodule and 2 (10%) cases were follicular adenoma, 3 (15%) cases were simple cysts and 6 (30%) cases were simple multinodular goiter (colloidal), 8 (40%) cases were in right lobe, 6 (30%) were in left lobe. tab. (2).

The lobe ranged from 1.75 to 4 cm with mean size of (3.38) and up to 8 cm in case of cystic nodule, the operative time ranged from 30 minutes to 90 minutes with mean operative time of (62.5±21.91 min) One case was converted to open method due to uncontrollable intraoperative bleeding, two (10%) cases were drained due to uncertain hemostasis, two (10%) cases developed seroma. There was one (5%) case with transient hypocalcaemia and another one case (5%) of transient hoarseness of voice due to concussion of recurrent laryngeal nerve. This resolved after 4 month of follow up. Tab. (3).

There was exact correlation between pre-operative fine needle aspiration biopsy and post-operative whole specimen pathological results except 2 cases (10%) with fine needle were follicular lesion but after end result pathological they were colloid goiter.

There were insignificant changes between the pre and post-operative values of the serum calcium and parathyroid hormone, where the mean serum calcium level pre-operative was 10.21+0.60 and post-operative mean value was 10.33+0.68 with p value was 0.377.

The pre-operative mean value of parathyroid hormone was 33.45+7.01 and the pos operative mean value of was 32.25+7.29

with p value was 0.502.

Pain in (MIVAET) was evaluated by using (visual analogue scale) there were moderate pain in 9 (45%) cases and severe pain in 11 (55%) cases after 6 hours of surgery, mild pain in 6 (30%) cases and no pain in 14 (70%) cases after 24 hours, where the mean pain value 6 hours post operatively was (6.4-1.60) with range 4-9 and the mean value of postoperative pain at 24 hours was (0.65+1.09) with range 0-3. We used NSAID (non-steroidal anti-inflammatory drugs) AS a pain killer in sever and moderate pain.

The dose and frequency of analgesic increased with severity of the pain.

The pain free return to activity ranged from one to three days with mean value of (1.35 ± 0.59) and the pain free return to work ranged from 6 days to 14 days with mean value of (8.55 ± 2.061).

From the cosmetic point of view, all cases were so satisfied due to small and unremarkable scar which ranged from 2 to 4 cm except one cases converted to open procedures reached to about 10 cm.

All patients had from good to excellent cosmetic appearance of postoperative scar. Cosmetic results are evaluated with a verbal response scale. Which had 4 options: 1= poor, 2= acceptable, 3= good and 4= excellent. The patients were asked to grade the cosmetic appearance of their wound 1 month after surgery[11] where the 17 (85%) were grade 4 (excellent), two cases (10%) were grade 3 (good) due to scars of the drains and one case (5%) was grade 1(poor) due to conversion to open surgery (the grade ranged from 1 – 4, the mean value was 3.75 and SD was 0.72).

Identification of the recurrent laryngeal nerve and the relation of the nerve to the inferior thyroid artery (whether deep or superficial to the inferior thyroid artery shown in the table or in between the branches of the inferior thyroid artery found in one left solitary nodule case), Identification of the inferior thyroid artery and identification of middle thyroid vein (which was obscured on right side in one case of multinodular goiter) and totally absent in another case.

Table (1): Gender of the patients underwent MIVAET.

	No	%
Male	8	40
Female	12	60

Table (2): Pathological results of solitary thyroid nodule.

	No	%
Colloidal nodule	9	45%
Follicular adenoma	2	10%
Cysts	3	15%

Table (3): Operative complications.

	No	%
Seroma	2	10%
Bleeding	1	5%
Recurrent laryngeal nerve injury	1	5%
Conversion to open technique	1	5%
Hypoparathyroidism	1	5%
Esophageal injury	0	0%
Tracheal injury	0	0%

Table (4): comparison between the pre and postoperative values of serum calcium and Parathyroid hormone.

	Preoperative	Postoperative	P value
Serum Ca	10.21 ± 0.60	10.33 ± 0.68	0.377
Serum PTH	33.45 ± 7.01	32.25 ± 7.29	0.502

Table (5): The range and the mean values according to VAS of pin in 6 and 42 hours.

	6 hours post-operative	24 hours postoperative	P value
Mean ± SD	6.4 ± 1.60	0.65 ± 1.09	< 0.0001
Range	4 – 9	0 – 3	

Table (6): Pain free return to activity and to work.

	Pain free return to activity	Pain free return to work
Mean ± SD	1.35 ± 0.59	1 – 3
Range	8.55 ± 2.061	6 – 14

Table (7): identification of inferior thyroid artery, middle thyroid vein, and recurrent laryngeal nerve in MNG cases.

Items / No	Bilateral Identify. of inferior thyroid artery	Bilateral Identify. of middle thyroid vein	Rt. Recurrent laryngeal nerve	Lt. recurrent laryngeal nerve
1	Identified	Identified	Deep to inf. thyr. art	Deep
2	Identified	Identified on right	Superficial	Deep
3	Identified	Identified	Superficial	Superficial
4	Identified	Not Identified	Deep	Deep
5	Identified	Identified	Deep	Superficial
6	Identified	Identified on left	Superficial	Deep

Table (8): identification of inferior thyroid artery, middle thyroid artery and recurrent laryngeal nerve in solitary thyroid nodules.

	Identify of inf. Thyr,artery	Identify of mid.thy.vein	Rt.rec.laryngeal nerve	Lt.rec.laryngeal nerve
1 Rt	Identified	Identified	Iden.deep to the nerve	
2 Lt	Identified	Identified		Iden.deep
3 Lt	Identified	Identified		Iden.deep
4 Rt	Identified	Identified	Iden.superfacial	
5 Rt	Identified	Identified	Iden.deep	
6 Lt	Identified	Identified		Iden.in between
7 Rt	Identified	Identified	Iden.deep	
8 Lt	Identified	Identified		Iden.superfacial
9 Rt	Identified	Identified	Iden. Deep	
10 rt	Identified	Identified	Iden.superfacial	
11 rt	Identified	Identified	Iden.superfacial	
12 lt	Identified	Identified		Iden.deep
13rt	Identified	Identified	Iden.deep	
14 lt	Identified	Identified		Iden.superfacial

Table (9): collective data of minimal invasive video assisted endoscopic thyroidectomy.

Case	Gender, age	Duration of oper.	Path. and size	Postop compl	Cosmetic result	Hospital stay	s.calcium level		s.PTH	
							Pre-op.	Post-op.	Pre-op.	Pos-op.
1	F 29 y	120 min	Colloid 4.5cm Rt	No	Small scar 4 cm	24 h	10mg/dl	10.25mg/dl	30pg/ml	32pg/ml
2	M 36 y	90 min	Colloid (multinodular goiter), Rt 2,75 Lt 3cm	Transient hypoparathyroidism	4 cm	72h	9.39mg/dl	8mg/dl	40pg/ml	8pg/ml
3	F 51 y	30 min	Colloid (MNG) Rt 2, Lt 1.75	Seroma	3.5 cm	48 h	10.25mg/dl	11mg/dl	35pg/ml	35pg/ml
4	F 48 y	60 min	Colloid 3cm Lt	Drained due to uncertain hemostasis	4 cm	24 h	11mg/dl	10.25mg/dl	44pg/ml	40pg/ml
5	M 46 y	30 min	Colloid 3.5 Rt	No	4 cm	24 h	10.5mg/dl	11mg/dl	34pg/ml	33pg/ml
6	F 39 y	90 min	Cystic nodule 7.5 cm Lt	Conv.to open due to bleeding	Long scar	48 h	9.25mg/dl	10mg/dl	26pg/ml	30pg/ml
7	F 54 y	60 min	Follicular adenoma 2.75 Rt	Drained due to uncertain haem	3.5 cm	48 h	10mg/dl	10.5mg/dl	32pg/ml	33pg/ml
8	M 34 y	30 min	Colloid 3 cm Lt	Infected seroma	3 cm	48 h	11mg/dl	11mg/dl	25pg/ml	27pg/ml
9	M 34 y	50 min	Colloidal (MNG) Rt 2.5 Lt 3cm	No	3 cm	24 h	10.5mg/dl	10mg/dl	30pg/ml	29pg/ml
10	F 55 y	45 min	Colloid 2.57cm Rt	No	3cm	24 h	10mg/dl	10mg/dl	23pg/ml	30pg/ml
11	F 45 y	30 min	Follicular adenoma 3.5 Lt	Trans dypsnea on exertion	2.5cm	48 h due to partial inj. of rec lar. nerve	11mg/dl	10.75mg/dl	44pg/ml	40pg/ml
12	F 30 y	50 min	Colloid 2.5 Rt	No	3 cm	24 h	9.75mg/dl	10mg/dl	35pg/ml	33pg/ml
13	F 37 y	30 min	Colloid (MNG) Rt 3, Lt 2.75	No	2cm	24 h	9.5mg/dl	10.5mg/dl	45pg/ml	40pg/ml
14	M 39 y	60 min	Colloid 2.25 cm Rt	No	3 cm	24 h	10mg/dl	10mg/dl	22pg/ml	25pg/ml
15	F 40 y	30 min	cystic nodule 4.5 cm Rt	No	3.5cm	24 h	10.5mgldl	11mgldl	42pg/ml	39pg/ml
16	M 45 y	90 min	Cystic nodule 8 cm Lt	No	3.5cm	24 h	11mg/dl	10.75mg/dl	37pg/ml	39pg/ml
17	F 50 y	30 min	Colloid(MNG) Rt 2.75 Lt 3.5	No	3 cm	24 h	10.25mg/d	10mg/dl	28pg/ml	30pg/ml
18	M 33 y	50 min	Colloid3.5cm Lt	No	2.5cm	24 h	11mg/dl	11mg/dl	33pg/ml	35pg/ml
19	F 52 y	60 min	Colloid (MNG) Rt 3, Lt 2cm	No	2.75cm	24 h	9.25mg/d	10mg/dl	35pg/ml	37pg/ml
20	M 40 y	30 min	Colloid 4cm Rt	No	3 cm	24 h	10mg/dl	10.5mg/dl	29pg/ml	30pg/ml

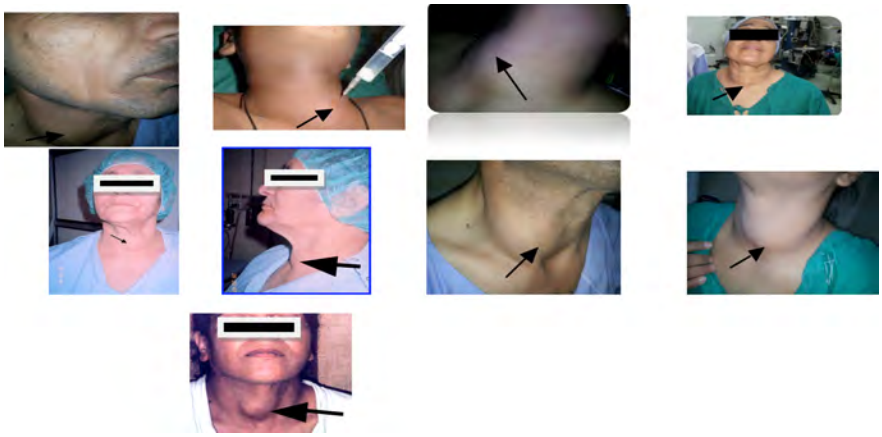

Fig. (1): Preoperative assessment (the arrow show the thyroid swelling).

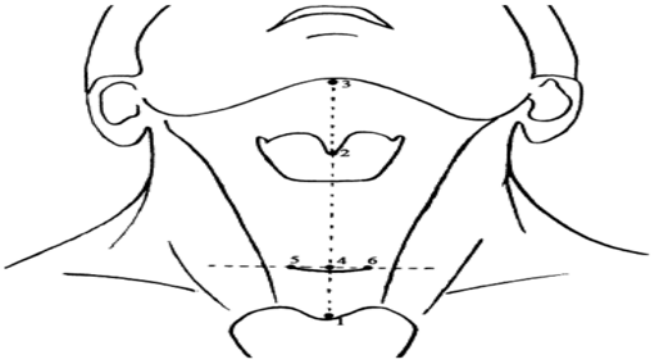

Fig. (2): incision design for MAVAET.

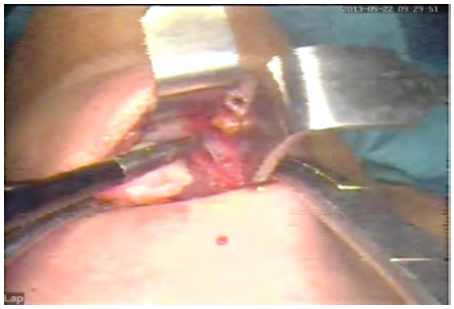

Fig.(3): Dissection using conventional and endoscopic tools.

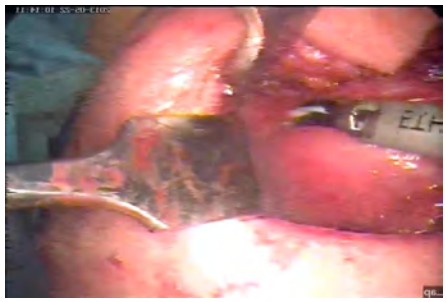

Fig.(4): Dividing the middle thyroid vein with the ultrasonic scalpel.

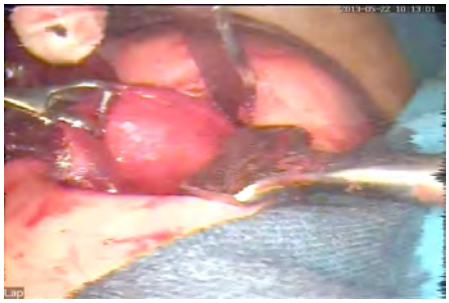

Fig.(5): Opening of potential space between superior thyroid lobe and crico-thyroid muscle.

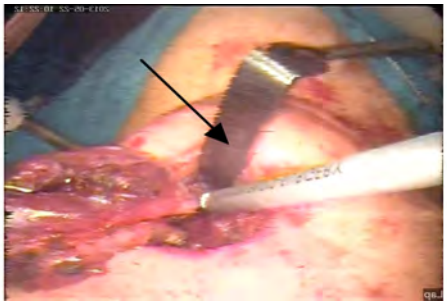

Fig.(6): Division of the superior thyroid pedicle.

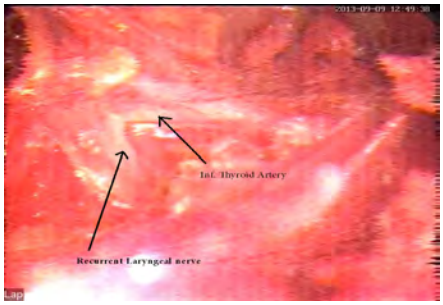

Fig.(7): Exposure of the recurrent laryngeal nerve.

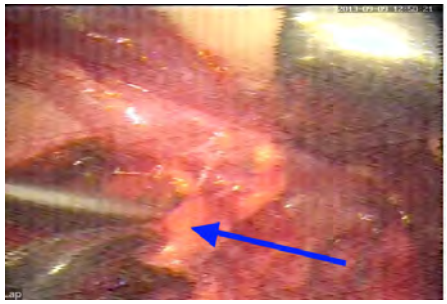

Fig.(8): Identification of the superior parathyroid gland in relation to the superior thyroid pole.

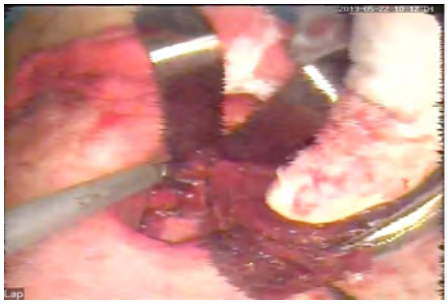

Fig.(9): traction of the lobe downward to facilitate exposure and division of the upper pole.

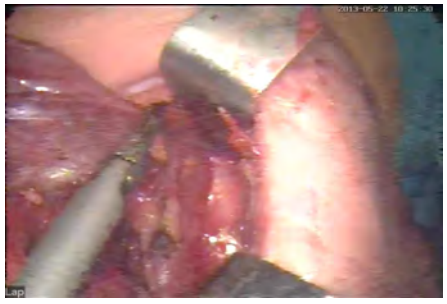

Fig.(10): Division of the thyroid isthmus using the ultrasonic scalpel.

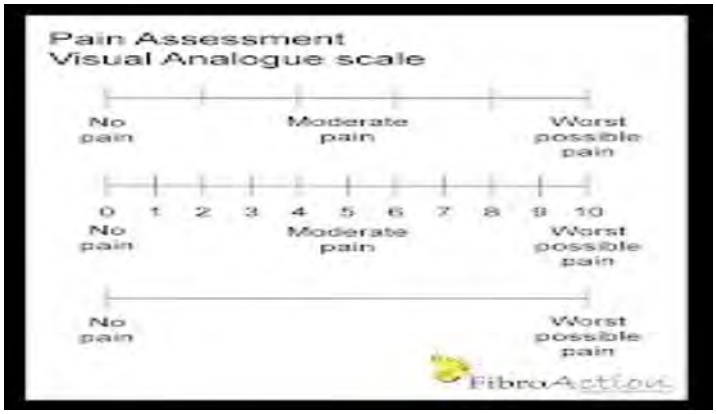

Fig. (11): Visual analogue scale **(VAS)**.

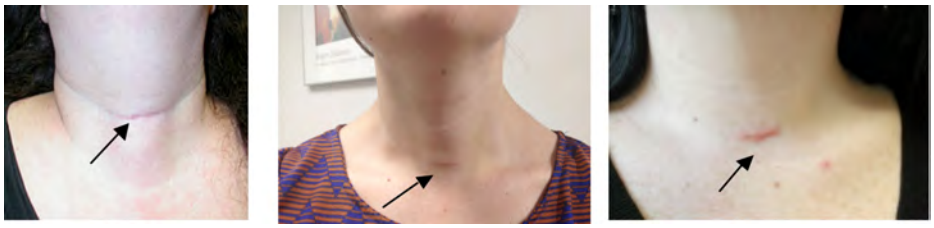

Fig. (12): Scar of the (MIVAET) 2weeks postoperative.

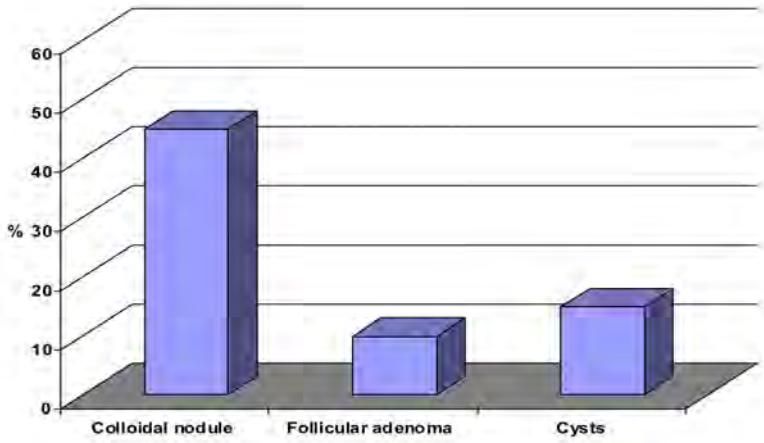

Fig. (13): Percentage of pathology.

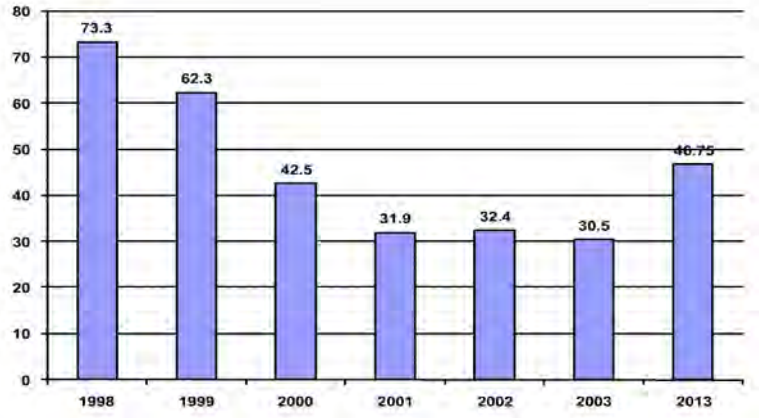

Fig. (14)

Discussion

Conventional thyroid surgery has developed dramatically in the19th century, especially in the French school of medicine where the mortality of this procedure has been greatly reduced partly by Theodore Kocher's emphasis on attention to detail and antisepsis. today the figures of mortality and morbidity are close to zero and less than 1%, respectively[12], current goals are now focusing on the additional outcome of cosmoses and functionality through the development of minimally invasive Endoscopic and video-assisted techniques that reduce the level of surgical invasiveness and result in an improved cosmetic appearance, where the first reported case was published in 1997[13]. Consequently, these surgical techniques are rapidly being applied to neck surgery. However, these procedures were time-consuming, and their complexity made them difficult to reproduce in all surgical backgrounds[14]. However our approach has major facilities like: it is small incision; the field is magnified by the endoscope and using ultrasonic scalpel. these all make the procedure less time consuming and less complications specifically recurrent laryngeal nerve and parathyroid gland injuries and leads to a very small scar which made this technique has now the superiority to the conventional one.

On the other hand, an invasive approach for neck surgery, such

as the mini-incision approach, seems to be more suitable for thyroid disease than others, since this procedure is characterized by a single skin incision in the neck and the use of conventional instruments, retractors, and a surgeon's finger. Improvement in finger eye coordination simplifies the procedure, enabling the procedure to be less technically demanding and time consuming. Total thyroidectomy and clearance of the central compartment neck nodes can be additionally performed using same technique through the mini-incision[15].

Twenty cases were included in our study, where the pathological examination reviled simple colloidal goiter in 15 cases (75%), follicular adenoma in 2 cases (10%), and simple cysts in 3 cases 15% in comparison to the other studies conducted[16] had studied 13 cases of simple goiter. Correlation between pre and postoperative pathological result is so important because this will determine if there were more surgical steps or not.

The procedure differs from conventional thyroidectomy in requiring a smaller skin incision and no rising of the skin flap. Therefore, this technique can obviate damage to the subcutaneous tissue, and the potential risk of complications related to a large skin incision and rising of the skin flap, such as hypoesthesia or parathesia in the neck and discomfort while swallowing, Furthermore, the small skin incision and small working space result in a good cosmetic status, less invasiveness. And less traumatization of the tissue so less incidence of post-operative hematomas nerves, vessels, and parathyroid gland injuries[15].

Identification of blood and nerve supplies were facilitated by magnifying effect of using endoscope. Where identifying these structure bilaterally in mutlinodular goiter and unilaterally in case of simple solitary nodules.

Where the superior and inferior thyroid arteries and middle thyroid vein were identified except one case of simple multinodular goiter where the middle thyroid vein was not identified on the right side .But there was a com-

plete identification of the recurrent laryngeal nerves, similarly there were complete identification of the nerves and blood vessels in all cases as reported by[17] where there were 39 cases (34 cases of solitary thyroid nodule and 5 cases of grave's disease.

MIVAET ranged from 30 to 90 minutes with mean time 53.25 minutes and this time was long at the beginning then it became shorter by the time where our skills got perfect at the end of this study. In comparison to 94 minutes as mean time reported by[18]. While that of conventional surgery ranged from 60-150 minutes with mean time was 90 minutes in study of[19]. Although a large goiter can be respected by this technique, a tendency towards a longer operation time was noted.

Comparison of the median operative time of our study of video-assisted thyroidectomy in 2013 which was (30) to Values represent median operative times (minutes) per year from 1998 to 2003[9]. We found that the operative time deceased steadily as follow: the median times were (73.3 in 1998), (62.3 in 1999), (42.5 in 2000), (31.9 in 2001), (32.4 in 2002), (30.5 in 2003) fig. (14).

Learning curve of video-assisted thyroidectomy. Values represent median operative times (minutes) per year from 1998 to 2003[9] in comparison to our thesis results in 2013.

Only one case (5%) case was drained because of uncertain hemostasis, one case (5%) was converted to open because of bleeding, and there was one case (5%) of transient hypoparathyroidism, and another one (5%) of recurrent laryngeal nerve transient injury. Where the difference between the pre and post-operative values of serum calcium and parathyroid hormone showed insignificant changes. In comparison to the results of similar studies[20] was acting on 39 with patients with benign thyroid disease). Where there were one case of transient recurrent laryngeal nerve concussion and another case of transient hypoparathyroidism.

In our study the patients were hospitalized for 24 hours, Al-

though the duration of the hospital stay is mainly determined according to the surgeon, clinical assessment and by general conditions factors, patients well and the patients family often actively participate in decisions regarding the hospital discharge date in Japan. Therefore, the patients were hospitalized for much longer periods than patients with similar conditions in the US[18]. All patients in this study discharged 24 hours postoperative except those with complications.

Postoperative pain is assessed by means of visual Analog Scale (VAS).this scale is consisted of a 100-nm line with the words no pain on the left side and worst pain imaginable on the right side. All patients were asked to evaluate their pain 6 and 24 hours after operation by indicating its level on the line .they were also asked to described the location of the pain, in particular, incision pain versus back or neck pain[9] from Portenoy & Tanner[21].

In our study all scars of MIVAET are satisfying to the patients and the surgeons, which the main aim of our study except that case which converted to open surgery where the average length of conventional thyroidectomy (10-15cm) while in MIVAET was (2-4cm). Cosmetic results are evaluated with a verbal response scale (VRS). Which had 4 options: 1= poor, 2= acceptable, 3= good and 4= excellent. The patients were asked to grade the cosmetic appearance of their wound one month after surgery[11]. Cosmetic evaluation was done by patients only one month after the operation, the better satisfaction after MIVAET may be attributed to lower postoperative pain and complications and better cosmetic results compared to conventional thyroidectomy[22] who carried out 80 cases of benign and malignant thyroid swelling.

The follow up period in this study was six months to evaluate the complications which may be happened in this period and how to deal with each one like: the recurrent laryngeal nerve injury (one case of dyspnea on exertion which appeared immediately post-operative and recovered early within three weeks), (one case of hypothyroid-

ism appeared in the form of circum-oral numbness and tingling in extremities which was dealt with by intravenous calcium gluconate in first 24 hours and oral calcium for two weeks and reevaluate the parathyroid hormone PTH), follow up the pain as described before, follow up of the scar after one month as described before, follow up of thyroid hormones two weeks post-operative this was monitored by thyroid stimulating hormone TSH and adjusted with oral thyroxin) and follow up of the recurrence within the six month where there were no cases with recurrence because we made total lobectomy or total thyroidectomy in solitary thyroid nodule and multinodular goiters respectively.

All previous researches concluded that this maneuver were difficult, time consuming, and expensive but by the time and with the progression of surgical facilities which have been available made this maneuver the one of choice in the near future and this was proved from the following meta-analysis where thy described these difficulties as criticisms, including.

The 1st criticism is represented by the higher cost comes from use of a Harmonic Scalpel. The Ultracision shears are disposable, so they must be considered an additional cost, but this is not peculiar to video-assisted and endoscopic thyroid surgery as reported by[9]. In fact and as reported by Meurisse et al.[23] this device is used more and more in conventional neck surgery because it has proved in several experiences to significantly reduce operative time).

Cost seems to be the most important point against minimally invasive thyroidectomy due to use of Harmonic Scalpel, but this is balanced by the shorter operative time, shorter hospital stay, smooth postoperative period, and lower incidence of complications[9] and now the harmonic scalpel got less expensive and available by different brands.

The 2nd criticism was represented by the scarce number of patients who fulfill the criteria to undergo this kind of procedure and this limits its clinical impact[9] but by the time and with increased the experience the size of the swelling

increased from small nodule to multinodular goiter and including more variety of pathology like toxic and malignant ones.

The 3rd criticism was the lack of feedback from palpation. In addition, the restricted criteria for performing the procedure makes it difficult to get satisfactory experience with the technique. So, we started initially with larger incisions (3.5-4-cm) and gradually reduce the size of the incision as experience grow[9] but from our study curve on the 20 cases there is great improve in our experience in time of operation and the size of the swelling, cosmetic results, postoperative complications ,and stay in hospital.

The final criticism of this access is that it requires a 3rd surgical assistant on the operative team, because retractors must be held. It is important to avoid trauma to the skin edges, particularly contact burn injury from the ultrasonic dissector. Other skin injury may result from excessive skin traction, which may result in hypertrophied scars particularly in young females who may be prone to this problem. To prevent this happening, it is important to emphasize to the 3rd surgical assistant that a lifting rather than pulling retraction motion is required[22], finally the our study will not show new but applying already present one.

Conclusion

From this study, it is shown that, the minimal invasive video assisted endoscopic thyroidectomy (MIVAET) is a safe and feasible technique that can be done for thyroidectomy in simple nodular goiter through small neck incision (2-3 cm). Although the number of patients that we have treated in this manner is still small, we believe that our new procedure constitutes a useful surgical treatment for thyroid disease.

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MINIMAL INVASIVE VIDEO ASSISTED ENDOSCOPIC THYROIDECTOMY (MIVAET) STUDY OF 20 PATIENTS

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MASPIN EXPRESSION IN BREAST CARCINOMA AND ITS RELATION TO CD34-ASSESSED ANGIOGENESIS

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Abstract

Objective: *Breast cancer is the most common female malignancy in Egypt and morbidity and mortality continue to increase. Therefore, there is a great need for assessment of patients by new markers able to predict better prognosis for patients with this type of cancer. Maspin is a serine protease inhibitor with a reported tumor suppressor property attributable to inhibition of cell motility, induction of apoptosis and inhibition of angiogenesis. Contradictory results have been reported concerning the role of maspin in human breast cancer. The aim of this study is to assess the maspin expression in breast carcinoma and its relation to microvessel density (MVD).* ***Material and methods:*** *This study was conducted on 100 cases of breast carcinoma obtained from surgical pathology files of Oncology Center in Mansoura University during the period from 2011 to 2013. Immunohistochemical staining for maspin and CD34 was performed.* ***Results:*** *Invasive duct carcinoma (IDC) was diagnosed in 79 cases (79%), invasive lobular carcinoma (ILC) was diagnosed in 15 cases (15%), medullary carcinoma and mucinous carcinoma were diagnosed in 3 cases each (3%). In IDC, maspin expression was detected in 37 cases (46.8%) and was significantly correlated with tumor size (P=0.0001). Also there was significant positive correlation between tumor grade and score of maspin expression (P=<0.0001). Strong maspin expression was significantly correlated with triple negative (TN) phenotype in IDC (P=0.0004). The maspin staining was limited to the cytoplasm in 35 cases (94.6%) and in both nucleus and cytoplasm in the remaining 2 cases (5.4%). In ILC, out of 15 cases included*

in this study, only 2 cases (13.3%) showed maspin expression. In medullary carcinoma, one case (33.3%) was exhibited maspin staining while the remaining 2 (66.7%) were negative. All the three cases of mucinous carcinoma (100%) were negative for maspin expression. The relationship between maspin expression and MVD was insignificant. ***Conclusion:*** *Maspin expression is upregulated during malignant progression and that the IHC detection of maspin is an indicative of aggressive tumors characterized by higher grade, larger size and a TN phenotype. This should be taken in consideration in therapeutic strategies implicating maspin as a target.* ***Key words:*** *Maspin-MVD-IDC-ILC-TN.*

Introduction

Breast cancer is the most common malignant tumor in female worldwide and the morbidity and mortality continue to increase despite remarkable progress in the field of early diagnosis and adjuvant therapy 0[1]. Breast carcinoma is a heterogenous disease such that they may have different prognosis and respond to therapy differently despite similarities in histological types, grade and stage[2].

Recently, a number of molecular markers (ER, PR, Ki 67 and HER2/neu) have been identified in patients with breast cancer with an immediate impact on therapeutic strategies. Although, the expression of these markers as demonstrated by immunohistochemistry and/or genetic analysis were considered in routine examination of breast cancer, the molecular profile of these tumors is far to be complete[1].

Maspin is a relatively novel member of serine protease inhibitor family with a reported tumor suppressor function that has been widely studied in vitro and in animal models[3]. The propable mechanisms underlying this function is a combination of increased cell adhesion and apoptosis and decreased invasion and angiogenesis[4]. Although the tumor suppressor function is well documented experimentally, its expression and prognostic impact in human cancers is still controversial. Maspin expression may predict a better prognosis for several types of carcinomas including

prostate, colon and oral squamous cell carcinoma. However, in other malignancies such as pancreatic, lung, thyroid, ovarian and endometrial cancers, maspin expression were paradoxically increased in malignant cells compared to their normal cells of origin. Concerning the role of maspin in breast cancer, contradictory results have been reported[5]. On the light of some investigations, maspin expression is a potentially good prognostic factors[6] while on the other hand maspin overexpression in breast cancer is associated with aggressive behavior and poor prognosis in breast cancer[7].

Angiogenesis is critical for growth and progression of various human solid tumors because it enables delivery of oxygen and nutrition[8]. Tumor angiogenesis can be understood as a disruption of normal control mechanism of endothelial cell growth and function resulting in the formation of new vessels[6]. These newly senthesized vessels provide a close, direct route to the systemic circulation for tumor cells to spread[9].

The common pathologic approach to assess angiogenesis involves microscopic estimation of microvessel density (MVD) which can be detected on paraffin sections by immunohistochemical methods with specific antibodies directed against endothelial cell antigens[10]. MVD has been reported to be an independent prognostic indicator of outcome in a variety of human malignancies, including breast cancer, with increased MVD correlating with malignancy progression, shorter overall and relapse-free survival[11].

Considering the conflicting data about maspin in breast cancer, the aim of this study is to assess maspin expression in breast carcinoma using immunohistochemistry and to correlate its expression with established prognostic factors and MVD.

Material and Methods

Paraffin-embedded tissue blocks were collected from 100 patients with primary breast cancer. The patients had been operated on from 2011-2013 by modified radical mastectomy.

All breast cancers were histo-

logically classified according to the criteria of the World health Organization (WHO).

The histological grade was assigned according to the criteria of Elson and Ellis[12]. Cancers were staged according to the TNM classification. They were also classified into 3 categories (luminal, HER2(+) and triple negative) according to the modern molecular classification.

Immunohistochemistry:

Formalin-fixed, paraffin-embedded sections of tumor tissue and their adjacent normal breast tissue obtained from resected breast specimens were cut into 4 microns thick sections. Antigen retrieval was performed by using EDTA. The slides were then incubated 30 minutes with monoclonal anti-maspin immunohistochemistry (prediluted, clone EWA24, Lab vision) and with anti-CD34 (prediluted, clone QBEnd/10, Lab vision). This is followed by biotinylated secondary antibody and streptavidin-peroxidase conjugate. DAB was used as chromogenic substrate. Brown staining was identified as positive.

In case of maspin, positive staining was identified in the cytoplasm, nucleus or in both, and the expression level was determined by assessing the percentage of positive cells as well as the staining intensity. The percentage of positive cells were rated as 0 point: no positive cells, 1 point: 0-5% positive cells, 2 points: 6-50% positive cells and 3 points: >50% positive cells. The staining intensity was rated as 0 point: no staining, 1 point: weak intensity, 2 points: moderate intensity and 3 points: strong intensity. Points for intensity and percentage of positive cells were added and an overall maspin score (OMS) 0-6 was assigned as follow:

Score 0 (negative): ≤5% cells stained regardless of intensity.

Score 1 (weak expression): 2-3 points.

Score 2 (moderate expression): 4-5 points.

Score 3 (strong expression): 6 points.

OMS 2 and 3 were considered overexpression[13].

For CD34, each slide was first scanned at low power (X40) and the area with the higher number

of new vessels is identified (hot spot) and then scanned at X250 and three fields were analysed and the number of immunostained blood vessels was counted. A countable vessel was judjed as immunostained endothelial cells or endothelial cell cluster that was separate from adjacent microvessel, tumor cell and other connective tissue element. Results were expressed as the highest number of microvessels identified and counted within any single X250 HPF of the three fields counted.

The expression of both markers was correlated with positive (myoepithelial cells surround normal breast ducts for maspin and mature blood vessels in the non tumorous area for CD34) and negative controls. The expression of maspin was correlated with the clinicopathological data collected. For this purpose, the patients were divided into two age groups (≤50 years & >50 years), 3 size categories (≤2, >2-5 & >5 cm). tumors were graded according to the criteria of Elson and Ellis[12], staged according to the TNM classification and classified into 3 groups according to hormonal status: luminal, HER2(+ve) and TN tumors. Then the relation between maspin and MVD was identified in the 100 cases included in this study. Statistical analysis of data was done by using the PRISM 6 (GraphPad software Inc., san Diego, CA). $P<0.05$ = significant.

Results

Immunohistochemical expression of maspin:

There were positive maspin staining limited to the myoepithelial cells surrounding ducts and acini in normal breast tissue neighboring the tumor tissue and was taken as positive control. The staining was detected in both the nucleus and the cytoplasm (Figure 1). There was 60 (60%) maspin negative cases that showed no evidence of staining or staining of less than 5% of cells. 40 cases (40%) were maspin positive.

As regards IDC, 42 cases (53.2%) were maspin negative (score 0), 37 cases (46.8%) were positive. Six cases (7.6%) were score 1 (Figure 2), 19 cases (24%) were score 2 (Figure 3) while 12 cases (15.2%) were score 3 (Figure 4). Thirty five cases (94.6%) of

maspin positive tumors showed staining limited to the cytoplasm while only 2 cases (5.4%) showed staining in both the nucleus and the cytoplasm.

As regard ILC, 2 cases (13.3%) were maspin positive and 13 cases (86.7%) were negative. Of these two cases, one case showed cytoplasmic OMS-1 (Figure 5) and the other showed OMS-2 where maspin staining was detected in both nucleus and cytoplasm (Figure 6).

As regards IDC of special types, 2 cases (66.7%) of medullary carcinoma were negative and only one case (33.3%) showed cytoplasmic moderate level of maspin expression (OMS-2) (Figure 7). All cases of mucinous carcinoma were maspin negative (Figure 8).

Correlation between maspin expression and clinicopathological data:

In IDC, as shown in table 2, 23 cases (58.9%) of patients of 50 years or less show positive staining of maspin and 19 cases (48.7%) of them show overexpression (33.3% score 2 and 15.4% score 3), in contrast to 14 cases (35%) of patients of more than 50 years who were positive and only 12 cases (30%) of them show maspin overexpression (15% score 2 and 15% score 3). The difference in maspin expression between two age groups was statistically insignificant (P=0.1319). In relation to size, most positive maspin cases are more than 5 cm in size (17 cases; 94.3%), of them 22.2% in score 3. In tumors ranging in size between >2-5 cm, only 20 cases (44.9%) are maspin positive with only 14% in score 3. No cases less than 2 cm were positive for maspin. The relation between maspin expression and tumor size were statistically highly significant (P=.000). Most of positive cases for maspin were grade III,11 cases (50%) of them are in score 3 while 2 cases (9.1%) are in score 2 and only 4 cases (18.2%) are in score 1. Grade II tumors showed 19 positive cases, one case of them (1.9%) are in score 3. Only one case (33.3%) of grade I tumors are positive which was not in high score. There was significant positive relation between tumor grade and maspin scores (P=0.0001).

As regard lymphovascular invasion, maspin expression and also the high level of expression (OMS-3) were more prevalent in tumors with lymphovascular invasion. The results were statistically insignificant (P=0.1786).

Maspin staining was detected in 28 cases (50.9%) of node-positive tumors versus 9 cases (37.5%) of node-negative tumors. The difference in maspin expression between two lymph node status groups was statistically insignificant (P=0.077). In this study, maspin expression was insignificantly related to pathologic stage of studied IDC cases.

Most cases (72.7%) of TN tumors were maspin positive, 45.5% of them showed strong expression (score 3) versus 10% of HER2(+) and 2.1% of luminal tumors. The relation between maspin expression scores and receptor status was statistically significant (P=0.0004).

As regards ILC, the case with weak level of maspin expression (OMS-1) was 44 years old, with tumor 3 cm in size, no lymph node metastasis, stage III and it was of luminal phenotype. The case with moderate maspin expression (OMS-2) was 53 years old, with tumor size >5 cm, lymph node metastasis (N1), stage III and it was of luminal phenotype.

As regards medullary carcinoma, the maspin positive case was 42 years old, with tumor size 2.5 cm, LN involvement (N3), stage III and it was TN phenotype. As regards mucinous carcinoma, all the 3 cases (100%) were maspin-negative.

Correlation between maspin expression and MVD in 100 studied cases:

MVD was higher in IDC than ILC (Figures 9&10). The correlation between both maspin positivity and OMS and MVD in the 100 studied cases was insignificant as shown in tables (3&4).

Table (1): Maspin expression in the studied cases.

Variables	IDC	ILC	Medullary carcinoma	Mucinous carcinoma
Maspin staining No. (%):				
Negative	42 (53.2%)	13 (86.7%)	2 (66.7%)	3 (100%)
Positive	37 (46.8%)	2 (13.3%)	1 (33.3%)	-
Staining intensity of positive cases No. (%):				
weak	6 (7.6%)	1 (6.6%)	-	-
moderate	16 (20.3%)	1 (6.6%)	1 (33.3%)	-
strong	15 (18.9%)	-	-	-
OMS No. (%):				
0	42 (53.2%)	13 (86.7%)	2 (66.7%)	3 (100%)
1	6 (7.6%)	1 (6.6%)	1 (33.3%)	-
2	19 (24%)	1 (6.6%)	-	-
3	12 (15.2%)	-	-	-
Subcellular localization No. (%):				
Cytoplasmic	35 (94.6%)	1 (50%)	1 (100%)	-
Nuclear	-	-	-	-
Both	2 (66.7%)	1 (50%)	-	-

Table (2): Relation between maspin expression and clinicopathological parameters in 79 cases of IDC.

Variables	Overall Maspin Score (OMS)				total	p-value
	0	1	2	3		
1- age groups:						
≤50 years	16 (41%)	4 (10.1%)	13 (33.3%)	6 (15.4%)	39	0.1319
>50 years	26 (65%)	2 (5%)	6 (15%)	6 (15%)	40	
2- tumor size:						
≤2 cm	4 (100%)	-	-	-	4	
>2-5 cm	37 (64.9%)	5 (8.7%)	7 (22.2%)	8 (14%)	57	< 0.0001
>5 cm	1 (5.5%)	1 (5.5%)	12 (66.6%)	4 (22.2%)	18	
3- tumor grade:						
I	2 (66.7%)	-	1 (33.3%)	-	3	
II	35 (64.8%)	2 (3.7%)	16 (29.6%)	1 (1.9%)	54	< 0.0001
III	5 (22.7%)	4 (18.2%)	2 (9.1%)	11 (50%)	22	
4- lympho-vascular invasion:						
present	4 (28.6%)	1 (7.1%)	5 (35.7%)	4 (28.6%)	14	0.1786
absent	38 (58.5%)	5 (7.7%)	14 (21.5%)	8 (12.3%)	65	
5- LN status						
Negative	15 (62.5%)	1 (4.2%)	2 (8.3%)	6 (25%)	24	0.077
positive	27 (40.1%)	5 (9.1%)	17 (30.9%)	6 (10.9%)	55	
6- number of Involved LNs:						
N0	15 (62.5%)	1 (4.2%)	2 (8.3%)	6 (25%)	24	
N1	10 (50%)	1 (5%)	6 (30%)	3 (15%)	20	0.3880
N2	10 (58.8%)	2 (11.8%)	4 (23.4%)	1 (5.9%)	17	
N3	7 (38.9%)	2 (11.1%)	7 (38.9%)	2 (11.1%)	18	
7- tumor stage:						
I	3 (100%)	-	-	-	3	
II	22 (59.5%)	2 (5.4%)	6 (16.2%)	7 (18.9%)	37	0.3197
III	17 (43.6%)	4 (10.3%)	13 (33.3%)	5 (12.8%)	39	
8- IHC-based Molecular types:						
Luminal	29 (61.7%)	5 (10.6%)	12 (25.5%)	1 (2.1%)	47	
HER2(+)	7 (70%)	-	2 (20%)	1 (10%)	10	0.0004
TN	6 (27.2%)	1 (4.5%)	5 (22.7%)	10 (45.5%)	22	

LN = lymph node TN = triple negative. P<0.05 = significant.

Table (3): Relation between maspin expression (negative or positive) and MVD in the 100 studied cases.

Maspin expression	mean	SD	minimum	maximum	median	total	p-value
Negative	40.3	15.5	7	80	40	40	0.95
posive	40.2	17.5	6	90	40	60	

Table (4): Correlation between MVD and maspin expression in the 100 studied cases.

OMS	MVD			
	number	mean	Standard deviation	significance
0	60	40.3	15.5	r = 0.6
1	7	37.9	14.4	p-value=0.416
2	21	40.8	19.3	
3	12	40.7	17.3	

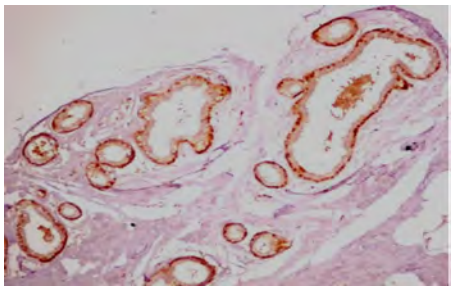

Fig. (1): Normal breast ducts showing maspin staining limited to myoepithelial cells in both nucleus and cytoplasm (immunohistochemical staining for maspin x100).

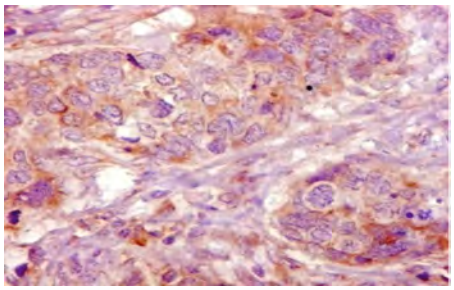

Fig. (2): IDC showing cytoplasmic OMS-1 (immunohistochemical staining for maspin x200).

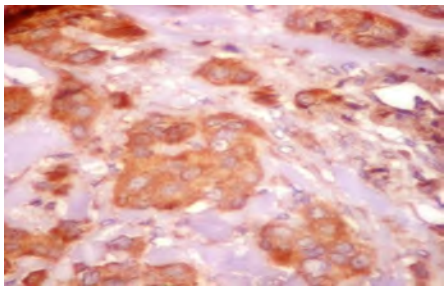

Fig. (3): IDC showing cytoplasmic OMS-2 (immunohistochemical staining for maspin x200).

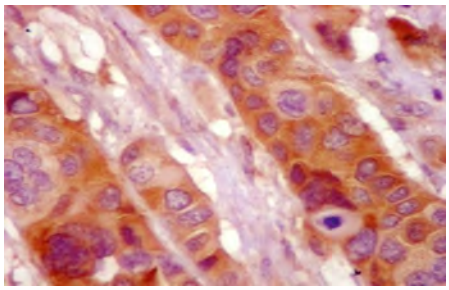

Fig. (4): IDC showing cytoplasmic OMS-3 (immunohistochemical staining for maspin x200).

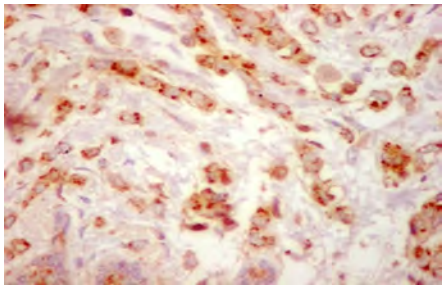

Fig. (5): ILC showing cytoplasmic OMS-1 (immunohistochemical staining for maspin x200).

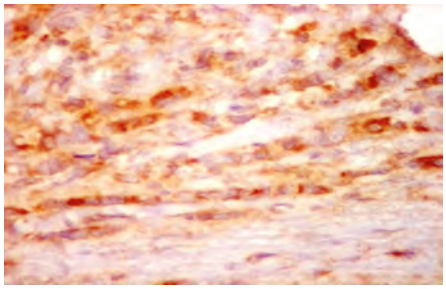

Fig. (6): ILC showing OMS-2, some cells show nuclear in addition to cytoplasmic staining (immunohistochemical staining for maspin x200).

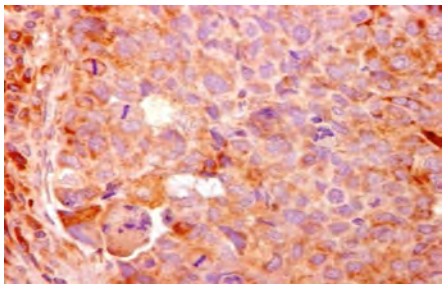

Fig. (7): Medullary carcinoma showing cytoplasmic OMS-2 (immunohistochemical staining for maspin x200).

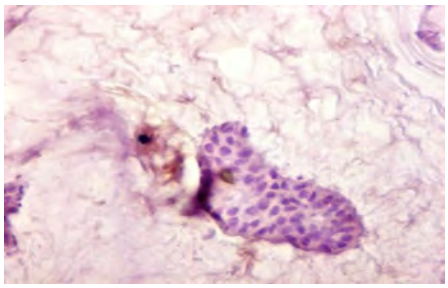

Fig. (8): Mucinous carcinoma showing negative maspin (immunohistochemical staining for maspin x100).

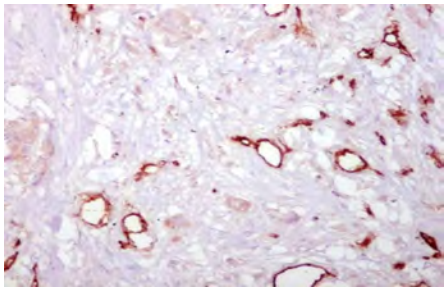

Fig. (9): IDC showing higher MVD (immunohistochemical staining for CD34 x200).

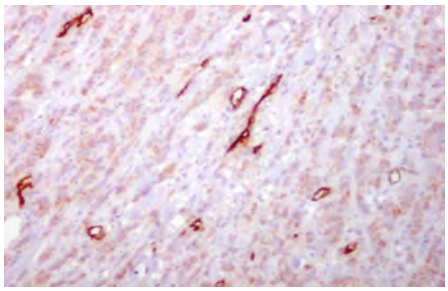

Fig. (10): ILC showing lower MVD (immunohistochemical staining for CD34 x100).

Discussion

Maspin is a 42 KD protein with tumor suppressor property attributable to inhibition of cell motility, promotion of apoptosis and inhibition of angiogenesis, subsequently the expression of maspin was shown to correlated with a better recurrence free survival[10]. Meanwhile, several other studies suggest that the correlation of maspin expression with breast cancer progression may not be so straight forward. In some cases, a higher level of maspin expression correlated with or predicted a poor prognosis of breast cancer[5].

Concerning expression of maspin in invasive duct carcinoma, 37 cases (46.8%) were maspin positive. This percentage lies in between the percentage reported by Sopel et al.[13] who found that maspin expression in 50.73% and that is reported by Lee et al.[14] who detected maspin expression in 31.3% of their studied IDC cases. The variation in maspin expression could be explained by the method used for interpretation of maspin staining.

Regarding the subcellular localization of maspin protein, in the present study, the majority of positive IDC cases (94.6%) showed maspin staining confined to the cytoplasm of cancer cells. This result agrees with Sopel et al.[13] and Lee et al.[14] who demonstrated that maspin expression has always regarded to be limited in the cytoplasmic compartment of tumor cells.

Panou et al.[5] reported that the presence of maspin expression did not show a significant association with age of the patients and tumor size, grade, lymph node positivity and stage. However, other studies showed strong association between maspin expression and large tumors and higher grade (Lee et al.[14]), lymph node positivity (Tsoli et al.[15]).

In our study, there is no correlation between maspin expression and age while strong maspin expression was positively correlated with tumor size and grade while the difference in maspin scores and lymph node status was insignificant. As regard the receptor status, there is strong relation between strong maspin (score 3) ex-

pression and TN tumors. Umekita et al.[16] reported the same finding as they observed positive rate of maspin was 85.9% of TN tumors and this positivity was significantly correlated with basal markers and they suggested that maspin could be a candidate for a basal marker in TN breast carcinoma. However, nalysis of gene expression array is required to conclude that maspin is a marker of the basal-like subtype.

From the previous discussion, the present study demonstrated that maspin expression was upregulated during ductal malignant progression and that the IHC detection of maspin expression in carcinoma cells was significantly correlated with an aggressive phenotype that was characterized by large size, grade and TN phenotype.

Three hypothesis were thought for the aberrant expression of maspin in breast cancer cells: maspin gene alteration with loss of activity, a high intracellular density of maspin resulting in auto-inhibition of its function, myoepithelial cell differentiation in cancer cells could contribute to more aggressive phenotype[17].

Concerning the expression of maspin in other variants of breast carcinoma included in this study, it has been showed that compared to IDC, ILC showed lower percentage of maspin positive staining as it was detected in 2 (13.3%). Similarly, Turashvilli et al. (2005)[17] reported that this kind of breast carcinoma has a low incidence (7.1%) of maspin expression. No informations in the available literature are found regarding the relation of maspin expression and clinicopathological variables in ILC. In the present study, one of the two maspin-positive cases showed weak expression (OMS-1), it was 44 years old with tumor 3 cm in size, nodal-negative, in stage III and it was of luminal phenotype. The other one showed moderate expression (OMS-2), it was 53 years old, with tumor size >5 cm, nodal-positive (in N1), in stage III and it was of luminal phenotype.

As regards the medullary carcinoma and mucinous carcinoma, no available informtions about maspin expression in these variant. In the

present study, one case (33.3%) was positive for maspin protein while all the three cases (100%) of mucinous carcinoma were negative for maspin expression.

Further study is needed to assess maspin expression and its relation to the clinicopathological variables of ILC and other special types of breast carcinoma.

On studying the correlation between maspin and MVD, it revealed statistically insignificant difference between maspin-positive cases and maspin-negative cases (P=0.95). This finding seemed to be contrary to the expected results that maspin has an angiogenesis inhibition function. This can be explained on the light of Goulet et al.[18]. They surmised that nuclear maspin could function as a tumor suppressor through regulation of genes involved in tumor growth and metastasis. When maspin was excluded from the nucleus via a nuclear exclusion signal, it no longer functions as a metastasis suppressor. The authors concluded that the nuclear localization of maspin is required for its tumor and metastasis suppressor function in vivo and suggests that its mechanism of action involves, in part, direct association of maspin with target genes. Therefore, it is not surprising that in this study, maspin was especially cytoplasmic, so, it is no longer associated with genes involved in metastasis; some of these genes may be those involved in angiogenesis.

Conclusion

The present study demonstrated that maspin is not downregulated through malignant progression and that the immunohistochemical detection of maspin in carcinoma cells may be helpful for selecting the group of breast cancer patients with an aggressive phenotype.

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MASPIN EXPRESSION IN BREAST CARCINOMA AND ITS RELATION TO CD34-ASSESSED ANGIOGENESIS

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THE EFFECT OF ZINC ON CADMIUM-INDUCED TOXICITY IN ALBINO RAT LIVER: HISTOLOGICAL AND HISTOCHEMICAL STUDY

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Abstract

Cadmium (Cd) is currently one of the most important pollutants that accumulate mainly in the liver causing many metabolic and histological changes. Zinc (Zn) is one of the ubiquitous metals in the environment that have a role in the regulation of liver functions. Therefore, the current study has been performed to evaluate the effect of Zn administration on Cd-induced toxicity in adult male albino rat liver. Thirty adult male albino rats were used and divided into 3 groups (10 rats /group). Group A was the control rats. Group B included Cd-treated rats. Group C included Cd & Zn treated rats. After 6 weeks, blood samples were obtained for the biochemical evaluation of serum albumin and liver enzymes; aspartate aminotransferase (AST) and alanine aminotransferase (ALT), while the liver was obtained and prepared for the histological and histochemical study. Compared to group A, haematoxylin and eosin stained sections in group B rats showed thickened portal tracts and interlobular septa, mononuclear inflammatory cellular infiltration, dilated blood sinusoids and hepatocytes with vacuolated cytoplasm and condensed nuclei. Group C showed vesicular nuclei and non vacuolated cytoplasm in most of hepatocytes. Mallory's trichrome stain revealed abundant collagenous fibers in portal tracts and in the thickened interlobular septa of group B, while in group C collagen fibres were less in portal tracts and in the thin short interlobular septa. Periodic Acid Schiff reaction showed a strong positive reaction in the cytoplasm of

most of the hepatocytes in group A, a weak positive reaction in almost all of the hepatocytes in group B and a strong positive reaction in some of the hepatocytes in group C. Succinic dehydrogenase enzyme activity in group A showed a strong enzyme activity in the hepatocytes around the portal tracts. However, the hepatocytes around the central veins revealed a moderate activity. Group B revealed marked decrease in succinic dehydrogenase activity in most of hepatocytes, while in group C strong activity was seen in the hepatocytes around portal tracts with some areas of weak activity in hepatocytes around central veins. The serum level of liver enzymes AST and ALT showed significant increase while the serum level of albumin was significantly decreased in group B as compared to group A. On contrary, the serum level of AST and ALT were significantly decreased in group C as compared to group B, but still significantly higher than group A. Moreover, serum level of albumin was significantly increased as compared to group B, but still significantly lowers than group A. ***In Conclusion:*** *Zn partially prevented Cd induced hepatotoxic changes in adult male albino rats, suggesting a possible hepatoprotective role of Zn.*

Keywords: *Cadmium, Light microscope, Liver, Zinc.*

Introduction

Cd is an industrial and environmental pollutant, arising primarily from battery, electroplating and galvanizing, pigment, plastic, fertilizer, metallurgical, fungicides and mining industries[1,2].

Cd has also found its place in the rapidly expanding field of nanotechnology, which is therefore likely to become another source of its toxicity. Fertilizers continue to be contaminated with Cd as a result of the recycling of industrial wastes for agricultural and home use fertilizers. The uptake of Cd from the soil results in elevated concentrations in vegetables, fruits, and grains, with the highest levels in wheat, rice, leafy greens and potatoes. Cigarette smoke is another source of non-occupational exposure[3].

Cd has been shown to be toxic even at low doses, since the metal accumulates with a long biological

half-life (>20 years) after ingestion or inhalation[4]. Chronic exposure to Cd results in accumulation of the metal mainly in the liver causing many metabolic and histological changes. It has been demonstrated that Cd stimulates free radical production, resulting in oxidative damage to various macromolecules and initiating various pathological conditions in humans and animals[5]. Oxidative stress is due to excessive production of reactive oxygen species (ROS)[2,6].

Cd disrupts the hepatic antioxidant defence mechanism within 15 days[7]. Cd accumulation in liver of rats is associated with a decrease in the level of reduced glutathione (GSH), and reduction of the activities of different antioxidant enzymes as superoxide dismutase (SOD), GSH peroxidase (GPx) and thioredoxin reductase in liver[1]. Cd has been reported to cause alterations in the serum and tissue concentrations of some lipid compounds, including cholesterol, high and low density lipoprotein cholesterol causing hypercholesterolemia and hyperlipidemia in various experimental models[8]. In addition, Cd exposure was shown to alter carbohydrate metabolism in liver[9].

Zn is one of the ubiquitous metals in the environment and is abundantly present in water and air[10]. It is an essential dietary element which is involved in many cellular functions[11]. Zn is an important antioxidant that decreases ROS production[12]. It is an essential element of more than 200 metalloenzymes, including some of the antioxidant enzymes like SOD[13]. Zn may function as an antioxidant by preventing the production of hydroxyl and superoxide ion radicals by protecting SH groups against oxidation[14].

There is sufficient evidence that Zn supplementation provides significant hepatoprotection under different toxic conditions[15]. Studies have also indicated the efficacy of Zn in the regulation of liver functions in various animal models of increased oxidative stress[16].

Cd has been recognized as an antimetabolite of Zn[17]. Interactions between Zn and Cd can occur at different stages of their me-

tabolism i.e. absorption from the gastrointestinal tract, distribution, retention in the organism, and excretion from the body[18]. Numerous studies have shown that Zn supply may reduce Cd absorption and accumulation[17].

Therefore, the current study has been performed to evaluate the effect of Zn administration on Cd-induced toxicity in adult male albino rat liver by histological and histochemical methods.

Materials and Methods

Animal Studies:

The current study was carried out on thirty adult male albino rats weighing 200-230 grams. The animals were kept in adequate ventilation and temperature with regular 12 h light / 12 h dark cycle in plastic cages and were allowed a free access to standard laboratory food and water ad-libitum. The animals were equally divided into three groups (10 rats each). Group A was the control rats that were fed on the basal diet and received 0.5 ml of distilled water (the solvent of Cd and Zn) daily by gavage. Group B included rats treated with cadmium chloride ($CdCl_2$) (obtained from LOBA CHEMIE PVT. LTD, Egypt) in a daily oral dose of 20 mg/kg body weight, dissolved in distilled water, by gavage[19]. Group C included rats treated with $CdCl_2$ (as in group II) and zinc chloride ($ZnCl_2$) (Al-Gomhoria Co-operation-Egypt) in a daily dose of 25 mg/kg body weight, dissolved in distilled water by gavage[20].

Obtaining the Samples:

After six weeks from the beginning of the experiment, the rats were anaesthetized, intraperitoneally, with sodium pentobarbital (40 mg/kg). For biochemical study of liver function, blood samples were taken by exposing the heart in each rat to obtain 1 ml of blood through its apex. After that, the liver was dissected out and prepared for histological and histochemical studies.

Histological and Histochemical Studies:

Small pieces from the liver of each rat were dissected and fixed in 10% neutral-buffered formalin and paraffin sections of 5 µm thickness were prepared and stained with: Hematoxylin and eo-

sin (H&E) stain,[21], Mallory's trichrome stain[22] and Periodic acid Schiff's (PAS) reaction[23]. Other small fragments of the liver from each rat were rapidly dissected and transferred immediately to the cryostat. Frozen sections of 10-15 µm thickness were prepared and processed for demonstrating succinic dehydrogenase enzyme activity[22].

Biochemical study:

The blood samples obtained from the rats in different groups of the study were centrifuged to separate the serum. The serum levels of liver enzymes; AST and ALT were measured by colorimetric method[24] using the commercial diagnostic kits produced by Randox Co., United Kingdom. The serum level of albumin was determined calorimetrically[25].

Statistical analysis:

Biochemical data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 9.05 to obtain:

A. Descriptive data:

Descriptive statistics were calculated in the form of mean and standard deviation (±SD).

B. Analytical statistics:

In the statistical comparison between the different groups, the significance of difference was tested using ANOVA (analysis of variance) to compare between more than two groups of numerical (parametric) data followed by post-hoc tukey for multiple comparisons. A P value <0.05 was considered statistically significant.

Result

I. Histological and Histochemical Results:

1- Haematoxylin and Eosin Stain (H&E stain):

Light microscopic examination of H&E stained sections in the liver of group A control rats showed it was divided into ill-defined classical hepatic lobules containing central veins in their centers. The portal tracts were located at the periphery of the lobules (Fig. 1). Each classical hepatic lobule was formed of plates of hepatocytes radiating from the central vein to the periphery of the lobule. The hepatocytes were polyhedral in shape and had finely vacuolated

acidophilic cytoplasm and central rounded vesicular nuclei, some of them were binucleated. The plates were separated by blood sinusoids which were lined by endothelial and Kupffer cells (KCs) (Fig. 2). The portal tracts contained branches of the hepatic artery, portal vein and a bile ductule (Fig. 3).

H&E stained sections in the liver of group B (Cd-treated rats) revealed congested blood vessels, thick portal tracts and interlobular septa. Marked mononuclear inflammatory cellular infiltration was evident in both interlobular septa and portal tract areas (Figs. 4, 5 and 6). The hepatocytes showed heterogeneous appearance. Many hepatocytes exhibited vacuolated cytoplasm, some of them showed pyknotic nuclei. Few small hepatocytes with deep acidophilic cytoplasm and small pyknotic nuclei were seen (Figs. 5 and 6). Some areas of hepatic lobule showed intralobular mononuclear cellular infiltration (Fig. 7).

In H&E stained sections of the liver of group C (Cd & Zn-treated rats), blood vessels were still congested but mononuclear cellular infiltrations was less than that seen in group B and restricted to the areas of portal tracts and interlobular septa. The latter appeared thin and short. Few hepatocytes were seen with vacuolated cytoplasm. Few other hepatocytes had deep acidophilic cytoplasm and small pyknotic nuclei (Fig. 8).

2- Mallory's Trichrome Stain:

Light microscopic examination of liver sections of control group A showed that the classical hepatic lobules were not demarcated by connective tissue septa. Few collagenous fibers were seen mainly at the areas of portal tracts (Fig. 9).

The liver sections of group B (Cd-treated rats) showed abundant collagenous fibers in the portal tracts and in the thickened interlobular septa that demarcated hepatic lobules (Fig. 10).

The liver sections of group C (Cd & Zn-treated rats) showed collagenous fibers in the portal tracts that were less than that seen in group B, but slightly more than in group A (Fig. 11).

3- Periodic Acid Schiff's Stain:

PAS stained sections of the control group A liver revealed a strong PAS positive reaction in the cytoplasm of most of the hepatocytes which appeared magenta red. The reaction was stronger in hepatocytes at the periportal regions than those around the central veins (Figs. 12 & 13).

The liver of group B (Cd-treated rats) revealed weak PAS positive reaction in almost all of the hepatocytes with moderate reaction in few cells (Fig. 14).

The liver group C (Cd & Zn-treated rats) revealed a strong PAS positive reaction in some hepatocytes, while others showed a weak reaction (Fig. 15).

4- Succinic Dehydrogenase Enzyme Activity:

Frozen sections of control group A liver revealed a strong succinic dehydrogenase activity in the hepatocytes around the portal tracts. However, the hepatocytes around the central veins revealed a moderate enzyme activity (Fig. 16).

The liver of group B (Cd-treated rats) revealed marked decrease in succinic dehydrogenase activity in most of the hepatocytes as compared to control group A. The strong activity was confined to small areas around the portal tracts. Very weak activity was seen in most of the hepatocytes around the central veins (Fig. 17).

The liver of group C (Cd & Zn-treated rats) revealed that the strong succinic dehydrogenase activity was seen in the hepatocytes at the periportal areas around the portal tracts. Areas of weak activity were still seen in the hepatocytes around the central veins (Fig. 18).

II. Biochemical Results and Statistical Analysis:

The mean value, standard deviation and probability of serum levels of AST, ALT and albumin for all groups were shown in tables (1, 2 & 3), respectively. ANOVA test revealed significant changes ($P<0.001$ for each) among different groups of the experiment.

Post-hoc tukey test showed the following:

In group B (Cd-treated) rats,

serum levels of AST and ALT showed significant increase (P<0.001 for each) as compared to those of group A (control) rats. Serum level of albumin showed significant decrease (P<0.001) as compared to that of group A (control) rats.

In group C (Cd & Zn-treated) rats, serum levels of AST and ALT were significantly decreased (P<0.001 for each) as compared to those of group B (Cd-treated) rats, but still significantly higher than those of group A (control) rats (P<0.001 and =0.010 respectively). Serum level of albumin was significantly increased (P=0.009) as compared to that of group B (Cd-treated) rats, but still significantly lower than that of group A (control) rats (P<0.001).

Table (1): The serum levels of AST in control and experimental group rats:

	Group A (Control)	Group B (Cd)	Group C (Cd+Zn)	ANOVA P value
Mean (IU/L)	18.93	57.74	40.68	< 0.001
SD (IU/L)	2.72	5.89	10.81	
P1		< 0.001*	< 0.001*	
P2			< 0.001*	

Table (2): The serum levels of ALT in control and experimental group rats:

	Group A (Control)	Group B (Cd)	Group C (Cd+Zn)	ANOVA P value
Mean (IU/L)	89.08	182.56	124.70	< 0.001
SD (IU/L)	20.07	39.84	25.95	
P1		< 0.001*	0.010*	
P2			< 0.001*	

Table (3): The serum levels of albumin in control and experimental group rats:

	Group A (Control)	Group B (Cd)	Group C (Cd+Zn)	ANOVA P value
Mean (IU/L)	4.11	3.4	3.6	< 0.001
SD (IU/L)	0.16	0.18	0.16	
P1		< 0.001*	< 0.001*	
P2			0.009*	

SD: Standard deviation
P: Probability * is a significant change
P1-P2: Multiple comparisons with post-hoc tukey test.
P1: Comparison between group A and other groups (B and C).
P2: Comparison between group B and group C.

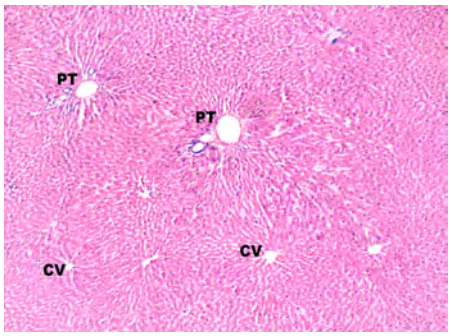

Fig.1: A photomicrograph of a paraffin section in the liver of group A control rat showing ill-defined hepatic lobulation. Portal tracts (PT) are seen at the periphery of hepatic lobules and central veins (CV) in their centers. (H&E X 100)

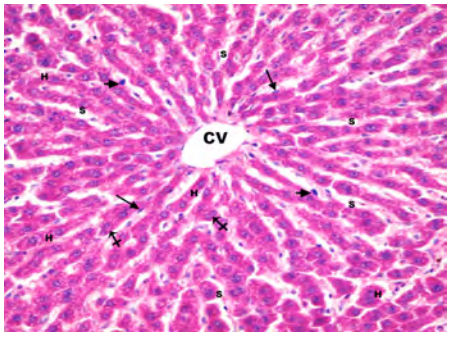

Fig.2: A photomicrograph of a paraffin section the liver of group A control rat showing polyhedral hepatocytes (H) with central rounded vesicular nuclei and acidophilic finely vacuolated cytoplasm, arranged in plates radiating from the central vein (CV). The plates are separated by blood sinusoids (S) lined by endothelial cells (arrows) and KCs (short arrows). Some hepatocytes are binucleated (crossed arrows). (H&E X 400).

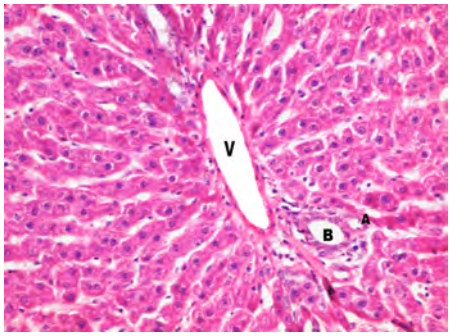

Fig.3: A photomicrograph of a paraffin section in the liver of group A control rat showing a branch of the hepatic artery (A), portal vein (V) and bile ductule (B) in a portal tract. (H&E X 400)

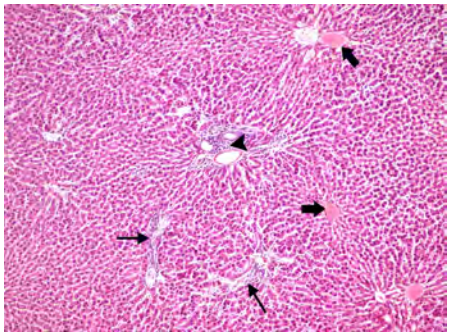

Fig.4: A photomicrograph of a paraffin section in the liver of group B Cd-treated rats showing marked cellular infiltration in the thickened interlobular septa (arrows) and in portal tract (arrow head). Note the congested blood vessels (thick arrows). (H&E X 100).

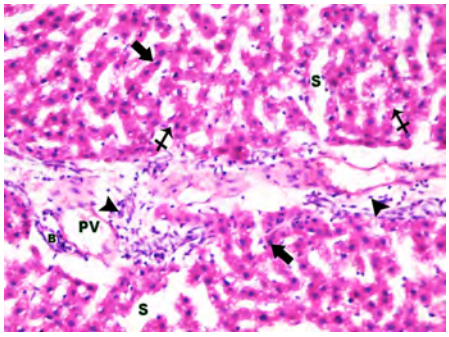

Fig.5: A photomicrograph of a paraffin section in the liver of group B Cd-treated rats showing a thick portal tract containing bile ductule (B) and portal vein (PV) with mononuclear cellular infiltration (arrow heads). The surrounding lobules show dilated blood sinusoids (S). Some hepatocytes show acidophilic cytoplasm and pyknotic nuclei (thick arrows) and others have vacuolated cytoplasm (crossed arrows). (H&E X 400)

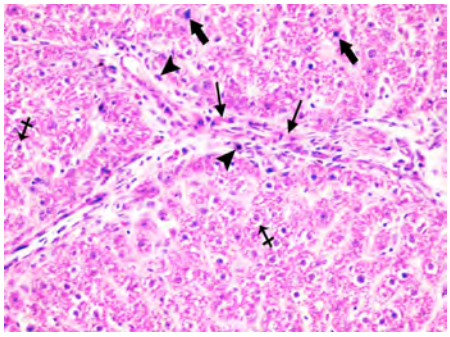

Fig.6: A photomicrograph of a paraffin section in the liver of group B Cd-treated rats showing thick interlobular septa (arrows) with mononuclear cellular infiltration (arrow heads). Hepatocytes show vacuolated cytoplasm (crossed arrows) and some of them have pyknotic nuclei (thick arrows). (H&E X 400)

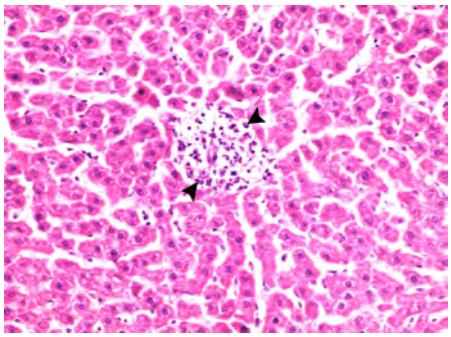

Fig.7: A photomicrograph of a paraffin section in the liver of group B Cd-treated rats showing an area of mononuclear cellular infiltration (arrow heads) in a part of hepatic lobule. (H&E X 400)

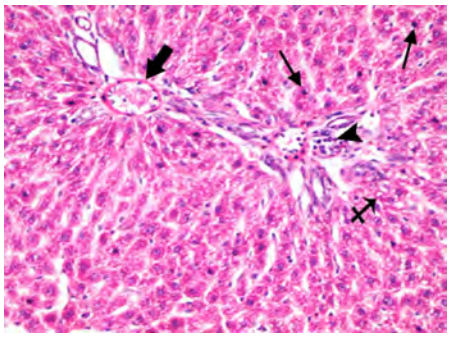

Fig.8: A photomicrograph of a paraffin section in the liver of group C Cd & Zn-treated rats. The portal tract shows congested blood vessel (thick arrow) and mononuclear cellular infiltration (arrow head). Few hepatocytes show vacuolated cytoplasm (crossed arrows) and others have small pyknotic nuclei (arrows). (H&E X 400)

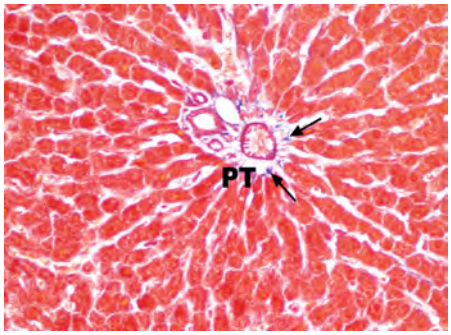

Fig.9: A photomicrograph of a paraffin section in the liver of group A control rat. Few collagenous fibers (arrows) are seen in the portal tract (PT). (Mallory's trichrome X 400)

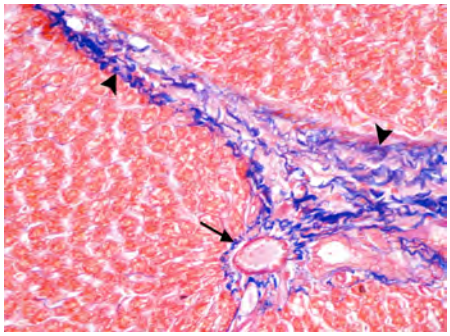

Fig.10: A photomicrograph of a paraffin section in the liver of group B Cd-treated rats showing abundant collagenous fibers in the portal tract (arrow) and in the interlobular septa demarcating the hepatic lobules (arrow head). Compare versus fig. (9). (Mallory's trichrome X 400)

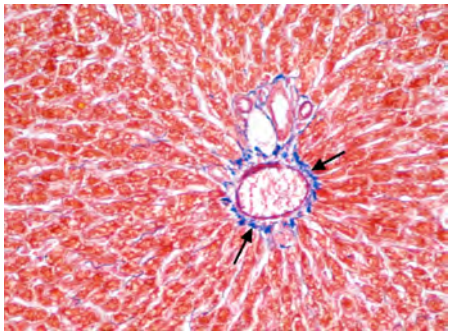

Fig.11: A photomicrograph of a paraffin section in the liver of group C Cd & Zn-treated rats showing some collagenous fibers (arrows) in the portal tract. Compare versus figs. (9 & 10). (Mallory's trichrome X 400)

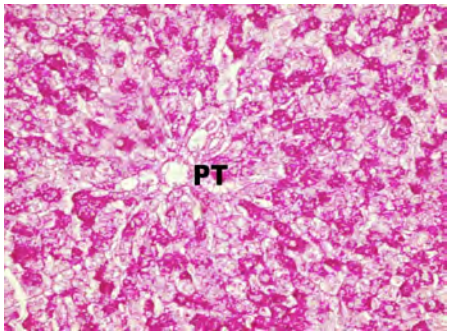

Fig.12: A photomicrograph of a paraffin section in the liver of control group A rat showing a strong PAS positive reaction in the cytoplasm of most of the hepatocytes around the portal tracts (PT). (PAS X 400)

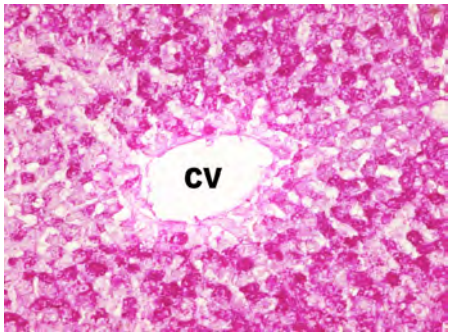

Fig.13: A photomicrograph of a paraffin section in the liver of group A control rat showing a strong PAS positive reaction in the cytoplasm of most of the hepatocytes with a weak reaction in those around the central vein (CV). (PAS X 400)

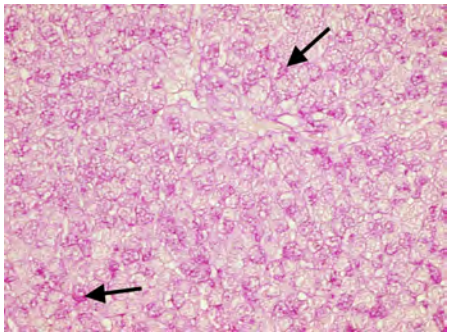

Fig.14: A photomicrograph of a paraffin section in the liver of group B Cd-treated rats showing a weak PAS positive reaction in most of the hepatocytes. Few hepatocytes show moderate reaction (arrows). (PAS X 400)

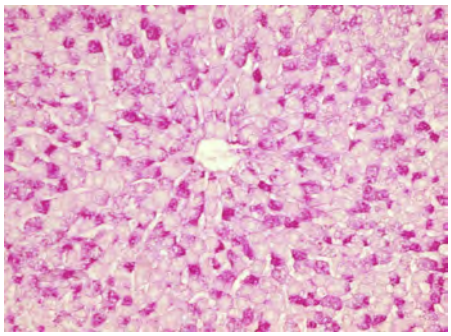

Fig.15: A photomicrograph of a paraffin section in the liver of group C Cd & Zn-treated rats showing a strong PAS positive reaction in some hepatocytes and a weak reaction in others. (PAS X 400)

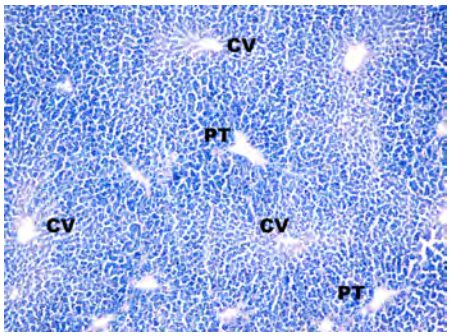

Fig.16: A photomicrograph of a frozen section in the liver of group A control rat showing a strong succinic dehydrogenase activity in the hepatocytes around the portal tracts (PT) with a relatively moderate activity seen around the central veins (CV). (Succinic dehydrogenase X 100)

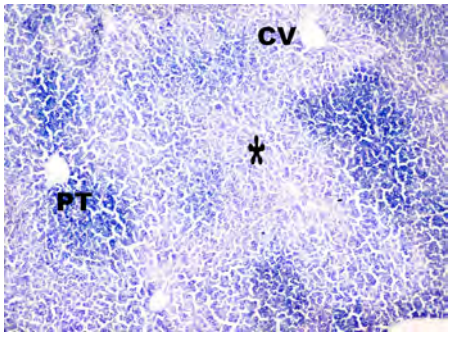

Fig.17: A photomicrograph of a frozen section in the liver of group B Cd-treated rats showing weak succinic dehydrogenase activity (asterisk) in most of the hepatocytes, especially around the central veins (CV). The strong activity is confined to a small area of hepatocytes around the portal tract (PT). Compare versus fig. (16). (Succinic dehydrogenase X 100)

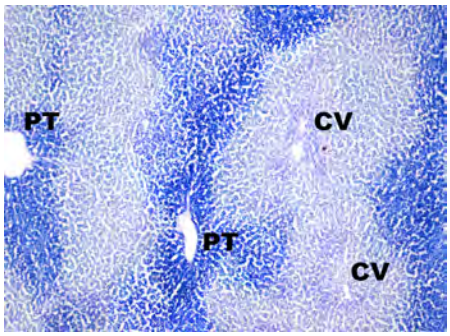

Fig.18: A photomicrograph of a frozen section in the liver of group C Cd & Zn-treated rats showing a strong activity in the hepatocytes around the portal tracts (PT). Note the areas of weak succinic dehydrogenase activity in the hepatocytes around the central veins (CV). Compare versus fig. (17). (Succinic dehydrogenase X 100)

Discussion

Cadmium (Cd) is a widespread occupational and environmental heavy metal and is recognized to be one of the most hazardous pollutants to human health[26-30]. It has been reported that even moderate treatment with this metal may cause serious pathological alterations in the liver[27-29,31].

Light microscopic examination of the control rat liver in this study showed ill-defined classical hepatic lobules. Each classical hepatic lobule was formed of plates of hepatocytes radiating from the central vein to the periphery of the lobule where portal tracts were located. The hepatocytes had finely vacuolated acidophilic cytoplasm and central rounded vesicular nuclei, some of them were binucleated. The plates were separated by blood sinusoids which were lined by endothelial cells and KCs. This microscopic picture was in agreement with that reported in previous studies[26,30,32-34].

PAS stained sections showed glycogen granules in the cytoplasm of most of the hepatocytes of group A control rat liver espe-

cially in the periportal regions. This finding was in accordance with those described by some authors[32-34].

Based on the results reported in this work, frozen sections of control rat liver revealed a strong succinic dehydrogenase activity in the hepatocytes at the periportal region and a moderate enzyme activity in the centrilobular hepatocytes. The same observation was shown in a previous study[32].

In group B rats of the present study, Cd resulted in alteration of the liver histoarchitecture. Portal tracts were thickened with an extension of the connective tissue as thick septa between hepatic lobules leading to demarcated hepatic lobulation. Excess collagen fibers were demonstrated by Mallory's trichrome stain in the connective tissue of the portal tracts and hepatic septa. It has been reported that the exposure to different doses of cadmium resulted in remodeling of liver stroma and enhance collagen synthesis [35]. Cd acts as a pro-fibrogenic agent in the liver as it induces collagen I and MT II gene responsible for fibrosis[36]. In a previous study, it has been reported that the liver of Cd-treated rats showed increased collagen fibers in between hepatocytes, around the central veins and in the portal areas[26].

In group B Cd-treated rats in the present study, blood vessels were congested and blood sinusoids among hepatocytes were obviously dilated. Similar results were previously reported[37]. Excess inflammatory cellular infiltrations were frequently detected in portal tracts, hepatic septa and nearby tissue in the periportal areas. It has been reported that Cd provokes influx and activation of leukocytes into the tissue leading to tissue inflammatory response that represents a primary hallmark of cadmium toxicity[38].

KCs were much more frequently encountered in group B Cd-treated rats of the current study compared to control group rats. Due to their location, KCs are the first cells to be exposed to toxins [39]. KCs are unique cells for their role in both the innate and acquired immunity. The primary functions of KCs are phagocytosis,

processing of ingested material, antigen presentation and secretion of biologically active products [40]. However, activated KCs play a role in liver injury and hepatocellular necrosis through the release of different mediators including cytokines such as tumor necrosis factor alpha (TNF-α) and interleukins. Reactive oxygen, nitrogen species, proteases, and lipid metabolites such as prostaglandins and thromboxane are also released. These mediators can act directly on hepatocytes to cause cell death[41]. In addition, Rikans and Yamano[42] have postulated that injury from Cd exposure was thought to occur from the activation of KCs and a cascade of events involving liver cells and a large number of inflammatory and cytotoxic mediators.

In the present study, most of the hepatocytes in the periportal regions of the liver in group B Cd-treated rats revealed vacuolated cytoplasm. In the pericentral regions, some hepatocytes showed more degenerative changes and developed deeply acidophilic cytoplasm and pyknotic nuclei. Focal areas of necrosis containing remnants of hepatocytes were also detected. Many studies reported that toxic injury is often prominent in the pericentral regions owing to enrichment in hepatocyte enzymes involved in drug detoxification and biotransformation[43,44].

Thick interlobular CT septa demarcating the hepatic lobules were observed in H&E stained sections of Cd-treated rat liver in the current study. This was confirmed by Mallory's trichrome stained sections that showed also abundant collagenous fibers in the portal tracts. This was in agreement with previously reported findings [26]. It has been reported that the exposure to different doses of Cd resulted in remodelling of liver stroma and enhancement of collagen synthesis[35].

In the present study, PAS stained sections of the liver of Cd-treated rats revealed weak PAS positive reaction in most of the hepatocytes. This result was in agreement with that of previous studies which reported that Cd administration has depleted liver glycogen[35,45,46]. However, it was stated that intoxication of rats by

Cd within 1-90 days interval reduced hepatocyte glycogen levels, compared to normal liver. While the prolongation of rat treatment with heavy metals for 90-365 days led to glycogen accumulation in hepatocytes[47]. In addition, it is reported that the Cd effect on liver glycogen is influenced by seasons[48].

In this study, frozen sections of the Cd-treated rat liver revealed marked decrease in succinic dehydrogenase activity in most of the hepatocytes as compared to control group. In one previous study, it has been reported that succinic dehydrogenase activities decreased in the liver after 1 month of Cd treatment[49] as Cd affects the mitochondria directly by the binding of Cd molecules to sulfhydryl groups on critical molecules in mitochondria. Sulfhydryl group inactivation causes oxidative stress, mitochondrial permeability changes, and mitochondrial dysfunction[42].

In this experiment, oral administration of Cd caused abnormal liver function in group B rats. The biochemical results and statistical analysis revealed that serum levels of AST and ALT of Cd-treated rats in group B showed significant increase when compared with those of group A control rats. Several authors have reported findings which were in agreement with those of the current study[28,30]. Cd induced liver injury caused loss of functional integrity of hepatic membranes and increased their permeability leading to leakage of hepatic enzymes into the bloodstream and abnormal liver function[31,50]. In addition, exposure to Cd was observed to cause impairment of hepatocyte functions in dose dependent manner[28].

In the current experiment, the serum level of albumin in group B Cd-treated rats showed significant decrease as compared to that of group A control rats. Similarly, it was reported that Cd induced a significant decrease in serum total protein and albumin levels[51]. This may be explained by the concept that one of the targets of Cd is the system of protein synthesis. Cd ions at high concentrations can inhibit both the rate and the level of translation[52].

Oxidative stress appears to play a major role in chronic Cd-induced hepatotoxicity[29]. The oxidative damage of Cd to cellular organelles is based mainly on the generation of ROS, exhaustion of GSH and inhibiting antioxidant defences[29]. The ROS lead to lipid peroxidation in cellular membranes leading to their destruction and a loss of membrane functions [6,8,50]. Cd also caused damage to the hepatocytes by disrupting their tight junction resulting in a damaged cell membrane[53].

In the present experiment, Zn acted as hepatoprotective agent which partially protected the liver from Cd-induced toxicity. Light microscopic examination of H&E stained sections of Zn-treated liver revealed less interlobular mononuclear cellular infiltration. Few hepatocytes were seen with vacuolated cytoplasm, while others had deep acidophilic cytoplasm and small pyknotic nuclei. These finding were in agreement with a previous study which reported that Zn has a protective effect on histological damage by maintaining membrane integrity due to its direct action on free radicals[26]. It has been reported that in Cd-Zn co-treatment, there was improvement in the Cd-induced damage in the liver represented by absence of nucleus fragmentation and decrease in sinusoidal widening and mononuclear cell infiltrations[37].

In the current work, light microscopic examination of liver sections of Zn-treated rat liver stained with Mallory's trichrome stain showed a slight increase in collagenous fibers in the portal tracts when compared to control group. A marked reduction in the distribution of collagen fibres has been reported previously in rats treated with Cd and Zn[26].

In this study, light microscopic examination of PAS stained sections of Zn-treated rat liver revealed moderate PAS positive reaction in most of hepatocytes. Zn has been reported to antagonize the Cd-induced alteration in glycogen metabolism in the liver[54].

Frozen sections of Zn-treated rat liver revealed that the strong succinic dehydrogenase activity was seen in the hepatocytes at the

periportal areas. Areas of weak activity were still seen in the hepatocytes around the central veins. This result was in agreement with a previously reported data[31]. The inhibition of succinic dehydrogenase activity by Cd decreased when the metal is mixed with Zn and iron[55].

In the current experiment, the biochemical results and statistical analysis revealed that serum levels of AST and ALT of group C (Cd & Zn-treated rats) were significantly decreased as compared to those of group B Cd-treated rats, but still significantly higher than those of group A control rats. Also, serum level of albumin was significantly increased as compared to that of group B (Cd-treated rats), but still significantly lowers than that of group A control rats. It was found that the supplementation with Zn during the exposure to Cd totally or partially prevented the Cd-induced increase in the serum activities of ALT and AST[31]. Co-exposure of liver to Cd and Zn results in recovery of protein synthesis intensity in comparison to Cd induced suppression[52].

Zn is one of the most important nutritional factors influencing the metabolism and toxicity of heavy metals, including Cd[14]. Zn administration to the Cd-exposed animals has been reported to decrease the toxic metal accumulation in the organism and protect from some of its hepatotoxic effects[52,56].

One of the main reasons for the action of Zn against Cd toxicity may be the interaction between the two elements in the absorption and distribution. Enhanced consumption of Zn may decrease Cd absorption and accumulation in the organism[26,56].

The beneficial Zn influence seems to be mainly concerned with its ability to induce the biosynthesis of metallothionein which is widely implicated in the sequestration of Cd, and as a result it may prevent its absorption and toxic action at the cellular level, as well as by this bioelement antioxidative properties[14,31,57]. However, it was reported that Zn pretreatment resulted in accumulation of Cd in mouse liver at the level as without pretreat-

ment. Therefore, one may assume that in vivo Zn protects liver against Cd toxicity not by decreasing its uptake[52].

The other possible hepatoprotective Zn actions against Cd may be related to its antioxidant and anti-inflammatory properties[18,56]. Zn has also the ability to stimulate regenerative processes in the liver[31]. The supply of Zn was effective in the prevention of oxidative damage induced by Cd. One of the mechanisms involved in the protective role of Zn against Cd-induced toxicity is connected with inhibition of Cd-induced ROS formation[58]. It was suggested that Zn, by preventing lipid peroxidation, may protect against the Cd-induced oxidative damage to the cellular membranes and stabilize them[31]. Protective action of Zn regarding the Cd-induced lipid peroxidation might be concerned with its impact on the antioxidant glutathione peroxidise enzyme activity[59].

Conclusion

On the basis of the current results, it can be concluded that administration of Zn during Cd exposure partially prevented the histological, histochemical and biochemical hepatotoxic changes induced by Cd.

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THE EFFECT OF ZINC ON CADMIUM-INDUCED TOXICITY IN ALBINO RAT LIVER: HISTOLOGICAL AND HISTOCHEMICAL STUDY

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ROLE OF ANTEROLATERAL RETROPERITONEAL APPROACH FOR NEURAL DECOMPRESSION AND ANTERIOR RECONSTRUCTION OF LUMBAR BURST FRACTURES

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Abstract

Introduction: *Anterior column reconstruction of the lumbar spine to treat spinal trauma is now becoming increasingly popular due to the unfavorable results obtained with exclusively posterior surgical treatment of ventrally located cord-compressive fractures and to the inadequate durability of kyphosis correction in patients with significant ventral column destruction*

Material & Methods: *This study was conducted upon twenty patients operated in Mansoura Emergency Hospital by a surgical team including both neurological and vascular surgeons. The study started in November 2011, and the operated cases were followed up to 12 months. Our study included patients with lumbar spine fractures that need surgical stabilization and anterior reconstruction.*

Results: *Mean age of patients ranged from 23-49 years, 60% males and 40% females, 80% were victims of fall from height, 15% from motor vehicle, 5% road traffic accidence, L1 fracture representing 90% of cases, L2 5% & L3 5%. Radiological follow up of cases revealed 12 months correction of the Cobb angle from 25 degree to 11 degree in average.*

Discussion: *With the improvements in access technology, interest in approaching the upper lumbar vertebrae with the aid of the surgical microscope has evolved. Therefore, various anterolateral transthoracic and transabdominal approaches with mini-open modifications has been developed as an attempt to achieve the same efficacy and minimize the drawbacks of the invasiveness of traditional approaches.*

Conclusion: *At the end, we should say that mini thoracotomy is a*

good, ascending technique at least in our locality. Although we used it effectively in reconstruction of the lumbar fractures, we still did not use it in an exclusive anterior approach. Anterior corpectomy with cage insertion and placement can be used to reconstruct cases of lumbar fractures with intact posterior complex.

Introduction

Anterior column reconstruction of the lumbar spine to treat spinal trauma is now becoming increasingly popular due to the unfavorable results obtained with exclusively posterior surgical treatment of ventrally located cord-compressive fractures and to the inadequate durability of kyphosis correction in patients with significant ventral column destruction(1).

With the improvements in access technology, interest in approaching the upper lumbar vertebrae with the aid of the surgical microscope has evolved. Therefore, various anterolateral transthoracic and transabdominal approaches with mini-open modifications has been developed as an attempt to achieve the same efficacy and minimize the drawbacks of the invasiveness of traditional approaches(2).

Patients

This study was conducted upon twenty patients operated in Mansoura Emergency Hospital by a surgical team including both neurological and vascular surgeons. The study started in November 2011, and the operated cases were followed up to 12 months. Our study included patients with lumbar spine fractures that need surgical stabilization and anterior reconstruction. These included cases having lumbar burst fractures with clinical instability . However, extremes of age, cases with multilevel fractures, cases with osteoporosis or any other bony lesions were excluded.

Methods

(1) Preoperative evaluation:

Patients who enter the study, should undergo good preoperative evaluation as regard history taking, neurological examination, and radiographic studying.

(a) History taking:

A good history provides impor-

tant insights into the pathomechanics of the injury. Determining which force vectors predominates helps identify unstable spinal columns and thus dictates the most efficient construct to provide stability.

(b) Neurological examination:

Full neurological examination is done for all patients at the time of presentation. The American Spinal Injury Association (ASIA) Impairment Scale (table 3) is used in documenting, monitoring, and treating neurologic injuries.

(c) Radiographic studies:

Initial radiographic assessment includes anteroposterior (AP) and lateral spine films. The AP film should be examined for loss of vertical body height, fracture of the oval-shaped pedicles, increased interpedicular distance, transverse process, malalignment of vertebral bodies, or spinous processes without a history of scoliosis. The lateral radiograph is examined for loss of body height, widening of interspinous distance, and subluxation or angulation of vertebral bodies. The percentage loss of the vertebral body height is calculated by dividing the height of the fractured vertebra by the average height of the cephalad and cauded vertebrae and then multiplying this number by 100%. Segmental kyphosis is determined by measuring Cobb angle between the superior endplate of the vertebral body 1 level above the injured vertebra to the inferior endplate of the vertebral body 1 level below. This method was found to exhibit significantly higher interobserver and intraobserver reliability, in a study comparing 4 different methods for measuring lumbar kyphosis.

Computerized tomography (CT) is used to reveal the degree of canal compromise, degree of separation of bone fragments, and pedicular diameter for screw selection. The percentage of obstruction of the spinal canal was estimated on the preoperative computed tomography scan by dividing the available anteroposterior diameter of the spinal canal at the injured level by the average of the diameter of the canal space at the two uninjured vertebrae cephalad and caudal to the injured level.

Magnetic resonance imaging

(MRI) is used in evaluating those patients with neurologic injury that cannot be accounted for by osseous disruption on plain radiographs and a CT scan, to role out injury to the spinal cord, ligaments, annulus fibrosis, disc herniations, and epidural hematomas. The MRI images can also help in detection or prediction of injuries to the posterior ligamentous complex (PLC).

Operative technique:

Anterior approach (retroperitoneal exposure):

The anterior retroperitoneal approach exposes the lumbar spine through to the first sacral vertebra. This approach uses the potential space anterior to the quadratus lumborum and psoas muscles and behind the peritoneum and renal fascia.

(a) Positioning and Incision Placement:

A left-sided approach is generally used. This avoids retroperitoneal dissection around the overlying liver on the right side as well as dissection in proximity to the thin-walled inferior vena cava. The patient may be positioned supine for limited exposure and anterior interbody fixation or semilateral position lying on the right side midway between lateral and supine. Flexing the table in the semilateral position allows the body to flex away opening the space between the twelfth rib and the iliac crest. Unbend the table when correcting spinal deformity. Flexing the hips will relax the psoas muscle for retraction away from the spine.

Incision placement depends on the level of access desired. For a direct anterior approach, the incision is orientated in a longitudinal fashion slightly to the left of the midline. L1 exposure generally involves an incision above the umbilicus. L2 and L3 are at the level of the umbilicus and slightly below. Exposure of levels L4 and L5 generally lie below the umbilicus. An incision for exposure to S1 extends close to the pubis. A variation of this approach includes a cosmetic Pfannenstiel incision, transversely above the pubic symphysis, which affords a limited exposure to L5-S1 exposure.

The classic incision lies trans-

versely from the left side toward the midline. It begins at or below the twelfth rib on the patient's left side and proceeds transversely or obliquely to the anterior abdomen. The obliquity of the incision depends on the level of the spine requiring exposure. Incisions for the upper lumbar spine begin at or just below the twelfth rib and proceed transversely around to the anterior abdomen above the level of the umbilicus. Exposure of the lower lumbar spine is suited by an incision beginning below the costal margin or midway between the twelfth rib and the iliac crest on the left and sloping obliquely and anteriorly below the umbilicus. An extended exposure of the entire lumbar spine should begin over the twelfth rib on the left and course obliquely over the abdomen below the umbilicus.

(b) Superficial Dissection:

For the anterior midline approach, a longitudinal incision is made through the skin and subcutaneous tissues. Control bleeding and coagulate as needed branches of the paraumbilical veins and superficial epigastric artery and veins. Small anterior cutaneous branches of the intercostal nerve are also encountered. Divide the anterior layer of the rectus sheath, which is the first fascial layer, encountered. Bluntly dissect around the lateral border of the rectus abdominus muscle. The inferior epigastric artery and vein arises laterally deep and inferior to the arcuate line and runs longitudinally behind and within the rectus muscle. It may be necessary to proceed medial to the rectus muscle if the exposure involves a more caudal incision to avoid the inferior epigastric vessels. Behind the rectus muscle, a second fascial layer is encountered which is the posterior rectus sheath. The posterior rectus sheath extends down to the arcuate line. Beneath the posterior rectus sheath is a layer of transversalis fascia. Below the arcuate line, the transversalis fascia is encountered without an overlying posterior rectus sheath layer of fascia. Below this layer is the peritoneum. The delicate peritoneal layer is bluntly dissected away from the fascial layer above. Proceed around to the left exposing the potential retroperitoneal space.

A classic incision begins on the left side. The oblique incision crosses through the thoracoepigastric vein and branches of the deep circumflex iliac artery in the region of midaxillary line. Small lateral cutaneous branches of the intercostal nerve are encountered. The muscle layer is divided through the lateral edge of the latissimus muscle and transversely through the external abdominal oblique, internal abdominal oblique, and transverses abdominis muscles. Anteriorly the field of dissection extends to the lateral border of the rectus muscle. Below the muscle layer, divide the transversalis fascia. The next layer is the peritoneum and extraperitoneal areolar tissue. Bluntly develop the retroperitoneal space around posteriorly. Repair any violations of the peritoneum. Pack away the peritoneum protecting it with a lap pad. Insert a rib-spreading or Finochietto retractor and Deaver retractors.

(c) Deep Dissection:

The retroperitoneal space is developed bluntly by dissecting the peritoneum around exposing the psoas muscle and anterior lumbar spine. The ureter traverses the field of dissection lying atop the psoas muscle. The ureter is occasionally confirmed with gentle pressure producing peristaltic contraction. Other important structures include the genitofemoral nerve coming through and overlying the psoas muscle and the sympathetic trunk lateral to the vertebral body. The lumbar arteries branch out from the aorta at each vertebral level transversely crossing the middle of the vertebral body at each level. These are ligated and divided at each level necessary for mobilization of the aorta. This may require division of lumbar arteries one or two levels above and below the instrumented vertebral levels. These vessels are best divided closer to the aorta to preserve collateral sources of circulation among branches closer to the neuroforamen. Corresponding lumbar veins are similarly divided. Note that there is also an ascending lumbar vein running longitudinally and parallel to the aorta and vena cava interconnecting the lumbar veins at each level. Branches of the aorta in the region of the lumbar spine from proximal to distal include the infe-

rior phrenic arteries below the diaphragm, the celiac trunk, superior mesenteric artery, superior, middle, inferior suprarenal arteries, bilateral renal arteries, bilateral testicular or ovarian arteries, inferior mesenteric artery. Lumbar arteries arise posteriorly at each vertebral level from level 1 through 4 of the lumbar spine. The aorta bifurcates into the common iliac arteries in the vicinity of the L4 vertebra. The middle sacral artery arises within the bifurcation traversing distally along the L5 vertebra and sacrum. The exact level of bifurcation varies, as does the fifth lumbar artery, which may arise from the middle sacral artery.

Exposure of the spine is accomplished after mobilization and protection of the aorta and inferior vena cava. Subperiosteal dissection and elevation of the psoas muscle exposes the lateral aspect of the vertebra and the neuroforamen. This region requires attention to and protection of the sympathetic trunk, lumbar nerve roots, and lumbosacral trunks. The lumbar spine is now exposed for appropriate spinal procedures.

Result

Twenty cases of lumbar burst fractures were undergo decompression and reconstruction via a retroperitoneal approach.

The age of patients included in our study ranged from 23 - 49 years, with mean age 35.73 year and a standard deviation of ± 9.05. Three fifth of the patients in this study were males (60%), and two fifth were females (40%).

Eighty percent of cases (16 patients) were victims of fall from height, constituting the most common cause of trauma. The second common cause was motor vehicle accidents which affected three patients (15%). However road traffic accidents constituted the least common cause of lumbar burst fractures in our study affecting only one patient (5%).

As regard the level of fractures in the lumbar spines, the first lumbar vertebra shows the majority of fractures of cases (18 cases representing 90%) followed by lumbar 2 fractures in one case (5%) and lumbar 3 fracture in one case (5%).

The cases were prepared for surgery as early as possible. Depending on the patient condition and some administrative roles, the timing of the surgery varied from the sixth to the tenth day posttraumatic. Fourteen cases of our patients were operated on their six day, four cases were operated at the eighth day, while two cases were delayed to the tenth day posttraumatic. The mean timing of cases were (8±2 days).

No major post operative complications were noticed and there were no mortality cases in our study, but three cases in our study developed superficial wound infection which was swapped and cultered, the patients were given the proper antibiotics, two of them passed conservatively and only one patient underwent wound depridement after that the wound also showed good healing response

Also one patient developed CSF leakage postoperative as intraoperatively there was dural and ligamentous tear, the patient was medicated by dieuretics and showed stoppage of leak.

All cases entered out study were surgical candidate according to the thoraco-lumbar injury classification and severity score (TLICS). This means that all patients included had at least TLICS score of 5 or a score of 4 with preference of surgery over conservative treatment. Only two cases with score 4 were included while the rest were >5. The mean TLICS score of patients in our study was 6.25.

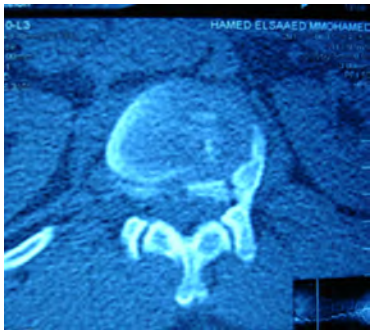
All cases with lumbar fractures necessitate anterior reconstruction because those fractures were severely comminuted anterior and middle column as the anterior column will not be able to share in load bearing.

We relied on the load sharing classification to determine the cases in need for anterior reconstruction and all cases in our study were in need for anterior reconstruction (>7). The mean score of the patients was 7.1.

Radiological follow up of cases was directed to evaluate the Cobb angle immediately postoperative, at 3 months, 6 months and at 12 months, in addition to evaluate the fusion at 12 months. At the

end of the 12 months follow up period two cases were found with no radiological singe of fusion which were 2 early cases in the study. The other 18 cases showed radiological signs of fusion at the end of 12 months.

The Cobb angles registered preoperatively ranged from 25-38 degree. After surgical correction and fixation the angles ranged from 5 to 18 degrees in the immediate postoperative images, giving a significant correction of 11-28° (18.7±5.68) being achieved. All (18) cases except the two non fused case retained more or less the same angle at the end of 12 months. The two cases showed an increase of about 4 degrees in the Cobb angle at 3 months compared to the immediate postoperative angle but it retained the same angle during the follow up period till the end of 12 months.



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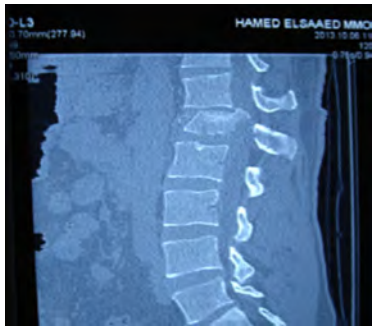

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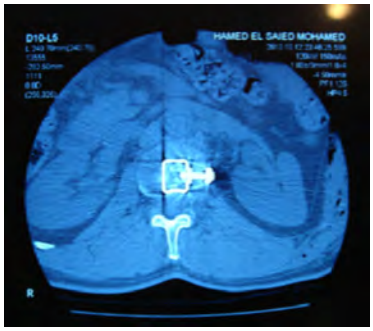

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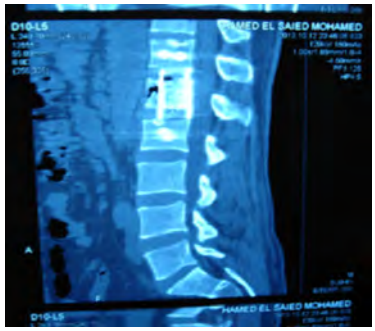

(D)

Fig.(1): a & b Axial and sagittal CT scan showing L1 fracture with retropulsed segment inside the canal, c & d follow up CT scan revealing total clearance of the canal with corrected Cobb angle.

Discussion

With the improvements in access technology, interest in approaching the upper lumbar vertebrae with the aid of the surgical microscope has evolved. Therefore, various anterolateral transthoracic and transabdominal approaches with mini-open modifications has been developed as an attempt to achieve the same efficacy and minimize the drawbacks of the invasiveness of traditional approaches[2].

About 73% of vertebral fractures admitted during the period of our study were located in the upper lumbar region. This makes the upper lumbar region the most commonly affected region among spinal fractures. In a study by Scoliosis Research Society upper lumbar region, was reported to be the most frequently affected site (52%) in spine injuries, followed by lumbar site (L2-L5) with a rate of 32% and thoracic site (T1-T10) with a rate of 16%. Krompinger et al.[3] reported that most frequently injured sites were upper lumbar region (48%), lower lumbar region (34%) and thoracic region (18%), respectively. Gertzbein[4] upper lumbar region was once more the site most frequently involved in a study by Keen[5] and a another study done by Erturer et al.[6] where upper lumbar region was involved in about 57.2% of spinal fractures.

Our results thus agree with others', despite in different ratios, that upper lumbar region is the most common site of spinal fractures. The percentage of upper lumbar region involvement is higher (73%) in our study

In our study, fall from height was the most common mode of injury (80%), followed by motor vehicle accidents (15%), while road traffic accident was represented by only one patient (5%). In Indian studies, fall from height is reported as the most common mode of injury[7], while in western literature, several studies reported that motor vehicle accidents were by far the most common etiology of spinal fractures[8].

Surgical candidacy in our study was based on a novel classification proposed by the Spine Trauma Study Group called the

Thoracolumbar Injury Classification and Severity score (TLICS). This classification system was suggested first by Vaccaro et al.[9] as the Thoracolumbar Injury Severity Score (TLISS) in a trial to compensate for the problems of the previous classification systems and satisfy the requirements of an ideal classification system. The TLISS classification scores three variables that are considered to be directly associated with the stability of the vertebral body and the prognosis. These variables are composed of the mechanism of injury as determined by radiological tests, the presence of an injury in the PLC and the neurological status, and all of these are scored to help decide on the treatment plans. Afterward, the TLISS was modified to the TLICS that includes the radiological injury pattern (the morphology and the fracture pattern), which could be classified objectively instead of subjectively for determining the mechanisms of injuries.

In our study we relied on load sharing classification to select cases which is less likely to fulfill successful vertebral fusion. This scale can predict cases of vertebral fractures that will lack effective fusion and thus more likely to develop progressive collapse and increase in kyphotic deformity. In this scale, the more the initial collapse, the displacement of fragments, and the correction in kyphotic angle with hyperextension, the larger will be the score, and the more the possibility of late kyphotic deformity. So, we selected cases with big scores (7 and more) to be our candidates for anterior reconstruction (procedure candidacy).

As regard the neurological and functional outcome, we found that cases with initial complete neurological deficit (ASIA A) gained no neurological benefit from surgery at 12 months follow up period. Although it has been reported that up to 3-8% of such patients can reach an ambulatory status,[10] we found no improvement among them during the period of follow up. This may be due to several factors. The small patient sample available for analysis may not be truly representative. Moreover, the lack of an effective transport system especially in rural areas, the

lack of highly trained paramedical personnel, the increased incidence of patient transfer by relatives or accident attendants, and the unavoidable prehospital delay with more than one transport between health-care centers, are all significant factors that may lead to secondary spinal cord injury and thus reducing the chance of recovery.

Fehlings and Sekhon[11], also reported that 30-40 % of patients with partial spinal cord injury can achieve neurological recovery to the ambulatory status. In our study 11 cases had partial neurological deficit (ASIA B, C, D). 8 cases (72%) reached an ambulatory status with or without walking aids (ASIA D and E) of them 1 case (12.5%) achieved complete recovery (ASIA E). If we add to the 8 cases who reached an ambulatory status another 3 cases which was improved from ASIA B to ASIA C but did not achieve ambulation, we can say that 81% of cases with partial neurological deficit in our study had the potential for neurological improvement. The difference in score improvement between our study and other studies was not statistically significant. Those cases who were initially neurologically intact remained so immediately postoperative and during the period of follow up. Sasso et al.[12] found that will be at least one grade improvement in ASIA score in cases with good decompression and removal of stenotic elements from the spinal canal after lumbar trauma.

Twelve patients (60%) in our study were totally or almost pain-free (f4 and f5 in Prolo rating scale). If we add those patients with low level of daily pain (f3), we will have about 16 patients (80%) with minimal tolerable pain. All those patients were found to have solid fusion on follow up radiological studies. Schnee and Ansell[13], reported that postoperative pain was minimal in 78% of their patients who underwent anterior and combined approaches, whereas patients who underwent posterior approaches had persistent pain. Danisa and colleagues,[14] also found increased pain relief after anterior procedures (67% with minimal pain vs. 35% after posterior operation). When we compare those patients who are almost

pain-free between groups, we will find 15 cases in our study. This is not a significant difference indicating equivalent effectiveness various techniques.

In our study we succeeded in achieving a significant correction in the kyphotic deformity in the immediate postoperative radiographs. The mean angle of correction was about 18.07º (11-28º), which compares favorably with the literature dealing with isolated anterior approaches (mean angle of correction 15.1°)[15].

Finally, although many studies reported shorter hospital stay with the use of minimally invasive spinal surgeries, we found no statistically significant difference in the mean hospital stay between various studies. The mean hospital stay was 6.3 days. We think that other studies compared their experience with mini thoracotomy with old conventional approaches of their team or of others. However, in our study we are prospectively comparing our recent results of the newly applied mini-open modifications of the conventional approaches with the results of the literated studies. There is no doubt that application of mini-open modifications of the conventional anterior approach to the upper lumbar region, have revolutionized the results of our group not only as regard the hospital stay, but also in terms of postoperative pain, functional outcome, and cosmosis.

At the end, we should say that mini thoracotomy is a good, ascending technique at least in our locality. Although we used it effectively in reconstruction of the lumbar fractures, we still did not use it in an exclusive anterior approach. Anterior corpectomy with cage insertion and placement can be used to reconstruct cases of lumbar fractures with intact posterior complex. Further, applications of anterolateral approach for management of various spinal disorders can be an open field in the near future.

Conclusion

At the end, we should say that mini thoracotomy is a good, ascending technique at least in our locality. Although we used it effectively in reconstruction of the lum-

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BENHA MEDICAL JOURNAL

ROLE OF ANTEROLATERAL RETROPERITONEAL APPROACH FOR NEURAL DECOMPRESSION AND ANTERIOR RECONSTRUCTION OF LUMBAR BURST FRACTURES

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COMPARATIVE STUDY OF HYALURONIC ACID VERSUS LIVER BIOPSY FOR THE ASSESSMENT OF FIBROSIS IN CHRONIC HEPATITIS C

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Abstract

OBJECTIVE: *There are many studies that have described the use of non invasive methods for assessment of the degree of liver fibrosis. We performed a prospective study to evaluate whether hyaluronic acid can be used as a surrogate marker for liver biopsy in patients with chronic hepatitis C.*

SUBJECTS AND METHODS: *The study was conducted on 100 patients with chronic HCV and 70 healthy control persons, all were subjected to through history taking, clinical examination, laboratory investigation including complete blood count, serum bilirubin, ALT, AST, serum albumim and serum hyaluronic acid, abdominal ultrasound and liver biopsy was taken for pathological assessment of fibrosis.*

RESULTS: *Significant increase of hyaluronic acid in patients than control persons, there were direct correlation between hyaluronic acid and platelets count, also significant direct correlation between hyaluronic acid activity and fibrosis score by Metavir and Histopathological Activity Index.*

CONCLUSION: *Serum hyaluronic acid increased in patients with chronic HCV and this increase is correlated with the degree of liver fibrosis.*

Abbreviations: *HCV: Hepatitis C Virus, ALT: Alanine Aminotransferase, AST: Aspartate Aminotansferase.*

Key words: *hyaluronic acid, chronic hepatitis, liver biopsy.*

Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease. The long term hepatic impact of HCV infection is highly variable, from minimal changes to chronic hepatitis, extensive fibrosis, and cirrhosis with or without hepatocellular carcinoma[1] Approximately 10%-20% of patients with chronic HCV infection have cirrhosis at the first clinical presentation, and nearly 20%-30% of those who do not present with liver cirrhosis will eventually develop this illness and its complications[2].

The prognosis and management of chronic liver disease often depend on the degree of liver fibrosis. This is particulary true in chronic hepatitis C virus infection[3]. Untill recently, liver biopsy (LB) examination was the only way of evaluating liver fibrosis[4]. However, LB examination is invasive[5] and may have life threatening complications[6]. The accuracy of LB examination for assessing fibrosis also has been questioned because of sampling errors and interobserver variability that may lead to over or under staging of fibrosis[7].

Thus, there is a need for accurate non-invasive methods of measuring the degree of liver fibrosis, proposed approaches include physical examination, routine biochemical and hematologic tests[8] surrogate serum fibrosis markers and hyaluronic acid[9].

Hyaluronic acid levels increase in chronic liver disease. In patient with chronic HCV, these levels increase with the development of fibrosis and correlate with the severity of cirrhosis[10].

Aim of The Work

To evaluate the diagnostic value of hyaluronic acid as surrogate non-invasive marker for liver biopsy in patients with chronic hepatitis C.

Subjects and Methods

This study was carried out on 100 patients with chronic HCV infection. Patients were studied from Mansoura University Hospital (MUH) and Mansoura International Hospital (MIH). The study was performed from May 2012 to July 2013, and their ages ranged from 25 to 55 years. In addition, 70 apparently healthy controls of matched age

with patients were included.

Excluded from the study Patients with: decompensated cirrhosis; autoimmune hepatitis; hepatitis B infection; HIV infection;previously treated patients by interferon or ribavirin or patients with sever comobrid disease.

All patients and controls were subjected to medical history taking, clinical examination and complete laboratory investigations in the form of complete blood count, liver function tests (including: serum albumin, Serum bilirubin, ALT, AST, Alkaline phosphatase, INR), serum creatinine, Hepatitis C virus antibodies, polymerase chain reaction (PCR), Serum Hyaluronic acid, Abdominal ultrasound and liver biopsy.

Results

Clinical parameter: there was no significant difference between patients and control regarding the demographic data. In patient group there were hepatomegaly in 38 patients while splenomegaly was found in 44 patients. 64 patients were classified as child A while 36 were child B. There was no significant difference between patients with splenomegaly and hepatomegaly regarding serum hyaluronic acid.

Laboratory data: there was significant difference between control group and patient group as regard haemoglobin, platelets, AST, ALT, Albumin, Bilirubin, Alkalin phosphatase, INR and creatinin. Hyaluronic acid was significantly high in patients in comparison to controls, no significant correlation between hyaluronic acid and haemoglobin, WBCs, AST, ALT, Albumin, Bilirubin, Alkaline phosphatase, INR and Creatnine, but significant direct correlation with platelets. Hyaluronic acid was significantly higher in child B group when compared with child A.

Pathological data: according to Metavir scorig system 58 patients had significant fibrosis >2 while 42 had non significant fibrosis <2. There was statistically significant direct correlation between hyaluronic acid and Metavir A and F score so patients with significant fibrosis had significantly higher hyaluronic acid than those with non-significant fibrosis.

Table (1): Correlation between hyaluronic acid and the laboratory data.

Laboratory parameter	Hyaluronic acid	
	r	p
Hb(gm/dl)	0.16	0.11
WBCs(n/cmm)	0.12	0.23
Platelets(n/cmm)	0.72	0.0001*
AST(IU/L)	0.13	0.17
ALT(IU/L)	-0.071	0.47
Albumin(gm/dl)	0.16	0.095
Bilirubin(mg/dl)	-0.094	0.34
Alkalinephosphatase(IU)	0.019	0.84
INR	-0.16	0.10
Creatinine(mg/dl)	-0.073	0.46

This table shows no significant correlations between hyaluronic acid and the laboratory data. However, there a was a statistically significant direct correlation between hyaluronic acid and platelets count.

Table (2): Relation between hyaluronic acid and Child pugh classification.

	Hyaluronic acid	Student t test	
		t	p
Child A	145.4 ± 115.2	-12.8	0.0001*
Child B	385.9 ± 72.1		

This table shows that patients with Child B score had significantly higher HA levels when compared with patients with Child score A.

Table (3): Comparison between hyaluronic acid in patients with significant fibrosis and patients with non-significant fibrosis.

	Hyaluronic acid	Student t test	
		t	p
Significant fibrosis	273.9 ± 121.6	3.16	0.002*
Non-significant fibrosis	174.2 ± 175.6		

This table shows that patients with significant fibrosis had significantly higher HA level when compared with patients with non-significant fibrosis.

Table (4): Cut off level, sensitivity, specificity, positive predictive value and negative predictive value of hyalouronic acid for diagnosis of fibrosis.

Cut off	90.0
Sensitivity	98.0 %
Specificity	59.5 %
Positive predictive value	77.0 %
Negative predictive value	96.0 %

This table shows the diagnostic value of HA for significant liver fibrosis. At a cut-off of 90.0, the test had a sensitivity of 98.0%, specificity of 59.5%, positive predictive value of 77.0% and negative predictive value of 96.0%.

Discussion

Determining the degree of liver fibrosis (LF) in chronic viral hepatitis is necessary in determining a patient's prognosis and indications for therapy. Although liver biopsy is a gold- standard procedure in determining the stage and grade of LF[11], it can be associated with complications (e.g. bleeding and pain at the biopsy site, and a prolonged hospital stay), high hospital expenses[12], false sample recording, and in some cases contraindications during the procedure, and dependence on pathologists' skills in examining samples[13].

Considering the aggressiveness of the procedure, repeating it for further evaluation and follow-up to determine treatment response is not recommended[14]. From a clinical perspective, the use of a non-invasive procedure in diagnosing and determining the presence and degree of LF, and as a tool in following up on the effects of anti-viral and antifibrotic medicines on the liver is needed[12].

LF is a complex and dynamic process with an increased extracellular matrix, increased activity of the matrixproducing cells, release of cytokines and, ultimately, structural tissue changes[11].

Hyaluronic acid (HA) is a polysaccharide with a high molecular weight found in the extracellular spaces. Through the bloodstream, it enters the lymphatic system where it is quickly degraded and removed by the sinusoidal endothelial cells[15]. Recent studies have shown that HA levels are elevated in patients with LF[16].

Bases on these data, the present study aimed to evaluate the diagnostic value of hyaluronic acid as surrogate non-invasive marker for liver biopsy in a group of Egyptian patients with chronic HCV.

To get this target accomplished 100 HCV patients were included in the study. They were subjected to careful history taking, thorough clinical examination and laboratory investigations including liver profile and serum hyalouronic acid. In addition, there were age and sex matched 70 controls.

In our study, comparison be-

tween hyaluronic acid levels in patients and controls had shown statistically significant higher levels of hyaluronic acid in patients when compared to controls. This is in accordance with the study of Isman et al., 2007 who investigated whether hyaluronic acid and hyaluronidase can be used as non-invasive markers of disease activity in hepatitis C by studying 89 with chronic hepatitis C and 32 healthy controls. In their study, HCV patients had significantly higher HA levels when compared with controls[17]. The association between HA and Metavir score were reported in multiple studies. In the study of Avila et al., 2010, higher plasma levels were significantly correlated to significant liver fibrosis (METAVIR > F2)[18]. Also, in the study of Rohrbach et al., 2013, HA correlated significantly with the histological METAVIR-stage[19].

Regarding to the diagnostic value of HA for significant liver fibrosis, we found that at a cut-off of 90.0, the test had a sensitivity of 98.0%, specificity of 59.5%, positive predictive value of 77.0% and negative predictive value of 96.0%. In the relevant literature, there were mixed results.

In the study of Xie et al., 2003, the cutoff value of serum HA for detecting patients with significant fibrosis were 90 micrograms/L. sensitivity was 80.4%, specificity was 70.2%, positive predictive values (PPV) were 86.7% and negative predictive values (NPV) were 59.8%[20].

In another study, Iushchuk et al., 2005 HA test proved highly sensitive and specific (in HA 100 ng/ml sensitivity was 100%, specificity 84.6%), but this method cannot stage hepatic fibrosis[21]. Also, the study of Yilmaz et al., 2007 found that A cut-off value of 63 ng/ml for HA had a 100% specificity for diagnosing fibrosis score > or =1 in chronic hepatitis [22].

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COMPARATIVE STUDY OF HYALURONIC ACID VERSUS LIVER BIOPSY FOR THE ASSESSMENT OF FIBROSIS IN CHRONIC HEPATITIS C

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RENAL FUNCTIONS IN PRESCHOOL CHILDREN WITH CONGENITAL ANOMALIES OF THE KIDNEY AND URINARY TRACT

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Abstract

Background: *To detect renal function in children from 2-5 years with congenital anomalies for early diagnosis of renal injury either UTI or early stage renal failure in order to prevent irreversible renal damage and renal scarring*

Patient and Methods: *This study was conducted on 100 children suffering from congenital anomalies of the kidney and urinary tract, they were subjected to renal function testing by doing urine analysis, blood urea and serum creatinine for all cases. Also, GFR was calculated to determine the stage of renal disease (stage 1: GFR 90, stage 2 GFR 60-89, stage 3 GFR 30-59, stage 4 GFR 15 - 29 and stage 5 GFR below 15). These cases were divided into 5 groups: group 1: cases of VUR (38 cases), group 2: cases of PUJO (33 cases), group 3: cases of PUV (19 cases), group 4: cases of uretrocele (4 cases) and group 5 cases of horseshoe kidney. All data was analyzed by using statistical method*

Results: *In this study we found 28 cases with renal failure, 3 cases were in end stage renal disease, 40 cases were in stage 3, 28 cases were in stage 2 and only 1 case in stage 1. Urine analysis showed protinurea was found in 14 cases, hematuria was found in 2 cases, pyuria was found in 66 cases, pyuria and hematuria was found in 1 case and urine analysis was free in 17 cases. Regarding urine culture it was positive in 66 cases (66%) and negative in 34 cases (34%). The most common organism was E.coli in 55 cases (55%) followed by Psudomonas in 6 cases (6%) and Klebsiela in 5 cases (5%). The mean urea was 45 for case of PUJO, 65 for cases of PUV, 44 for cases of VUR, 47 for cases of uretro-*

cele and 48 for cases of horseshoe kidney. The mean creatinine for cases of PUJO was 0.98, 2.96 for cases of PUV, 1.27 for cases of VUR, 1, 43 for cases of Horseshoe kidney and 1.73 for cases of Uretrocele.

Conclusions: Early detection of renal function in children with congenital anomalies and urinary tract to reduce the number of patient with End Stage Renal Diseases (ESRD).

Keywords: Pelvi ureteric junction obstruction (PUJO), Posterior uretheral valve (PUV), Vesico ureteric reflux (VUR), Renal function.

Introduction

Congenital urinary tract anomalies are relatively frequent and may be found in 1 - 2% of all pregnancies and in about 3 - 4% of the population[1]. Also, they account for 20 - 30% of all anomalies identified in pre-natal period[2]. Congenital anomalies of the kidney and urinary tract are major cause of morbidity and collectively represent a significant cause of chronic kidney disease in children and young adult[3]. The prevalence of congenital urological abnormalities is roughly the same in various geographical locations being 45% in Japan and 33% in North America[4].

Congenital anomalies of the urinary tract are well causes of urinary tract infection (UTI) in children as about 40% of infants and children with symptomatic UTI are reported to have veiscoureteric reflux (VUR) and 20% have other associated anomalies in urinary tract[5]. UTIs have been considered important risk factor for the development of renal insufficiency or end stage renal disease in children[6]. About 10 - 30% of children with febrile UTIs develop renal scarring which is thought to a risk factor for hypertension and renal insuffiency in longer term[7]. Congenital nephropathies and uropathies constitute a diverse group of disorders that account for more than one third of end stage renal disease much of the morbidity and mortality linked to these entities can be minimized by early correct diagnosis[8]. Therefore, early diagnosis of renal function in children with congential urinary tract anomalies is crucial. As potential targeted therapy either conservative or surgical,

which in role prevent irreversible damage of renal parenchyma[4], is depending on early detection of renal function. Renal function in children with congenital anomalies will help us to diagnose many subclinical and clinical renal diseases early as it is cheap, reliable and can be performed in many local hospitals.

Subjects and Methods

Subjects: This study was conducted on 100 preschool children with congenital renal anomalies 68 male (68%) and 32 female (32%).

Methods: All the studied children were subjected to the following

1. History taking (complete history taking):

• Prenatal history with emphasis on the following:

• History of the risk factors such as; diseases of the mother as (DM), medications intake during pregnancy, exposure to irradiation or infections and history oligohydramnios

• Postnatal ultrasound and its findings

• Natal history with emphasis on the method of delivery.

• Postnatal history with emphasis on urine voiding (time of 1st urine voiding and the urine stream).

• Family history of the children including congenital anomalies of the kidney and the urinary tract in the parents and siblings.

2. Urine analysis:

All cases were subjected to microscopic urine examination. A red blood cell count of five or more per high power field (HPF), one or more plus protein, one or more plus glucose, pus cells count of five or more per high power field (HPF) and crystals were considered as abnormal urine findings.

3. Urine culture:

Urine culture was done for children with pus cells count of 20 or more per high power Field[9].

4. Serum creatinine was done to all cases.

5. Blood urea was done for all cases.

6. GFR was calculated using Schwartz formula[10].

GFR = k * height (cm) /S. Creatinine (mg/dL) where K is a constant. 0.55 for children and 0.7 for adolescents.

Statistical methods: The collected data were tabulated and analysed by the suitable statistical tests using the SPSS computer software.

Results

Our study was conducted on 100 children with congenital anomalies of the kidney and urinary tract. Their age ranged from 2 to 5 years. These cases were tested for renal functions by doing urine analysis, urine culture, blood urea and serum creatinine. We also calculated GFR using Shwartez formula. These cases were divided into 5 groups (Table 1):

• **Group 1 (cases of primary vesico ureteric reflux):**

38 cases were diagnosed as primary VUR, their mean age was 37.11±9.71 months, mean urea was 45.79±14.2, mean creatinine was 1.27±1.01and the mean GFR was 53.29±19. There was male predominance and regarding laterality it was bilateral in 23.7% and unilateral in 66.7% more commonly on the right side. The most common organism in urine culture was E. coli Sibilings of cases of VUR were affected in 25% and 5% of cases were in end stage renal disease. Based on GFR classification, stage 2 was found in 15 (39.4%), stage 3 in 15 cases (39.4%), stage 4 in 6 cases (15.7%) and stage 5 in 2 cases (5.2%).

• **Group 2 (cases of PUJO):**

33 cases were diagnosed as having PUJO. Their main age was 37.45±9.34 months, mean urea was 45.09±11.5, mean creatinine was 0.98±0.28 and mean GFR was 56.45±16.1. There was male predominance. According to laterality it was bilateral in 21.1% and unilateral in 78.2% more commonly on the left side. 6 cases (18%) were associated with MCDK and 5 cases (15%) were associated with VUR. The most common organism in urine culture was E.coli. Renal failure was found in 6 cases (18%). Based on GFR classification, stage 1 was found in one case (3%), stage 2 was found in 9 cases (27%), stage 3 in 17 cases (51%), stage 4 in 6 cases (18%). One third of cases (11 cases) were

associated with other congenital renal anomalies classified as 6 cases associated with MCDK (18%) and 5 cases with VUR (15%).

• **Group 3 (cases of PUV):**

19 cases of PUV were diagnosed. Their mean age was 32.0±5.74 months, mean urea was 65.53±8.5, mean creatinine was 2.96±0.81and mean GFR was 21.68±6.1. 12 cases (63%) were in renal failure), 1 case (5%) was in ESRD and the rest of cases had some degree of renal impairment. 25% of cases were associated with VUR. One case was presented by end stage renal disease with GFR below 15. Five cases were associated with VUR (25% of cases).

• **Group 4 (cases of uretrocele):**

4 cases of uretrocele were diagnosed. Their mean age was 34.25±9.95 months, mean urea 45.34±12.4, mean creatinine 1.73±0.602 and mean GFR was 48.25±15.8.Their sex distribution showed female predominance. The most common presenting symptom was recurrent UTI.2 cases (50%) were associated with double ureter.the most common organism in urine culture was E.coli.

According to GFR, one case was in stage 2 and three cases were in stage 3. Two cases were associated with double ureter.

• **Group 5 (cases of horseshoe):**

Six cases of horse shoe kidney were diagnosed. Their sex distribution showed male predominance. The mean age was 40.33±10.7 months, mean creatinine was 1.43±0.82, mean urea was 48.83±12.5 and mean GFR was 54.83±22.5. The main presenting symptom was recurrent UTI then abdominal mass and renal failure. 2 cases (33%) were associated with VUR. The most common organism in urine culture was E.coli. According to GFR classification, 3 cases were in stage 2, 2 cases in stage 3 and one case in stage 4.

Table (1): Stages of chronic kidney disease among studied groups based on KDOQI Classification.

Cases	PUJO (33)	PUV (19)	VUR (38)	HSK (6)	Uret (4)	P value
Stage 1 (GFR 90)	0	0	0	0	0	0.001 HS
Stage 2 (GFR 60 - 89)	27	0	39.4	50	25	
Stage 3 (GFR 30 - 59)	51.7	15.7	39.4	33.4	75	
Stage 4 Renal failure (GFR 15 - 29)	18.3	79	15.8	16.6	0	
Stage 5 End stage renal des (GFR blow 15)	0	5.3	5.4	0	0	

Discussion

Patients with congenital kidney disease have few signs and symptoms early in the disease course; laboratory evaluation may be the only way of detecting disease[11]. The outcome for patients with severe obstructive nephropathy has not improved significantly over the past two decades despite advances in antenatal diagnosis and therapeutic intervention[12].

Cases of VUR:

In our study, we found five siblings with VUR representing 26.2% of all cases. The results are in accordance with Lim[13] who reported that Siblings of children with reflux are an important group. Even when asymptomatic, VUR has been reported in 25-40% of these siblings.

In our study, we found two cases of end stage renal disease representing 5.25% of all cases. Those two cases had grade five reflux. Our findings are coordinate with Murer et al.[14] who reported that 8% of patients affected by VUR develop end-stage renal failure as a result of reflux nephropathy, which accounts for 25% of end-stage renal disease. Many individuals with VUR hidden in the population have renal dysplasia and/or hypoplasia, which represent the major cause of end-stage renal failure. It is still controversial whether renal dysplasia is due to poor nephrogenic differentiation resulting from the back pressure effect of urine reflux on the developing fetal kidney[14].

Vesico-ureteric reflux (VUR).

There are, as yet, no long-term studies devoted specifically to the late outcome of children with prenatally detected VUR. Nevertheless, some consistent themes are beginning to emerge[15].

Cases of PUJO:

Ismail et al.[16] reported their experience of 234 children with prenatally detected PUJ obstruction managed over a 13-year period but did not define the mean or median duration of follow up for this cohort of patients. These authors performed early pyeloplasty (predominantly for reduced differential function), in 52 children (22%), whilst 182 children were initially managed conservatively. Of these, 45 (25%) subsequently underwent delayed pyeloplasty for either deteriorating function (42 patients) or urinary infection (3). In this series, the mean age at delayed pyeloplasty was 18 months; 137 kidneys showed no deterioration in differential function during the (unspecified) period of conservative management, and indeed mean differential function was reported to have improved marginally from 45.7% to 48.2% by the end of the follow up period. In another retrospective long-term study, Chertin et al.[17] evaluated outcomes of 343 children with prenatally detected PUJ obstruction who had been managed over a 16-year period (1988-2003). In total, 179 children (52.2%) underwent pyeloplasty. Virtually all the children who required delayed pyeloplasty did so in the first 4 years of life with only two children (1%) requiring surgery beyond the age of 4 years. From the available evidence it is now possible to formulate some broad predictions of the long-term outcome for infants with prenatally detected PUJ obstruction. Thus, for an infant with PUJ obstruction characterized by renal pelvic diameter >2 cm or SFU Grade 4, the available evidence points to an overall 50%e55% probability that pyeloplasty will be required according to objective criteria (as opposed to surgeon's subjective preference, etc.) before 16 years of age. Conversely there is a 40%-45% overall probability that conservative management will be successful, with complete resolution or sustained improvement in the dilatation/ obstruction by the age of 16 years. Finally, there is a 5% probability

of stable but significant persisting dilatation at the age of 16 years. For this relatively small group of patients the outcome in later adult life is, as yet, unknown[18].

Cases of PUV:

The nature of renal injury in boys with PUV is complex and appears to have 2 distinct components. Some damage, described as obstructive uropathy (glomerular and tubular injury) is caused by persistent high pressure, but it is potentially reversible if the high pressure is relieved. Other damage, termed renal dysplasia, results from either increased pressure during kidney development or abnormal embryologic development. Renal dysplasia is not reversible and therefore the degree of dysplasia is critical in determining eventual RF in valve patients[19]. There is a large subset of children treated for PUV, as many as 25% in some long-term series, who do well initially after treatment, but later show progressive deterioration in RF in the prepubertal and pubertal years, culminating in CRF or ESRD[20]. Smith et al.[21] reported an incidence of ESRD of 10% at 10 years and 38% at 20 years, with evidence of CRF in 34% and 51% at the same ages. In their study on long-term outcome (10-23 years) of 25 prenatally-detected PUV patients, Kousidis et al.[20] showed that those patients who survive beyond the neonatal period have a 20-30% risk of progressing into ESRD. In a study by Pereira et al.[19]; of 77 PUV cases diagnosed in their Paediatric Urology Department before 6 months of age and followed for an average of 11.1±7 years, thet have seen that 27 (35.1%) had CRF, 14 of whom were in ESRD. Of these 27 boys in CRF, 22 had gone into CRF in the first 3 years of life: 4 at 15-16 years of age and 1 at 20 years of age. Sarhan et al.[22] reported that prenatal diagnosis is unlikely to alter outcome in the first decade of life (since renal dysplasia is almost certainly the crucial determinant in this age range).

Cases of Ureterocele:

Ureterocele occur in about 1 in 500 to 1 in 4000 people. Ureteroceles are equally common in both left and right side ureters. Children with ureterocele often present with severe UTI. The prog-

nosis varies but in unilateral cases, renal failure is uncommon because the other kidney usually continues to work as normal[23].

Conclusio

• Screening of children who have 1st degree relative with VUR. 5 - 8% of cases of VUR develop end stage renal disease as a result of reflux nephropathy

• UPJO is the most common cause of obstructive nephropathy in children and it is the most common cause of antenatal hydronephrosis. Most urologists manage cases of PUJO conservatively reserving surgery for cases in which there is deterioration of renal function

• Renal failure was the most common presentation of cases of PUV denoting delayed diagnosis. 10 - 30% of cases of PUV progress to end stage renal disease in their lifetime. No difference in renal outcome among children with PUV who presented before and after infancy.

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EVALUATION OF THE NEURODEVELOPMENTAL OUTCOME OF PEDIATRIC OPEN CARDIAC SURGERY PERFORMED DURING THE FIRST YEAR OF LIFE, USING TRANSCRANIAL DOPPLER

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Abstract

Objectives: *To evaluate the neurodevelopmental outcome of open pediatric cardiac surgery performed during the first year of life and to assess the relation between this outcome and the intraoperative transcranial doppler (TCD) findings.*

Methods: *This study was conducted on 38 children who underwent open cardiac surgery during their first year of life. All patients were subjected to preoperative neurodevelopmental assessment using Denver II test as a baseline, Then, the patients underwent open heart surgery with intraoperative neuromonitoring by transcranial doppler to assess cerebral blood flow and presence of cerebral air emboli. Six months postoperatively, the Denver II test was reperformed to assess the neurodevelopmental outcome after surgery and this was compared to the preoperative one. The relation between the TCD finding and the neurodevelopmental outcome was evaluated.*

Results: *There was an improvement in the overall neurodevelopmental outcome of our patients postoperatively (27 patients, 71.1% became totally normal postoperatively versus 6 patients, 15.8 % preoperatively), (p-value<0.001). Also, in personal social development (35 patients,*

92.1% became normal postoperatively versus 19 patients, 50% preoperatively), (p-value=0.036). In fine motor adaptive (36 patients, 94,7% became normal postoperatively versus 13 patients, 34.2% preoperatively) (p-value<0.001). In language development (33patients, 86.8% became normal postoperatively versus 21 patients, 55.3% preoperatively), (p-value=0.003). And in gross motor (30 patients, 78.9 % became normal postoperatively versus 15 patients, 39.5% preoperatively) (p-value=0.001). There was no statistical significance in the relation between presence of air emboli detected by TCD and the neurodevelopmental outcome (p>0.05).

Conclusion: *There is an improvement in the overall neurodevelopmental outcome, and in every developmental area following pediatric cardiac surgery performed during the first year of life. There is no statistical significance between presence of air emboli and the neurodevelopmental outcome.*

Keywords: *Neurodevelopmental Outcome, Open cardiac surgery, Neuromonitoring, Transcranial doppler*

Introduction

Children with congenital heart diseases are highly exposed to neurodevelopmental delay secondary to altered cerebral blood flow and impaired cerebral oxygen delivery[6,10]. There is a significant debate about the neurodevelopmental outcome of those children after open heart surgery as this surgery has itself a negative neurological impact of 2- 25%[11]. On the other hand, those patients may be still at higher risk of neurodevelopmental delay if they do not undergo open heart surgery to correct such congenital anomalies because of chronic hypoxemia or because of palliative or reparative surgeries that they may undergo later in childhood[8]. Neuromonitoring is considered as a new strategy that could prevent harmful events during pediatric cardiac surgery. There is evidence that both hypo- and hyperperfusion with consequent compromise of oxygen delivery, as well as the development of microemboli are the major causes of brain injuries. Transcranial doppler (TCD) is an effective neurological monitoring

modality in detecting different types of abnormalities before, during, and after surgery[1].

We did this research to evaluate the neurodevelopmental outcome of open pediatric cardiac surgery performed during the first year of life and to assess the relation between this outcome and the intraoperative transcranial doppler (TCD) findings.

Patients and Methods

This study was conducted on 38 children who underwent open cardiac surgery using cardiopulmonary bypass during their first year of life, in the congenital and pediatric cardiac surgery unit, Mansoura University Children Hospital, after approval by the local ethical committee and obtaining written informed consent from parents of the patients prior to enrollment.

Inclusion criteria:

Any child who underwent open heart surgery (requiring cardiopulmonary bypass) with age up to one year.

Exclusion criteria:

Any child with previous history of neurological disease, apparent genetic syndromes including Down syndrome, preoperative cerebral organic lesion or previous history of cardiac operation that required cardiopulmonary bypass. Also, any child required total circulatory arrest or selective cerebral perfusion. Cases of mortality or lost follow up before the postoperative six months follow up were excluded.

All patients were subjected to:

• Full clinical history and examination including demographic data and the main symptoms and signs.

• Preoperative full neurodevelopmental assessment using Denver Developmental Screening Test-II.

The neurodevelopmental outcome was assessed in the terms of:

1- Over all assessment: the case that was completely normal (has no delaying in the four developmental areas) or a case that was delayed (that has any delay in any of the four developmental areas).

2- Assessment for each of the developmental areas:

a- Personal social development.

b- Fine motor development.

c- Language development.

d- Gross motor development.

• Preoperative CT brain (to exclude cerebral organic lesion).

• Preoperative Transcranial Doppler (TCD) as a baseline parameter: Detection of air emboli with calculation of its number per second via counting the cerebral embolic signals.

• Total repair (using cardiopulmonary bypass) with the following:

- continuous flow.

- Cooling range between 28-32 c.

- Max. CPB time, 180 min.

-ABG management (PH stat).

• Intraoperative monitoring by TCD.

• Postoperative assessment (within 24 hours) by TCD.

• Early postoperative assessment (during hospital stay) including; ICU data, neurological and other complications and hospital stay.

• Follow up (after six months) including full neurodevelopmental assessment using Denver Developmental Screening Test-II.

Statistics:

Statistical analysis was carried out via Statistical package for social Science (SPSS) version 17 program on windows 7. Qualitative data were represented in the form of number and frequency, while quantitative data were represented in the form of mean ± standard deviation (mean±SD). Kolmogrov-smirnov test was used to test normality of quantitative data. Both chi square and McNemar tests were used to compare qualitative data. While, quantitative data were analyzed by both paired and unpaired student's t tests. Results were considered significant if p value is less than or equal 0.05.

The results were analyzed in the term of:

• Preoperative demographic data.

• Data of preoperative neurodevelopmental assessment.

• Intraoperative data (Cardiopulmonary bypass duration, cross clamp time, TCD findings).

• Six months postoperative neurodevelopmental assessment.

• Relation between the preoperative and postoperative neurodevelopmental outcome. Also, the relation between presence of air emboli and the postoperative neurodevelopmental outcome.

Results

1- Preoperative Data:

- The study included 38 children. Demographic data are demonstrated in table (1).

The diagnosis of the cases was variable with highest incidence for tetralogy of Fallot. table (2)

2- Intraoperative Data: Table (3)

- Cases underwent Glenn shunt were operated on beating heart (Aortic cross clamp time = zero).

TCD Findings (presence of air emboli): Table (4)

- Air emboli were detected in different stages with maximum appearance during removal of aortic cross clamp. The amount of gas bubbles was increasing with increasing heart contractility on systole.

Intraoperative complications:

- There were interaoperative complications in four cases in the form of defective deairing, 2 (5.2%) cases, heart block, temporary pace maker, 1 (2.6%) case and hypoxia, 1 (2.6%) case with no hazardous effect on the patient and didn't cause hemodynamic instability.

3- Postoperative Data: Table (5)

Early postoperative complications:

Occurred in 11 cases (28.9%) in an overlapping manner in the form of Localized Muscle twitches in 2 (5.3%) cases, Supra ventricular tachycardia in 3 (7.9%) cases, Temporary Heart block in 1 (2.6%) case, Bleeding and re-exploration in 1 (2.6%) case, wound infection in 2 (5.3%) cases, chest_infection in 5 (13.2%) cases, hypoxia+ re-intubation in 1 (2.6%) case and hypertensive pulmonary crisis in 2 (5.3%) cases.

These complications were managed early with minimal or no affection on the hemodynamics or the central nervous system.

- Late complication (after 4 months):

Late complication (4 months post discharge), occurred only in one case in the form of severe pneumonia which required mechanical ventilation.

- Relation between preoperative neurodevelopmental assessment and postoperative neurodevelopmental outcome: Table (6)

There was statistical significant improvement in the overall neurodevelopmental outcome, and in every developmental domain. (p-value<0.05).

- Relation between TCD presence of air emboli and neurodevelopmental outcome: Table (7)

- There was no statistical significance between presence of air emboli detected by TCD, their number or duration and the neurodevelopmental outcome (p-value > 0.05).

Table (1): shows the demographic characteristics of the studied cases(total number=38).

	Mean ± SD	Range(minimum-maximum)
Age (months)	8.4 ± 3.5	1-12
Weight (kg)	7.5±1.8	3.5-10.8
Preoperative O2 saturation(%)	80 ± 16	42-100
Gender	Male, 26 (68.4 %)	Female, 12 (31.6 %)
cyanosis	**Present,** 18 (47.4 %)	**Absent,** 20 (52.6 %)

Table (2): shows the diagnosis and performed operations for the studied cases.

Diagnosis	Number (%)	Operation	Number (%)
VSD	7 (18.4 %)	Total repair,	7 (18.4 %)
TOF	12 (31.6 %)	Total repair	12 (31.6 %)
D.TGA	3 (7.9 %)	Arterial switch Senning operation	1 (2.6 %) 2 (5.3 %)
Truncus arteriosus	2 (5.3 %)	Total repair	2 (5.3 %)
AP window	3 (7.9 %)	Total repair	3 (7.9 %)
RV tumor	1 (2.6 %)	Resection	1 (2.6 %)
VSD,MS	1 (2.6 %)	VSD repair + MVR	1 (2.6 %)
VSD,PS	2 (5.3 %)	Total repair	2 (5.3 %)
DORV,PS	1 (2.6 %)	Rastelli operation	2 (5.3 %)
DORV,PA	1 (2.6 %)		
DILV,TGA,Mitral atresia	1 (2.6 %)	IP banding,IAS	2 (5.3 %)
DOLV,VSD,ASD	1 (2.6 %)		
DIORV,PDA stent	1 (2.6 %)	On pump bidirectional Glenn shunt	3 (7.9 %)
DORV,PA,TGA,VSD	1 (2.6 %)		
DORV,Tricuspid atresia,TGA,PS	1 (2.6 %)		
Total	38 (100 %)	Total	38 (100 %)

Table (3): shows the Cardiopulmonary bypass data in the studied cases

	Mean ± SD	Range(minimum-maximum)
Cardiopulmonary bypass time (min)	86 ± 36	30-160
Aortic cross clamp time (min)	60 ± 34	0-120

Table (4): shows the presence, average number and duration of air emboli in the studied cases.

	Mean ± SD	Range
Average number of air emboli(signals/second)	3 ± 2	0-8
Average duration(min)	6.1 ± 5.2	0-21
Presence of air emboli(no. of cases, Total 38)	Yes, 32 (84.2 %)	No, 6 (15.8 %)

Table (5): shows the postoperative mechanical ventilation, ICU, Hospital duration.

	Mean ± SD	Range(minimum-maximum)
mechanical ventilation time (hour)	29 ± 14	4-240
ICU length of stay(day)	3.6 ± 2.9	1-15
hospital length of stay(day)	7.4 ± 3.7	5-21

Table (6): Shows the relation between preoperative neurodevelopmental assessment and postoperative neurodevelopmental outcome

	Preoperative (n=38) n(%)	Postoperative (n=38) n(%)	*p-value*
Overall assessment			
Delayed	32 (84.2 %)	11 (28.9%)	<0.001 *
Normal	6 (15.8%)	27 (71.1%)	
Personal social			
Delayed	19 (50%)	3 (7.9%)	0.036 *
Normal	19 (50%)	35 (92.1%)	
Fine motor			
Delayed	25 (65%)	2 (5.3%)	< 0.001 *
Normal	13 (34.2%)	36 (94.7%)	
Language			
Delayed	17 (44.7%)	5 (13.2%)	0.003 *
Normal	21 (55.3%)	33 (86.8%)	
Gross motor			
Delayed	23 (60.5%)	8 (21.1%)	0.001 *
Normal	15 (39.5%)	30 (78.9%)	

(NS: Non-significant, *: Significant)

Table (7): shows the relation between presence of air emboli and postoperative neurodevelopmental outcome

		presence of air	P value
Postoperative overall neurodevelopm ental outcome	**Normal (n=27)**	23(85.2%)	0.79 NS
	Delayed (n=11)	9(81.8%)	
personal-social Post	**Normal (n=35)**	30(85.7%)	0.43 NS
	Delayed (n=3)	2(66.7%)	
fine motor Post	**Normal (n=36)**	31(86.1%)	0.24 NS
	Delayed (n=2)	1(50.0%)	
language	**Normal (n=33)**	28(84.8%)	0.79 NS
	Delayed (n=5)	4(80.0%)	
gross motor	**Normal (n=30)**	26(86.7%)	0.44 NS
	Delayed (n=8)	6(75.0%)	

(NS: Non-significant, *: Significant)

Discussion

The present study was conducted with objectives to evaluate the neurodevelopmental outcome in children who undergo open cardiac surgery during their first year of life and to assess the value of TCD in prediction of such neurodevelopmental outcome.

We chose to operate our patients in their first year of life as during this period, the child's brain is rapidly developing and easily affected by different variables of pediatric cardiac surgery in addition to the already existing cardiac defect, the concept which was confirmed by Fuller's study and his colleagues[4]. In addition to that, we were aiming to a study with short term follow up as a first step-to our knowledge- to handle this subject in Egypt. So, the first year of life was an ideal period at which we can observe rapid change in the neurodevelopmental milestones enabling us to get a suitable idea about the neurodevelopmental outcome after pediatric cardiac surgery with a postoperative follow up of only six months.

For that purpose, we prospectively analyzed the neurodevelopmental outcome of 38 children who underwent open cardiac surgery using cardiopulmonary bypass during their first year of life.

We found statistically significant postoperative improvement in the overall neurodevelopmental assessment, personal social development, fine motor -adaptive development, language development and gross motor development in relation to the preoperative one (p-value <0.05).

These results don't cope with the study of Walter and his colleagues[23] which included 101 patients who underwent infantile cardiac surgery with follow up 4-6 months postoperatively. Walter found decline in all neurodevelopmental domains than population norms postoperatively using the "Bayley Scales of Infant Development-II" test. Sananes and his colleagues[18] found significant gross motor and fine motor difficulties in infants who underwent open cardiac surgery with a postoperative follow up for 24-month. Also, Fuller's and his colleagues[4] study at which neurodevelopmen-

tal outcome was evaluated at one year of age in 359 Infants with complex CHD who underwent open cardiac surgery. He found decline in all neurodevelopmental domains than normal postoperatively using the Bayley Scales of Infant Development-II test. But, he said that the patient-specific factors such as birth weight, ethnicity, and the presence of a genetic syndrome were the most important determinants of neurodevelopmental outcome after cardiac surgery in neonates and infants in his study. He added that Specific operative management strategies had little impact on the neurodevelopmental outcome. Moreover, neither the use nor the duration of DHCA was associated with a worse outcome at one year of age.

Our study was different from the previously mentioned ones in that we tried to fix patient specific factors and exclude the extremes which were claimed as the major predictors for the neurodevelopmental outcome after pediatric cardiac surgery in the previous studies.

On the other hand, Michael and his colleagues(12) studied the neurodevelopmental outcome in two groups, the first group underwent repair of acyanotic CHD in school-aged children using CPB (CPB+) and the other group of a same age underwent repair of pectus excavatum with thoracic incision under similar anesthetic, duration of hospitalization, and pain management strategy(CPB-). He found mild improvement in postoperative neurodevelopmental scores in both groups 6 months after repair with presence of scores for both groups within normal range at baseline and its increasing postoperatively. He concluded that there was no clear hazardous effects of CPB on neuropsychological status of acyanotic CHD in school-aged children. Also, Van der Rijken and his colleagues[22] performed neurocognitive testing before and 1 year after surgery in a heterogeneous cohort of school-aged children, including some children with more complex CHD. The investigators reported no differences between the study groups when compared with a non-surgical control group that was composed of children who underwent transcatheter ASD closure.

In fact, we take into consideration that these studies were applied in different less risky age groups and in patients with different diagnosis (most of them were acyanotic). But, if we look at these studies from a different point of view by stating that the CPB has no or limited negative neurological effect with the fact that the cyanosis in our cases was improved postoperatively. So, we can put the possibility of neurodevelopmental improvement secondry to improved oxygenation in the cyanotic patients in our study.

Inspite of the relatively different outcome of our study than the most of the literature, we consider the step that we had taken would be a step in a new perspective toward the positive effect of early pediatric cardiac surgery on the neurodevelopmental outcome. We believe that although the increasing body of literature reporting negative outcomes after surgical repair of CHD from various cohorts, generalization is problematic since there is heterogeneity amongst these reports and our study in regards to surgical/ medical era, genetic composition, patient population, type of cardiac lesion, age at follow up, and type of assessment tool.

Moreover, we noticed in multiple studies that multiple confounding risk factors for neurodevelopmental decline have been described in children with complex CHD, including a relatively high incidence of genetic syndromes, abnormal brain development, perinatal instability, and need for multiple operations[2,9]. Consequently, it has been difficult to delineate the relative contributions of CPB and the surgery themselves on neurodevelopmental outcomes in this population.

What we tried to do is to control and exclude most of the non CPB factors and to apply intraoperative neuromonitoring to evaluate the effect of CPB and the cardiac surgery themselves on the neurodevelopmental outcome.

Thus, such improvement in our cases and the relative difference between our study and other studies may attribute to several points:

- Exclusion of preoperative patients' risk factors: All patients

had no preoperative organic neurological problems, abnormal brain development, apparent genetic syndromes, perinatal problems, and exposed to CPB only once (no redo cases). All patients had preoperative normal CT brain.

- All patients were operated during their first year of life with mean age of 8.4±3.5 months. So, they relatively have not been exposed to the haemodynamic instability and/or hypoxemia caused by the congenital heart disease for long time.

- The mean preoperative O2 saturation was 80% ± 16 which is relatively not low which led to exposure of low number of our cases to preoperative hypoxia.

- Intraoperative neuromonitoring with TCD played a major role in correction of some dangerous situation that may expose the patient to post operative neurological problems.

TCD helped us to correct the position of the aortic cannula after discovery of absent blood flow in the left common carotid artery due to total entrance of the aortic cannula into the innominate artery. Also, TCD helped us to discover a missed PDA after going on bypass and opening of right atrium by alarming presence of massive cerebral air emboli and sudden decrease of cerebral blood flow.

- Follow up of our cases was for only 6 months postoperatively rather than other studies with follow up for several years up to and beyond school age. That made them capable of discovery of delayed outcome in certain neurodevelopmental domains which couldn't be examined in the early age of our cases. Also, inspite of exclusion of presence any organic brain lesion preoperatively by CT brain, researchers from the Children's Hospital Zurich demonstrated that morphological changes of the brain can be detected many years after open-heart surgery and can have a long-term impact on brain development which may not appear in such small age during follow up of our cases[20].

- Follow up of our cases was for once which may lack –at the end of this study- the observation of any change that may occur after

that by further improvement of the still delayed cases or delay in or appearance of new defects in some patients who appeared as normal in some neurodevelopmental domains. Valerie and colleagues[21] stated that neurodevelopmental abnormalities might not become apparent until months after hospital discharge. And on the contrary, early impairment may also resolve over time. So, close developmental follow-up of our patients is highly recommended.

- The cognitive and executive functions were less tested in our study. That made our study less comparable to other studies in the literature at which cognitive and executive functions were considered significantly in the assessment of the neurodevelopmental outcome.

- We used "Denver II" test as a simple tool for neurodevelopmental assessment in our study. Other studies used different neurological tests which arise the importance of taking the difference in scoring systems and criteria of assessment between the tests into consideration. So, we recommend further use of "Denver II" test in larger studies to make our study more comparable.

- Low incidence of serious intraoperative and postoperative complications which were managed early with minimal affection on the hemodynamics.

In our study, all cases were neuromonitired intraoperatively by TCD. There was no significant alteration of cerebral blood flow, but TCD recorded presence of cerebral air emboli in 32 (84.2%) cases. Inspite cerebral microemboli were claimed as a major cause of neurological injury after cardiac surgery[1,17,19], there was no association between the presence or the number of air emboli and the impairment of the neurodevelopmental outcome in our study. This result copes with the study conducted by Naik[14] who stated that the number of cerebral air emboli detected during infant cardiac surgery by TCD was not shown to be associated with worse neurodevelopmental outcomes on follow up.

The insignificant effect of cerebral microemboli on the neurodevelopmetal outcome in our study

may be attributed to the intraoperative cooling effect of CPB which has a neuroprotective role[3,13], thus preventing the negative effect of air emboli. Also, methylprednisolone was highly recommended as a neuroprotective agent by Kirkham[7] which was used routinely in all our cases.

There is no doubt that TCD plays an important role in neuromonitoring during pediatric cardiac surgery. However, Inability to find a relation between the intraoperative findings of TCD regarding air emboli and the neurodevelopmental outcome made us conclude -at the level of our study- that we can not use TCD as a tool to predict the neurodevelopmental outcome.

Absolutely, larger studies at our community are probably necessary to evaluate the potential influence of cerebral emboli on neurodevelopmental outcome in children after infantile cardiac surgery and to asses the role of TCD in predicting such outcome.

Limitation of the study:

- Relatively limited number of our cases in relation to other studies in the literature.

- Lack of information about birth weight, parents education and genetic study which are important factors in determining the neurodevelopmental outcome. We only excluded patients with the apparently genetic syndromes like Down syndrome.

- Short follow up (only six months) which may be a relatively early time to put a final conclusion about the neurodevelopmental outcome.

- Inability to measure the size of air emboli with the used ultrasound device hindered our ability to assess the relation between the size of air emboli and the neurodevelopmental outcome.

Conclusions

• Pediatric cardiac surgery performed during the first year of life is associated with improvement in the postoperative neurodevelopmental outcome after six months follow up.

• There is no significant relation between presence of cerebral air emboli and the neurodevelopmental outcome.

• Intraoperative neuromonitor-

ing using TCD could not be used as a tool to predict the neurodevelopmental outcome in infantile cardiac surgery.

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BENHA MEDICAL JOURNAL

EVALUATION OF THE NEURODEVELOPMENTAL OUTCOME OF PEDIATRIC OPEN CARDIAC SURGERY PERFORMED DURING THE FIRST YEAR OF LIFE, USING TRANSCRANIAL DOPPLER

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STUDY OF MESENCHYMAL STEM CELLS VERSUS NILOTINIB IN MANAGEMENT OF CARBON TETRACHLORIDE INDUCED LIVER FIBROSIS IN SPRAGUE DAWELY RATS

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Abstract

Introduction: *Hepatic fibrosis is a reversible wound-healing response to either acute or chronic cellular injury that reflects a balance between liver repair and scar formation. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension and often requires liver transplantation. These problems have highlighted the need to explore other sources of cells such as stem cells and antifibrotic drugs to solve this major problem. For studying this issue, carbon tetrachloride (CCl4)-induced hepatic fibrosis and cirrhosis in rodents which was a well established and widely accepted experimental model for the study of liver fibrosis and cirrhosis.*

Aim of the work: *induction of CCl4 mediated liver fibrosis at 2 different stages (early at 2 weeks, late at 6 weeks) with studying he role of mesenchymal stem cells (rat bone Marrow derived and warton jelly derived) and nilotinib in the revervsal of hepatic fibrosis.*

Materials and methods: *Eighty female Sprague Dawely rats were used for the experimental study, they were divided into 4 main groups, each group consists of 20 rats and each group divided into 2 subgroups (subgroup a up ta stage 3 andsubgroup b from stage 4 up to stage 8). Rats were injected by CCl4 by dose 0.5 mg/kg in olive oil in ratio of 1:1 intraperitonially twice per week for 2 weeks and 6 weeks and rats were sacrificed 2 weeks after treatment (mesenchymal stem cells and niloti-*

nib). Each group was submitted for the following laboratory investigations: alanine-amino transferase (ALT), aspartate-amino transferase (AST), international normalized ratio (INR) and serum albumin. Also, liver was fixed by formalin 10% for histopathological examination by heamtoxlin and eosin and maisson trichrome.

Results: *Our results revealed that there were marked elevation in the levels of ALT and AST in the control group with dramatic reduction in those levels in groups treated with BM stem cells and warton jelly derived mesnechymal stem cells with no significant difference in the other laboratory parameters. For the fibrosis score, the best results was obtained with nilotinib , which was confirmed by using the image J software. The regeneration score was high in the groups treated with the warton jelly derived mesenchymal stem cells when compared with the other treated groups by dtetection of human albumin gene by RT-PCR technique.*

Conclusion: *Nilotinib is a potent antifibrotic drug irrespective to the duration of fibrosis, and warton jelly derived mesenchymal stem cells were better than the BM derived stem cells in reversal of hepatic fibrosis.*

Introduction

Hepatic fibrosis is reversible wound-healing response to either acute or chronic cellular injury that reflects a balance between liver repair and scar formation. During acute injury, the changes in liver architecture are transient and reversible. With chronic injury, there is progressive substitution of the liver parenchyma by scar tissue. Despite ongoing injury, the liver has a remarkable regenerative capacity, and, as a result, patients often progress slowly to cirrhosis over decades[1]. It consists of a fibrous scar that is constituted by many extracellular matrix proteins (ECMs) including type I collagen. In all clinical and experimental liver fibrosis, myofibroblasts are the source of the ECM constituting the fibrous scar. Myofibroblasts express a-smooth muscle actin (α-SMA) and type I collagen and are only found in the injured, but not the normal, liver. Thus, activation and proliferation

of hepatic myofibroblasts is a key mechanism in development of liver cirrhosis[2].

A key discovery in understanding fibrosis has been that the hepatic stellate cell (HSC) is the primary effector cell, orchestrating the deposition of ECM in normal and fibrotic liver. HSCs are resident perisinusoidal cells in the subendothelial space between hepatocytes and sinusoidal endothelial cells. They are strategically positioned to intimately interact with hepatocytes, endothelial cells, and nerve endings through their numerous processes extending across the space of Disse[3].

Additionally, the HSC plays a pivotal role in activating the immune response through secretion of cytokines and chemokines and interacting with immune cells. HSC also contributes to angiogenesis and the regulation of oxidant stress[4].

Activation of the HSC into a myofibroblast like phenotype can be provoked by a range of chronic injuries to the liver, amongst which are viral hepatitis, toxins, non alcoholic steatohepatitis and autoimmune disorders[5].

Activation consists of two major phases, initiation [also called a "preinflammatory stage"] and perpetuation, followed by a resolution phase if the injury subsides[6]. Initiation refers to early changes in gene expression and phenotype that render the cells responsive to other cytokines and stimuli shortly after injury occurs. The initial paracrine stimulation, including exposure to lipid peroxides and products of damaged hepatocytes and signals from Kupffer and endothelial cells, drive early activation, as well as changes in surrounding extracellular matrix. Once the cell is primed for activation, perpetuation results from the effects of these stimuli on maintaining an activated phenotype and generating fibrosis. Sustained activation involves at least seven discrete changes in cell behavior: proliferation, chemotaxis, fibrogenesis, contractility, matrix degradation, retinoid loss, and WBC chemoattractant/cytokine release. The net effect of these changes is to increase accumulation of extracellular matrix.

During this phase there is a release of proinflammatory, profibrogenic and promitogenic stimuli that act in an autocrine and paracrine manner. Resolution of fibrosis refers to pathways that cause either HSC apoptosis, senescence, or quiescence[7].

Additional cellular sources of ECM have emerged from more recent studies. As well as HSCs, portal myofibroblasts and bone marrow-derived cells contribute to fibrosis, in addition to the growing potential of epithelial mesenchymal transition (EMT). Portal myofibroblasts, whereas similar to HSCs, have unique characteristics that include a distinct response to transforming growth factor β (TGFβ) and a prominent role in responding to matrix stiffness[8,9].

Finally, EMT is emerging as yet another source of injury-associated mesenchymal cells, derived either from resident hepatocytes or biliary epithelial cells[10]. Signaling pathways underlying this intriguing process are being clarified, with particular emphasis on hedgehog and its receptors[11].

Aim of the work

Induction of CCl4 mediated liver fibrosis at 2 different stages (early at 2 weeks , late at 6 weeks) with studying he role of mesenchymal stem cells (rat bone Marrow derived and warton jelly derived) and nilotinib in the revervsal of hepatic fibrosis.

Materials and Methods

They were 4 groups of experimental work, each group consists of 20 rats and each group divided into 2 subgroups (a up ta stage 3, b stage 4 up to stage 8)-Rats were injected by carbon tetrachrolide (CCl4) by dose 0.5 mg/kg in olive oil in ratio of 1:1 intraperitonially twice per week for 2 weeks and 6 weeks.In each group was submitted for the following laboratory investigations: alanine-amino transferase (ALT), aspartate-amino transferase (AST), international normalized ratio (INR) and serum albumin, liver was fixed by formalin 10% for histopathological examination by heamtoxlin and eosin and maisson trichrome stains. Rats were sacrificed 2 weeks after treatment (stem cells and nilotinib).

Control groups 1) Negative

control (control vehicle): rats were injected by olive oil only equivalent to their body weight and they were scarificed at 2 and 6 weeks and blood samples and liver were taken for histopathological examination. 2) Positive control: rats were injected by CCl4 with the above mentioned dose and rates were sacrificed at the end of 2 and 6 weeks. They were exposed to anathesia using thiopental sodium and blood samples were taken for biochemical analysis for AST, ALT, INR and serum albumin and the liver was fixed by formaline 10% and taken for histopathological examination using both hematoxillin and eosin and maisson trichrome for staining the fibrous tissue.

Assessment of fibrosis and regeneration:

A modification of the staging system adopted by Zhao et al based on ishak score for fibrosis was used[12]. Liver fibrosis was classified into 8 stages:

Stage (1): Central fibrosis in some lobules.

Stage (2): Central fibrosis in most lobules.

Stage (3): Short fibrous septa.

Stage (4): Central to central fibrosis (long fibrous septa).

Stage (5): Nodules corresponding to portal lobules.

Stage (6): Focal sub segmentation of portal lobules.

Stage (7): Diffuse subsegmentation of portal lobules.

Stage (8): Small and large separate cirrhotic islets separated by wide fibrous septa.

Bone marrow mesenchymal stem cells:

Isolation of Mesenchymal stem cells from bone marrow:

Eight-week-old male Sprague-Dawley rat was sacrificed by cervical dislocation and their femurs and tibiae were carefully cleaned from skin by pulling toward the foot, which is cut at the ankle bone. Remove the muscle and connective tissue from both the tibia and the femur by scraping the diaphysis of the bone clean then pulling the tissue toward the ends of the bone. Cut the ends of the tibia and femur by sharp scissors. Insert a 27-gauge needle and flushing with Dulbecco's Modified Eagle's Medium (DMEM) and collect in a 15-ml tube. Filter the cell suspension through a 70-mm fil-

ter mesh. Culture BM cells in DMEM+10% FBS+1%1% antibiotic–antimycotic solution (Thermo scientific, USA) in 25 cm2 flask. Incubate flask at 37 0C with 5% CO2.

Cell culture:

After 1 day, nonadherent cells were removed by two to three washes with phosphate-buffered saline PBS (Thermo scientific, USA) and adherent cells further cultured in complete medium. The medium was changed every 3 days until the monolayer of adherent cells reach 70-80% confluence. Then, we made trypsinization for cell splitting by trypsin-EDTA solution (0.25%, sigma Aldrich, USA) for passage 1. Evaluate the number of cells by hemocytometer and cellular viability by the Trypan Blue exclusion test. Inoculate each 250-300x103 cells in 75cm2 culture flask that were incubated at 37°C and 5% CO2. Cell cultivation was maintained up to the 3th passage.

Flow cytometry analysis:

Cells were characterised using cell surface markers by fluorescence-activated cell sorting (FACS) analysis. The cells were stained with different fluorescently labeled monoclonal antibodies (mAb) (eBioscience). In brief, 5*105 cells (in100 µl PBS/0.5% BSA/2mmol/LEDTA) were mixed with 10 µl of the fluorescently labeled mAb and incubated in the dark at 2-8 °C for 30 min. Washing with PBS containing 2% BSA was done twice and the bellet was resuspended in PBS and analyzed immediately on flow cytometry. Using the mouse anti-rat CD45-FITC mAb, CD29-PE mAb and CD 90-PCY5. The fluorescence intensity of the cells was evaluated by EPICS-XL flow-cytometry (Coulter, Miami, Fl, USA).

CFU-F assay:

For colony forming unit-fibroblast (CFU-F) assays, plate about 100 cells per 100-mm tissue culture dish (Falcon) in complete culture medium. Incubate cells for 10-14 d at 37°C in 5% humidified CO2, and wash with PBS and fixed in 95% ethanol for 5 minutes, and then the cells were incubated for 20-30 minutes at room temperature in 0.5% crystal violet (sigma Aldrich, USA) in 95% ethanol.then wash plate

twice with distilled H2O. The plates were dried and the CFU-F units counted.

Harvest passage 3 BMSCs by trypsin digestion as described above, the cells was Counted and seeded at a density of (5x104 per well) in a 6-well plate then at 80% confluent add to 4 wells Osteogenesis differentiation media: DMEM supplemented with 10% FBS, 0.1μM dexamethasone, 50μM Ascorbic acid, 10mM β-glycerol phosphate; and add to other 2 well complete culture Media as negative control. Change the medium twice per week for 2-3 weeks. The differentiation potential for osteogenesis was assessed by 40 mM Alizarin Red (pH 4.1) after fixation in 10% Neutral buffered formalin.

Harvest passage 3 BMSCs by trypsin digestion as described above, Count the cells and seed at a density of (10x104 per well) in a 6-well plate then at 100% confluent to 4 wells Adipogenesis differentiation media: DMEM supplemented with 10% FBS, 1μM dexamethasone, 500 μM isobutylmethylxanthine (IBMX) 5μg/ml insulin, 200μM Indomethacin. And add to other 2 well complete culture Media as negative control Change the medium twice per week for 2 weeks. The differentiation potential for Adipogenesis and formation of intracellular lipid droplets were assessed by Oil-red-O after fixation in 10% Neutral buffered formalin.

Results

- Immunophenotypic characterization:

Cultures of BMSCs were analyzed for expression of cell-surface markers. BMSCs were negative for the hematopoietic lineage marker CD45 with percentage 1.72. BMSCs were positive for CD29 and CD90 with percentage 98.4 and 93.7 respectively.

- Human umbilical cord stem cells (warton jelly):

Isolation of UC-MSC:

Fresh human umbilical cords were obtained after full-term births (cesarean section) with informed consent from Mansoura Universty Hospital.

The umbilical cords were washed with phosphate-buffered

saline (PBS) containing 1% antibiotic–antimycotic solution (Thermo scientific, USA). The washing step is repeated until cord become clear. UC cut longitudinally then minced into very small pieces 1-2 mm3 fragments. UC incubated with enzyme cocktail of 0.1% collagenase and 0.25% trypsin at 37°C with shaking 100 rpm for 45 min. After digestion, Neutralize enzyme activity by adding equal volume of DMEM containing 10% fetal bovine serum (Thermo scientific, USA). Then the mixture passed through a 100µm filter to obtain cell suspensions. Then Centrifuge the sample at 2000 rpm for 5 min at room temperature. Then culture the cells in DMEM with 10% fetal bovine serum (FBS) and 1% antibiotic antimycotic solution in 25 cm2 culture flask and maintained in an incubator supplied with humidified atmosphere of 5% CO2 at 37°C.

Cell cultivation:

After 1 day, nonadherent cells were removed by two to three washes with PBS and adherent cells further cultured in complete medium. The medium was changed every 3 days until the monolayer of adherent cells reach 70-80% confluence. Then make trypsinization for cell splitting by trypsin-EDTA solution (0.25%, sigma Aldrich, USA) for passage 1. We evaluate the number of cells by hemocytometer and cellular viability by the Trypan Blue exclusion test. We inoculate each 250-300x103 cells in 75cm2 culture flask that were incubated at 37 0C and 5% CO2. Cell cultivation was maintained up to the 3th passage.

Flow cytometry analysis:

Cells were characterised using cell surface markers by fluorescence-activated cell sorting (FACS) analyses. The cells were stained with different fluorescently labeled monoclonal antibodies (mAb) (eBioscience). In brief, 5x105 cells (in100 µl PBS/0.5% BSA/2mmol/LEDTA) were mixed with 10 µl of the fluorescently labeled mAb and incubated in the dark at 2-8 0C for 30 min. washing with PBS containing 2% BSA was done twice and the bellet was resuspended in PBS and analyzed immediately on flow cytometry.

Using the mouse anti-human

CD90- PCY5 mAb, CD105-PE mAb, CD29- FITC mAb, CD13-FITC mAb, CD34-PE mAb, CD11b- FITC mAb, CD19- PCY5 mAb, CD45- PCY5 mAb, CD14-FITC mAb. The fluorescence intensity of the cells was evaluated by EPICS-XL flowcytometry (Coulter, Miami, Fl, USA).

CFU-F assay:

For colony forming unit-fibroblast (CFU-F) assays, plate about 100 cells per 100-mm tissue culture dish (Falcon) in complete culture medium. Incubate cells for 10-14 d at 37°C in 5% humidified CO2, and wash with PBS and fixed in 95% ethanol for 5 minutes, and then the cells were incubated for 20-30 minutes at room temperature in 0.5% crystal violet (sigma Aldrich, USA) in 95% ethanol. Then wash plate twice with distilled H2O. The plates were dried and the CFU-F units counted.

UC-MSC Differentiation Capability:

Osteogenic Differentiation:

Harvest passage 3 MSCs by trypsin digestion as described above, the cells were Counted and seeded at a density of (5x104 per well) in a 6-well plate then at 80% confluent add to 4 wells Osteogenesis differentiation media: DMEM supplemented with 10% FBS, 0.1μM dexamethasone, 50μM Ascorbic acid, 10mM β-glycerol phosphate; and add to other 2 well complete culture Media as negative control. Change the medium twice per week for 2-3 weeks. The differentiation potential for osteogenesis was assessed by 40 mM Alizarin Red (pH 4.1) after fixation in 10% Neutral buffered formalin.

Adipogenic Differentiation:

Harvest passage 3 MSCs by trypsin digestion as described above, the cells were Counted and seeded at a density of (10x104 per well) in a 6-well plate then at 100% confluent to 4 wells Adipogenesis differentiation media: DMEM supplemented with 10% FBS, 1μM dexamethasone, 500 μM isobutylmethylxanthine (IBMX) 5μg/ml insulin, 200μM Indomethacin. And add to other 2 well complete culture Media as negative control Change the medium twice per week for 2 weeks. The differentiation potential for Adipogene-

sis and formation of intracellular lipid droplets were assessed by Oil-red-O after fixation in 10% Neutral buffered formalin.

- Cell culture:

The cultures were observed by using an invert light microscope. Attachment of spindle-shaped cells to tissue culture plastic flask was observed after 1 days of culture. Primary cultures reached 70-80% confluence in approximately 5 days.

- Immunophenotypic characterization: Cultures of Third passage WJ-MSC were analyzed for expression of cell-surface markers. WJ-MSC were negative for CD34, CD11b, CD19, CD45 and CD14 with percentage 0.18, 1.02, 0.06, 0.03 and 0.5 respectively. WJ-MSC were positive for CD90, CD105, CD29 and CD13 with percentage 97.3, 99.8, 99.5 and 76.9 respectively.

After complete preparation of the cells, 1.000.000 cells in 10 ml medium were injected into the lower pole of the spleen, the rats received postoperative care as of subcutaneous saline into the rat to avoid its dehydration and IM amoxicillin 15 mg/kg bid was used to prevent infection for 5 days.

• Scarification of the treated rats:

After 2 weeks, rats of all groups were sacrificed in the following steps:

1) Deep anaesthesia with ketamine 8 mg/100g body weight and xylazine 1 mg/100 g body weight injected IP.

2) Body weight was measured.

3) Blood was taken through percutaneous puncture of the heart.

4) Long midline incision was performed followed by longitudinal cutting of the sternum.

5) The dorsal vein was exposed and a cannula was inserted into it.

6) Mesenteric, hepatic, and renal vessels were punctured then saline was infused through the dorsal vein to remove any remaining blood.

7) Formalin was infused into the dorsal vein.

8) Liver was dissected, weight was measured, and fixed in paraffin for histopathology.

Comparing control group with all groups (control, BM stem cells, warton jelly derived mesenchymal stem cells and nilotinib) there was significant elevation of ALT and AST with no significant changes in other laboratory parameters between all groups.

When we compare between the BM, warton jelly derived mesenchymal stem cells and nilotinib treated group there was significant improvement in s. albumin, regeneration score in warton jelly derived mesenchymal stem cells group and significant reduction in area of fibrosis percent in nilotinib group.

We found that the regeneration score was higher in the group treated by the warton jelly derived mesenchymal stem cells in comparison with other treated groups.

Table (1): Comparison of all treated groups at week 2 (BM, mesenchymal and Nilotinib) by Chisquare test:

parameter	X^2	P
ALT	6.415	0.04
AST	1.625	0.444
INR	1.429	0.489
Albumin	9.094	0.01
Fibrosis score	0.806	0.668
Regeneration score	8.642	0.013
Area fibrosis percent	19.228	0.000

Comparing between the BM, warton jelly derived mesenchymal stem cells and nilotinib treated group there was significant improvement in s. albumin, regeneration score in warton jelly derived mesenchymal stem cells group and significant reduction in area of fibrosis percent in nilotinib group.

Table (2): Comparison of the treated groups (BM, mesenchymal and Nilotinib) at week 6 by Chisquare test:

parameter	X^2	P
ALT	6.667	0.36
AST	1.344	0.511
INR	0.340	0.844
Albumin	6.193	0.45
Fibrosis score	2.548	0.280
Regeneration score	2.920	0.232
Area of fibrosis percent	20.116	0.000

There is significant reduction only at the level of the area of fibrosis percent in nilotinib group.

Table (3): Comparison of warton jelly derived mesenchymal stem cells groups at week 2 versus week 6 (total no. = 20):

Parameter	Z	P
ALT	0.000	1
AST	0.000	1
INR	0.000	1
Albumin	0.000	1
Fibrosis score	-1.866	0.062
Regeneration score	-1.774	0.076
Area of fibrosis percent	-2.160	0.031

There is significant difference in the warton jelly derived mesenchymal stem cellsbetween week 2 and week 6 at the level of fibrosis score, regeneration score and area of fibrosis percent in week 6 and no significant difference at the level of biochemical results.

(a) (b)

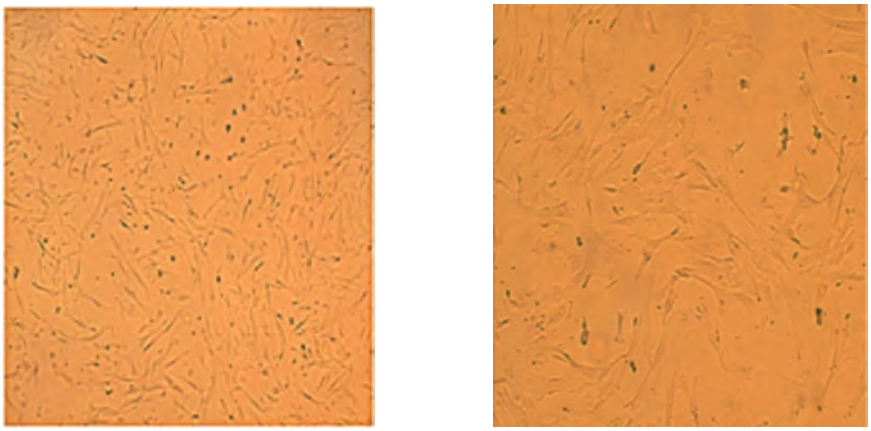


Fig. (1): (a) Image of rat BMSCs in passage 3 with70- 80% confluence Scale bar=100 µm. **(b)** Image of rat BMSCs in in passage 3 Scale bar=200µm.

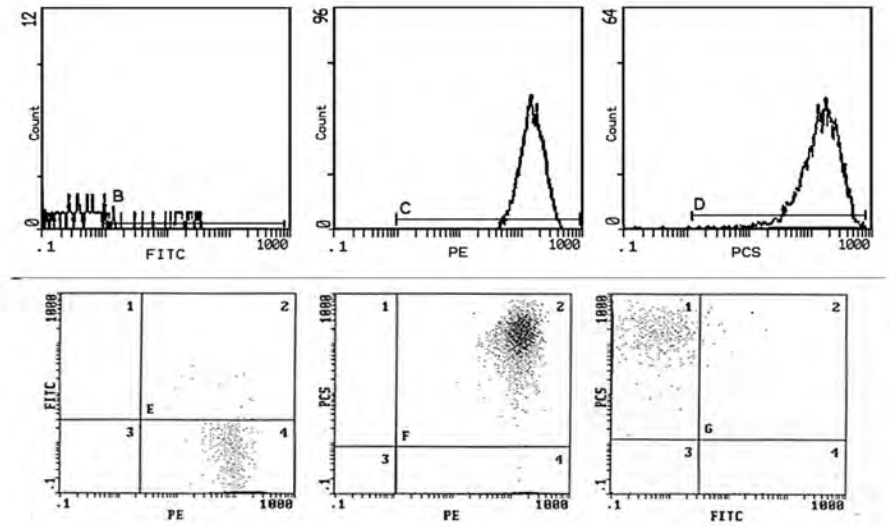


Fig. (2): Histogram representing the flow cytometry performed on the BMSCs. The BMSCs were stained with monoclonal antibodies conjugated to FITC, PE and PCY5 against the following markers: CD45, CD 29 and CD90 respectively. 97.1% of cells positive for CD 29 and negative for CD45 93.7% of cells positive for both CD29 and CD 90. 93.1% positive for CD 90 and negative for CD45.

Marker	%
CD90	93.7
CD29	98.4
CD45	1.72

(a) (b)

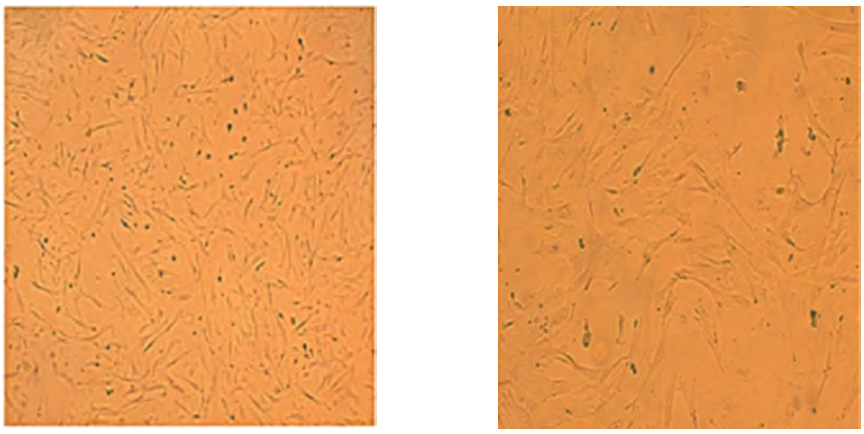


Fig. (3): Image of UCMSCs in passage 3 with 80% confluence **(a)** Scale bar=100 µm. **(b)** Scale bar=200 µm.

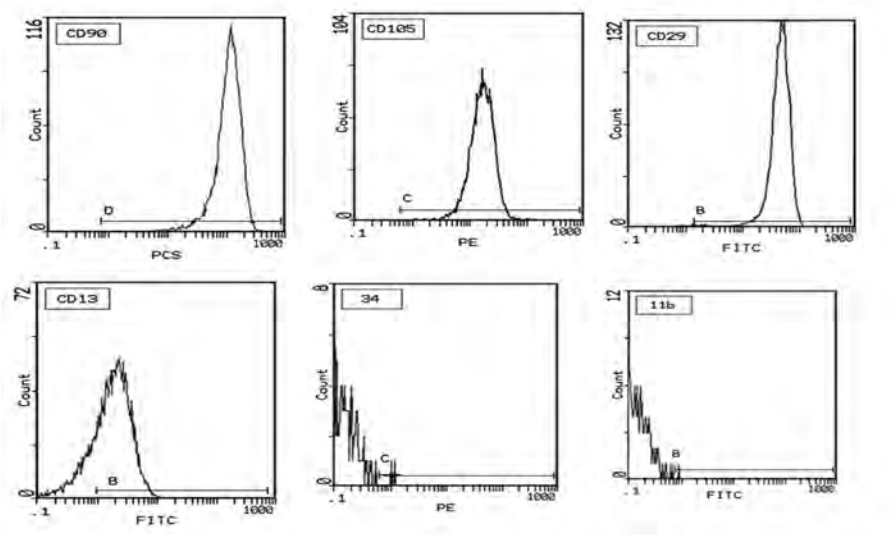


Fig. (4): Immunophenotypic analysis of UC-MSCs. Histogram representing the flow cytometry performed on the UCMSCs .The UC-MSCs were stained with monoclonal antibodies conjugated to FITC, PE and PCY5.

Antibody	Conjugate	WJ-MSC%
CD90	PCY5	97.3
CD105	PE	99.8
CD29	FITC	99.5
CD13	FITC	76.9
CD34	PE	0.18
CD11b	FITC	1.02
CD19	PCY5	0.06
CD45	PCY5	0.03
CD14	FITC	0.5

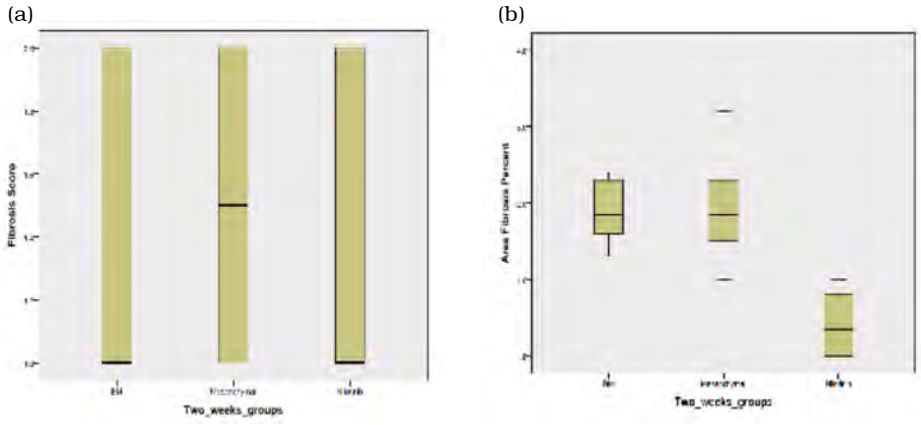


Fig. (5): a) fibrosis score at week 2 **b)** area of fibrosis percent at week 2.

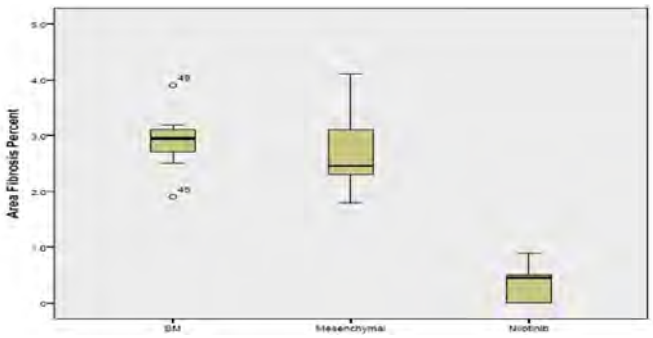
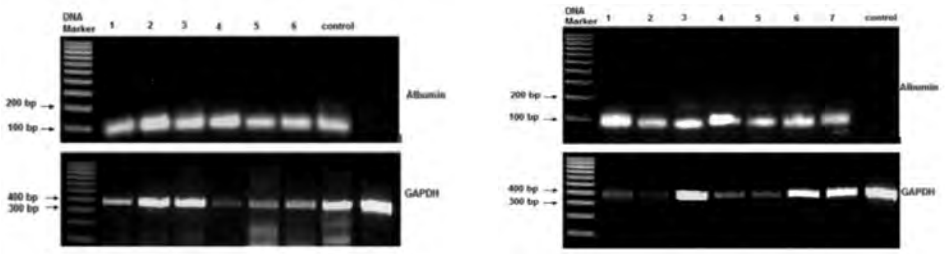


Fig. (6): area of fibrosis percent at week 6.



Human albumin gene at week 2 Human albumin gene at week 6

Fig. (7): For the warton jelly derived warton jelly derived mesenchymal stem cells, we have detected the human albumin gene in the liver tissue of the rat by RT-PCR technique.

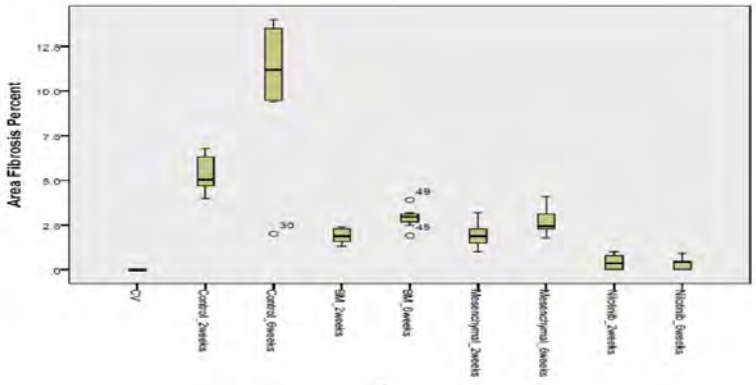


Fig. (8): Comparison between all groups (control & treated) at week 2 and week 6 showing significant reduction of fibrosis with more evident antifibrotic effect in nilotinib treated groups.

Discussion

Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension and often requires liver transplantation[5].

Worldwide, 130-170 million persons are living with chronic hepatitis C virus (HCV) infection, which, if left untreated, can result in cirrhosis and liver cancer. Egypt has the largest burden of HCV infection in the world, with a 10% prevalence of chronic HCV infection among persons aged 15-59 years[13].

All these problems have highlighted the need to explore other sources of cells such as stem cells that could be isolated, expanded to yield sufficiently large populations and then induced to differentiate into functional hepatocytes.

Our study was carried out to investigate the potential anti-fibrotic effects of mesenchymal stem cells (BM & warton jelly derived) and nilotinib and to compare between them in reversal of experimental liver fibrosis induced by CCl4 in rats.

Our results revealed that there were marked elevation in the levels of ALT and AST in the control group with dramatic reduction in those levels in groups treated with BM stem cells and warton jelly derived mesnechymal stem cells with no significant difference in the other laboratory parameters.

For the fibrosis score, there was evident fibrosis detected histopatholgicaly at week 2 and week 6 in the control group with significant decrease in the fibrosis score with the BM and warton jelly mesenchymal stem cells treated groups with best results obtained with nilotinib treated group.

These results were confirmed by quantitave assessment of the liver fibrosis by using the image J software reflected in area of fibrosis percent (mean area of fibrosis was 5% in control group at week 2 and 10.7% at week 6, 2.5% in BM stem cells treated group at week 2

and 3.9% at week 6, 1.703% in warton jelly derived stem cells at week 2 and 2.65% at week 6, 0.31% in nilotinib group at week 2 and 0.37% at week 6).

The regeneration score was significantly high in warton jelly mesenchymal treated group compared with the control group, BM treated group and nilotinib group.

At week 2, there was significant improvement in s. albumin ($p \leq 0.01$), regeneration score in mesenchymal stem cells group ($p \leq 0.013$) and significant reduction in area of fibrosis percent in nilotinib group ($p \leq 0.000$) with no significant difference between the BM and warton jelly derived mesenchymal stem cells treated groups in both histopathological examination and laboratory parameters. When we compared between the BM stem cell group and Nilotinib group at week 2, we found that there was significant elevation at the level of albumin ($p \leq 0.002$), regeneration score ($p \leq 0.005$) and area of fibrosis percent ($p \leq 0.000$) in nilotinib group. While, comparison between warton jelly derived mesenchymal stem cells and Nilotinib at week 2 revealed significant reduction of ALT in mesenchymal stem cell group than that in group received nilotinib ($p \leq 0.015$) with statistically significant improvement in area of fibrosis in nilotinib group ($p \leq 0.000$). In our study, there was no statistical significance between BM mesenchymal stem cells and warton jelly derived mesenchymal stem cells at week 2.

At week 6, there was significant improvement in the area of fibrosis percent between the BM, warton jelly mesenchymal stem cells and nilotinib treated groups with significant reduction in fibrosis with nilotinib group ($p \leq 0.000$) with no statistical significance between the BM and warton jelly derived mesenchymal stem cells [14,15].

Our study found that the warton jelly derived mesenchymal stem cells have higher regenerative power compared with the remaining treated groups confirmed by detection of human albumin gene by RT-PCR technique indicating homing of transplanted stem cells with development of ef-

fective functioning hepatocytes in the rat's liver (Tsai et al., 2009). Nilotinib is a 2nd generation BCR-ABL inhibitor, it was developed for treatment of imatinib resistant chronic myeloid leukemia.

Beside being used in treatment of leukemia, it was discovered that it inhibited c-Kit, PDGFR and the TEL-PDGFRα and FIP1L1-PDGFRα fusion proteins[16].

Based on Shaker et al., 2011, in our study, nilotinib was given orally to the rats at week 2 and week 6 of liver fibrosis in a dose 20mg/kg, it exerted significant antifibrotic effect specially at week 2 when compared with the control vehicle group.

Despite of its effectiveness as antifibrotic medication, the regenerative power of the liver was not significantly different as we have detected with the warton jelly deived mesenchymal stem cells.

Our study was conducted to ensure the potency of mesenchymal stem cells or nilotinib as antifibrotic, we concluded that the warton jelly derived stem cells has the most regenerative power when compared with either BM derived stem cells or nilotinib, and we consider that nilotinib is a potent antifibrotic drug in vivo which will give the hope to many patients with liver fibrosis in complete cure.

Conclusion

Hepatic fibrosis is reversible wound-healing response to either acute or chronic cellular injury that reflects a balance between liver repair and scar formation. During acute injury, the changes in liver architecture are transient and reversible. With chronic injury, there is progressive substitution of the liver parenchyma by scar tissue. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension and often requires liver transplantation.

Chronic HCV is a major health problem in Egypt with a 10% prevalence of chronic HCV infection among persons aged 15-59 years, which, if left untreated, can result in cirrhosis and liver cancer.

Eighty female Sprague Dawely rats were used for the experimen-

tal study, they were divided into 4 main groups, each group consists of 20 rats and each group divided into 2 subgroups (a up ta stage 3, b stage 4 up to stage 8). Rats were injected by carbon tetrachrolide (CCl4) by dose 0.5 mg/kg in olive oil in ratio of 1:1 intraperitonially twice per week for 2 weeks and 6 weeks and rats were sacrificed 2 weeks after treatment (mesenchymal stem cells and nilotinib). Each group was submitted for the following laboratory investigations: alanine-amino transferase (ALT), aspartate-amino transferase (AST), international normalized ratio (INR) and serum albumin. Also, liver was fixed by formalin 10% for histopathological examination by heamtoxlin and eosin and maisson trichrome stains which are summarized as follows:

1. There was significant elevation in AST and ALT levels in control groups with their significant reduction in those values In groups treated by BM stem cells, warton jelly derived mesenchymal stem cells and nilotinib with no significant difference in other laboratory parameters.

2. There was significant reduction in fibrosis score in the treated groups when compared with the control groups with best results obtained with the nilotinib treated group.

3. The results obtained by histopathological examination were confirmed by quantitative assessment of the fibrosis using image J software with best results with nilotinib treated group.

4. For the regeneration score, it was significantly higher in group treated with warton jelly derived mesenchymal stem cells.

5. At week 2, there was significant improvement in s. albumin, regeneration score in warton jelly derived mesenchymal stem cells group and significant reduction in area of fibrosis percent in nilotinib group with no significant difference between BM and warton jelly mesenchymal stem cells.

6. At week 6, there was only significant improvement in the area of fibrosis percent with best results obtained with the nilotinib treated group.

When we compared between

the control groups at week 2 and week 6 there is significant elevation in fibrosis score, regeneration score and area of fibrosis percent in week 6 with no statistical difference in the laboratory parameters.

For the warton jelly derived mEsenchymal stem cells, we have detected that the transplanted cells transdifferentiated into functioning hepatocytes by detection of human albumin gene by RT-PCR technique.

For the nilotinib treated goup, there was no significant difference between the treated group at week 2 and those which were treated at week 6.

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I would like to express my deep gratitude to Prof. Dr. Gamal Shiha professor of internal medicine (Head of Hepatology and Gastroenterology Unit, faculty of medicine, Mansoura University), Prof. Dr. Mohamed Abd Al Kader Sobh professor of internal medicine (Head of medical experiental research center (MERC), faculty of medicine, Mansoura University) for their great support in this work. Also, I would like to thank Prof. Dr. Maha Maher professor of internal medicine for her great effort in helping, writing, improving the manuscript and her sincere supervision and her advice.

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BENHA MEDICAL JOURNAL

STUDY OF MESENCHYMAL STEM CELLS VERSUS NILOTINIB IN MANAGEMENT OF CARBON TETRACHLORIDE INDUCED LIVER FIBROSIS IN SPRAGUE DAWELY RATS

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RELATIONSHIP BETWEEN PLASMA CELL DENSITY AND STAGE OF FIBROSIS IN PEDIATRIC AUTOIMMUNE HEPATITIS

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Abstract

Background/Aim: *Autoimmune hepatitis is a chronic inflammatory disease of the liver, which frequently results in cirrhosis. The present study aimed to verify the relationship between plasma cells and stage of fibrosis in children with autoimmune hepatitis.*

Methods: *We analysed the clinical and histological features of 24 pre-treatment, patients with autoimmune hepatitis. Nine out of 24 had a second liver biopsy performed post-treatment to evaluate the response to therapy. Haematoxylin and eosin (H&E), and Masson's trichrome stain were carried out on liver biopsies.*

Results: *A positive significant correlation was observed between plasma cells and stage of fibrosis (the stages were evaluated according to METAVIR's staging system for chronic hepatitis). Additionally, we also observed a reduction in the number of plasma cells, and fibrosis in patients who had successfully been treated and had a second post-treatment liver biopsy.*

Conclusion: *Our findings support that the number of plasma cells can be a surrogate marker for the severity of liver disease, reflecting the stage of fibrosis.*

Keywords: *Autoimmune hepatitis, plasma cells, Liver fibrosis, Liver biopsy, Portal infiltrate*

Introduction

Autoimmune hepatitis is a chronic liver disease of unknown etiology which can affect patients of all ages, races, and both sexes, characterized by interface hepatitis, hypergammaglobulinaemia - mostly of the IgG class and circulating autoantibodies[1].

Autoimmune hepatitis (AIH) is characterized by autoimmune injury to the hepatocytes, resulting in hepatic fibrosis and cirrhosis. It more frequently affects young patients (including children) and females[2]. The response to immunosuppressive therapy is excellent in most patients, including cases with significant regression of fibrosis, although definitive cure is rarely (if ever) achieved[3].

Diagnosis of AIH is based on a series of positive and negative criteria defined by the International AIH Group. These criteria have been produced mainly for research purposes[4]. Only in 2008 a simplified scoring system has been devised for wider applicability in routine clinical practice. This simplified scoring system includes just four criteria: autoantibody detection, IgG levels, liver histology, and exclusion of viral hepatitis[5].

Histological features of AIH can be varied, but all patients display some degree of interface hepatitis. Typical features of AIH are rosettes, plasma cell enrichment and conspicuous emperipolesis by lymphocytes (emperipolesis: active penetration by one cell into and through larger cell)[6].

Interface hepatitis is the sine qua non of classic AIH. Plasma cells are typically abundant at the interface and may be present throughout the acinus. Thirty-four percent of patients with autoimmune hepatitis have few or no plasma cells, and their absence does not preclude the diagnosis[7].

Progression to cirrhosis occurs in 40-80% of cases. Inflammatory activity often subsides when cirrhosis has developed, making it difficult to distinguish end-stage AIH from other causes of cirrhosis. The presence of cirrhosis at the time of presentation has also been associated with an adverse prognosis[8].

Follow-up biopsies should be considered because there is 5% per year progression to cirrhosis and this is much higher in patients with continuing inflammation. Twenty-five percent of patients with normal transaminase levels have inflammatory lesions on biopsy. Also it is important to repeat biopsy for confirmation of remission prior to a trial of treatment withdrawal. Patients in remission with minimal inflammatory activity on biopsy have a higher chance of successful treatment withdrawal[9].

The pathologist is the key arbiter of treatment response, and the histologic interpretation may affect treatment duration. The biopsy findings should be compared with those of any previous biopsy specimen. Restoration of normal hepatic architecture during treatment is associated with a 20% frequency of relapse after drug withdrawal; the presence of portal hepatitis is associated with a 50% frequency of relapse; and the presence of interface hepatitis of any degree or progression to cirrhosis is associated with an 87% to 100% frequency of relapse[10].

Plasma cells originate from B-lymphocytes that undergo cellular differentiation in response to antigenic stimulus. They are able to produce and release a high amount of antibodies that are fundamental to humoral immune response. Nevertheless, they are also involved in undesirable situations such as autoimmune diseases[2]. A study Verma et al., showed that globulin levels and cirrhosis are independent predictors of poor outcome in development of AIH, and plasma cells were associated with relapse[11].

Thus, it is not clear if the number of plasma cells in liver biopsies has significance in AIH. The present study aimed to verify the relationship between Plasma cells and stage of fibrosis in patients with AIH.

Material and Methods

The study involved 24 children diagnosed with AIH. Nine out of 24 had a second liver biopsy performed post-treatment to evaluate the response to therapy. A total 33 tru cut liver biopsy were collected from our Mansoura University Children's hospital pathology laboratory.

A) Clinical and laboratory data:

Routine clinical examinations, biochemical and serological studies were performed for all patients at the time of first admission to the Pediatric Gastroenterology and Hepatology unit. These data were available in the hospital files. Biochemical studies performed for all patients included Alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ glutamyl transpeptidase (GGT), alkaline phosphatase (AP), total and direct serum bilirubin, serum albumin and prothrombin time.

Medical records were reviewed for serologic findings, including results of serum levels of IgG, anti nuclear (ANA), anti smooth muscle (SMA), anti liver kidney microsomal type 1 (LKM-1) antibody and anti-mitochondrial antibody (AMA).

None of the patients had a previous or current drug history. Cases with Chronic hepatitis C, B or genetic liver diseases (Wilson's disease, α1-antitripsin deficit and hereditary haemochromatosis) were excluded.

B) Radiologic evaluation:

Ultrasonographic evaluation of the abdomen and MRCP (magnetic resonance cholangiopancreatography) were done for assessment of hepatobiliary system.

C) Histological evaluation:

Paraffin embedded blocks of the selected 33 tru cut liver biopsy was retrieved. We used 5 μm thick serial sections that were rewashed, rehydrated and stained with haematoxylin-eosin (H&E), and Masson's trichrome stain. Only biopsies at least 10mm long and containing more than six portal tracts were evaluated.

We investigated the typical histological findings of AIH, such as mononuclear and plasma cells infiltration of the portal spaces expanding into the liver lobule with interface hepatitis; and rosette formation[6]. We also searched for conspicuous emperipolesis by lymphocytes (emperipolesis: active penetration by one cell into and through larger cell) as a part of typical histological features of AIH according to the simplified scoring system[5].

We counted the number of

plasma cells per high power field (HPF) in the three most representative portal tracts for each specimen, calculated the mean values of the number of plasma cells present in the three most representative portal tracts. The number of the plasma cells were graded as minimal (1-2/HPF), mild (3-5/HPF), moderate (6-10/HPF) and marked (>10/HPF) (figure 1A-D).

Finally, the severity of the disease, as regards the degree of fibrosis (staging) was evaluated according to the METAVIR's staging system.

D) Statistical methods:

The statistical analysis of data was done by using Excel program and statistical package for social science (SPSS) program version 16. Continuous variables were summarized as mean±standard deviation (SD), and categorical variables as frequency and percentage. Significant differences were calculated using the ANOVA. Correlation coefficients were calculated by Spearman's test. Differences were considered significant for p values of <0.05.

Results

All 24 untreated pediatric patients ranged in age from 2 to 14 years old with mean age 8.25±3.87, including 18 females and 6 males. The clinical, biochemical, serological and histological features of patients at baseline were shown in table 1.

All patients were classified as having a 'definitive' diagnosis of AIH according to the simplified scoring system.

Plasma cell densities with its different grades were evaluated. A positive significant correlation between plasma cell density and stage of fibrosis was found (p=0.007). It was noticed that the more density of plasma cell infiltrate, the higher the stage of fibrosis (table 2, figure 2).

Nine cases underwent follow-up liver biopsy after one to two years from starting treatment. The mean age of these patients was 10.11±3.33, including 6 females and 3 males.

The changes in the biochemical, serological and histological

features from the pre to post treatment state were summarized in table 3. There was no difference in autoantibodies positivity along treatment course.

Eight out of 9 patients met clinical, laboratory, and histological criteria for response to treatment. Six patients had a reduction in liver fibrosis. The variables of sex, age, and presence of autoantibodies, were not able to predict if the AIH patients would have or have not a reduction in stage of fibrosis after treatment. On the other hand, the liver specimens from patients who had reduction in fibrotic stage also presented a significantly decreased number of plasma cells after therapy (p= 0.005) (table 4, figure 3 & 4). Patients with no reduction in the fibrotic stage of liver fibrosis did not present a significant decrease in number of plasma cells after therapy. Although it is only one case that showed progression of fibrosis, we observed increased number of plasma cell (figure 5).

Table (1): Clinical, biochemical and histological features of pre-treated 24 patients.

	AIH (n=24)
Mean age (years)	8.25±3.87
Female gender	18(75%)
Abdominal enlargement	20(83.3%)
Jaundice	8(33.33%)
Associated autoimmune disorders	0
ALT (n:0–35 U/L)	367.38±219.32
AP (n: 30–120 U/L)	127.42±60.23
GGT (n:6–19 U/L)	44.63±28.21
Serum IgG (mg/dL)	3173±1184.95
Auto-antibodies	24(100%)
ANA alone	5(20.8%)
ASMA alone	7(29.2%)
ANA+ASM A	9(37.5%)
anti- LKM-1	3(12.5%)
AMA	0
None detected	0
Viral hepatitis (B & C) negative	24(100%)
Histology	
Bile ductular proliferation	9(33.5%)
Cholangiolitis	2(8.3%)
METAVIR staging	
F1	3(12.5%)
F2	4(16.7%)
F3	14(58.3%)
F4	3(12.5%)
Plasma cell density	
Minimal	1(4.2%)
Mild	10(41.7%)
Moderate	7(29.1%)
Marked	6(25%)

* Continuous variables: mean±standard deviation; categorical variables: absolute value (%).

Table (2): Correlation between plasma cell density and stage of fibrosis according to METAVIR staging system (One Way ANOVA).

Fibrosis	N	Mean ± Std. Deviation	*P*
F1	3	1.67 ± 0.58	(p=0.007)
F2	4	2 ± 0.0	
F3	14	3.1 ± 0.83	
F4	3	3.3 ± 0.58	

Table (3): Biochemical, serological and histological features of patients in the pre and post treatment state.

	Pre-treatment	Post-treatment
	Mean ± Std. Deviation	**Mean ± Std. Deviation**
Serum IgG (mg/dL)	2898.22 ± 846.6	1954.67 ± 757
ALT (n:0–35 U/L)	503.22 ± 237.65	181.56 ± 92.54
AP (n: 30–120 U/L)	332.67 ± 278.03	150 ± 102.81
GGT(n:6–19 U/L)	131 ± 92.75	61.56 ± 43.62
Auto-antibodies		
ANA alone	5	5
ASMA alone	0	0
ANA+ASM A	4	4
anti- LKM-1	0	0
AMA	0	0
None detected	0	0
Histology		
Bile ductular proliferation	5(55.6%)	1(11.1%)
Cholangiolitis	2(22.2%)	0
Plasma cell density		
Minimal	0	1
Mild	3	6
Moderate	4	1
Marked	2	1

* Continuous variables: mean±standard deviation; categorical variables: absolute value (%).

Table (4): Correlation between plasma cell density and stage of fibrosis according to METAVIR staging system in post-treatment group (One Way ANOVA).

Fibrosis	N	Mean ± Std. Deviation	*P*
F1	2	1.5 ± 0.71	(p =0.005)
F2	5	2 ± 0.0	
F3	1	3 ± 0.0	
F4	1	4 ± 0.0	

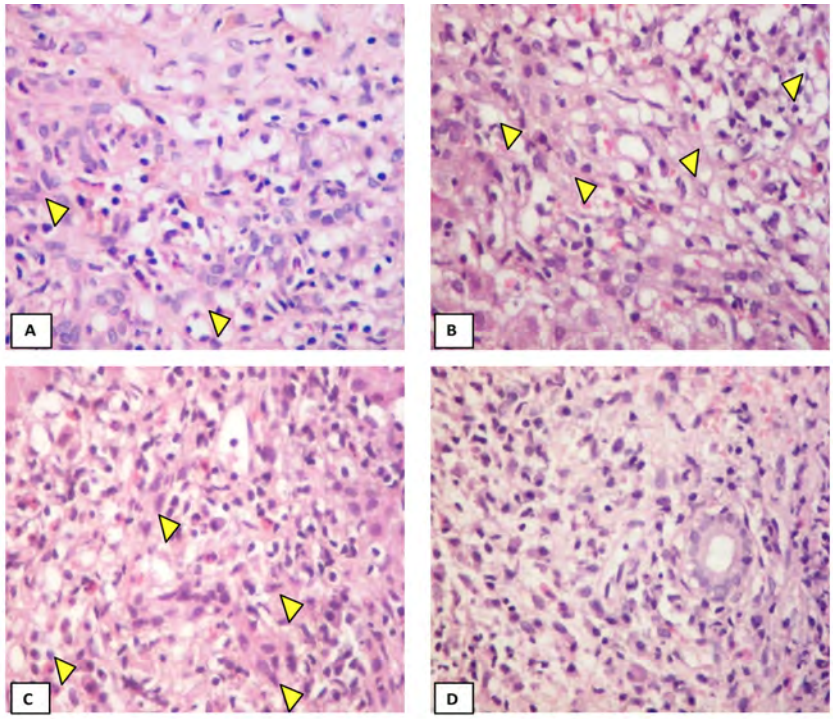

Fig. 1: Different grades of plasma cell density; H&E: x400. **(A)** Photomicrograph of minimal plasma cells (1-2/HPF) (arrow head). **(B)** Photomicrograph of mild plasma cells (3-5/HPF) (arrow head). **(C)** Photomicrograph of moderate plasma cells (6-10/HPF) (arrow head). **(D)** Photomicrograph of marked plasma cells (>10/HPF).

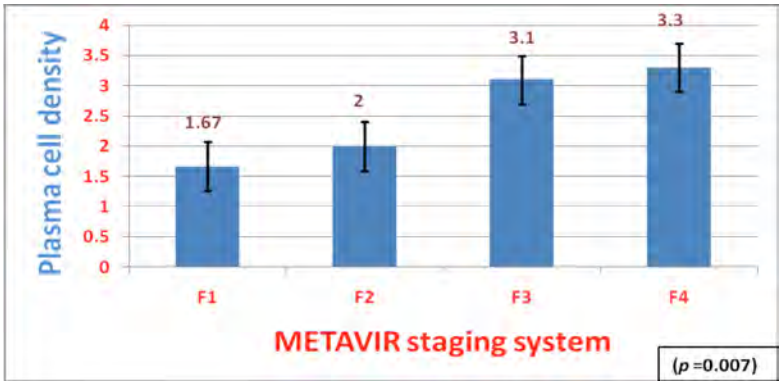

Fig. 2: Correlation between plasma cell density in different stage of fibrosis according to METAVIR staging system.

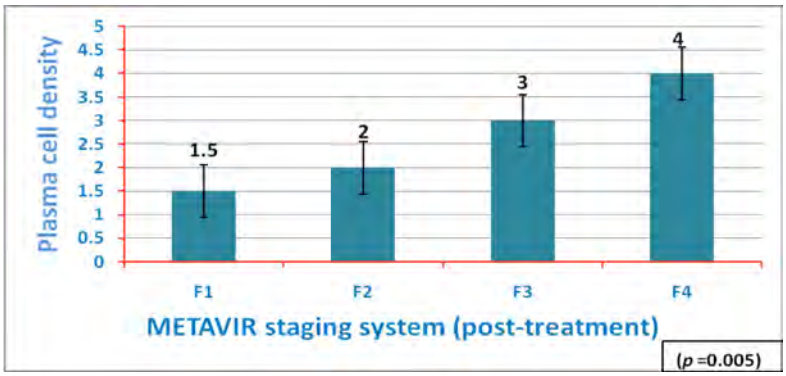

Fig. 3: Correlation between plasma cell density and stage of fibrosis according to METAVIR staging system in post-treatment group.

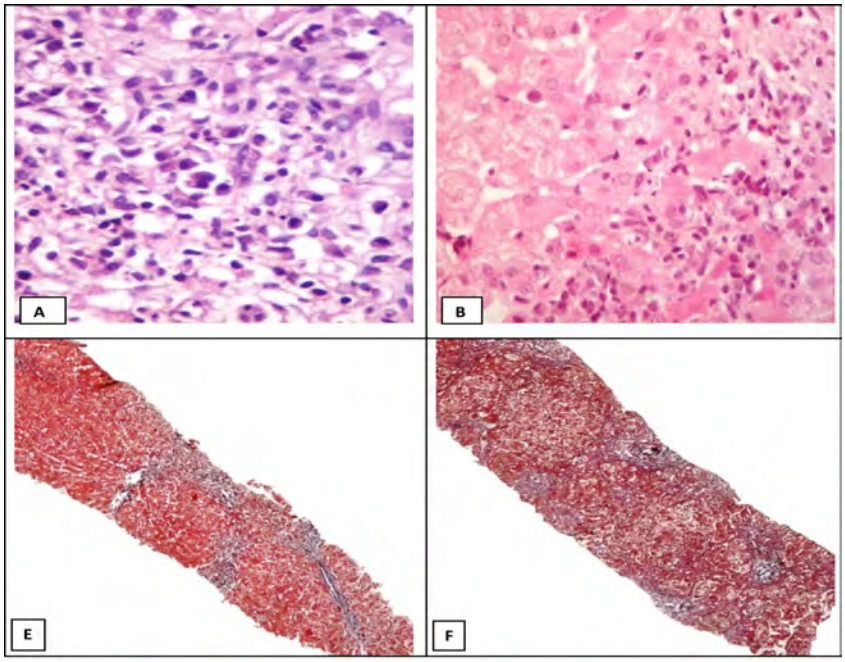

Fig. 4: Histological features of pre and post-treatment liver biopsy in patient with AIH. **(A)** Photomicrograph of pre-treatment portal tracts showing moderate plasma cell infiltrate (6-10/HPF); H&E: x400. **(B)** Photomicrograph of post-treatment portal tracts showing mild plasma cell infiltrate (3-4/HPF); H&E: x400. **(C)** Photomicrograph of pre-treatment portal tracts showing early bridging fibrosis; Masson's trichrome: x40 and **(D)** Photomicrograph of post-treatment portal tracts showing fibrous expansion; Masson's trichrome: x40.

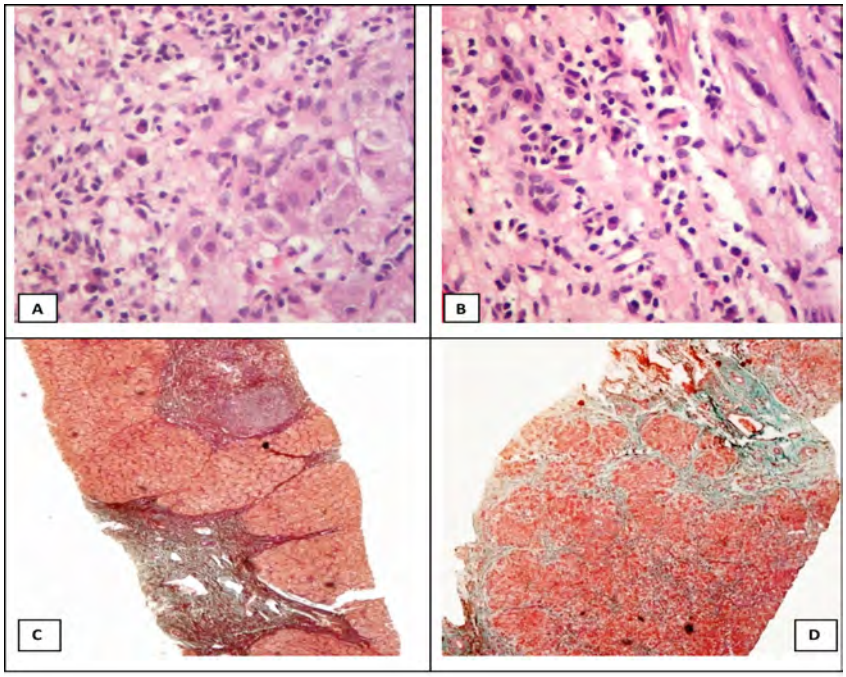

Fig. 5: Histological features of pre and post-treatment liver biopsy in patient with AIH. **(A)** Photomicrograph of pre-treatment portal tracts showing moderate plasma cell infiltrate (6-10/HPF); H&E: x400. **(B)** Photomicrograph of post-treatment portal tracts showing marked plasma cell infiltrate (>10/HPF); H&E: x400. **(C)** Photomicrograph of pre-treatment portal tracts showing marked bridging fibrosis with occasional nodule formation; Masson's trichrome: x40 and **(D)** Post-treatment portal tracts showing established cirrhosis; Masson's trichrome: x40.

Discussion

Chronic inflammatory infiltrate, often plasma cell-rich, is a common feature in autoimmune hepatitis. The analysis of liver biopsies from patients with AIH showed a variable number of plasma cells but conistantly present. This finding emphasized the importance of the plasma cell as one of the main criteria that favor the diagnosis of AIH[2]. The number of plasma cells in AIH patients was positively correlated to stage of fibrosis. Thus, a greater number of plasma cells could imply a more amount of fibrosis.

When follow-up liver biopsies from treated patients were examined comparing the number of plasma cell before and after thera-

py, we observed that patients who presented reduction in fibrotic stage had a concomitant decrease in the number of plasma cell. In contrast, in those without reduction of fibrosis, no reduction in the number of plasma cell was observed. Although it is only one case that showed progression of fibrosis, we observed increased number of plasma cell. Therefore, the number of plasma cell may serve as a prognostic marker in the biopsy after therapy. This finding supports the idea that there is an intimate relationship between numbers of plasma cell and liver fibrosis in most cases of AIH.

A study by Verma et al.,[11] showed that plasma cell is not an independent predictor of poor outcome in the development of AIH and that the number of plasma cell is not predictive of response to therapy.

A recent study by Brandão et al.,[12] performed on 33 patients diagnosed with AIH showed a significantly higher number of plasma cell and hepatic stellate cells (HSC) compared to normal livers. Moreover, they have detected frequent co-localization of plasma cell and activated HSC in liver biopsies from AIH patients. However, they did not confirm if plasma cell has a role in the activation of HSC, or if the plasma cell number is a result of the influence of the same stimulus that acts on HSC.

In people being followed up after treatment, the presence of mild portal inflammatory changes frequently persist and do not appear to be associated with an adverse outcome. However the presence of portal plasma cells has been associated with an increased risk of relapse after drug withdrawal[13].

In conclusion, we found a close correlation between plasma cells and liver fibrogenesis in AIH. The number of plasma cell was correlated to the severity of liver fibrosis in liver biopsies obtained before AIH treatment. After AIH treatment, we observed a concomitant reduction in plasma cell and liver fibrosis. Collectively, these findings point to the importance of plasma cell number as a surrogate marker of liver-disease severity indicated by the amount of fibrosis.

List of abbreviations:

AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibody; ANA, antinuclear antibody; AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; H&E, haematoxylin and eosin; HPF, high power field; HSC, hepatic stellate cells; LKM-1, liver kidney microsomal type 1 antibody; MRCP, magnetic resonance cholangiopancreatography; SMA, smooth muscle antibody.

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RELATIONSHIP BETWEEN PLASMA CELL DENSITY AND STAGE OF FIBROSIS IN PEDIATRIC AUTOIMMUNE HEPATITIS

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RESVERATROL ENHANCES THE NEUROPROTECTIVE EFFECT OF DIAZEPAM ON PILOCARPINE INDUCED SEIZURES IN RATS

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Abstract

In this study, we aimed to investigate the potential enhancement effects of natural an antioxidant anti-inflammatory drug (resveratrol) on the anticonvulsant effect of one of established commonly used antiepileptic drug (diazepam) on pilocarpine induced seizure in rats. The study was carried out in 50 rats weighing 150-200 g subdivided into five groups each of which consisted of 10 rats. Group1: Rats were given saline 0.9 % intraperioneally and served as control group. Group2: Rats were given pilocarpine dissolved in normal saline and the pH adjusted to 7.4 and was given in a single dose of 400mg.Group3: Received resveratrol single dose of 40 mg/kg i.p. administered 30 min prior to pilocarpine.Group4: Received diazepam single dose of 10mg/kg administered 30 min prior to pilocarpine. Group5: Received diazepam and resveratrol administered 30 min prior to pilocarpine in a dose as given in 3, 4.Then animals were decapitated under ether anesthesia, and the hippocampus was collected and homogenized, the homogenates were centrifuged and the supernatant was used for determination of cytokines (Interleukin-1beta (IL-1β), Interleukin-6 (IL-6), Tumor necrosis factor alpha (TNFα).nitric oxide (NO), malondialdhyde level (MDA), superoxide dismutase, catalase (CAT), and glutathione peroxidase activity were done. The results of this work revealed that resveratrol lowered efficiently brain cytokines, NO, MDA while increase brain superoxide dismutase, CAT, and glutathione peroxidase activity on and this was reflected by delaying onset of convulsion and mortality rates in rats. In conclusion, The study showed that resveratrol potentiate the anticonulsant effect of diazepam and this may be of benefit in lowering incidence of resistance to diazepam and also may allow decrease its dose and side effects in further studies.

Introduction

Epilepsy is the most frequent neurodegenerative disease after stroke. It affects more 50 million people worldwide[1]. More than 30% of patients with epilepsy continue to have inadequate control of their seizures despite medical treatment[2]. Currently, more than 20 antiepileptic drugs (AEDs) approved by the U.S. Food and Drug Administration for the treatment of epilepsy. However, all of these medications are associated with side effects. Commonly encountered adverse side effects include gastrointestinal disturbance, central nervous system symptoms such as somnolence, dizziness, and mood/ behavioral disturbance. Furthermore, potentially serious side effects may occur with some AEDs: these include bone marrow suppression, Stevens-Johnson syndrome, and teratogenicity[3]. Though several two-AED combinations are useful for patients with refractory epilepsy [4]. Approximately 14% of epileptic patients are remain refractory to AED combination[2]. Therefore, a need was felt for some novel combinations of AEDs or a combination of AEDs with natural products possessing anti seizures activity for suppressing of epileptic attacks.

A great deal of the knowledge that has improved our understanding of epileptic disorders has derived from appropriate animal models[5]. Experimental models of seizures and epilepsy have been valuable for understanding of the basic mechanisms underlying ictogenesis and epileptogenesis as well as for developing new therapeutic options[6]. This is certainly the case in temporal lobe epilepsy (TLE), the most common type of partial complex seizure in adulthood[7]. One of the earliest and most frequently used models for evoking experimental status epilepticus and subsequent epilepsy is the systemic injection of pilocarpine in rodents[8]. Pilocarpine is a cholinergic agonist for developing experimental models of intractable epilepsy with seizures resembling status epilepticus or complex partial seizure in human on study of seizure-induced brain injury[9].

Evidence supports the hypothesis that experimental epilepsy is mediated by oxidative stress[10]. It

is also known that oxidative stress occurs as a result of seizure[11]. The high rate of oxidative metabolism, coupled with the low antioxidant defenses and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage[12].

Clinical and experimental studies have provided proof-of-concept evidence that brain inflammation is an important factor in epilepsy, In particular, high levels of proinflammatory cytokines [e.g. interleukin (IL)-1beta, tumour necrosis factor (TNF)-alpha[13]. The involvement of cytokines in the pathogenesis of epilepsy has been suggested by the evidence that limbic seizures increase messenger RNA (mRNA) of inflammatory cytokines in rodent forebrain[14]. In addition, the release of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) from rat hippocampal slices is enhanced by seizures[15]. Moreover, cytokine expression in immature brain is associated specifically with cell injury rather than with seizures per se, suggesting that proinflammatory cytokines may contribute to the occurrence of status epilepticus induced hippocampeal damage[16].

Resveratrol is a non-flavonoid polyphenolic compound found in grapes and the red wine prepared from them. Resvertrol shows pleiotropic health beneficial effects, including anti-oxidant, anti-inflammatory, anti-aging, cardioprotective and neuroprotective activities[17]. Resveratrol has been shown to modulate lipoprotein metabolism[18], eicosanoid synthesis[19], lipid oxidation[20] and platelet aggregation[21]. Resveratrol also suppresses the induction of nitric oxide synthase and disrupts arachidonic acid metabolism by inhibiting cyclooxygenase-2[22] The ability to enter the brain after a peripheral administration and no adverse effects on the brain or body are other features that are appealing for using this compound as a therapy for brain injury or neurodegenerative diseases[23].

Based on the consideration above, the objectives of the present study were to evaluate the potential enhancement effects of natural an antioxidant anti-

inflammatory drug (resveratrol) on the anticonvulsant effect of one of established commonly used drug (diazepam) on pilocarpine induced seizure in rats in an attempt to increase the efficiency or decrease the dose of diazepam.

Material and Methods

Chemicals:

1. Thiobarbituric acid: (Sigma chemical CO., USA).

2. Nitric oxide kits (R & D System, inc., USA).

3. Total protein kits: Diamond Biodiagnosis, Egypt.

4. Ethanol 99.9%: Diamond Biodiagnosis, Egypt.

5. Saline 0.9%.

6. Cytokines kits (Interleukin-1beta (IL-1β), Interleukin-6 (IL-6), Tumor necrosis factor alpha (TNFα) ((Sigma chemical CO).

7. Superoxide dismutase, catalase and glutathione peroxidase kits (Randox Laboratories Ltd., UK).

Drugs:

1. Pilocarpine hydrochloride (Sigma chemical CO., USA).

2. Resveratrol: Purchased from the Sigma Chemical Co. and was dissolved in normal saline and given in a dose of 40 mg/kg i.p.[24].

3. Diazepam: Purchased from Roche/Egy and given in a dose of 10 mg/kg i.p[25].

Animals:

Fifty albino rats obtained from local animal house weighing 150-200 g were use throughout this study, animals were housed in standard cages under a12-hour-light/12-hour- dark cycle and had free access to food and water.

Experimental design:

Animals were subdivided into five groups, each of which consisted of ten rats as follow:

Group1: Rats were given saline 0.9 % intraperioneally and served as control group.

Group2: Rats were given pilocarpine dissolved in normal saline and the pH adjusted to 7.4 and was given in a single dose of 400mg[26].

Group3: Received resveratrol single dose of 40 mg/kg i.p. administered 30 min prior to pilocarpine[27].

Group4: Received diazepam single dose of 10mg/kg[28] administered 30 min prior to pilocarpine.

Group5: Received diazepam and resveratrol administered 30 min prior to pilocarpine in a dose as given in 3, 4.

Rats were put into individual cages and then had been observed for 24h following pilocarpine injection for behavioral changes. The behavioral changes observed were similar to those recorded by Turski et al. 1983[27] where animals were motionless for 5-10 min after pilocarpine administration and subsequently displayed oro-facial movements, salivation, eye-blinking, twitching of vibrissae, and yawning. This activity persisted up to 45 min. Then, discontinuous seizures were observed 30 min after injection and lasted up to 90-150 min, before giving way to limbic motor seizures with intense salivation, rearing, upper extremity clonus, and falling. Such seizures were observed every 5-15 min, presenting maximal frequency after 1-2 h. SE spontaneously remitted 5-6 h after pilocarpine administration, and the animals entered post-ictal coma, lasting 1 days. . The time from injection of each chemical to the first appearance of seizure activity was measured for each animal and is referred to as the seizure latency. Only animals with seizure activity were considered to calculate the latency to the onset of seizures. The latency to death was observed in the cut- off 24 hour.

Then animals were decapitated under ether anesthesia, and the hippocampus was collected and homogenized in 2 volumes of chilled 50 Mm potassium phosphate buffer PH 7.0) for further assay. Homogenates were centrifuged at 3000 g for 10 minutes at 4C. The supernatant was transformed to chilled Eppendorf tubes and used for determination of:

• Cytokines (Interleukin-1beta (IL-1β), Interleukin-6 (IL-6), Tumor necrosis factor alpha (TNFα).

Nitric oxide measurement was done by ELISA using total NO/ Nitrite/Nitrate assa[28].

• Malondialdhyde level was measured based on method of Draper and Hadley 1990[29].

• Superoxide dismutase activity assay was analyzed using the methods of Tsai et al. 2010[30].

• Catalase activity assay was

analyzed using the methods of Aebi 1984[31].

• Glutathione peroxidase activity was analysed using the method of Paglia and Velentine 1967[32].

Statistical analysis:

All results are expressed as mean ± standard error of mean. Comparisons between the groups were performed by one-way ANOVA followed by Student t-test. Differences were considered significant at P<0.05.

Results

a- Administration of pilocarpine single dose of 400 mg intraperiotneally produced significant increase in number of animals having tonic/clonic convulsion, significant increase in mortality rate (p<0.05), significant increase in onset of convulsion with significant decrease in interval until death (p<0.05), significant increase in tissue levels of Interleukin-1beta (IL-1β), Interleukin-6 (IL-6) and Tumor necrosis factor alpha (TNFα) (p<0.05) together with significant increase in tissue levels of NO an MDA levels p<0.05) while produced significant decrease in tissue levels of superoxide dismutase, catalase and glutathione peroxidase activity (p<0.05) when compared to control group .

b- Administration of resveratrol 40 mg/kg i.p.30 min prior to pilocarpine produced significant decrease in number of animals having tonic/clonic convulsion, significant decrease in mortality rate (p<0.05), significant delay in onset of convulsion with significant increase in interval until death (p<0.05), significant decrease in tissue levels IL-1β, IL-6 and TNF-α (p<0.05), significant decrease in tissue levels of NO an MDA levels p<0.05),significant increase in tissue levels of superoxide dismutase, catalase and glutathione peroxidase activity (p<0.05) when compared to pilocarpine group.

c- Administration of diazepam 5mg/kg 30 min prior to pilocarpine produced significant decrease in number of animals having tonic/clonic convulsion with significant decrease in mortality rate (p<0.05), significant delay in onset of convulsion with significant increase in interval until

death ($p<0.05$), insignificant changes in il-1β, IL-6 and TNF-α, NO, or MDA ,superoxide dismutase, catalase orglutathione peroxidase levels when compared to pilocarpine group

d- Administration of both resveratrol and diazepam 30 min prior to pilocarpine produced significant decrease in number of animals having tonic/clonic convulsion with significant decrease in mortality rate ($p<0.05$), significant delay in onset of convulsion with significant increase in interval until death, significant decrease in tissue levels IL-1β, IL-6 and TNF-α ($p<0.05$), significant decrease in tissue levels of NO an MDA levels $p<0.05$), significant increase in tissue levels of superoxide dismutase, catalase and glutathione peroxidase activity $p<0.05$) when compared to pilocarpine group.

Table (1): Incidence of convulsions and death among animals of different studied groups during the observation period.

Groups	Number of animals having tonic/ clonic convulsion	Percentage of animals having tonic/ clonic convulsion (%)	Mortality (number of dead animals)	Mortality rate (%)	Onset of convulsion (min)	Interval until death(min)
Group 1 (control)	0	0	0	0	0	0
Group 2 (pilocarpine)	10^{*}	100^{*}	10^{*}	100^{*}	$14 \pm 0.61^{*}$	$17 \pm 0.66^{*}$
Group 3 (Resveratrol)	$5^{\neq}$	$50^{\neq}$	$5^{\neq}$	$50^{\neq}$	$30 \pm 0.81^{\neq}$	$35 \pm 0.86^{\neq}$
Group 4 (Diazepam)	$3^{\neq}$	$30^{\neq}$	$3^{\neq}$	$30^{\neq}$	$45 \pm 0.51^{\neq}$	$50 \pm 0.97^{\neq}$
Group 5 (Resveratrol plus diazepam)	$1^{\neq}$	$10^{\neq}$	$1^{\neq}$	$10^{\neq}$	$56 \pm 0.71^{\neq}$	$58 \pm 0.57^{\neq}$

Number of animals in each group = 10
The observation time was 24 hours
***Significant when compared to control group ($P<0.05$).**
≠ Significant when compared to pilocarpine group ($P<0.05$).

Table (2): Effect of resveratrol, diazepam and both on Interleukin-1beta (IL-1β), Interleukin-6 (IL-6), Tumor necrosis factor alpha (TNFα), nitric oxide, malondialdhyde level, Superoxide dismutase, Catalase and glutathione peroxidase activity.

Groups	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)	NO (µM/ mg protein)	MDA (µM/ mg protein)	Superoxide dismutase (U/mg protein)	Catalase (U/ mg protein)	Glutathione peroxidase (U/mg protein)
Control	12 ±0.68	21±0.74	57±1.48	15.1±1.1	3.4 ±0.4	6.9 ±0.31	3.5 ±0.14	0.48 ±0.02
Pilocarpine	136 ±1.48*	249±1.48*	613±3.1*	49±1.39*	9.2 ±0.2*	4.16±0.15*	2.1 ±0.21*	0.28 ±0.01*
Resveratrol	30 ±0.68≠	80±1.15≠	210±2.7≠	18.7±1.3≠	4.4±0.2≠	4.9 ±0.21≠	2.87±0.1≠	0.42 ±0.01≠
Diazepam	135 ±1.26	250±1.96	610 ±2.62	47± 1.74	9.1±0.14	4.1 ± 0.14	2.2 ±0.14	0.26 ±0.03
Resveratrol plus diazepam	32 ±1.05≠	77 ±1.28≠	205±2.4≠	19±1.05≠	4±0.34≠	4.91±0.2≠	3.3 ±0.22≠	0.4 ±0.01≠

Each value represents Mean ± SEM
Number of animals in each group = 10
The observation time was 24 hours
***Significant when compared to control group (P<0.05).**
≠ Significant when compared to pilocarpine group (P<0.05).

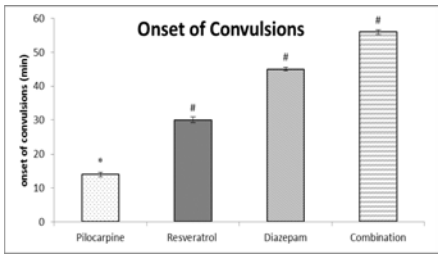


Fig. 1: *Significant when compared to control group (P<0.05).
≠Significant when compared to pilocarpine group (P<0.05).

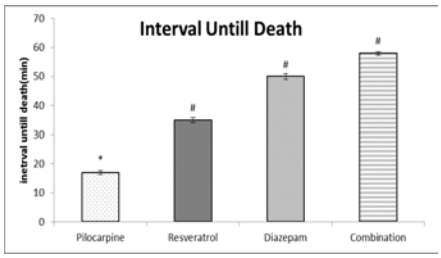
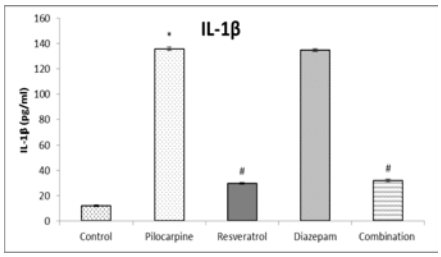


Fig. 2: *Significant when compared to control group (P<0.05).
≠Significant when compared to pilocarpine group (P<0.05).



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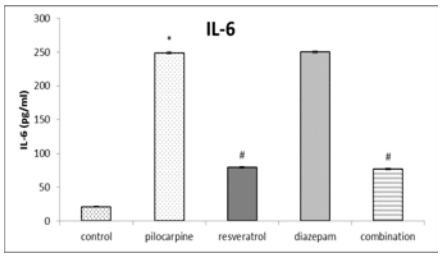

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≠Significant when compared to pilocarpine group (P<0.05).

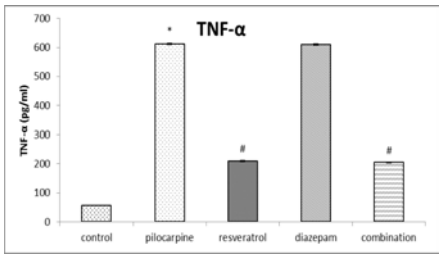

Fig. 5: Significant when compared to control group (P<0.05).
≠ Significant when compared to pilocarpine group (P<0.05).

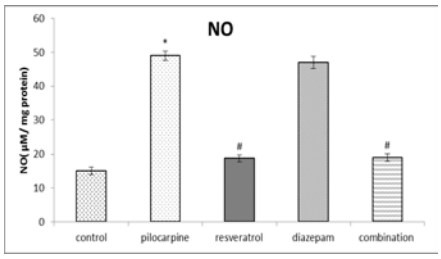

Fig. 6: *Significant when compared to control group (P<0.05).
≠Significant when compared to pilocarpine group (P<0.05).

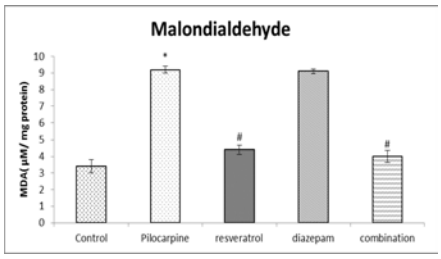

Fig. 7: *Significant when compared to control group (P<0.05).
≠Significant when compared to pilocarpine group (P<0.05).

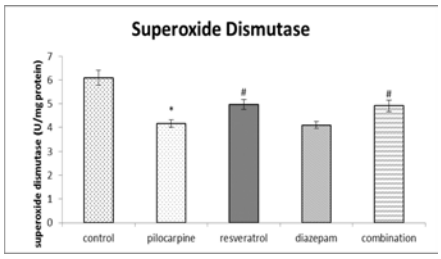

Fig. 8: *Significant when compared to control group (P<0.05).
≠Significant when compared to pilocarpine group (P<0.05).

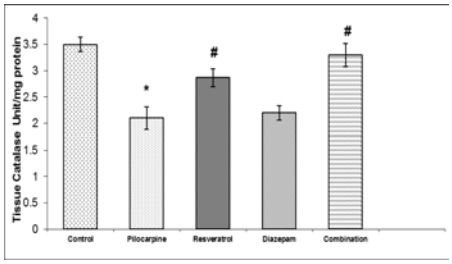

Fig. 9: *Significant when compared to control group (P<0.05).
≠Significant when compared to pilocarpine group (P<0.05).

Discussion

The anticonvulsive mechanisms of conventional and newly introduced drugs vary considerably. The most common actions were shown on ion channels, GABA-ergic and glutamatergic metabolism, receptors or secondary messengers[33]. Extensive efforts have been made to achieve neuroprotection through effective seizure suppression with anticonvulsants and new compounds that may be neuroprotective through mechanisms other than anticonvulsant actions. Neuronal hyperexcitability and excessive production of free radicals have been implicated in the pathogenesis of a considerable range of neurological disorders, including epilepsy. The high rate of oxidative metabolism, coupled with the low antioxidant defenses and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage[34].

In the present study, administration of pilocarpine single dose of 400mg intraperiotneally produced significant increase in number of animals having tonic/clonic convulsion together with significant increase in mortality rate. This is in accordance with Jope et al. 1986[35]. They reported that pilocarpine single dose of 400 mg intraperitoneally induce SE in 83% of animals and mortality was 100%. Morover, Curia et al. 2008 [36]. They stated that mortality increased to 50% and 100% with 350 and 400 mg/kg of pilocarpine, respectively with occurrence of motor limbic seizures more rapidly in the animals treated with the highest pilocarpine dose.

The ability of pilocarpine to induce SE is likely to depend on activation of the M1 muscarinic receptor subtype, since M1 receptor knockout mice do not develop seizures in response to pilocarpine [37]. In addition, pilocarpine-induced SE can be blocked by systemic administration of the muscarinic antagonist atropine[38]. Once seizures are initiated, however, their maintenance depends on other mechanisms since atropine becomes ineffective[38]. Some findings raise the possibility that pilocarpine-induced seizures are not only the result of direct cholinergic system activation in the brain, but also are derived from

primary proinflammatory actions of pilocarpine involving the periphery where pilocarpine causes acute peripheral proinflammatory changes manifested by elevated serum levels of IL-1β together with reduction in the number of CD4-expressing cells leading to blood-brain barrier (BBB) leakage, shortly after pilocarpine injection and prior to the onset of status epilepticus[39].

The concept that peripheral inflammatory reactions contribute to lower seizure threshold is in agreement with Sayyah et al. 2003[40] who stated that systemic lipopolysaccharide (a component of the bacterial wall of Gram-negative bacteria mimicking infection) reduces the threshold to seizures in adult rodents. Further studies established by Galic et al. 2008[41] who demonstrated that lipopolysaccharide or intracolonic administration of 2, 4, 6-trinitrobenzene sulfonic acid (in a modelof bowel disease), delivered to immature rats (postnatal day 7 or 14), was reported to induce long-lasting increases in seizure susceptibility and seizure-associated brain damage.

Regarding to the effect of pilocarpine on inflammatory markers in our present work, it was found that pilocarpine significantly increase tissue levels of IL-1β, IL-6 and TNFα. This in accordance with othe results that postulated that IL-1β is elevated in serum early after pilocarpine or litium chloride administration and considered to be an etiological factor in BBB breakdown[42]. Moreover, IL-1β possesses proconvulsant activity when applied to the brain [43]. This was further supported by a previous study who documented that, antagonism of peripheral inflammation reduces the severity of status epilepticus[6]. Several authors described increased expression of mRNA for IL-1β, IL-6, iNOS and TNFα after seizures, and showed that specific inflammatory pathways are chronically activated during epileptogenesis and they persist in chronic epileptic tissue, suggesting that they may contribute to etiopathology of TLE[44]. Increased level of NO after pilocarpine injection in our work is in collaborance with Van Leeuven et al., 1995[45] that emphasized that administration of 7-Nitroindazole, an inhibitor of neuro-

nal nitric oxide synthase, attenuates pilocarpine-induced seizures.

In the present work, administration of pilocarpine induced significant decrease in antioxidant parameters represented by decrease in tissue catalase, superoxide dismutase, glutathione peroxidase and this was accompanied by significant elevation in MDA and nitrite levels. This is in agreement with Tsai et al. 2010[30] who recorded that rats received pilocarpine show significant increase in MDA level together with significant decrease in catalase, superoxide dismutase levels. On the other hand, some authors reported that, rats injected with pilocarpine 400 mg i.p showed a significant increase in lipid peroxidation and nitrite levels together with increase the catalase activity with insignificant changes on superoxide dismutase tissue level[46]. Increased inflammatory markers have been detected in serum, CSF, and brain of people with epilepsy. There are relevant findings of increased IL-6 following recent tonic-clonic seizures[47,48].

In the present work, administration of resveratrol induced significant decrease in number of animals having tonic/clonic convulsion with significant decrease in mortality rate and increase in latency to convulsion. These results were accompanied by significant reduction in inflammatory markers IL-1β, IL-6 and TNF-α. This is in accordance with another study stated that resveratrol decreased the frequency of spontaneous seizures and inhibited the epileptiform discharges when applied to kainate-induced temporal lobe epilepsy (TLE) in rat[49].

Inhibition of the lipid peroxidation by RESV has been demonstrated in several studies[50,51]. They demonstrated that intraperitoneal administration of RESV in a healthy normal rat decreased brain malondialdehyde (MDA) levels and increased brain superoxide dismutase, catalase and peroxidase activities. Moreover, some studies showed that resveratrol reduced ipid peroxidation as indicated by decrease in MDA levels to nearly those measured in control rats when compared with rats injected with ethanol alone intraperitoneally[52].

Another study using neuronal cell cultures demonstrated that RESV treatment induces heme oxygenase 1 activity with no detectable toxic effects[50]. Because heme levels increase inside cells after stroke and heme (iron-protoporphyrin IX) is considered a pro-oxidant, its rapid degradation by heme oxygenase is believed to be neuroprotective. From this perspective, increased heme oxygenase activity is likely one of the mechanisms by which RESV functions as a neuroprotective compound. Thus, RESV exerts neuroprotective properties by regulating several detoxifying enzmes. A study examined the effects of RESV administration on nitric oxide and tumor necrosis factor-alpha (TNF-alpha) production in cultured microglia that are activated through lipopolysaccharide (LPS) treatment[53]. While the microglial cultures exposed to LPS alone exhibited increased levels of TNF-alpha and NO, microglial cultures exposed to LPS and RESV displayed no significant increases in TNF-alpha and NO. Similar results are recorded by previous studies They found that LPS stimulated the expression of TNF-alpha, IL-1beta, IL-6, and iNOS in murine microglia and astrocytes and the use of RES was associated with inhibition of LPS-induced expression and release of TNF-alpha, IL-6 and iNOS/NO in both cell types with more potency in microglia, and inhibited LPS-induced expression of IL-1beta in microglia but not astrocytes[54].

In the present study, administration of diazepam i.p produced significant decrease in number of animals having tonic/clonic convulsion with significant decrease in mortality rate and increase in latency to convulsion but showed insignificant changes in tissue levels of inflammatory markers (IL-1β, IL-6 and TNF-α, MDA,NO) and antioxidant parameters (catalase, superoxide dismutase and glutathion peroxidase). This is in accordance with another study[55], reported that administration of diazepam reduced the percentage of epileptic animals. The mode of action of diazepam was explained by modulating the GABAA receptor. First, they increase the frequency of opening of the associated chloride ion channels and hyperpolarize the membrane[56,57]. These

changes facilitate the inhibitory effects of the available GABA, and leads to sedatory and anxiolytic effects. Second, different benzodiazepines can have different affinities for GABAA receptors[56,58]. On the contrary, some studies reported increase in activity of nitric oxide synthase (NOS) and the concentration of NO in rat brain 15 min after administration of anticonvulsant doses of diazepam (which is known to activate GABA A receptor), diazepam enhanced both NOS activity and the concentration of NO in a dose-dependent manner[59]. Another study reported that chronic administration of diazepam caused an increase in malondialdehyde (MDA) levels and a decrease in glutathione (GSH) content in rat liver[60].

Conclusion

The ability of antioxidants for reducing the seizure manifestations and the accompanying biochemical changes (i.e., markers of oxidative stress) further supports a role of free radicals in seizures and highlights a possible role of antioxidants as adjuncts to antiepileptic drugs for better seizure control. Our findings strongly support the hypothesis that oxidative stress occurs in during pilocarpine-induced seizures, indicating that brain damage induced by the oxidative process plays a crucial role in seizures pathogenic consequences, which implies that strong protective effect could be achieved using antioxidants like resveratrol. Hence, The replenishment of SOD and CAT activities might be useful in protecting brain against prolonged-seizure-induced neuronal death. Resveratrol potentiate the anticonulsant effect of diazepam and this may be of benefit in lowering incidence of resistance to diazepam and also may allow decrease its dose and side effects in further studies.

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RESVERATROL ENHANCES THE NEUROPROTECTIVE EFFECT OF DIAZEPAM ON PILOCARPINE INDUCED SEIZURES IN RATS

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DEPRESSION AND ANXIETY SYMPTOMS AMONG CHILDREN SUFFERING FROM OBESITY

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Abstract

Objective: *The aim of our study was to explore the presence of anxiety and depressive symptoms among children suffering from obesity.*

Background: *Overweight or obese youth are at increased risk for a multitude of medical comorbidities affecting almost every major body system. Certain psychiatric and psychological disorders are also more common in overweight or obese youth.*

Methods: *All children in the study were classified according to their BMI into two groups:*

Patient group: 100 obese children they comprised 60 male and 40 female with a mean age of (12.3±1.96).

Control group: include 100 healthy normal weight matched for age and sex with obese children, they comprised 58 male and 42 female with a mean age of (12.17±1.62). Children Body Mass Index: BMI, calculated as weight in kilograms divided by the square of height in meters)

The triceps and subscapular skinfold thickness were measured using skinfold caliper according to American College of Sports Medicine guidelines for skinfold measurement - Anxiety and Depression Assessed by Children's Depression Inventory scale and children manifest anxiety scale -Social Background Assessed by Fahmy and Sherbini sheet 1986 Results: Our data show a significant increase in Both depression and anxiety symptoms in obese children as compared with healthy controls. Depression score P >0.05. Anxiety score P<0.001.

Conclusion: *Obesity affects psychosocial adjustment of children raising the importance of early detection and prevention of obesity in the form of nutritional and health awareness programs and training of school health personnel.*

Key words: *obesity, depression, anxiety in children.*

Introduction

Obesity means having too much body fat. It is not the same as overweight, which means weighing too much.(1)

The prevalence of childhood obesity has been rapidly increasing dramatically during the past two decades.(2)

Some of the most commonly reported and striking consequences of pediatric obesity are psychosocial.(3)

There is a common belief that the overweight children are unhappy with their weight and experience most psychosocial distresses, particularly depressive symptoms.(4)

Depression is a syndrome (group of symptoms) that reflects a sad, blue mood exceeding normal sadness.(5)

When child seems depressed or sad, it does not necessarily mean they have depression. But if these symptoms become persistent, disruptive and interfere with social activities, interests, schoolwork and family life, it may indicate that he or she is suffering from depression.

Obese, Anxiety is a common emotion amongst children at various ages. When it becomes severe, however, it may be indicative of more serious, psychological problems.(6)

Children and adolescents who are overweight or obese are at increased risk for certain psychiatric disorders mostly depression anxiety and impaired psychological functioning.

Patients and Methods

This study was conducted during year 2010-2011 in four preparatory schools in Menofyia Governorate After gaining the

approval of local educational authorities.

• **The schools were divided into 2 groups according to locality of each:**

1. Urban schools:

2 Schools were selected randomly in Shebin El-Koum city.

2. Rural schools:

They included the other2 schools, which located in villages around the capital the 1st was from Meat Khalaf village the 2nd was from Al Rahib village.

Inclusion criteria:

Ages from 9-15 years.

Both sex are included.

BMI equel or more than 85 percentile for age and gender based on the Egyptian growth reference charts 2002.

A consent was taken from parents of these children before being included in this study.

All children in the study were classified according to their BMI into two groups:

1. Patient group: 100 obese children they comprised 60 male and 40 female with a mean age of (12.3±1.96).

2. Control group: include 100 healthy normal weight matched for age and sex with obese children, they comprised 58 male and 42 female with a mean age of (12.17±1.62).

Every child was subjected to:

• Complete medical history.

• Complete nutritional history.

Clinical examination (chest, heart, abdominal and neurological examination).

Children having congenital anomalies or chronic diseases were excluded from the study.

Age of the children was recorded from birth certificates that were already present in school files.

A- Body Mass Index:

Weight: Children's weight was measured in kilograms using a standardized portable scale, and measured twice to the nearest 0.1kg without shoes or outer clothing and the mean was used

in the analysis.

Height: was measured in meters by a direct reading using stadiometer. Height was measured twice to the nearest centimeter and the mean of the two measures was used in the analysis.

Children Body Mass Index: BMI, calculated as weight in kilograms divided by the square of height in meters).

BMI is the preferred measure of children adiposity for epidemiological and clinical studies.

B- Skinfold Thickness:

The triceps and subscapular skinfold thickness were measured using skinfold caliper according to American College of Sports Medicine guidelines for skinfold measurement.

C- Anxiety and Depression Assessment:

1- Children's Depression Inventory scale:

The Arabic children's Depression Inventory (ACDI) derived from Kovacs[7] and constructed for Egyptian children by Abdel Khalek 1999[8] was used.

Children's Depression Inventory cover the symptoms of juvenile depression: it conation 27 items in which the children have to respond by themselves through three alternatives i.e. Rarely, sometimes and often.

2- Personality Assessment Sheet For Assessment OF Anxiety:

The anxiety sheet is derived from children manifest anxiety scale it contain 36 items in which the children have to respond by themselves through two alternatives. Yes or no.

The Scale is a test of anxiety as a personality trait. It was developed by Janet Taylor in 1953 to identify subjects who would be useful in the study of anxiety traits (Taylor, Janet 1953[9] The test is for adults but in 1956 a children's form was developed (Castaneda 1956).[10] The test was very popular for many years after its development but is now used infrequently (Antony 2001)[11].

D- Social Backgrounde:

Socioeconomic status of the family (education of parents, work

of father, income, sanitation, family size).

• The social classification was divided into 4 status according to the research of Fahmy and Sherbini 1986.

1. High social status.
2. Middle social status.
3. Low social status.
4. Very low social status.

Results

Table 1 showed that there were no significant difference found between patient and control group regarding age, P>0.05 and sex P>0.05.

Age of obese children rang between 8-15 years with mean age (12.3±1.96) years while those of control range between 9-15 years with mean age (12.17±1.62) years.

In patient group there were 60% males and 40% females while those of control 58% males 42% females.

60% of patients were from urban area compared to only 40% were from rural area. P<0.001.

There were highly statically significant difference between two groups regarding parental educational level income and socioeconomic state. P<0.001.

Table 2 Body Mass Index and skinfold thickness showed significant differences between groups

Table 3 shipping breakfast was frequent in 58% in patients and 24% in controls, consumption of energy dense food and food consumption during TV watch. Were more than 80% in patients and 16% in controls.

Physical activity showed significant difference between two groups **Table 4** showed that 25% of patients had pallor, 35% had dyspnea and 44% had hepatomegaly compared to 0% in controls

Table 5 this table showed significant difference in depression scores and highly significant difference in anxiety scores between two groups.

Table (1): Comparison of the demographic characteristics between the studied groups.

	Patients N=100	**Controls N=100**	**Student t test**	**P value**
Age in years	12.3 ± 1.96	12.17± 1.62	0.38	> 0.05
X ±SD				
Range	9-15	9-15		
Sex			Chi square test	P value
Males	60(60%)	52(52%)	1.30	> 0.05
Females	40(40%)	48(48%)		
	Patients N=100	**Controls N=100**	**Chi square test**	**P value**
Residence				
Urban	60(60%)	36(36%)	11.54	**<0.001**
Rural	40(40%)	64(64%)		
Father education				
Illiterate	18(18%)	12(12%)	37.69	**<0.001**
Primary	52(52%)	16(16%)		
Secondary	24(24%)	60(60%)		
University	6(6%)	12(12%)		
Mother education				
Illiterate	28(28%)	24(24%)	35.67	**<0.001**
Primary	50 (50%)	16 (16%)		
Secondary	18(18%)	52(52%)		
University	4 (4%)	8(8%)		
Income				**<0.05**
More than sufficient	19	6	9.77	
Sufficient	69	72		
Less than sufficient	12	22		
Socioeconomic state				
Low	25(25%)	52(52%)	17.43	**<0.001**
Middle	40(40%)	32(32%)		
High	35(35%)	16(16%)		

Table (2): Anthropometric measurement among the studied group.

Anthropometry	**Patient**	**Control**	**T test**	**P value**
Height "cm"	145.93±16.62	143.92±13.13	0.338	>0.05
Weight kg	57±17.3	40.25±11.74	2.83	**<0.001**
BMI	27.58±2.19	21.69±1.87	15.74	**<0.001**
Skin fold	25.59±1.95	15.04±1.27	39.8	<0.001

Table (3): Comparison between nutritional patterns and Physical activity in patients and controls.

		Patients N=100	Controls N=100	Chi square test	P value
Skipping breakfast	Rare Infrequent Frequent	10(10%) 32(32%) 58(58%)	12(12%) 64(64%) 24(24%)	24.95	**<0.001**
Consumption of energy dense food	Rare Infrequent Frequent	4 (4%) 14 (14%) 82 (82%)	24(24%) 60(60%) 16 (16%)	87.33	**<0.001**
Food consumption during TV watch	Rare Infrequent Frequent	6 (6%) 6 (6%) 88(88%)	28 (28%) 52 (52%) 16 (16%)	89.63	**<0.001**
Physical activity	Absent Playing in summer Regularly	60(60%) 20(20%) 20(20%)	12(12%) 52(52%) 36(36%)	50.79	**<0.001**

Table (4): Clinical data of the studied group.

Clinical data	Patient		Control		Chi square	P value
	No	%	No	%		
General exam						
Normal	75	75	100	100	28.57	**<0.001**
Pallor	25	25	0	0		
Cyanosis	0	0	0	0		
Chest						
Free	65	65	100	100	42.40	**<0.001**
dyspnea	35	35	0	0		
CVS					--------	----------
Free	100	100	100	100		
Abdominal						
Normal	10	10	100	100	189.15	**<0.001**
Hepatomegaly	44	44	0	0		
Full flanks	72	72	0	0		

Table (5): Comparison between depression and anxiety scale scores in cases and controls.

	Patients N=100	Controls N=100	Student t test	P value
Depression score	11.26±4.39	8.84±2.75	3.55	**<0.05**
Anxiety score	19.72±6.58	15.68±3.22	5.04	**<0.001**

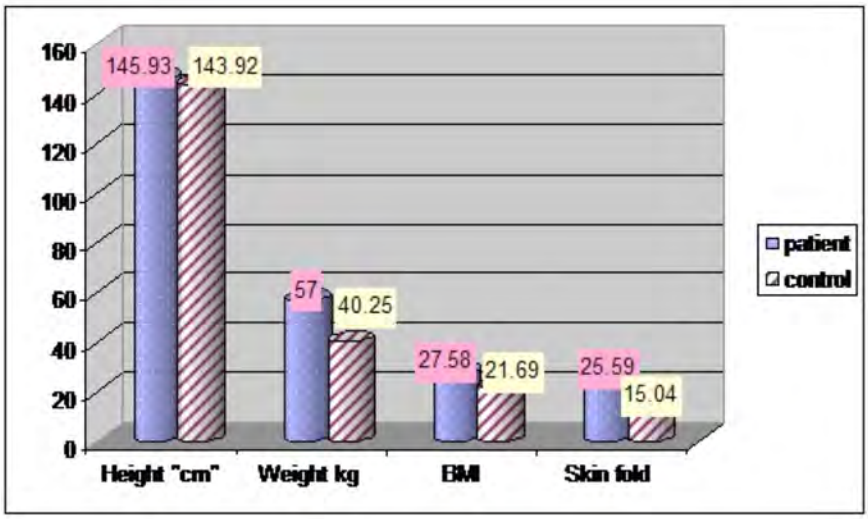

Fig. 1: Anthropometric measurement among the studied group.

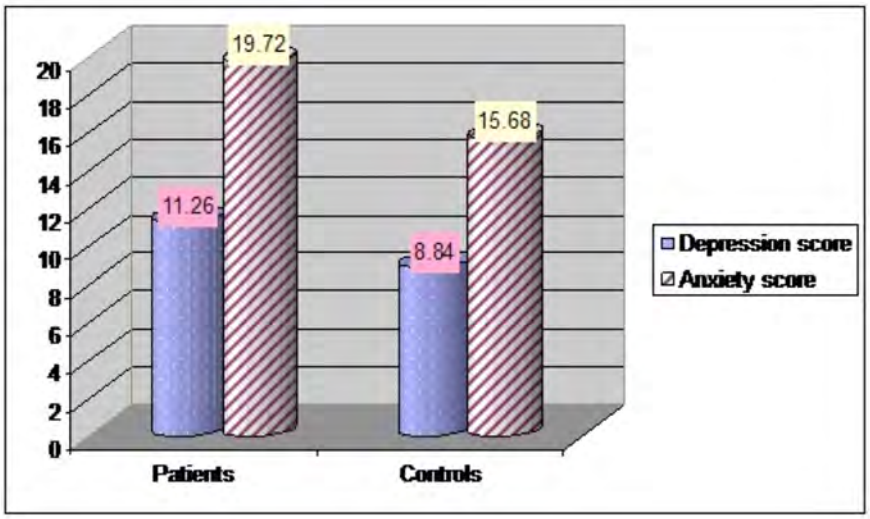

Fig. 2: Comparison between depression and anxiety scale scores in cases and controls.

Discussion

Obesity has become an increasing important medical problem in children and adolescents.[12]

The prevalence of overweight children in the United States has significantly increased over the past decade. More than 1 in 5 children are overweight or at risk for obesity. It is easy to blame the advancement of technology, the increase in sedentary activities, or other environmental aspects of our current society for the expanding obese population. However, there is a complex relationship between genetic, cultural, and psychological factors.[13]

Children who are obese are at increased risk for emotional problems that last well into adulthood.[14]

The aim of our study was to explore the relationship between nutritional obesity and psychological behavior i.e depression and anxiety and to examine the effect of social background and other demographic factors in this relationship.

This study include 100 obese children (60 boys and 40 girls) with body mass index equal or more than 95th percentile and 100 normal weight children (58 boys and 42 girls) with body mass index (BM1) equal or less than 85th percentile as a control group In our study patients age ranged between 8-15 years with a mean age of 12.3±1.96 years of the included group patient. The control group included 100 children aged from 9-15 years with mean age of 12.17±1.62 years.

• In our study (there were no significant difference regarding age and sex between patients and control groups.

In agreement with our result previous study among Greek preschoolers found that no statistical differences in the prevalence of obesity between males and females.[15]

In contrast to our result Stark 2011[16] found that their was higher percentage of obesity among males children[17] also found increase prevalence of obesity among girls.

In our study 60% of patients

were from urban area compared to only 40% were from rural areas. In agreement with us previous studies found that their was higher prevalence of overweight and obesity in urban compared to rural areas in Greece.[18]

The Egyptian national survey found that the prevalence of overweight and obesity was 22.6% among urban adolescents versus 10.4% among rural adolescents in Egypt Giza governate El-Tawila 1999.[19] In contrast to our result Wang Y 2001[20] found that region of residence was not found to influence the risk of overweight, on the other hand in a study by Savva et al., 2005[21] conducted in Cyprus in preschool children has reported that overweight and obesity prevalence was higher in children living in rural areas (16.1%) in comparison to urban areas (12.8%).

Eagle, 2012[23] also found that the prevalence of overweight/ obese children rises in communities with lower household income.

So residence had variable effects in the prevalence of obesity and this are particularity attributable to difference in people's access to food and health services, physical activity of the patients and social status in rural and urban areas like in urban Egyptian which usually have higher family income better access to food especially meat and other high dense foods which are more, expressive than other food such as vegetable also have public services such as health care and transportation, they are also more likely to have sedentary lifestyle in contrast, American, rural and urban children and adolescents have similar access to food choices thanks to the well established food production and Distribution system.[23]

In Russia urban groups less likely to be obese than rural groups this is probably due to that in the past decade, living standards of urban groups have been seriously affected by the socioeconomic in difficulties occurring since the collapse of the farmer Soviet Union.[24]

Our study revealed that patients fathers and mothers had significantly lower educational lev-

el when compared with controls this is in harmony with Lamerz 2005[25] who found that one of the most risk. Factors for obesity in children and adolescents was the less educational parents.

An international group of experts from eight European countries have analyzed the relation between parents levels of education and prevalence of obesity among their children the study include Data from 14.426 children aged between two and nine from right European countries: Italy, Cyprus, Belgium, Sweden, Hurgar, Germany and Spain. Their results published in the journal public health Nutrition and discovered that Parents with a lower level of education feed their children food rich in sugars and fats more often than those parents with a higher level of education who feed their children more productions of a higher nutritional quality, including vegetables, fruits, pasta, rice and bread.[26]

In our study comparison between studied groups regarding socioeconomic status (SES) showed statistical significant differences as we found that high socioeconomic groups had a higher risk of obesity, and this is in accordance with other studies like. El Tawila who found that in Egypt. Giza governate the prevalence of obesity among high. SES adolescents was more than double that among low SES groups (7.0% versus 3.1%).[19]

In China high SES groups were at an increased risk of obesity.[27]

In contrast to our results Wang Y, 1998[28] show no significant association between SES and overweight in American boys and girls.

The Explanation for the different SES. and obesity relationship in developed countries such as the US. and developing countries like Egypt is that the influence of SES on people's lifestyles such as diet and physical activity which may differ. We can take food consumption patterns as an example in Egypt richer people have better access to meat and other energy dense food (which are much more expensive than other food such as vegetables)[29] while in the US,

higher-SES groups usually consume more vegetables and fruits, which are less energy-dense, than low-SES groups.[30]

In this study 35% of patients had dyspnea compared with 0% in controls.

In agreement with this result other studies report that obesity alone, without the presence of underlying lung disease, can be a cause of dyspnea at rest. The study involved 269 children from 6 to 17 years of age enrolled in the National Heart, Lung, and Blood Institute Severe Asthma Research Program the results found that 60% of obese children had dyspnea.[31]

In comparison between patients and control group regarding gastroenterology symptoms we found statistical significant difference between two groups as 44% of patients had hepatomegaly in agreement with this result other studies report that.

Some experts think as many as 10 percent of all children and half of those who are obese may suffer from fatty liver disease and its corresponding symptoms like hepatomegaly and some of these children are at risk of developing non alcoholic steatohepatitis.[32]

Discussing the nutritional patterns in the current research declared a distinctive pattern of the obese children when compared with controls as they. Skip breakfast.in the present study 58% of patients skipping their breakfast.

This is in accordance with other studies, which found that skipping breakfast was associated with overweight/obesity in children like. Study, involving 4,326 secondary school children. United kingdom research has found that Children who skip breakfast are more likely to be obese and inactive.[33]

Clinic Cleveland 2013[34] found that breakfast is the most important meal of the day for kids it gives kids the energy to start their day, and it improves learning and behavior as well and prevents childhood obesity.

Our findings suggest that a dietary pattern characterized by high

consumption of fruits, vegetables, poultry, and legumes is associated with lower risk of general and central obesity, while a dietary pattern with high amounts of refined grains, red meat, butter, processed meat, and high-fat dairy products and low amounts of vegetables and low-fat dairy products is associated with increased risk of these conditions. This is consistent with previously reported findings in American[35] and European[36] studies. Other studies found that a dietary pattern characterized by low-fat dairy, grains, and fruit was inversely associated with changes in BMI and waist circumferance in women.[37]

In the present work there were statistical significant difference regarding to physical activity between obese children and normal control children as we found inverse relationship between obesity and physical activity in agreement with our study[38] found that children whose physical activity are low associated with increased BMI and combustion of high fat, high sugar or high caloric food.

Our study showed that highly relation between depression anxiety and obesity in children and adolescents we found in the present study that depression is higher about 0.26 times in obese persons than in normal and anxiety is higher about 0.35 times in obese persons than in normal.

In agree with this finding previous studies investigate the relation between obesity, depression, shame, and psychosocial and economic status in a population-based sample of 4703 Swedish adolescents. The results showed that BMI was associated with depressive symptoms. Moreover, those adolescents who belonged to the group with the highest BMI (obese group) significantly more often suffered from major depression according to the DSM-IV A-criterion, which were used in this study.[39]

Erickson 2000[40] in a study of 868 children, found a modest but significant relation between BMI and depression, which was moderated by weight concerns.

Subanalysis by Luppino and colleagues found that the effect of

obesity on the development of depression was stronger in American studies. They highlighted the possibility of a biological link between overweight, obesity, and depression.(41)

Conclusion

Our findings suggests that:

• The prevalence of obesity varied remarkably with difference socioeconomic development levels.

• Obese children had a characteristic nutritional pattern like skipping breakfast and consumption of energy dense food.

• Obesity affects psychosocial adjustment of children.

• There were an association between adolescent obesity and both depression and, anxiety in children and adolescents and this complex relationship affected by many factors like Gender differences, age and physical activity of patients.

• Social and environmental approaches should be considered, to effectively fight the global obesity epidemic.

• The clinical treatment of obesity not just is a matter of diet and exercise but also psychological evaluation.

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DEPRESSION AND ANXIETY SYMPTOMS AMONG CHILDREN SUFFERING FROM OBESITY

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TERATOGENIC EFFECT OF PRENATAL EXPOSURE TO CYCLOPHOSPHAMIDE: EVALUATION OF THE AMELIORATIVE EFFECT OF SUPPLEMENTAL FOLIC ACID

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Abstract

Objectives: *To evaluate impact of prenatal exposure to cyclophosphamide (CP) on morphology, skeleton and viscera of rats and to evaluate the effect of folic acid (FA) supplementation on these changes.*

Materials & Methods: *The study included 30 pregnant female rats divided equally into three groups: Control group, CP group injected intravenously by CP 7 µgm/gm body weight (BW)/day and CP+FA group injected intravenously by CP 7 µgm/gm BW/day and received FA 100 ug/gm/day orally. Drug therapy was given daily from the 6^{th} to 9^{th} days of gestation. On the 20^{th} day of gestation, all female rats were delivered by labarotomy. The uterus with the two ovaries were removed and examined for the number of corpora lutea of pregnancy and the number of implantation and resorption sites in each uterine horn. Crown rump length, head length and biparietal diameter were measured and BW was recorded. Apparent external gross abnormalities were detected. Alizarine red stained skeletons were studied for counting the number of ribs and vertebrae and examination for any skeletal abnormalities. Skeletal specimens were examined for scoring of ossification as being complete, delayed or absent. Hematoxylin and eosin stained skeletal sections were examined.*

Results: *CP induced significant reduction of number of corpra lutea and live fetuses compared to both control and CP+FA groups. All body measurements were significantly smaller compared to control group with non-significantly larger measurements in CP+FA group. BW was*

significantly lower in study groups compared to control group with significantly higher BW in CP+FA group. External examination of CP-treated rats showed large blood clots indicating late post-implantation resorption, macerated fetuses indicating prenatal fetal death and a large abdominal hematoma. Alizarine red stain showed evident failure of ossification of clavicle, sternum, metacarpal, metatarsal and caudal vertebrae; incomplete ossification of skull bones, scapula, hip, femur, vertebrae, metacarpal and metatarsal bones and irregular mandible, scapula, fused ribs, short 13th rib and extra-ossification centers in the vertebrae are seen in CP group. In CP+FA group, folic acid partly improved most of the skeletal changes. In CP-treated animals, razor sections showed thick nasal septum and malformed brain and bilaterally dilated lateral ventricles. Histological sections showed malformed mandible, rudimentary maxillae and hypertrophied nasal septum, bilaterally dilated lateral and third ventricles and malformed spinal cord and vertebrae.

Conclusion: *Prenatal exposure to CP induces manifest teratogenic effects especially altering the growth levels with multiple skeletal abnormalities. Additionally, CP induced significant brain and spinal cord detrimental effects. FA supplementation could ameliorate CP-induced changes but could not be a grantee for safe pregnancy outcome.*

Keywords: *Cyclophosphamide, Prenatal exposure, Folic acid supplementation*

Introduction

Cyclophosphamide (Cytoxan®, $C_7H_{15}Cl_2N_2O_2P$) is a nitrogen mustard alkylating agent, from the oxazophorines group. It adds an alkyl group (C_nH_{2n+1}) to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring. It is a prodrug that must be activated in the liver to form the active aldophosphamide that have chemotherapeutic activity. Cyclophosphamide (CP) is used clinically to treat a wide range of cancers and as immunosuppressive therapy following organ transplants or as a treatment for autoimmune disorders. Metabolism of CP takes place in the liver and undergoes metabolic activation by

cytochrome P450 isoenzyme 2B. CP-induced nucleic acid damage may lead to DNA mutations that result in cytotoxicity, carcinogenicity, teratogenecity, and reproductive toxicity following chronic exposure[1,2,3].

Folic acid, an essential vitamin converted to functional, naturally occurring metabolites via the action of dihydrofolate reductase. Folates are necessary in eukaryotic cells for single carbon transfer reactions and a multitude of steps in de novo nucleotide synthesis. Folic acid and its reduced derivatives, including 5-methyltetrahydrofolate and 10-formyltetrahydrofolate, are transported via two widely expressed facilitative transporters, the reduced folate carrier and the proton-coupled folate transporter, and via a family of glycosylphosphatidylinositol-anchored receptors with limited expression profiles generally described as folate receptors[4,5,6].

The decidua possesses nutritional functions during embryo organogenesis, as well as immunological functions in the regulation of trophoblast invasion and the formation of the placenta. Experimental studies have shown that folic acid supplementation highly reduces the induction of congenital malformations in human pregnancies. Indeed, in human studies, folic acid supplementation reduces the induction of congenital defects with doses ranging 0.4-4 mg/day by 50-70%. During development, antioxidant and anti-apoptotic effects of folic acid have been described and possibly related to their protective effects during embryo organogenesis. Moreover, folic acid has been shown to prevent neural tube defects caused by excessive nitric oxide. Both nitric oxide and reactive oxygen species are positive regulators of matrix metalloproteinases activities that have been found to be clearly related to relevant developmental processes such as implantation, decidualization, embryo organogenesis and placental formation[7,8,9,10].

The current prospective comparative study aimed to evaluate the impact of prenatal exposure to cyclophosphamide (CP) on morphology, skeleton and viscera of the rats and to evaluate the effect

of folic acid (FA) supplementation on these changes.

Materials & Methods

Animals:

Fifty albino rats; 30 females and 20 male rats of about 120 days in age and weight range of 160-200 gm. Rats were purchased from the laboratories of Ministry of Agriculture. Males and females rats were kept in separate animal cages, under standard conditions, temperature 20°C, humidity 60% and 12-hs day/night cycle and maintained on a balanced diet (bread, barely, carrots, lettuce, milk) and fresh-water supply. Each female was placed over night with one or two males and vaginal smear was examined, next morning, for presence of sperms and that day was considered the first day of gestation.

Groups & drugs:

The pregnant female rats were divided into three groups: Control group (Group C) included 10 pregnant female rats and two study groups; CP group included 10 rats injected intravenously by CP 7 µgm/gm body weight/day and CP+FA group included 10 rats injected intravenously by CP 7 µgm/gm body weight/day and received folic acid (FA) 100 ug/gm/day orally by gastric intubation through a urethral catheter with flexible adapter[11]. Drug therapy was given daily from the 6^{th} to the 9^{th} days of gestation.

Methods:

On the 20^{th} day of gestation (12-24 hours before the expected day of delivery), all female rats were anaesthetized by ether inhalation and the fetuses were immediately delivered by labarotomy to prevent the mothers from devouring any abnormal offspring.

Gross external examination of ovaries and uterine horns:

The anterior abdominal wall was incised and the uterus with the two ovaries were photographed and removed. The number of corpora lutea of pregnancy in each ovary was counted. The number of implantation and resorption sites in each uterine horn was counted. The percentage of the pre-implantation loss was calculated as the difference between number of corpora lutea and number of implantation sites. The

percentage of post implantation loss was calculated as the difference between the number of implantation sites and the total number of placentations of living, dead or resorped fetuses.

Gross internal examination of uterine horns:

The uterine horns were then cut longitudinally to deliver each fetus inside its amniotic sac and the crown rump length (CRL), head length (HL) and biparietal diameter (BPD) were measured using a calibrated metallic gauge and the weight (Wt) of the fetuses was recorded. Using magnifying lens each fetus was carefully examined for any apparent external gross abnormality in an ordinary manner from the head to the tail with counting of the number of digits in each limb[12].

Fetal examination:

1. Two fetuses delivered from each individual maternal rat were dehydrated and fixed by immersion in 95% ethyl alcohol for >7 days for subsequent skeletal visualization according to Dawson[13].

2. The remaining fetuses were immersed for 7 days in Bouin's fluid for efficient decalcification to allow serial sections (1-mm thickness) in the soft fetal tissues[14]. Sections were examined using the binocular dissecting microscope for the presence of any gross abnormalities in the internal organs as adopted by Kotb[15].

3. Skeletal system staining and examination: stained skeletons were studied under binocular dissecting microscope for counting the number of ribs and vertebrae and examination of the skull, limb bones and sternum for any skeletal abnormalities[16].

4. Skeletal system evaluation: using the dissecting microscope each specimen was examined for scoring of ossification as being complete, delayed or absent. Delayed centers can be identified when the center is either lightly stained (partial ossification) or asymmetrically developed (incomplete ossification) and abnormal ossification was also recorded.

5. Light microscopic examination of Hematoxylin and eosin stained sections of control and ex-

perimental fetuses[17].

Statistical analysis:

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using Wilcoxon; ranked test for unrelated data (Z-test) and Chi-square test (X^2 test). Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. P value <0.05 was considered statistically significant.

Results

Exposure to CP, irrespective of being alone or with FA, induced significant reduction of number of corpra lutea of pregnancy by more than 50% in comparison to control group. Moreover, CP induced significant reduction of number of live fetuses in relation to number of copora lutea compared to both control (X^2=46.775, p=0.0001) and CP+FA (X^2=11.95, p=0.0009) groups. However, FA supplementation significantly improved the chance of getting live fetus with a frequency non-significantly higher than in control group, in relation to number of corpora lutea, (Table 1, Fig. 1).

All body measurements of fetuses of CP and CP+FA groups were significantly smaller compared to control groups with non-significantly larger measurements of fetuses of CP+FA group compared to that of CP group (Fig. 2). Concerning BW data, fetuses of both study groups showed significantly lower BW compared to control fetuses with significantly higher BW of fetuses of CP+FA group compared to CP group, (Table 2, Fig. 3).

External examination of control group showed normal corpra lutea of pregnancy appeared as large yellowish tinge color (Fig. 4). Normal pregnant uterus with live fetuses can be observed (Fig. 5). In CP treated rats, large blood clots are seen indicating a site of late post-implantation resorption (Fig. 6). Other uteri contained macerated fetuses indicating prenatal fetal death (Fig. 7). A large abdomino-pelvic hematoma and rudimentary hind-limbs are seen in a fetus of CP-treated rat (Fig. 8). Fetuses of CP and CP+FA groups were of lower size compared to control fetuses. The ameliorative effect of FA was manifested as evident

difference in the extent of physical growth in CP+FA group compared to that of CP group (Fig. 9).

Alizarine red stain was used to detect skeletal deformities; in control group, most of the bones are ossified, while in CP group, anterior viewing showed evident failure of ossification of the clavicle, sternum, metacarpal and metatarsal bones and maxilla (Fig. 10). Posterior viewing showed incomplete ossification of all skull bones, scapula, hip bone, femur, vertebrae. Fused ribs, short 13^{th} rib and extra-ossification centers in the vertebrae are seen (Fig. 11). Lateral viewing showed normally ossified humerus, radius, ulna and femur, but incomplete ossification of metacarpal bones, tibia, fibula and metatarsal bones can be seen with absence of caudal vertebrae. Brachygnathia, irregular mandible, scapula and ribs are also seen. (Fig. 12).

Alizarine red stain in CP+FA group showed that folic acid improved most of the skeletal changes. Anterior viewing showed normally ossified mandible and clavicle but incomplete ossification of maxilla, sternum, and metacarpal and metatarsal bones were seen (Fig. 13). Posterior viewing showed incomplete ossification of all skull bones, hip bone and femur. Also, irregular ribs, short 13th rib and extra ossification centers are seen in lumbar and sacral regions (Fig. 14). Posterior viewing showed normally ossified skull bones (frontal, lacrimal, maxilla and mandible), clavicle, scapula, radius, ulna, femur, tibia and fibula. But showed brachygnathia, incomplete ossification of metacarpal and metatarsal bones, irregular humerus and ribs with absence of caudal vertebrae (Fig. 15).

Razor sections were made at different levels for studying gross abnormalities in the internal organs. At the level of the angle of the mouth, normal nasal conchae and septum, palate, tongue and mandible are seen in control group, while in CP treated groups, thick nasal septum and malformed nasal conchae are seen (Fig. 16). At the level of the largest transverse diameter of the head, control fetuses showed normal lat-

eral and third ventricles with regular subarachnoid space, while in CP-treated fetuses malformed brain, bilaterally dilated lateral ventricles and subarachnoid space were observed, (Fig. 17). At the level of the abdominal region just above the umbilicus, control fetuses showed normal spinal cord, kidney, stomach and liver. CP-treated fetuses showed normal spinal cord, stomach and liver but showed large malformed kidneys, (Fig. 18).

Histological sections made at the level of the angle of the mouth, showed normal nasal septum, vomeronasal cartilage, conchae, maxilla, palate, tongue and mandible in control fetuses. In CP-treated fetuses, there are normal conchae, vomeronasal cartilage and palate, but malformed mandible, hypertrophied nasal septum, narrow nasal cavity and rudimentary maxilla, (Fig. 19). At the level of the largest transverse diameter of the head, control rat fetus showed normal lateral and third ventricles, subarachnoid space, mandible and tongue. In CP-treated rats there is dilated lateral and third ventricles and subarachnoid space, (Fig. 20). At the level of the root of the neck, control fetus showed normal spinal cord, vertebra, esophagus, trachea, carotid sheath, thyroid and thymus glands. CP-treated fetus showed normal trachea, carotid sheath and thyroid gland but showed malformed spinal cord and vertebra, esophagus is deviated to the right and the thymus gland is rudimentary, (Fig. 21).

Table (1): Live fetuses, pre implantation loss and post implantation loss and resorption

Group	Live fetuses		Pre-implantation loss		Post implantation loss and resorption		Total number of corpus lutea of pregnancy
	No.	%	No.	%	No.	%	
Control	80	88.88	10	11.12	0	0	90
CP	25	56.81	12	27.29	7	15.90	44
CP+FA	38	88.4	5	11.6	0	0	43

Table (2): Mean (±SD) of fetal measurements of CP and CP+FA treated groups compared to control group.

Parameter	Group	Measurements	P value
CRL (cm)	Control	5.5±0.37	P1=0.005
	CP	2.60±0.28	P2=0.002
	CP+FA	3.30±0.14	P3=0.089
HL (cm)	Control	1.30±0.05	P1=0.020
	CP	0.95±0.07	P2=0.004
	CP+FA	1±0.02	P3=0.423
BPD (cm)	Control	1.25±0.05	P1=0.021
	CP	0.90±0.07	P2=0.027
	CP+FA	0.95±0.07	P3=0.55
BW (gram)	Control	5.63±0.002	P1=0.0004
	CP	1.73±0.02	P2=0.001
	CP+FA	2.90±0.09	P3=0.003

Data are presented as mean±SD; CRL: crown-rump length; HL: head length; BPD: biparietal diameter; BW: body weight; CP: cyclophosphamide; FA: folic acid; P1: significance of difference between CP and control groups; P2: significance of difference between CP+FA and control groups; P3: significance of difference between CP and CP+FA groups.

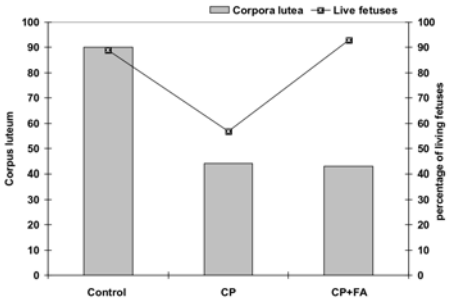

Fig. 1: Number of corpora lutea and percentage of living fetuses recorded for each group.

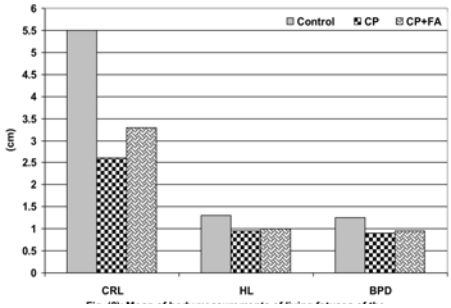

Fig. 2: Mean of body measurements of living fetuses of the studied groups.

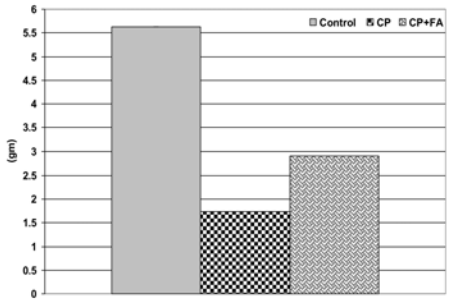

Fig. 3: Mean (±SD) body weight of living fetuses of studied groups.

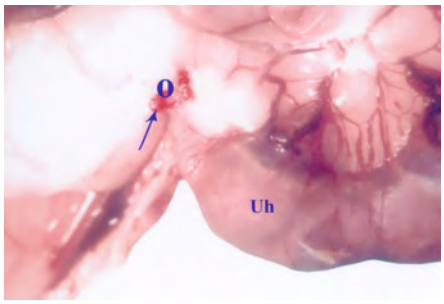

Fig. 4: A photograph showing a control pregnant rat with the ovary (O) containing normal corpus lutea of pregnancy (arrow) which appears large, with yellowish tinge color.

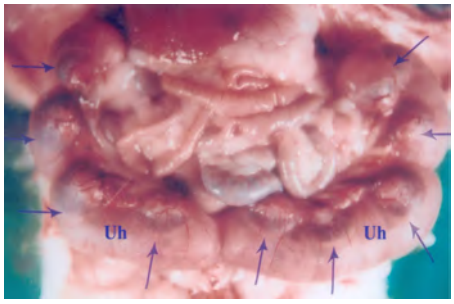

Fig. 5: A photograph of the control female pregnant rat showing four fetuses (arrows) in right uterine horn (Uh) and five fetuses (arrows) in the left horn.

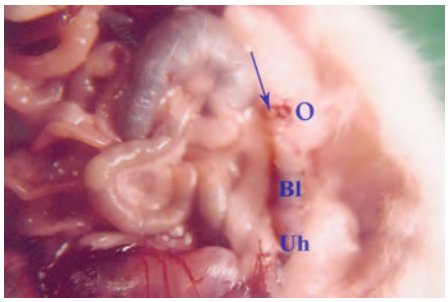

Fig. 6: A photograph of CP-treated female rat with pregnant uterus showing large blood clot (Bl) in one of the uterine horns (Uh) indicating a site of late post implantation resorption. Also, corpus lutea of pregnancy (blue arrow) in the ovary (O) can be seen.

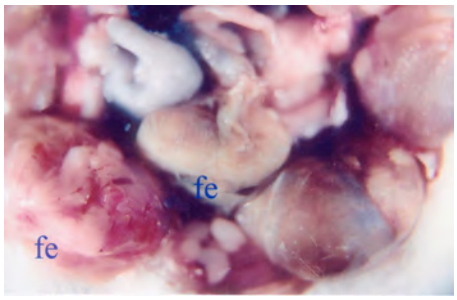

Fig. 7: A photograph of CP-treated female rat with opened uterine horns showing maceratd malformed fetuses (fe).

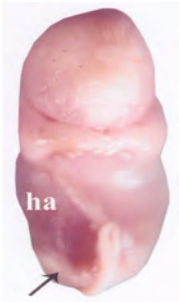

Fig. 8: A photograph of the fetus of CP-treated rat (CP) showing a large abdominopelvic haematoma (ha) and rudimentary hindlimbs (arrow).

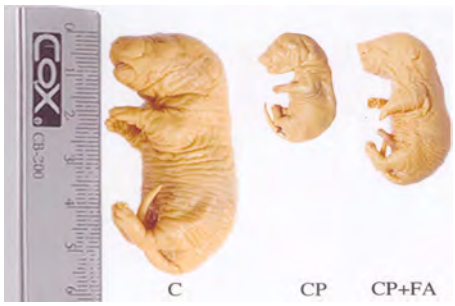

Fig. 9: A photograph showing the length of living control rat fetus (C), CP-treated rat fetus (CP) and CP+FA-treated rat fetus.

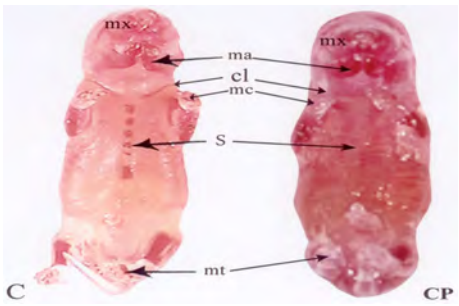

Fig.10: A photograph showing anterior view of control (C) and CP-treated rat fetuses showing absence of the clavicle (cl), sternum (S), metacarpal (mc) metatarsal (mt) and maxilla (mx) in CP-treated fetus, but normal skull bones and mandible (ma) can be seen. (Alizarine red stain).

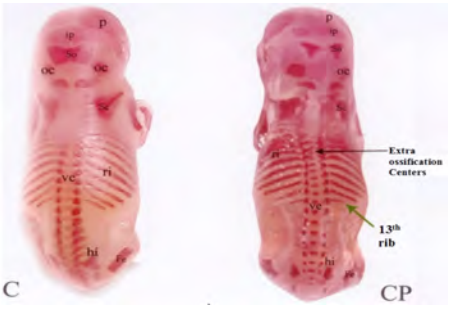

Fig.11: A photograph showing posterior view of control (C) and CP-treated rat fetuses showing incomplete ossification of all skull bones (parietal (p), interparietal (ip), supraoccipital (So) and occipoital (oc) bones) , scapula (Sc), hip bone (hi), femur (Fe) and vertebrae (ve). Also, irregular fused upper ribs (ri), short 13th ribs (green arrow) and extraossification centers (black arrow) (Alizorine red stain).

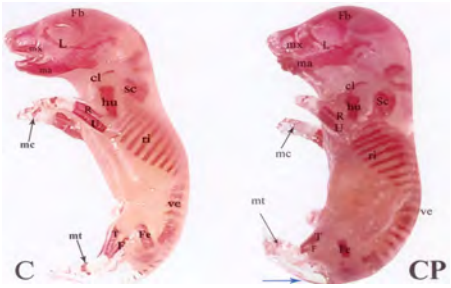

Fig.12: A photograph showing lateral view of control (C) and CP-treated rat fetuses showing brachygnathia, irregular mandible (ma), scapula (Sc), and ribs (ri). Incomplete ossification of metacarpal (mc), tibia (T), fibula (F) and metatarsal (mt) bones can be seen. There is absence of caudal vertebrae (blue arrow). Normally ossified humerus (hu), radius (R), ulna (U) and femur (Fe) can be seen (Alizarine red stain).

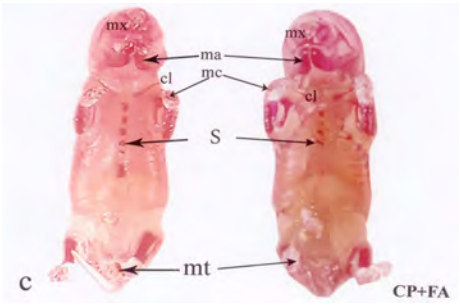

Fig.13: A photograph showing anterior view of control (C) and CP+FA treated rat fetuses showing incomplete ossification of sternum (S), metacarpal (mc), metatarsal (mt) bones and maxilla (mx). Normally ossified mandible (ma) and clavicle (cl) can be seen. (Alizarine red stain).

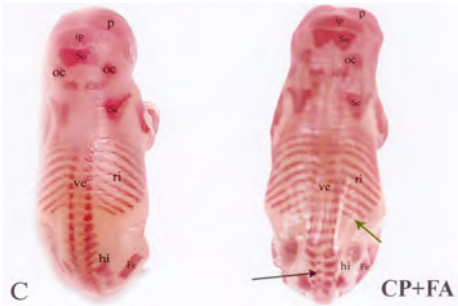

Fig.14: A photograph showing posterior view of control (C) and CP-treated rat fetuses showing incomplete ossification of all skull bones (parietal (p), interparietal (ip), supraoccipital (So) and occipital (oc) bones], hip bone (hi) and femur (Fe). Also, irregular ribs (ri) and short 13th rib (green arrow) can be seen. Extra ossification centers are seen in lumbar and sacral regions (black arrow). (Alizarine red stain).

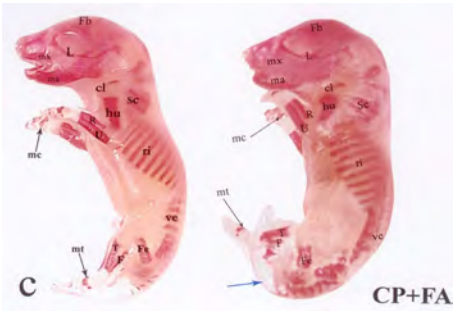

Fig.15: A photograph showing lateral view of control (C) and CP-treated rat fetuses showing brachygnathia and incomplete ossification of metacarpal (mc) and metatarsal (mt) bones. Irregular humerus (hu) and ribs (ri) can be seen. Also, there is absence of caudal vertebrae (blue arrow). Normally ossified skull bones (frontal (Fb), lacrimal (L), maxilla (mx) and mandible (ma)), clavicle (cl), scapula (Sc), radius (R), ulna (U), femur (Fe), tibia (T) and fibula (Fi) can be seen (Alizarine red stain).

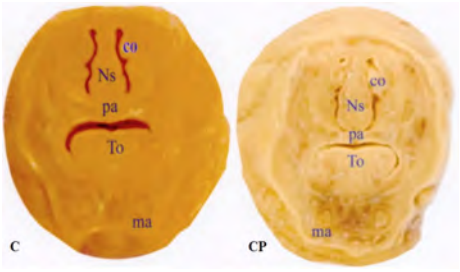

Fig.16: A photograph of section at the region of the angle of the mouth showing normal nasal conchae (co), nasal septum (Ns) palate (pa), tongue (To) and mandible (ma) in control fetus. In CP-treated fetus, it showed normal palate (pa), tongue (To) and mandible (ma), but malformed nasal conchae (co) and thick nasal septum (Ns). (Razor section).

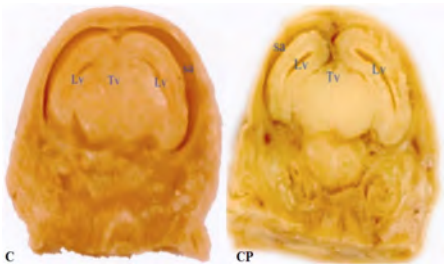

Fig.17: A photograph of section at the level of the greatest transverse diameter of the head showing normal lateral ventricle (Lv), third ventricle (Tv) and regular subarachnoid space (sa) in control fetus. In CP-treated fetus, it showed normal third ventricle (Tv), but showed malformed brain, bilaterally dilated lateral ventricles (Lv) and subarachnoid space (sa). (Razor section).

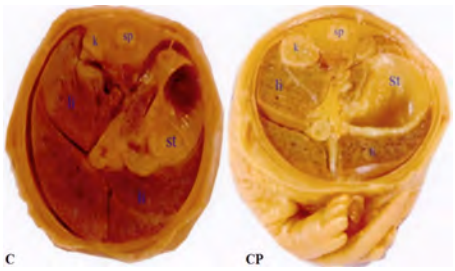

Fig.18: A photograph of section at the level of the abdominal region just above the umbilicus showing normal spinal cord (sp), kidney (k), stomach (St) and liver (li) in control fetus. In CP-treated fetus, it showed normal spinal cord (sp), stomach (St) and liver [li] but showed large malformed kidneys (k). (Razor section).

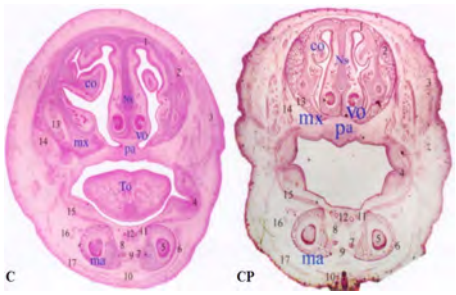

Fig.19: A photograph of section at the level of the angle of the mouth of control fetus showing normal nasal septum (Ns), vomeronasal cartilage (vo), conchae (co), maxilla (mx), palate (pa), tongue (To) and mandible (ma). In CP-treated fetuses, there are normal conchae (co), vomeronasal cartilage (vo) and palate (pa), but malformed mandible (ma), hypertrophied nasal septum (Ns), narrow nasal cavity and rudimentary maxilla (mx) are seen. (Hx & E X 10).
1: Nasal cartilage, 2: Ethmoid, 3: Temporoparietalis muscle, 4:Angle of the mouth, 5: Meckel's cartilage, 6: Depressor angluli muscle, 7: Hypoglossal nerve, 8: Genioglossus muscle, 9: Mylohyoid muscle, 10: Submental artery, 11:Sublingual duct, 12:Submandibular duct, 13-Maxillary sinus, 14: Levator anguli, 15: Buccinator muscle, 16: Facial artery, 17: Platysma muscle

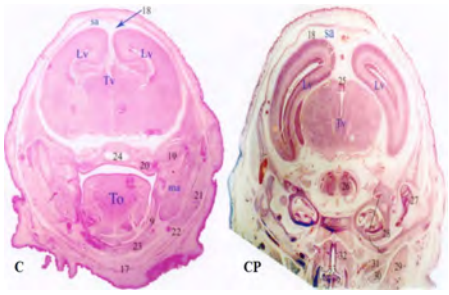

Fig.20: A photograph of section at the level of the largest transverse diameter of the head of control rat fetus showing normal lateral ventricle (Lv), third ventricle (Tv), subarachnoid space (sa), mandible (ma) and tongue (To). 18: Pia mater, 19: Lateral pterygoid muscle, 20: Tooth bud, 21: Masseter muscle, 22: Mandibular branch of facial nerve, 23: Anterior belly of digastric, 24: Nasopharynx. In CP-treated rats there is dilated lateral (Lv), third (Tv) ventricles and subarachnoid space (sa). 18: Pia mater, 25: Telachoroidae of 3rd ventricle, 26: Cerebellum, 27: Temporomandibular joint, 28: Inner ear, 29: Sternomastoid muscle, 30: Splenius capitis, 31: Longissimus capitis, 32: Pharyngeal constrictors, 33: Larynx. (Hx& E X 10).

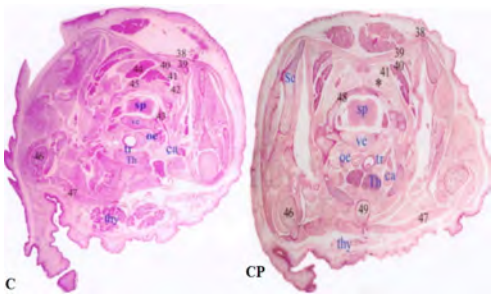

Fig.21: A photograph of section at the root of the neck of the control rat fetus showing normal spinal cord (sp), vertebra (ve), oesophagus (oe), trachea (tr), carotid sheath (ca), thyroid (Th) and thymus (thy) glands. In CP-treated fetus, it showed normal trachea (tr), carotid sheath (ca) and thyroid gland (Th) but showed malformed spinal cord (sp) and vertebra (ve), esophagus (oe) is deviated to the right and the thymus gland (thy) is rudimentary. (Hx& E X 10).
38: Trapezius, 39: Rhomboideus cervicis, 40: Splenius capitis, 41: Semispinalis capitis, 42: Longissimus muscles, 43: Dorsal root ganglion, 44: Semispinalis cervicis, 45: Multifidus, 46-Shoulder joint, 47: Pectoralis muscle, 48: Vertebral lamina, 49-Manubrium sterni, *(44& 45)-Semispinalis cervicis and multifidus.

Discussion

The current study reported significant reduction of body measurements and BW in fetuses exposed to CP during pregnancy and concomitantly there were multiple skeletal abnormalities detected in Alizarine red stained specimens and in Hx & E histological specimens. These findings indicated the teratogenic effects of CP exposure during pregnancy that go in hand with that previously reported in literature wherein Savion et al.[18] reported that in CP-treated female rats, a decrease in embryonic weight and increase in the resorption rate and the percentage of embryos exhibiting head malformations were noted with the appearance of apoptotic cells in the head but not in the liver and an increased expression of p53 in embryonic organs, while bcl-2 expression was decreased in the head and increased in the liver. Xiao et al.[19] examined neurocytotoxic effects of CP using scanning electron microscope and reported that compared with control group, the malformation incidence in the experimental groups was significantly higher and neural tube serial section of fetus showed apoptotic morphological features 24 h after CP administrated in the form of decreased growth and viability of neurons and damaged nuclear DNA.

Park et al.[20,21,22] found that CP reduced fetal and placental weights by 30-40% and induced malformations in live fetuses; 94.6%, 41.5% and 100% of the external (skull and limb defects), visceral (cerebroventricular dilatation, cleft palate and ureteric dilatation) and skeletal (acrania, vertebral/costal malformations and delayed ossification) abnormalities. Shreder et al.[23] observed that on CP prenatal exposure, intact rat embryonic and placental tissues have a characteristic ratio of cells with different degrees of DNA fragmentation and CP genotoxic effect was accompanied by a quantitative deviation from the control group ratio of different cell types with a relationship between the abnormal ratio of different cell types in the embryonic and placental tissues and the occurrence of embryonic morphological abnormalities.

In support of the deleterious ef-

fects of exposure to CP on skeletal development and growth, Olivera et al.[24] injected rats aged 26 days by CP and reported significantly reduced BW, body length and mandibular size and these parameters did not recover with time, which means that catch-up growth did not occur and that the overall growth of the body was permanently affected by the drug.

In trial to explore the mechanism underlying CP teratogenicity; Hao et al.[25] reported that eight hours after CP injection there was marked increase in brain macrophages/amoeboid microglia in different areas of the fetal brain as determined by lectin histochemistry, induction of tumor necrosis factor (TNF)-α mRNA, which was progressively increased with time, tissue growth factor (TGF)-β mRNA was undetectable until 24 h had elapsed, expression of interleukins 1 and 6 was undetectable at all stages and suggested that both TNF-α and TGF-β are involved in the progression of CP-induced neural damage in fetal brain. Luijten et al.[26] found that CP without metabolic activation is not teratogenic to rat embryos, as evidenced by a lack of effect on either embryonic growth or on morphological development, but after pre-incubation with Aroclor-induced hepatic microsomes, CP became highly embryotoxic in the whole embryo culture and concluded that CP is pro-teratogenic compound that require activation or induction of hepatic microsomes to be teratogenic.

The studies conducted by Park et al. supported the assumption that CP required activation to be teratogenic where Park et al.[20] reported that pre-treatment with green tea extract (GTE), CP-induced BW loss and abnormalities of fetuses were remarkably aggravated, and repeated treatment with GTE greatly increased mRNA expression and activity of hepatic cytochrome P-450 (CYP) 2B, which metabolizes CP into teratogenic acrolein and cytotoxic phosphoramide mustard, while reducing CYP3A expression (a detoxifying enzyme). Park et al.[21] found pretreatment with piperonyl butoxide greatly decreased mRNA expression and activity of hepatic CYP2B, and attenuated CP-induced BW loss and abnormali-

ties of fetuses. Moreover, Park et al.[22] found that pre-treatment with licorice, CP-induced BW loss and fetal abnormalities were remarkably aggravated and repeated treatment with licorice greatly increased mRNA expression and activity of hepatic CYP2B.

Supplemental folic acid in group CP+FA significantly improved BW and number of live births and could partly ameliorate external, skeletal and nervous system malformations. Thus indicating a beneficial effect of supplemental FA and goes in hand with Ma et al.[25] who found that CP prenatal exposure resulted in decreased weight and length of the fetuses, increased rate of neural tube defects and a retardation of ossification centers with a frequency of neural tube defects in group with no supplementation, supplemented with FA alone and supplemented with FA and vitamins A and E were 87.27%, 42.93% and 30.08% respectively and concluded that FA supplementation, alone or in combination with vitamins A and E, resulted in greater fetal weight and length and increased ossification of supraoccipital bone.

Xiao et al.[26] documented that FA should be the main protective factor of neural tube defects and isoflavone might reinforce this protective effect of FA by increasing its antioxidative ability, however, the effect is related with the ratio of the two factors. Zhao et al. [27] reported that the neuroprotective effect of FA or soybean isoflavone as solo drug was evident in the form of lower incidence of malformation, higher fetus' development indices, decreased damaged nuclear DNA, early apoptotic morphological changes and up-regulation of Bcl-2 and the down-regulation of Bax and P53 in embryo brains, but these effects were more superiorly manifested in combination versus solo-interventions.

Conclusion

Prenatal exposure to CP induces manifest teratogenic effects especially altering the growth levels with multiple skeletal abnormalities. Additionally, CP induced significant brain and spinal cord detrimental effects. FA supplementation could ameliorate

CP-induced changes but could not be a grantee for safe pregnancy outcome.

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BENHA MEDICAL JOURNAL

TERATOGENIC EFFECT OF PRENATAL EXPOSURE TO CYCLOPHOSPHAMIDE: EVALUATION OF THE AMELIORATIVE EFFECT OF SUPPLEMENTAL FOLIC ACID

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ROLE OF MULTI SLICE CT IN EVALUATION OF PORTO- SYSTEMIC COLLATERALS IN PORTAL HYPERTENSION

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Abstract

Objective: *Study the role of computed tomography in evaluation of collaterals in cases of portal hypertension.*

Background: *Liver cirrhosis can result in portal hypertension. Portosystemic collaterals are one of the severe complications. It may cause massive hemorrhage of the upper gastrointestinal tract. Therefore prompt evaluation of portosystemic collaterals is imperative.*

Methods: *This study included 80 patients, 63 males and 17 females with age distribution from 13 years to 73 years old. This study was conducted in the period (May 2012 to Feb 2014), from the outpatient clinic and the inpatient department of Hepatology department, National Liver Institute, Menofiya University. Patients who had finding of portal hyprtension on clinical examination underwent multislice CT.*

Results: *Multislice CT & CT portography has been shown to be useful for the evaluation of portosystemic collaterals in cases of portal hypertension.*

Conclusion: *CT and CT portography have become the tools for investigation of the liver and can detect the potentially problematic varices by detailing the course of these tortuous vessels. This knowledge is important in major operation as liver transplantation for detection of unexpected varix which can result in significant bleeding.*

Key Words: *Computed tomography, portal hypertension, collaterals.*

Introduction

Liver cirrhosis can result in portal hypertension. Gastric fundic and/or esophageal varices are one of the severe complications. It may cause massive hemorrhage of the upper gastrointestinal tract. The clinical management of gastric varices (GV) is related to their hemodynamics and locations. GV were classified into three types according to Sarin's Classification that is based on varices location by endoscopy.[1]

Precise and reliable assessment of the portal venous system in patients with hepatic cirrhosis and portal hypertension is essential before liver transplantation, non surgical Trans jugular shunting, or Surgical Porto systemic shunting. In patients with portal hypertension and a history of gastro esophageal bleeding, it is mandatory to determine whether the portal venous system is patent or the portal vein or its main branches has thrombosis[2].

Multidetector CT angiography has become powerful tool for investigation of the liver. The addition of a portal-phase acquisition with 3 dimensional vascular reconstructions can augment the Surgeon's perception of potentially problematic varices by detailing the course of these tortuous vessels. This knowledge is important not only for major operations such as liver transplantation but for more common procedures in which an unexpected varix can result in significant bleeding. With this tool, the radiologist can significantly affect patient care and alert a colleague to potential disaster[3].

Methods

This study included 80 patients, 63 males and 13 females with age distribution from 13 years to 73 years old over a period from (May 2012 to Feb 2014), from the outpatient clinic and the inpatient department of Hepatology department, National Liver Institute, Menofiya University.

All patients were subjected to the following:

- Full history sheet.
- Clinical examination.
- Laboratory investigation.
- Triphasic multislice CT scanning.

Upper endoscopy for 10 patients for sclerotherapy:

Multislice triphasic CT scanning.

CT was performed with a Siemens Somatome Definition scanner (20 detectors) in national liver institute.

Patient preparation:

- Fasting for 6 hours before scan.
- No oral contrast was used.
- Creatinin clearance should be >30.
- Vigorous Oral hydration.
- Intravenous cannula introduced through antecubital vein.

(Prtocol of Siemens Somatome Definition scanner (20 detectors) in national liver institute).

Patient position:

• The patient lies supine, scanning start from the lung bases down to the symphesis pupis in all phases.

• One scout was acquired in anteroposterior view. The examination is planned on these scouts from the level of the top of the right diaphragmatic copula (Hepatic Dome) till the symphesis pupis in the precontrast and post contrast sequences.

• The precontrast series is taken by using a 10mm section thickness, a slice pitch of 1.5 a gantry rotation period 0.6 second and a table speed of 15 mm per rotation. X-ray tube voltage was 120 KV, and the current was 240-280 mA.

• Multidetector CT scanner is applied to perform, arterial, portovenous& delayed phases on all patients. All patients received 120ml of non ionic material (ultravist 300) is introduced with an infusion rate 4.0ml/sec IV using single power injector.

• Arterial dominant phase images were acquired at 18 sec (collimation, 1.25 mm; pitch 0.6 ; kVp, 120; mA, 240-280).

• Portal dominant phase images were acquired at 60 sec (collimation, 2.5 mm; pitch 0.6; kVp, 120; mA, 240-280).

• Delayed phase images also then taken through the entire liver and were acquired at 200 sec (collimation, 2.5 mm; pitch 0.6; kVp, 120; mA, 240-280).

Image processing:

All further data were reconstructed with a standard algorithm, and post-processing was

performed on a commercially available workstation (Syngo work station) that equipped with soft ware tool that allow generation of 3D images.

We use MIP technique for 3D image reconstruction that is helpful in detection of details and orientation of vessels.

Image interptation:

The following features were recorded:

1. The portogrphy images& portal phase images were analyzed for portal vein patency& diameter.
2. Splenic vein diameter.
3. Signs of portal hypertension as liver cirrhosis, splenomegaly, ascites.
4. Prescence of collaterals, its sites and detection of its grading.

The dilated varices are classified into varices draining into the SVC as esophageal, paraesophageal & gastric varices. Varices draining into the IVC as splenic, perisplenic, linorenal& recanalized paraumblical vein. The dilated veins present within & outside the wall of the lower esophagus are termed the esophagealand paraesophageal respectively.

The dilated veins within the submucosal layer of the stomach are the gastric submucosal varices whereas that within the adventitial layer at the exterior wall of the stomach is termed the gastric adventitial varices. The dilated veins along the splenic hilum are termed the splenic varices whereas veins surrounding the spleen are termed the perisplenic. The recanalized paraumblical vein is the dilated vein at the site of the ligmantum teres & falciform ligament. On cross section it appears as circular or tubular structure.[4] (fig. 1)

Varices involving the splnic, linorenal and recanalized paraumblical vein were defined as vascular structure if diameter >3mm for the esophageal, paraesophageal & gastric collaterals the size criterion was 2mm in diameter. Detecting grading of varices by measuring the largest visible varix, according to the diameter of the largest varix and the number of varices on cross section images varices are graded on 5-point scale. If there more than 4dilated vessels on 2d cross section the va-

rices were graded one step higher.[4] (Table 1)

Image Display:

All images, including 3D reconstructed models, were sent to (Syngo work station) which found in National Liver Institute and permits interactive analysis.

Statistical Analysis:

Our data were collected, coded and processed by statistical software (SPSS) under professional XP and the following statistical tests were applied:

Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS version 20. Two types of statistics were done:

- Descriptive: e.g. percentage (%), mean and standard deviation SD.
- Analytical:
  - **Pearson's Correlation analysis:** It is used to show strength and direction of association between two quantitative variables.
  - P value.
  - Significant difference if P<0.05.
  - Non-significant difference if P>0.05.
  - Highly significant difference if P<0.001.

Results

- **Table 1 shows** CT grading scale of collaterals.
- **Table 2 shows** the perigastric collaterals group is the commonest type of collaterals draining into SVC present in 76.25% of cases.
- **Table 3 shows** splenic hilum& peri splenic collateral group is the commonest type of collaterals draining into the IVC present in 56.25% of cases.
- **Table 4 shows** shows upgrading of esophageal collaterals by CT in comparison with endoscope.
- **Table 5 shows** there is significant correlation between the PV diameter & number of collaterals as p=0.001. The increase in collaterals number is associated with decrease in PV diameter.

There is high significant correlation between the PV diameter & splenic vein diameter as well as the middle hepatic vein diameter as p>0.001. The increase in PV diameter is associated with increase in splenic vein diameter and decrease in middle hepatic vein diameter.

Table (1): CT grading of collaterals on 5 scale.

Varices	Largest Diameter of Varices (mm)
Esophageal, paraesophageal, and gastric submucosal varices grade	
0	< 2
1	2-2.9
2	3-6.9
3	≥ 7
4[a]	≥ 7
Gastric adventital, splenic, mesenteric, retroperitoneal varices grade	
0	< 3
1	3-4.9
2	5-9.9
3	≥ 10
4[a]	≥ 10

Table (2): Distribution of the studied patients regarding their collaterals draining into SVC.

Collaterals draining into SVC	Patients	
	No	%
Esophageal- Para esophageal		
Present	56	70.0
Absent	24	30.0
Perigastric		
Present	61	76.25
Absent	19	23.75

Table (3): distribution of the studied patients regarding their collaterals draining into Inferior Vena Cava (IVC):

Collaterals draining into IVC	Patients	
	No	%
Lino-Renal		
Present	23	28.75
Absent	57	71.25
Splenic hilum-Peri splenic		
Present	45	56.25
Absent	35	43.75
Para-umbilical vein		
Present	8	10.0
Absent	72	90.0
Intra-hepatic collaterals		
Present	0	0.0
Absent	80	80.0

Table (4): Distribution of the studied patients regarding their grading by CT and endoscope:

	Patients (n=10)			
	Endoscopic		CT	
	no	%	no	%
II	4	40.0	1	10.0
III	5	50.0	3	30.0
IV	1	10.0	6	60.0

Table (5): Correlation between PV diameter and Number of collaterals, splenic vein diameter and Middle hepatic vein diameter:

	PV diameter	
	R	P value
Number of collaterals	-0.407	0.001(S)
Splenic vein diameter	0.812	<0.001(HS)
Middle hepatic vein diameter	0.709	<0.001(HS)

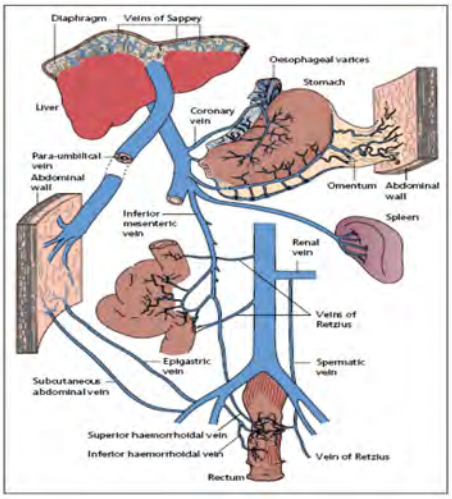

Fig. 1: Diagram shows Sites of portal-systemic collateral circulation.

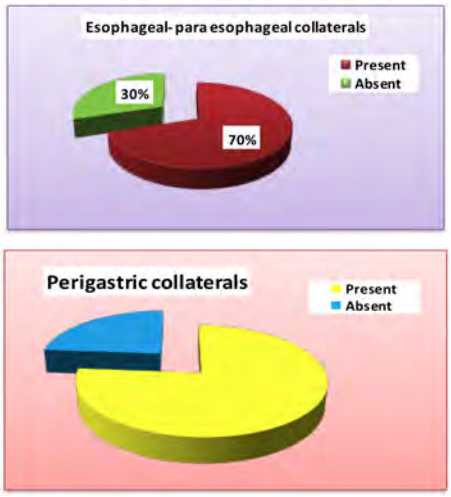

Fig. 2: Descriptive statics shows distribution of the studied patients regarding collaterals draining into (SVC).

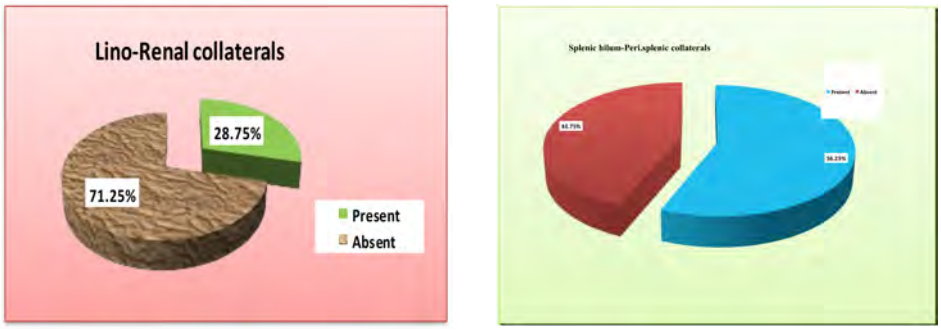

Fig. 3: Descriptive statics shows distribution of the studied patients regarding collaterals draining into (IVC).

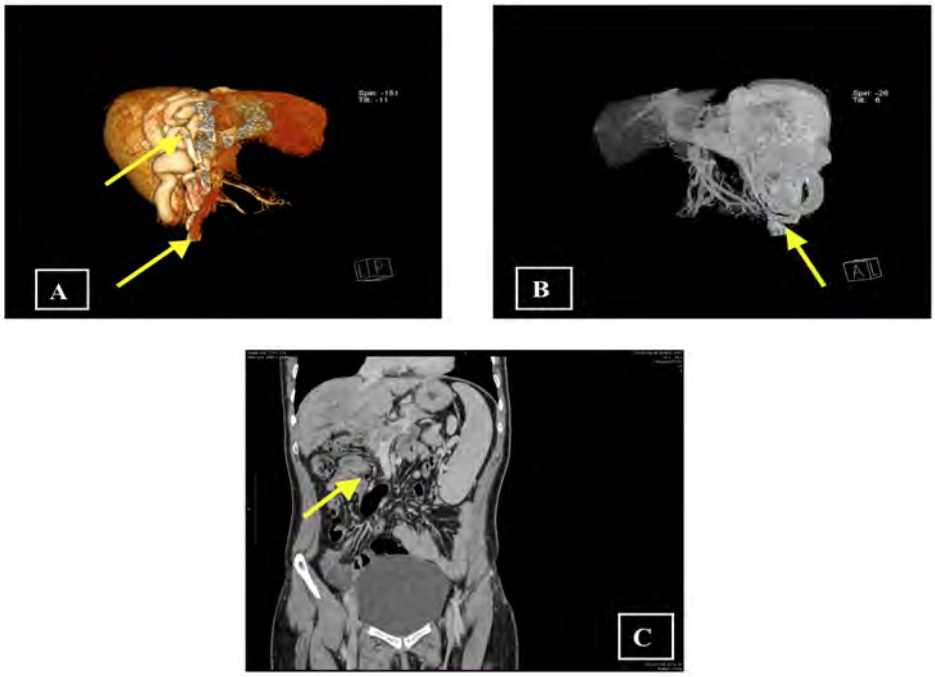

Fig. 4: A: Volume rendering technique image shows dilated linorenal & splenic collaterals **B:** CT portography shows dilated linorenal collaterals. **C:** CT cronal image shows HCC infiltrating the PV.

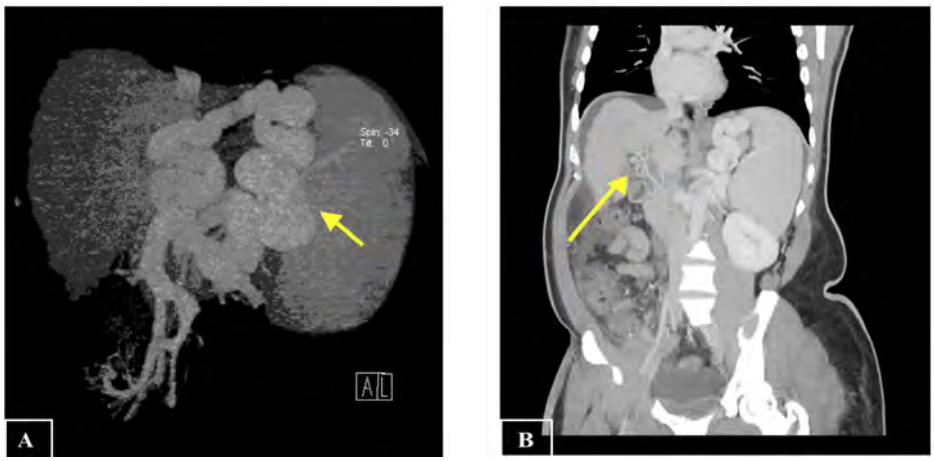

Fig. 5: A: CT portography image shows the dilated perigatric collateralS **B:** CT cronal image shows PV thrombosis recanalized by collaterals.

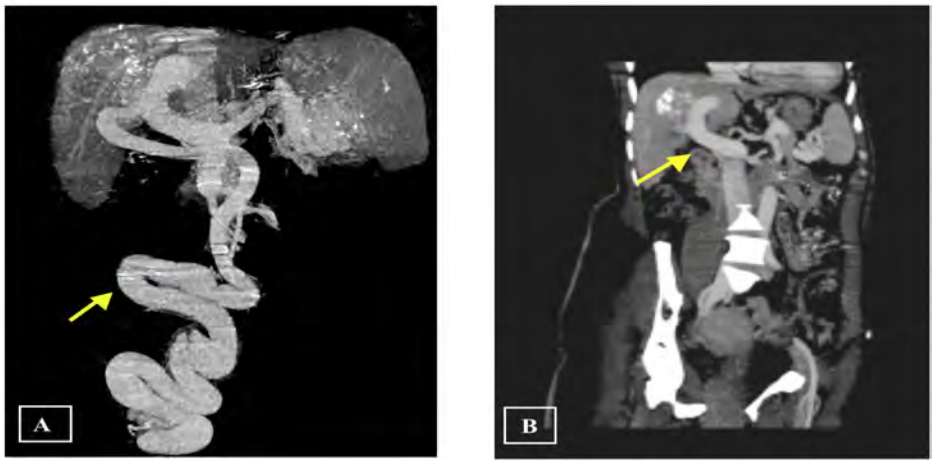

Fig. 6: A: CT portography image shows the recanalized paraumblical vein, **B:** CT cronal image shows dilated portal vein.

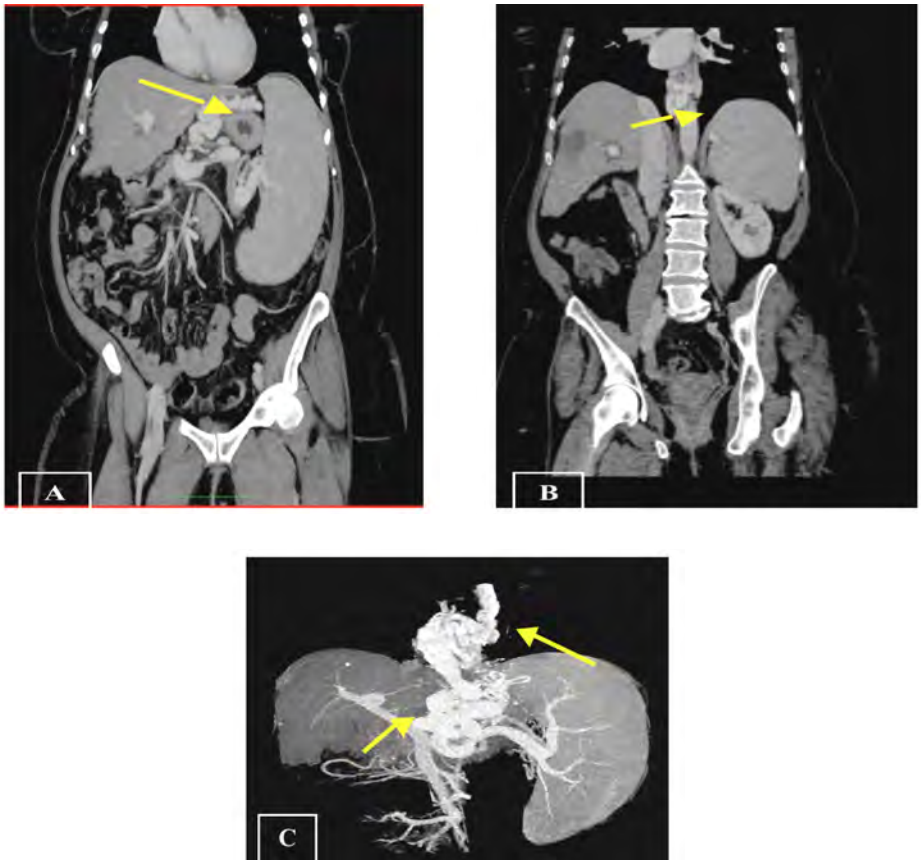

Fig. 7: A & B: CT cronal image shows the dilated perigastric & esophageal collaterls respectively. **C:** CT portography image shows the same dilated collaterls.

Discussion

The portal system consists of all veins that carry blood from the abdominal part of the alimentary tract except lower rectum and anal canal. It also receives drainage from the pancreas, spleen and gall bladder multidetector MDCT portal venography can determine the extent and the location of portosystemic collaterals in patient with portal hypertension.(5)

In cirrhotic patients with portal hypertension some blood in the portal venous system may reverse direction and pass through the portosytemic anastomoses in the systemic venous system.as aresult a variety of major hepatofugal collateral pathway can develop in patients with portal hypertension. For many years, angiography was considered the standard for detecting collaterals vessels.(6)

Multidetector computer tomography portal venography can display esophageal varices, gastric varices and related by bass circuits more especially because of the thinner slice and better spatial resolution of the computed tomography scanner.(1)

In agreement to our study(7) reported that CT-mip venography could be an effective and non invasive method for detecting the compensatory circulation resulting from decompensated portal hypertension.

With disagreement to our study computed tomography is a second line to ultrasonography with color doppler in patient with known portal hypertension not a primary.(8) However, in our study we consider CT is the first line for demonstration of all types of collaterals and hepatocellualr carcinoma.

Our study showed that the commonest type of collaterals draining into superior vena cava is the perigastric type as we detected esophageal & paraesophageal collaterals in 70% of cases, perigastric in 76.25%.

These result are in agreement to(9) who documented that the commonest type is the perigastric group as demonstrate the gastric fundic varices in 32 cases (97.0%) and esophageal varices in 27 (81.8%) and similarly to(7) who demonstrate gastric varices in 97% of cases and

esophageal varices shown in 83% and according to[8] who detected collaterals along the left gastric vein in 13%, esophageal collaterals shown in 6% of cases, This is due to similarity in the diseases leading to portal hypertension.

These results are with disagreement to[10] who detected esophageal collaterals in 90% of cases, gastric in 34% of cases. This is due to changes in the causes of portal hypertension.

According to collaterals draining into the inferior vena cava our study reported that the commonest type of collaterals was the splenic collaterals which shown in 56.25%, however the linorenal in 28.75%, recanalized paraumblical vein in 10% and no intahepatic collaterals detected. These results are in agreement to[7] who reported that the collaterals originated from the splenic vein shown in 67% and the paraumblical vein shown in 17%. This is due to similarity in the diseases leading to portal hypertension.

These results are in disagreement with[10] who detected collaterals at splenic hilum in 7%, recanalized paraumblical vein in 24% and mesenteric collaterals in 2%. This is due to changes in the causes of portal hypertension.

With comparison of 10 patient who underwent upper endoscopy and abdominal triphasic CT to detect esophageal varices grading it was found that there was upgrading of collaterals by CT.

This is in agreement to[11] who reported that endoscopic undergrading of high risk esophageal group will lead to overestimation of CT sensitivity for low risk esophageal varices & also in agreement to[12] reported that careful evaluation of high risk esophageal varices on a liver MDCT examination may be useful to avoid performing endoscopy. CT can be used a single non invasive surveillance tool for both esophageal varices and recurrent HCC.

Similarly[13] who reported that CT demonstrated high sensitivity for assessement of high risk gastric varices and in addition detected gastric varices in many patient in whom gastric varices not re-

ported in endocope & similarly according to[14] reported that in correlation to endoscope, MDCT is useful for prediction of high risk esophageal varices.

In our study it was found there is significant correlation between the portal vein diameter and number of collaterals as (P=0.001). The increase in number of collaterals is associated with decrease in portal vein diameter due to conversion of blood from portal vein to the collaterals.

Also high significant correlation between the portal vein diameter and splenic vein diameter as (P<0.001). The increase in portal vein diameter is associated with increase in splenic vein diamter and with correlation with middle hepatic vein diameter it was found that (P<0.001). The increase in portal vein diameter is associated with decrease in hepatic vein diameter

Conclusion

In Conclusion: At the end of this work, it can be concluded that multislice CT serves as an important non invasive imaging modality in diagnosis of collaterals in cases of portal hypertension. And also found that CT portography can replace endoscope in detection of varices.

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ROLE OF MULTI SLICE CT IN EVALUATION OF PORTO- SYSTEMIC COLLATERALS IN PORTAL HYPERTENSION

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ASSESSMENT OF CALCITONIN GENE RELATED PEPTIDE IN DIABETIC PATIENTS WITH CHARCOT FOOT

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Abstract

Introduction: *Charcot neuro-osteoarthropathy is a severely disabling complication of diabetes. It is characterised by progressive osteolysis, that lead to multiple fractures, joint destruction and joint dislocation which can results in severe deformity with ulceration, and eventually amputation. Abnormal calcitonin gene-related peptide may play a role in the development of Charcot foot.* ***Aim of the work:*** *studying the level of calcitonin gene-related peptide in Charcot neuroarthropathy diabetic patients and its relation to the pathogenesis.* ***Patients and Methods:*** *Patients were divided into four groups. Charcot patients according to criteria for the Eichenholtz classification system, were divided into acute and chronic groups (n=15 and 26) respectively, diabetic neuropathy group (n=15) and the healthy control group(n=15). Serum level of calcitonin gene related peptide were determined using radioimmunoassay.* ***Results:*** *Calcitonin gene related peptide levels in the acute,chronic Charcot and neuropathy groups were significantly higher than in the control group. The highest levels were observed in the Charcot group specially chronic. There were no significant differences in calcitonin gene related peptide levels between the Charcot and neuropathy groups. Calcitonin gene related peptide levels in the complicated ulcerative Charcot and neuropathy groups were significantly higher than in the noncomplicated group.* ***Conclusion:*** *Calcitonin gene related peptide may have a role in the pathogenesis of Charcot foot in patients with diabetes and may be an indicator of an accompanying osteomylitis.*

Introduction

Charcot neuro-osteoarthropathy (CNO) which was named after French neurologist Jean-Martin Charcot (1825-1893), is a progressive denervation-induced degeneration of the foot and ankle joints complicating of diabetes. It is characterised by progressive osteolysis, that lead to multiple fractures, joint destruction and joint dislocation which can results in severe deformity with ulceration, and eventually amputation[1,2]. CNO diagnosed cases of associated with diabetic patients range from 0.08% to 7.5%[3].

The exact pathogenesis of CNO remains elusive. Two theories (neurotraumatic and neurovascular) explain the pathogenesis of Charcot foot[4]. Calcitonin Gene Related Peptide (CGRP) is distributed throughout the central and peripheral nervous systems and exhibits a range of biological effects on tissues including those associated with gastrointestinal, respiratory, endocrine, and central nervous systems[5].

In the last twenty years, several studies have demonstrated that the nervous system is directly involved in bone remodelling and that both osteoblasts and osteoclasts express functional receptors for CGRP, substance P and VIP neuropeptides[6]. On top of that, abnormal calcitonin gene-related peptide and endothelial nitric oxide synthase activity may play a role in the development of Charcot foot as osteoclasts are recruited by RANKL in an unchecked fashion[7].

Aim Of The Work

The aim of the present study is to study the level of calcitonin gene-related peptide in Charcot neuroarthropathy diabetic patients and its relation to the pathogenesis.

Patients and Methods

The present study was carried out on patients selected from the diabetic foot clinic, Internal Medicine specialized hospital, Mansoura University, in the period between 15/7/2012 and 7/6/2014. This is a case control study that conforms to the medical Sciences Ethics Committee of Mansoura Faculty of Medicine and all the included patients have their written informed consent.

A total of 41 consecutive patients with Charcot neuroarthropathy (15 acute, 26 chronic, 22 men, 19 women; 1 with Type 1 diabetes, 40 with Type 2 diabetes) and 15 patients with diabetes, neuropathy and no history of Charcot neuroarthropathy (10 men, 5 women; all of 15 patients with Type 2 diabetes), as well as 15 healthy controls (6 men and 9 women) were included in the study. There is insignificant difference between acute, chronic Charcot, neuropathy and controls as regard age (54.80±7.66 years, 56.92±7.30 years, 59.07±6.63 years, 52.73±5.51 years respectively). In the non-Charcot group patients were required to have a vibration perception threshold (VPT) >25V and normal foot radiographs. The control group included unrelated individuals that worked at a hospital or were hospitalized for reasons other than diabetes, and who had never been diagnosed with diabetes (covering the same geographical area as the case group).

Charcot neuroarthropathy was diagnosed on the basis of the criteria for the Eichenholtz classification system and clinical presentation of a hot swollen foot and skin foot temperature more than 2_C higher than the contralateral foot and confirmed by typical radiological findings (destruction or fracture of bone, joint subluxation or destruction) on standard foot radiographs and MRI examination and divided into acute and chronic. Neuropathy was diagnosed on clinical grounds after quantitative assessment of vibration perception threshold determined as an average of three readings. A vibration threshold above 25 V was considered pathological[8]. Assessment of pressure sensation (10 g Semmes-Weinstein monofilament; and qualitative assessment of thermal threshold were also performed. In the patients with neuropathy Charcot neuroarthropathy was excluded on the basis of a lack of typical clinical presentation and normal radiographs and MRI.

Following the medical history and clinical examination, radiological evaluation by X ray and MRI examination was performed in the Radiology Department, Mansoura Faculty of Medicine. Study of cal-

citonin gene related peptide serum level and other laboratory investigations were conducted in the laboratories of the Clinical Pathology Department, Mansoura Faculty of Medicine.

Sample collection and Calcitonin gene related peptide measurement:

Three ml blood sample was drawn from the peripheral veins, and then collected in a serum separator tube (SST) and samples were allowed to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Subsequently, it was kept in a refrigerator at -20-80 C. The radioimmunoassay kits measuring the concentrations of serum CGRP were purchased from Wuhan EIAab, Wuhan, CHINA. The operational procedure was performed strictly according to the manufacture's instructions (Catalog No: E0876h).

Statistical analysis:

Statistical package for package SPSS (version 18.0, Echosoft Corp., USA, 2008) was used for data. Analysis was performed with statistica 8.0 software. Continuous variables were expressed as means±SD, and both number and percentage for categorized data. Variance analysis was used to evaluate the significance of difference among the groups. The means between the two groups were compared with the q test. Linear Pearson correlation analysis was performed to assess the relationship between the levels of serum CGRP and different patient parameters. A P value ≤0.05 was considered statistically significant. Quantitative variables were compared by means of a paired Student t test for two groups or ANOVA and Fisher's exact tests for comparison of qualitative values, P value ≤0.05 was considered statistically significant.

Results

The included patients have age range from 40-70 years; All patients are over 40 years old. 80% of acute Charcot patients are between 40-60 years old. 65% of chronic Charcot patients are between 40-60 years old. 53% of diabetic neuropathy patients are over 60 years old. The mean duration of diabetes was similar in the group of Charcot patients (acute

and chronic) and in the neuropathic patients (13.60±8.15, years 16.38±8.80 and 19.80±4.69 years, respectively; P=0.099), table (1).

There is significant difference between acute, chronic Charcot and neuropathic patients as regard BMI and this difference is mainly between chronic Charcot and the diabetic neuropathy patients as the former are obese than the later, p=0.042, table (1).

There is significant difference between acute, chronic Charcot and neuropathic patients as regard presence of HTN, p=0.021, table (2). All neuropathic patients are hypertensive for at least 10 years. The least hypertensive percent 65.4% is for chronic Charcot group. Eleven patients in the three groups have associated foot ulceration (3,4,4) patients with acute Charcot, chronic Charcot, and neuropathy respectively. Table (2).

There is no statistically significant difference between acute, chronic Charcot and neuropathic patients as regard DEXA scan performed at the femur, spine and forearm, table (3).

There is are statistically significant difference between acute Charcot patients and controls as regard HBA1c, CRP, ESR and CGRP, table (3). There is are statistically significant difference between chronic Charcot patients and controls as regard all parameters except uric acid, Alb/creat. Ratio and phosphorus, where the difference is statistically insignificant, table (3). There is no statistically significant difference between acute and chronic Charcot patients as regard all parameters, table (3). There is statistically significant difference between chronic Charcot and neuropathic patients as regard BMI, CRP, ESR, Cholesterol and Triglycerides, table (3).

There is statistically significant difference between neuropathic patients and controls as regard HBA1c, ESR, uric acid Alb/creat. ratio, table(3). There is statistically significant difference between acute, chronic Charcot, neuropathic patients and controls as regard CGRP, P=0.017 table (4). There is statistically significant difference between ulcerative and non ulcerative as regard CGRP. p=

0.046, table (5).

Calcitonin gene related peptide levels in the acute, chronic Charcot and neuropathy groups were significantly higher than in the control group. The highest levels were observed in the Charcot group specially chronic. There were no significant differences in calcitonin gene related peptide levels between the Charcot and neuropathy groups. Calcitonin gene related peptide levels in the complicated ulcerative Charcot and neuropathy groups were significantly higher than in the noncomplicated group, table (4,5).

There is significant correlation between CGRP and CRP and ESR. However, there is no significant correlation between CGRP and other parameters, table (6).

Table (1): Clinical parameters in acute, chronic Charcot, neuropathic patients and controls.

	Acute Charcot (n = 15) Mean ± SD	Chronic Charcot (n = 26) Mean ± SD	Diabetic neuropathy (n = 15) Mean ± SD	Control (n = 15) Mean ± SD	F	P
age	54.80±7.66	56.92±7.30	59.07±6.63	52.73±5.51	9.385	0.68
Sex						
Male	7 (46.7%)	15 (57.7%)	10 (66.7%)	6 (40%)	2.610	0.456
Female	8 (53.3%)	11 (42.3%)	5 (33.3%)	9 (60%)		
Occupation						
HW	7 (46.7%)	14 (53.8%)	5 (33.3%)	10 (66.7%)		
Employee	5 (33.3%)	3 (11.5%)	4 (26.7%)	1 (6.7%)		
Farmer	2 (13.3%)	5 (19.2%)	2 (13.3%)	0 (0%)	18.486	0.102
Worker	1 (6.7%)	2 (7.7%)	0 (0%)	3 (20%)		
Driver	0 (0%)	2 (7.7%)	4 (26.7%)	1 (6.7%)		
DM type						
I	1 (6.7%)	0 (0%)	0 (0%)		2.783	0.249
II	14 (93.3%)	26 (100%)	15 (100%)			
DM Duration	13.60±8.15	16.38±8.80	19.80±4.69		2.420	0.099
BMI	29.58±3.51	30.88 ± 3.03	28.07±3.01	29.88 ± 2.15	2.877	0.042

Table (2): History of HTN and foot ulcer in Charcot patients (acute and chronic), and neuropathic patients.

	Acute Charcot (n = 15)		Chronic Charcot (n = 26)		Diabetic neuropathy (n = 15)		χ^2 Chi-square	P
	No	%	No	%	No	%		
HTN +ve	13	86.7	17	65.4	15	100	7.738	0.021*
Ulcer	3	20	4	15.4	4	26.7	3.512	0.681

Table (3): parameters in acute, chronic Charcot, neuropathic patients and controls.

	Acute Charcot (n = 15)	Chronic Charcot (n = 26)	Diabetic neuropathy (n = 15)	Control (n = 15)	F	P
HBA1c	9.51±2.73	9.43±1.71	9.39 ± 1.88	5.47 ± 0.51	18.059	0.000
CRP	16.20±10.69	18.53±11.25	10.90 ± 7.47	7.13 ± 4.00	5.642	0.002
ESR	65.13±35.76	57.88±31.06	39.40±22.77	18.87±7.83	9.242	0.000
Calciton	222.40±86.99	252.46±163.08	224.93±97.17	126.47±63.09	3.639	0.017
Uric acid	6.32±1.67	5.90±1.35	7.21 ± 2.28	4.80 ± 1.23	5.647	0.002
Cholestrol	211.53±112.36	222.58±48.46	187.80±46.18	180.47 ± 56.42	1.591	0.200
Triglycerides	245.27±264.39	217.81±125.72	134.00±57.59	134.13 ± 38.52	2.473	0.069
Alb/creatrato	0.38±0.37	0.25±0.24	0.71 ± 0.82	0.20 ± 0.03	4.649	0.005
Dexa_Femor	0.03±1.66	-0.62±2.63	-0.59 ± 1.73	0.34 ± 0.42	0.951	0.421
Dexa_Spine	-0.32 ± 1.32	-0.30 ± 1.25	-0.21 ± 1.71	0.33 ± 0.62	0.908	0.442
Dexa_Foram	-0.27 ± 1.41	-0.29 ± 1.43	0.07 ± 1.10	0.15 ± 0.63	0.614	0.609
Calcium	9.56 ± 0.82	9.31 ± 1.03	8.87 ± 2.39	8.74 ± 0.57	1.164	0.330
Phosphrus	4.67 ± 0.79	4.57 ± 1.06	4.55 ± 0.93	4.73 ± 0.28	0.156	0.925

Table (4): Serum Calcitonin gene-related peptide in Charcot patients (acute and chronic), neuropathic patients and controls.

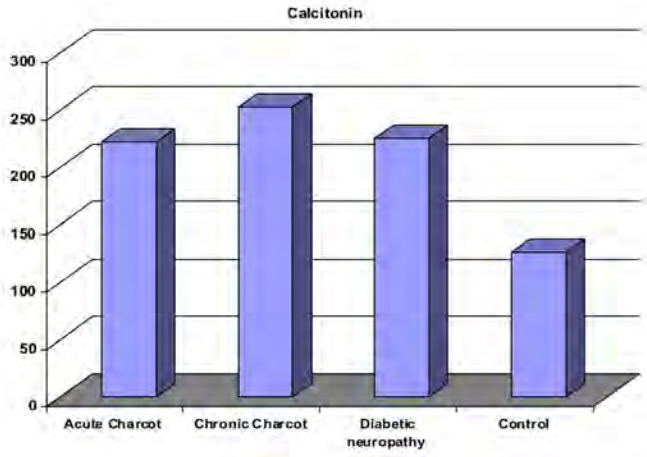
	Acute Charcot (n = 15)	Chronic Charcot (n = 26)	Diabetic neuropathy (n = 15)	Control (n = 15)	F	P value
CGRP pg/ml	222.40±86.99	252.46±163.08	224.93±97.17	126.47±63.09	3.639	0.017*

Table (5): Serum Calcitonin gene-related peptide in Charcot patients with and without ulcer.

	Negative ulcer (n = 34)	Positive ulcer (n = 7)	t	P
CGRP pg/ml	201.91 ± 58.02	433.57 ± 243.86	2.499	0.046*

Table (6): Correlation between serum Calcitonin gene-related peptide and different patient parameters in the Charcot group.

	CGRP	
	r	P
age	0.090	0.574
DM Duration	-0.183	0.252
BMI	-0.067	0.679
CRP	0.593	<0.0001*
ESR	0.342	0.029*
Uric acid	-0.033	0.837
Cholesterol	0.054	0.736
Triglycerides	0.051	0.751
Alb/creat. ratio	-0.109	0.496
HBA1c	0.181	0.258
Calcium	-0.164	0.304
Phosphorus	0.198	0.214
Dexa_Femur	0.029	0.857
Dexa_Spine	0.017	0.917
Dexa_Forearm	0.098	0.542



Discussion

CGRP, a 37-amino acid peptide, is one of the most potent vasodilator neuropeptides in vivo[9]. Existing extensively in the central / peripheral nervous systems, bone, cardiovascular, and lung tissues, it is capable of ensuring normal tissue perfusion and has a powerful cardiovascular protective[10] and immunomodulatory function [11]. Reduction in expression of calcitonin gene-related peptide and endothelial nitric oxide synthase activity in bone cells may play a role in the development of Charcot foot patients as osteoclasts are recruited by RANKL in an unchecked fashion[7].

The age of our Charcot patients ranged from 41-66 years for acute and 44-70 years for chronic Charcot, with all patients over 40 years and the majority between 40-60 years (80%, 65%) for acute and chronic Charcot respectively, tables (1). A finding that agrees with previous studies[12].

In this study males and females constituted (46.7, 53.3%) and (57.7%, 42.3%) for acute and chronic Charcot respectively with a mean (52.2%, 47.8) for male and females in the total Charcot group, table (1). This is closer to the literature[13], that documented that the prevalence of COA does not differ between men and women.

As regard the duration of diabetes 60% of acute Charcot patients and 84% of chronic Charcot patients are diabetic for at least 10 years, table (1). This coincide with previous studies[14] that reported 80% of Patients with COA are diabetic for at least 10 years, also Charcot osteoarthropathy is associated with a long standing duration of diabetes and peripheral neuropathy[15].

In our study the chronic Charcot patients have a significantly higher BMI compared to diabetic neuropathy patients ($p<0.006$), also the whole Charcot group (acute, chronic) have a significantly higher BMI compared to diabetic neuropathy patients ($p<0.018$), tables (1). Our findings agree with the previou reports that most patients are morbidly obese and obesity is significant risk factor for Charcot arthropathy in diabetic patients with peripheral neuropathy[16,17].

In our patients diabetic foot ulcer is present in (3,4,4) patients for the acute,chronic Charcot and nerurpathic groups respectively, table (2). This may be associated with underling osteomylitis as history, or the presence, of a local ulceration or a 'sausage toe' appearance is a characteristic of diabetic foot osteomyelitis[18].

In our study DEXA scan examination performed on the femur, spine, forearm revealed no difference in the BMD between Charcot (acute, chronic) patients, neuropathy and the control group, table (3). Similar findings were reported elsewhere[19]. Moreover CNO pathogenesis with bone quality, rather than bone density, and is a more important predictor of fracture than bone density[20]. However, these results disagree with previous data[21] that recorded significantly lower calcaneal BMD values obtained from the affected foot of Charcot patients. In the above recorded observations CNO is associated with normal BMD in in all bony areas with exception of the involved foot in some studies.So, we confirm that the CNO is a localized process.

In our study, Calcitonin gene related peptide levels in the acute, chronic Charcot and neuropathy groups were significantly higher than in the control group. The highest levels were observed in the Charcot group specially chronic. There were no significant differences in calcitonin gene related peptide levels between the Charcot and neuropathy groups, table (4). Calcitonin gene related peptide levels in the complicated ulcerative Charcot and neuropathy groups were significantly higher than in the noncomplicated, table (5). Moreover its level is positively correlated with the inflammatory markers as ESR and CRP.

In conclusion: Our study could provide a new evidence data that Calcitonin gene related peptide may have a role in the pathogenesis of Charcot foot in patients with diabetes and may be an indicator of an accompanying osteomylitis.

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ASSESSMENT OF CALCITONIN GENE RELATED PEPTIDE IN DIABETIC PATIENTS WITH CHARCOT FOOT

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CLINICOLABORATORY, COLONOSCOPIC, HISTOPATHOLOGICAL AND ANORECTAL MANOMETRY STUDY OF PATIENTS WITH NON SPECIFIC COLITIS

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Abstract

Introduction: *Inflammatory and non inflammatory colitides represent a heterogeneous group of diseases, often difficult to diagnose and classify, whose diagnostic criteria are not always uniform. Unfortunately, apart from infectious form of colitis, no data are available for non-IBD colitides. The term `non-specific colitis' has been linked in the past to chronic inflammatory bowel disease, and has also been used extensively in daily practice by many pathologists without strict definition.* ***Aim of the work:*** *To study the clinicolaboratory, colonoscopic, histopathological, immunohistochemical and anorectal manometry characteristics of patients with non specific colitis in order to optimize therapy.* ***Patients and Methods:*** *One hundred patients with lower gut symptoms and their colonoscopies were free are selected. Colonic biopsies were taken for histopathology and immunohistochemistry for TNF and IL-2. Anorectal manometry was done to all patients and compared to control.* ***Results:*** *73% of biopsies showed non specific colitis, 9% showed ulcerative colitis, 12% showed lymphocytic colitis and 8% collagenous colitis. TNF and IL-2 are significantly expressed in patients with non specific colitis relative to control. Anorectal manometry parameters are significantly changed in patients with non specific colitis relative to control.* ***Conclusion:*** *Non specific colitis has clinical and histopathological features intermediate between inflammatory bowel diseases and microscopic colitis. TNF and IL-2 play an important role in pathogenesis of non specific colitis and their effects on gastrointestinal muscles is reflected in anorectal manometry of these patients.*

Introduction

Inflammatory and non inflammatory colitides represent a heterogeneous group of diseases, often difficult to diagnose and classify, whose diagnostic criteria are not always uniform[1].

The diagnosis of various forms of colitides outside inflammatory bowel disease (IBD) embraces a miscellaneous group of entities that often represent a problem of differential diagnosis with IBD. Thus, the pathologist, in the daily routine practice, should keep well present these issues and try to give a precise classification in order to provide a correct diagnosis for a clinical targeted therapy[2].

It is of paramount importance that the clinician and the pathologist work as a well-integrated team; thus, the pathologist should receive accurate and detailed clinical information about the clinical problem, in addition to an adequate (at least three samples for each colonic segment, possibly including the terminal ileum, samples taken on both visible lesions and in the near, apparently normal mucosa) and correctly oriented number of biopsies[2].

The term `non-specific colitis' has been linked in the past to chronic inflammatory bowel disease and has also been used extensively in daily practice by many pathologists without strict definition. In the last few years it has become apparent that there is confusion amongst pathologists regarding the use of this term[3].

Non-specific colitis refers essentially to the histologic interpretation of an underlying colonic inflammation that has no characteristic qualitative and/or quantitative features to be properly classified as a specific form of acute or chronic colitis. Essentially, the pathologist does not found a specific cellular infiltrate or any characteristic changes in the gland architecture or epithelial cells that can be helpful in classifying the inflammation, or alternatively, despite adequate information the pathologist is unable to identify a specific form of colitis[4].

Irritable bowel syndrome (IBS) is probably by far the commonest

cause of non-specific colonic inflammation. Being aware that IBS is actually an inflamed colon compared to the colon of healthy people would prevent the pathologist from labeling IBS as a non-specific colitis. Unless complicated by another colonic disease, such as diverticulosis, histologic features of IBS are highly unlikely to change overtime[4].

The dilation of the rectum is reduced in patients with inflammatory bowel diseases due to an increase in the sensitivity of the rectum or a reduction in the compliance. In Crohn's disease patients, it would be of help to predict the function of the rectum remaining after an ileorectal anastomosis[5].

The increased sensitivity of rectum has also been related to the presence of inflammation. Accordingly an anti-inflammatory action could be beneficial resulting in restoration of anorectal sensitivity and tolerability to distension[6].

There is also a close association between rectal sensation and external sphincter contraction. As both urgency and diarrhea might be associated with an increased anal squeeze pressure due to a learned response to prevent fecal incontinence[7].

Aim of The Work

The aim of the present work is to study the clinicolaboratory, colonoscopic, histopathological, immunohistochemical and anorectal manometry characteristics of patients with non specific colitis in order to optimize therapy.

Patients and Methods

One hundred patients with vague lower gut manifestations (altered bowel habits, abdominal pain, flatulence and sense of incomplete evacuation) were randomly selected from attendants of the outpatient clinic of specialized medical hospital, Mansoura University. This is a case control study that conforms to the medical Sciences Ethics Committee of Mansoura Faculty of Medicine and all the included patients have their written informed conscent.

The study included both male and female patients aged between 18-60 years with non specific coli-

tis proved by histopathological examination of colonic biopsy.

We excluded patients with colitides of known organic disease (IBD, ischaemic, infectious and parasitic colitis). We excluded also patients with systemic disease affecting gastrointestinal motility as diabetes mellitus, systemic sclerosis and thyroid disorders.

All patients were undergo:

1- Complete history taking with stress on smoking, history of recurrent gastroenteritis, drug intake (NSAIDs, PPIs, rantidine, simvastatin and antineoplastic), history of pelvic surgery and prescience of dietary habits.

2- Laboratory investigations including CBC, liver function tests, serum creatinine, stool analysis, ESR and TSH).

3- Radiological investigations including abdominal ultrasound.

4- Complete colonoscopy, biopsy, histopathological examination using Hx &E and masson's trichrome stain and immunohistochemistry for inflammatory mediators (IL-2and TNF).

5- Lower GIT motility study (anorectal manometry).

Biopsy processing:

Biopsy specimens were fixed in sublimate formaldhyde, paraffin-embedded, cut at 4μm, step sectioned and stained with haematoxylin and eosin, masson's trichrome stain and immunohistochemistry for inflammatory mediators (IL-2 and TNF). From each biopsy specimen at least three slides, each containing six serial sectons, were examined.

Statistical analysis:

Statistical package for SPSS (version 18.0, Echosoft corp., USA 2008) was used for data analysis. Quantitative variables were summarized using Mean ± SD and both number and percentage for categorized data. The level of significance will be assessed in univariate analysis by using the x2 or Fisher's exact test for comparisons of qualitative values or the unpaired student's test for quantitative values. $P<0.05$ was considered to be significant, and the Bonferroni correction will be applied for multiple tests by multiplying the P value by the number of alleles compared (corrected P value). Differences in the means of continous variables will be assessed by

the student's paired test. The Mann-Whitney U test will be used to compare non parametric variables in independent samples. Multivariate analysis will be done by logistic regression. Adjusted Ors and 95% confidence intervals were derived from the coefficient of the final multivariate logistic model.

Results

The included patients have age range from 18 to 60 years; 46% of patients <40 years and 44% ≥40 years. Female represent 50% and male represent 50%. The mean age of patients is 39.77±9.31 and of control is 41.44±10.84. Of 100 patients 73 biopsy with a percentage 73.0% shows non specific colitis, 9 biopsies with a percentage 9.0% shows ulcerative colitis, 12 biopsy with a percentage 12.0% shows lymphocytic colitis and 6 biopsies with a percentage 6.0% shows collagenous colitis.

History of drug intake and history of gastroenteritis appear to be risk factors for non specific colitis (p is 0.032 and 0.041 respectively) where smoking and presence of previous pelvic surgery appear not (p is 0.422 and 0.698 respectively).

Colonoscopic findings do not differ in patients with non specific colitis relative to control (p=0.312). In 65 (89.0%) patients colonoscopy was free, small ulcers found in 4 (5.5%) patients, multiple superficial ulcers was found in 2 (2.7%) patients and pale mucosa 2 (2.7%) patients.

TNF is significantly expressed in biopsy specimens of patients relative to control ($p<0.05$). IL-2 is significantly expressed in biopsy specimens of patients relative to control ($p<0.05$).

Crypt density is shortened in patients relative to control ($p<0.05$). Of 73 patients Crypt density is shortened in 24 patient (32.9%). As regard crypt distortion, crypt was normal in 37 patient (50.7%). The distortion was focal in 27 patient (37.0%), mild in 2 patients (2.7%), moderate in 5 patients (6.8%) and severe in 2 patients (2.7%). Crypt inflammatory infiltrate is increased in patients relative to control ($p<0.05$). The crypt inflammatory infiltrate con-

sists mainly of lymphocytes (27.4%), lymphocytes and neutrophils (11.0%), lymphocytes, plasma and neutrophils (5.5%) and to less extent neutrophils alone (2.7%).

Lamina propria congestion, edema and cells are significantly present in biopsy specimens of patients with non specific colitis relative to control ($p<0.05$). The cells consists mainly of chronic inflammatory cells (38.4%) mainly lymphocytes and to less extent plasma cells esinophils and neutrophils.

Inflammatory infiltrate is significantly present in biopsy specimens of patients with non specific colitis relative to control ($p<0.05$). The cells consists mainly of lymphocytes and to less extent plasma cells esinophils and macrophages.

The maximum resting pressure is significantly decreased ($p=0.000$) and to less extent the minimum resting pressure ($p=0.003$) but the mean resting pressure is not significantly affected ($p=0.620$). The mean squeezing pressure is increased in patients with non specific colitis ($p<0.05$). Rectal sensation is increased while the maximum tolerable volume is decreased and there is no change in desire.

Table (1): Demographic data of patients and control

	Control (n = 30)	Patients (n = 100)	P
Age:	39.77±9.13	41.44±10.84	0.444
Sex:			
Male	15 (50%)	50 (50%)	1.0
Female	15 (50%)	50 (50%)	

There is no statistical significance between patients and control as regard age and sex (p = 0.444 and 1.0 respectively).

Table (2): Pathological results of biopsy specimens of patients and control.

Pathology	Control (n = 30)		Patients (n = 100)		P
	No	%	No	%	
Non specific colitis	0	0.0%	73	73.0%	0.000
Ulcerative colitis	0	0.0%	9	9.0%	
Lymphocytic colitis	0	0.0%	12	12.0%	
Collagenous colitis	0	0.0%	6	6.0%	
Normal	30	100.0%	0	0.0%	

Table (3): Relative risk factors for non specific colitis.

	Non specific colitis (n = 73)		Control (n = 30)		P	Odds ratio (95% CI)
	No	%	No	%		
Smoking	28	38.4	9	30	0.422	0.689 (0.277 – 1.715)
Drug intake	25	34.2	4	13.3	0.032	0.295 (0.093 – 0.941)
Gastroentritis	38	52.1	9	30	0.041	0.395 (0.160 – 0.977)
Pelvic surgery	10	13.7	5	16.7	0.698	1.260 (0.391 – 4.057)
Dietary habits	27	37.1	10	30	0.432	0.691 (0.282 – 1.723)

Table (4): Results of immunohistochemistry for TNF in biopsy specimens of patients and control

TNF	Control (n = 30)		Patients (n = 100)		P
	No	%	No	%	
Negative	24	80.0%	50	50.0%	0.000
less than 3%	6	20.0%	4	4.0%	
3-5 %	0	0.0%	22	22.0%	
5%	0	0.0%	10	10.0%	
5-10%	0	0.0%	14	14.0%	

Table (5): Results of immunohistochemistry for IL-2 in biopsy specimens of patients and control.

IL-2	Control (n = 30)		Patients (n = 100)		P
	No	%	No	%	
Negative	18	60.0%	34	34.0%	0.001
%5 mac	0	0.0%	4	4.0%	
less than 3% ly	12	40.0%	14	14.0%	
3%-5% ly	0	0.0%	8	8.0%	
5%-10 %ly	0	0.0%	4	4.0%	
15-20 ly	0	0.0%	6	6.0%	
5% mac,ly	0	0.0%	6	6.0%	
5%-10% mac, ly	0	0.0%	12	12.0%	
15-20 mac, ly	0	0.0%	8	8.0%	
20% mac, ly	0	0.0%	4	4.0%	

Table (6): Surface epithelial cells in biopsy specimens of patients with non specific colitis and control

Surface epithelial cells	Non specific colitis (n = 73)		Control (n = 30)		P
	No	%	No	%	
Normal	26	35.6%	30	100.0%	0.000
Intraepithelial lymphocytes	7	9.6%	0	0.0%	
Focal attenuation, ulcer	16	21.9%	0	0.0%	
Hyperplastic lymphocytes	8	11.0%	0	0.0%	
Focal erosions	8	11.0%	0	0.0%	
Villiform change	4	5.5%	0	0.0%	
Focal lymphocyte infiltration	2	2.7%	0	0.0%	
Hyperplastic, lymphocyte and neutrophil infiltration	2	2.7%	0	0.0%	

Table (7): Pathology of crypt in patients with non specific colitis and control.

	Non specific colitis (n = 73)		Control (n = 30)		P
	No	%	No	%	
Crypt density					
Normal	49	67.1%	30	100.0%	0.000
Short	24	32.9%	0	0.0%	
Crypt distortion					
Normal	37	50.7%	30	100.0%	
Focal	27	37.0%	0	0.0%	0.000
Mild	2	2.7%	0	0.0%	
Moderate	5	6.8%	0	0.0%	
Severe	2	2.7%	0	0.0%	
Cryptitis					
No	57	78.1%	30	100.0%	0.020
Cryptitis	12	16.4%	0	0.0%	
Crypt abscess	4	5.5%	0	0.0%	
Crypt inflammatory infiltrate No	37	50.7%	12	40.0%	
Lymphocytes	20	27.4%	18	60.0%	0.021
Neutrophils	2	2.7%	0	0.0%	
Ly and neut	8	11.0%	0	0.0%	
Ly, neut and plasma	4	5.5%	0	0.0%	
Acute and chronic cells	2	2.7%	0	0.0%	

Ly=lymphocytes, neut=neutrophils

Table (8): Pathology of lamina propria in patients with non specific colitis and control.

	Non specific colitis (n = 73)		Control (n = 30)		P
	No	%	No	%	
Congestion					
Mild	27	37.0%	12	40.0%	
Moderate	32	43.8%	0	0.0%	0.000
Severe	12	16.4%	0	0.0%	
Non	2	2.7%	18	60.0%	
Edema					
Focal	2	2.7%	0	0.0%	
Mild	31	42.5%	6	20.0%	0.000
Moderate	24	32.9%	0	0.0%	
Severe	16	21.9%	0	0.0%	
Non	0	0.0%	24	80.0%	
Cells					
Mild	14	19.2%	0	0.0%	
Moderate	5	6.8%	0	0.0%	
Lymphocytes	6	8.2%	24	80.0%	
Chronic inflammatory cells	28	38.4%	6	20.0%	0.020
Acute and chronic cells	8	11.0%	0	0.0%	
Lymphocytes and plasma	2	2.7%	0	0.0%	
Plasma, esinophils and neutrophils	8	11.0%	0	0.0%	
Lymphocytes and neutrophils	2	2.7%	0	0.0%	

Table (9): Anorectal manometric results of patients with non specific colitis relative to control

	Non specific colitis (n = 73)	Control (n = 30)	P
Minimum resting pressure	30.73±12.97	35.80±3.52	0.003
Maximum resting pressure	63.86±21.83	93.60±5.15	0.000
Mean resting pressure	41.79±14.41	42.70±3.78	0.620
Minimum squeezing pressure	59.26±23.85	50.50±2.46	0.003
Maximum squeezing pressure	112.97±39.81	150.50±15.14	0.000
Mean squeezing pressure	81.05±34.22	63.23±3.50	0.000
Rectal sensation	60.67±22.27	65.50±9.77	0.001
Desire	135.62±34.59	136.50±19.83	0.871
Maximum tolerable volume	188.22±53.06	276.00±52.89	0.000

Discussion

In our study, the included patients include 50 (50%) male and 50 (50%) female with a median age 39.77±9.13 year. 54 (54%) of patients were <40 years and 46 (46%) were > 40 years (table 1).

Minimum and maximum resting pressure are significantly decreased in patients relative to control (p=0.000) where there is less significant change in mean resting pressure (p is 0.008) and no change in minimum squeezing pressure (p=0.136). The mean squeezing pressure is significantly increased in patients (p<0.05). Rectal sensation and maximum tolerable volume are decreased in patients relative to control (p<0.05). In contrast there is no significant change in desire for defecation (p>0.05) (table 2).

In the present study, patients present mainly by alteration in bowel habits (56 patient present by diarrhea and 22 patients by constipation), 6 cases present by IBS like symptoms, 11 case present with abdominal pain and 5 cases with dysentery so that non specific colitis may have similar presentation to microscopic colitis suggesting that clinical presentation alone is not sufficient for diagnosis. This is in agreement with Stoicescu et al.[8], who showed that Collagenous colitis and lymphocytic colitis present with very similar symptoms and from a clinical perspective, there is no specific symptom or clinical feature that allows discriminating one or the other. Thus the differentiation between the two entities is made by histology only.

In the present study, risk factors for non specific colitis include history of recurrent gastroenteritis and drug intake but it appears not to be with smoking, previous pelvic surgery or dietary habits (table 3). A finding that runs parallel with Darkoh et al.[9], who showed that post-infectious (PI-IBS), which develops after a bout of acute diarrhea, gastroenteritis or bacterial infection. Several enteropathogens have been implicated in the pathogenesis of PI-IBS, including Shigella, Shiga toxinproducing E. coli, Campylobacter jejuni, Salmonella and enterotoxigenic E. coli. More importantly they showed that the concentrations of pro-

inflammatory cytokines including IFN-c, IL-1b, and TNF-a in both serum and fecal samples were higher in IBS patients than the healthy volunteers. However, the fecal and serum concentrations of anti-inflammatory cytokine IL-10 was significantly lower in the IBS groups. These results are consistent with previous reports of the levels of these cytokines in sera and mucosal biopsy samples of patients with IBS[10].

In the present study, TNF and IL-2 are significantly expressed in biopsy specimens of patients relative to control ($p<0.05$) (table 4, 5). This profile is similar to that found by Tagkalidis et al.[11], who found that however, sections of a normal colon demonstrated no visible staining for TNF, the biopsy specimens of 4 of 8 patients with lymphocytic colitis and of 6 of 10 patients with collagenous colitis showed positive cytoplasmic staining of subepithelial macrophages. They concluded that microscopic colitis demonstrates a T helper cell type 1 mucosal cytokine profile. A similar profile has been founded by Anderson et al. in 2009[12]. They have indicated that Th17 cells as well as Th1 cells play a major role in the pathogenesis of CD.

On the other hand, Beltran et al.[13], conducted a study to characterize the expression of IL-2 and IF-γ in patients at onset of IBD and still naive of treatment and compare the cytokines profiles in different stages of disease (activity vs endoscopic remission). They showed that both cytokines change significantly their concentrations during active IBD. However, while IL-2 gets back to control levels in inactive CD, it remains permanently inhibited in UC. On the contrary, IF-γ gets back to control levels in UC and remains permanently elevated in CD.

In the current study, we search for the pathological criteria of non specific colitis. As regard the crypt architecture, crypt density is shortened in patients relative to control ($p<0.05$). In non specific colitis, crypt was normal in 37 patient (50.7%). The distortion was focal in 27 patient (37.0%), mild in 2 patients (2.7%), moderate in 5 patients (6.8%) and severe in 2 patients (2.7%). Crypt inflammatory infiltrate is increased in patients

relative to control ($p<0.05$). The crypt inflammatory infiltrate consists mainly of lymphocytes (27.4%), lymphocytes and neutrophils (11.0%). Crypt abscess was found in 4 (5.5%) of patients and cryptitis was found in 12 (16.4%) of patients (table 7). In non specific colitis surface epithelial cells shows many changes as presence of intraepithelial lymphocytes, focal lymphocyte infiltration, hyperplastic lymphocytes, focal attenuation and ulcer, villiform change and hyperplastic epithelium (table 6).

Lamina propria congestion, edema and cells are significantly present in biopsy specimens of patients with non specific colitis relative to control ($p<0.05$). The cells consists mainly of chronic inflammatory cells (38.4%) mainly lymphocytes and to less extent plasma cells esinophils and neutrophils (table 8).

Inflammatory infiltrate is significantly present in biopsy specimens of patients with non specific colitis relative to control ($p<0.05$). The cells consists mainly of lymphocytes and to less extent plasma cells esinophils and macrophages (table 9).

These pathological features are similar to that found by DeRoche et al.[14], in UC. They found that UC usually exhibits a histological pattern of chronic active colitis. Activity is defined as the presence of neutrophil- mediated epithelial injury, which may take the form of neutrophils infiltrating crypt epithelium (cryptitis), collections of neutrophils within crypt lumens (crypt abscesses), or by infiltration of surface epithelium with or without mucosal ulceration. Chronicity is defined by crypt architectural distortion, basal lymphoplasmacytosis, or Paneth cell metaplasia in the left colon. Architectural distortion is represented by shortening and branching of the crypts. With spontaneous healing or medical treatment, UC may become inactive or quiescent. Histologically, quiescent colitis is characterized by marked architectural abnormalities in the absence of active inflammation[15].

Fraser et al.[16], has suggested the term “MC not otherwise specified” for a subgroup of patients with diarrhea, an increase in cellular infiltrate in the colonic lamina propria, and either an abnor-

mal collagenous layer or IELs short of fulfilling the criteria for CC and LC. Similarly, the term "paucicellular lymphocytic colitis" has been proposed for patients with typical clinical symptoms of MC and increased numbers of colonic IELs not fulfilling the diagnostic cut-off for LC. Others have also reported such patients with clinical characteristics indistinguishable from LC and CC. Uncontrolled data suggest that these patients respond to budesonide as well as patients with MC. Prospective studies are needed to describe this possible third subgroup of MC and the effect of medical treatment[17].

In our study, anorectal manometry of patients with non specific colitis showed that the maximum resting pressure is significantly decreased (p=0.000) and to less extent the minimum resting pressure (p=0.003) but the mean resting pressure is not significantly affected (p=0.620). The mean squeezing pressure is increased in patients with non specific colitis (p<0.05). Rectal sensitivity is increased while the maximum tolerable volume is decreased and there is no change in desire (table 10).

A similar results has been founded by Bassotti et al.[18], who studied pressure activity in the rectum and anal canal with a multilumen probe in 29 patients with UC (12 active, 11 quiescent, six studied during both phases) and 18 normal controls under resting conditions and during rectal infusion of saline. They found that resting motor activity was significantly decreased in patients with active colitis compared with quiescent colitis (p<0.05) and normal controls (p<0-001). Forty per cent of active colitis showed a featureless record compared with only one patient with quiescent colitis and one normal subject. The volume of saline infused before leakage occurred, and the total volume retained were significantly lower (p<0.001) in patients with active and quiescent colitis compared with normal controls.

Andersson et al.[19], reported that anorectal manometric variables and rectal sensation have been abnormal in CD patients, even in those with only microscopic involvement. In particular, low pressures due to anal involvement are strongly correlated with fecal

incontinence. Other authors, however, were unable to correlate urgency with anorectal variables or sensation[20].

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CLINICOLABORATORY, COLONOSCOPIC, HISTOPATHOLOGICAL AND ANORECTAL MANOMETRY STUDY OF PATIENTS WITH NON SPECIFIC COLITIS

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EVALUATION OF LAPAROSCOPIC SLEEVE GASTRECTOMY AS A ONE-STAGE PROCEDURE FOR MORBIDLY OBESE PATIENTS

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Abstract

Objectives: *This prospective study evaluated laparoscopic sleeve gastrectomy for its safety and efficiency as a one stage procedure in morbid obese patients.*

Patients and Methods: *The study included twenty morbidly obese patients for whom Laparoscopic sleeve gastrectomy (LSG) was done. Preoperative preparation included patient assessment for surgery, thromboprophylaxis, and antibiotics. Postoperative management included; close monitoringfoor mortality, morbidity, efficacy of the technique as regard weight loss.*

Results: *Twenty patients (16 women and 4 men) were included, with mean age of 37 years (range 18 to 55). Mean preoperative BMI was 59.40 ranged between 44.06-73.25 kg/m^2. The mean operative time was 105 minutes (range 95 to 180 min) and the mean hospital stay was 4.1 days (3 to 8 days). There was no postoperative mortality or need for a laparotomy conversion. One hemoperitonium, one late gastric leak, one gallstone cholecystitis and one incisional hernia were the major complications. After 18 months all patients had sufficient weight loss although one of them had insufficient results for us; she was satisfied of her weight loss. Two years after the initial laparoscopic sleeve gastrectomy, the EWL% was ranged between 68.02% and 95.45% with a mean of 81.30±7.28%.*

Conclusion: *The laparoscopic sleeve gastrectomy is a safe and efficient as a one stage procedure for treating morbid obesity.*

Introduction

There is an epidemic of obesity throughout the developed and much of the developing world during the last decades, probably due to a more sedentary lifestyle and changes in diets. Obesity is now considered to be the second leading cause of preventable death behind cigarette smoking[1].

Obesity is defined by the World Health Organization (WHO) as abnormal or excessive fat accumulation that may impair health. It is considered a chronic disease. It is typically defined by body mass index (BMI), this is calculated by dividing an individual's weight (in kilograms) by the square of the height (in meters), for both men and women. The acceptable or normal range of BMI is defined as 18.5-24.9 kg/m^2, underweight <18.5 kg/m2, overweight 25-29.9 kg/m^2, obesity 30-39.9 kg/m^2, and morbid obesity ≥40 Kg/m^2. However, overweight and obesity are not mutually exclusive, since obese persons are also overweight. In (1991), the National Institutes of Health[2] defined morbid obesity as a BMI ≥40 Kg/m^2 or >35 <40 kg/m^2 with comorbidity. Other non-WHO used definitions are morbid obesity BMI 40-50, super-obesity 50-60, and super-super-obesity ≥60 kg/m^2[1].

In Egypt 30.3% of the adult population are considered obese. The prevalence of obesity (BMI>30) in Egypt take the following figure; 40% of the adult urban women, 20% of adult urban men, 20% of adult rural women, and 6% of adult rural men. About 5% of women were severely obese (BMI≥40). Egyptian women have the highest prevalence rate of obesity at a national level worldwide. Obesity in Egypt takes the highest percentage of any country in the Mediterranean region and the 4th in the middle east only preceded by Saudi Arabia, Kuwait and the United Arab Emirates[3].

Morbid obesity is a lifelong, progressive disease of fat storage manifested by medical, physical, psychological, social and economic comorbidities and increases the risk of developing life threatening diseases. This phenomenon is known as obesity-related comorbidity. Comorbidities associated to morbid obesity are type 2 diabetes

mellitus (T2DM), hyper-tension, cardiovascular disease, obstructive sleep apnea syndrome, pulmonary insufficiency, thromboembolism, osteoarthritis and infertility. Furthermore, obesity is a risk factor for developing breast, endometrium, ovarian and kidney cancer. In a recently published review the relationship between obesity and certain types of gastrointestinal cancer (oesophageal, hepatic, gallbladder and colorectal cancer) has also been described[4].

There is strong evidence that non-surgical treatment among subjects with severe or morbid obesity with diet, exercise, behavior modifications, and medications rarely achieves adequate sustainable weight loss. On the contrary, bariatric surgery, achieves good long-term results including weight-loss maintenance, reduction of comorbedities and improvement of quality of life. Due to the increased demand for treatment of morbid obesity and the limitation of conventional therapies to accomplish and maintain substantial weight loss, surgical treatment of morbid obesity is gaining popularity, and there is obviously increased interest and acceptance of bariatric surgery[5].

Laparoscopic sleeve gastrectomy (LSG) has been advocated as the first of a 2- stage procedure for the high-risk, super-obese patient. More recently, LSG has been studied as a single procedure for weight loss in the morbidly obese. LSG has been shown in initial studies to produce excellent excess weight loss[6].

The aim of our study was to evaluate laparoscopic sleeve gastrectomy as a one-stage bariatric procedure for weight loss in morbidly obese patients regarding; outcomes of excess weight loss, changes in comorbidities; (hypertension, diabetes mellitus and hyperlipidaemia), and the complication rates.

Patients and Methods

This study have been conducted at department of surgery, Mansoura University Hospital on (20) morbidly obese patients, from January 2010 to March 2012. The Inclusion criteria were (1) MO patients with BMI greater than 40 kg/m^2 or 35 kg/m^2 with comor-

bidities. (2) Previous failed attempts at losing weight by diet and exercise (3) Cooperative and motivated patient (4) Patients ≥18 and ≤60 years of age.

All patients provided informed consent for inclusion in the study after explanation of the nature of the procedure and possible complications.The study was approved by Mansoura Faculty of Medicine ethical committee.

All patients were subjected to preoperative evaluation including careful history taking, clinical examination including body mass index (BMI), blood pressure and laboratory investigations including CBC, blood glucose (fasting and random), lipid profile (serum TG, and serum total cholesterol) and liver function tests. In addition to the routine preoperative assessment as for any other major abdominal surgery, the patient may undergo further assessment for pulmonary functions, endocrine disorders or gastro-esophageal disorders.

Patients were admitted to the hospital one day before surgery, at which time they underwent most of their preoperative tests. Upon admission, they were seen by an anaesthesiologist and an internist. Patients were given Enoxaparin (Clexan) 80 mg on the evening of admission, and daily thereafter during the hospital stay. Sequential compression stockings were used.

General anesthesia was administered with orotracheal intubation; the patient was placed in the supine split-leg position (French position).

Once pneumoperitoneum is achieved with 15-20mm Hg, a 30° optical laparoscopic viewing allowing for rapid diagnostic laparoscopy, then the remaining 4-5 trocars placed across the upper abdomen under laparoscopic visual control. Then the patient is placed in a steep reverse Trendelenburg position, if exposure is difficult because of a large amount of perigastric fat or a large liver, a sixth port can be placed in the left upper quadrant for the assistant. The laparoscopic sleeve gastorectomy was performed by transecting the greater curvature of the stomach distally to about 6cm

pre-pyloric and proximally to the angle of His; using ultrasonic shears. The stomach and fundus were fully mobilized during the dissection. The filmy posterior attachments were divided so the entire posterior surface of the stomach can be seen. Once this dissection was complete, the first stapler was placed tangentially across the antrum. We used green loads for the first two staple firings because of the increased thickness of the stomach in this area. The assistant flattened the stomach with lateral retraction and the anesthesiologist removed the orogastric tube before the first staple firing. The angle of the first firing was determined by the patient's anatomy, but care should be taken to not use an angle that will narrow the lumen at the incisura. Next 40 Fr. bougie was passed into the stomach along the lesser curve to the pylorus. If any concern existed that the lumen is too narrow at the incisura, the stapler was moved laterally before firing. Once the surgeon was satisfied with the lumen size, the stapler was fired. Care was taken to create a straight staple line and avoid anterior or posterior "spiraling" of the staple line. The position of the final staple firing was critical to avoid a leak by avoiding the gastro esophageal junction & esophagus during firing. Approximately 1cm of gastric serosa should be seen to the left of the stapler cartridge before the stapler is fired (Fig.1). On completion of creation of the sleeve an intraoperative leak test is done.

At the second postoperative day an upper gasterographin study (Fig. 2) and oral fluids start.

Results

Demographic data of all patients is shown in table (1):

The operation time ranged from 95 to 180 minutes with a mean operation time of 105±23.7 minutes. Postoperative hospital stay ranged from 3 to 8 days with a mean of 4.1±0.6 days. 2 (10%) cholecystectomies. No intraoperative complication occurred.

Postoperative complications (table 2):

Regarding the nutritional status, all patients in our study were given an iron supplementation in a multivitamin formula in amount

of 5 mg per day only for the first six months, and patients did not develop an iron deficiency during all the study period. Two of our patients developed symptoms of hair falling and dry skin 12 months postoperatively and found to have Zinc deficiency they controlled well with oral Zinc supplementation.

Durable weight loss is the one most important gain of bariatric surgery operations, and it is the parameter by which success or failure of weight-reducing techniques is measured. Success of treatment has been defined as weight loss >50% of excess weight, maintaining or even losing further after surgery[6].

The findings of excess weight loss percentage (EWL%) in each stage of follow up are summarized in table (3).

Also the findings of weight and BMI changes during follow up are summarized in table (4).

Most of comorbidities improved or resolved. Resolution/ improvement of comorbidities was 81.8% for hypertension, 100% for diabetes, 77.7% for dyslipidemia, 80% for OSA, 80%for GERD, and 66.6% for joint pain (Table 5).

Table (1):

Number of patients	20	100%
Sex (F/M)	16/4	80%/20%
Age in years	18-55 (33.75±10.56)*	
Height (cm)	145-180 (160.7±7.84)*	
BMI in kg/m²	44-73 (59.4 ± 7.77)*	
Morbid obese BMI ≥35 ≤50 kg/m²	3	15%
Super obese BMI ≥50 ≤60 kg/m²	6	30%
Super super obese BMI ≥60 kg/m²	11	55%

* = mean ± SD

Table (2): Early and late posoperative complications.

Early and late complications		N	%	Op BMI
Early complications	mortality	0	--	--
	hemoperitonium	1	5	60.55
	Staple line leakage	0	--	--
	Postoperative ventilator more than 24 hr	0	--	--
	Anemia	1	5	46.32
	Wound infection	1	5	65.47
Late complication	Postoperative GERD	1	5	51.20
	Late leakage	1	5	61.02
	Incisional hernia	1	5	51.2
	Gallstones	1	5	61.02
	Mortality	0	--	--
	Nutritional deficiency	2	10	51&61
Total		9	45	

Table (3): Excess Weight loss (EWL) after LSG.

Follow-up	3months	6months	12months	24months
Average EWL% in all 20 patients	26.2±5.51%	47.25±6.96%	65.87±7.54%	81.30±7.28%
Average EWL% in patients with BMI≥ 40 < 50 (3 patints)	29.57±4.88%	55.78±5.77%	74±7.79%	91.73±5.7%
Average of EWL% in patients with BMI ≥ 50 < 60 (6 patients)	28.14±4.74%	48.15±4.42%	65.81±6.59%	79.24±7.23%
Average of EWL% in patients with BMI ≥60 (11 patients)	24.21±5.63%	44.43±6.67%	63.69 ±7%	79.58±5.44%

Table (4): Weight and BMI changes during follow up (N=20):

	Preoperative (Mean±SD)	3months (Mean±SD)	6 months (Mean±SD)	12months (Mean±SD)	24months (Mean±SD)	P value
Weight (kg)	153.5±23.07	130.6±19.3	112.3±16.56	95.75±13.46	82.15±11.46	P1 < 0.001 P2 < 0.001 P3 < 0.001 P4 < 0.001
BMI (kg/m^2)	59.4±7.77	50.56±6.64	43.49±5.92	37.07±4.67	31.75±3.36	P1 = 0.018 P2 < 0.001 P3 < 0.001 P4 < 0.001

P1: preoperative versus 3months postoperatively; P2: preoperative versus 6months postoperatively;
P3: preoperative versus 12 months postoperatively; P4: preoperative versus 24months postoperatively.

Table (5): Improvement of co-morbidity.

Co-morbiditires	Improvement
Musculoskeletal problems (including decreased the need for analgesics for backache and knee pain)	66.6% (8 out of 12)
Diabetes	100% improvement (6 out of 6) With 50% stopped medications (3 out of 6)
Obstructive sleep apnea	80% (4 out of 5)
Dyslipidemia	77.7% (7 out of 9)
Hypertension	81.8% improvement (9 out of 11) with 18.2% stopped medications (2 out of 11)
Symptoms of GERD (heart burn-dyspepsia- dysphagia)	80% (8 out of 10)

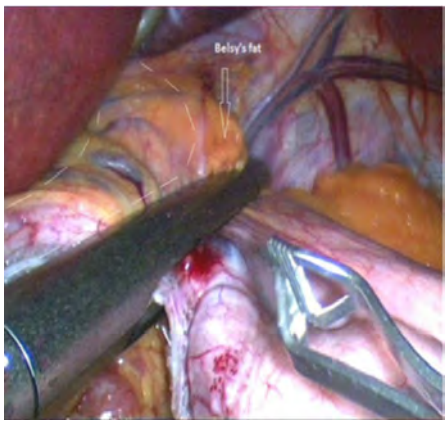

Fig. 1: Last firing 1cm lateral to GEJ.

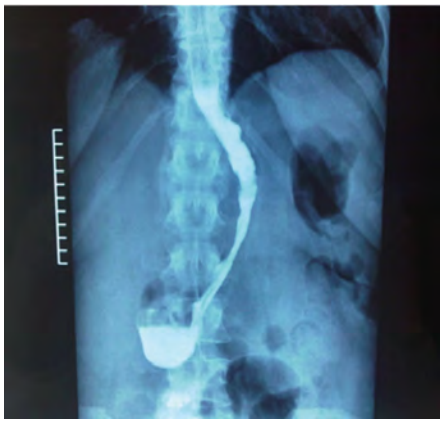

Fig. 2: Barium swallow in a patient 1 day after LSG.

Discussion

Probably, still there is no an ideal technique described, so efforts should be focused on achieving an effective, physiologic operation which has a low rate of postoperative complications. This may be applicable on LSG operation. Anticipating an appropriate weight loss comparable to that obtained after LRYGP which is considered by many authors to be the gold standard bariatric surgery [38], there are other theoretical benefits over it,: no anastomoses, preserved pylorus that controls gastric emptying, no dumping syndrome, reduction of operative time and possible lower rate of postoperative complications.

Our study evaluates the efficacy of SG as a one stage procedure in treatment of morbid obesity. The study included 20 patients; 16 (80%) females and 4 (20%) males. This sex distribution is in keeping with that in several studies by Bassiony et al, Cottam et al, Gluck et al, Al Khalifa et al and Boza et al 2012[7-11]. This sex differentiation cannot be just explained by higher incidence of morbid obesity among females; it seems that females are more prone to psychosocial problems; also women may be concerned about the cosmetic sequel of obesity, worried about marriage and the body image and infertility in some cases[11].

The mean operation time in our study ranged from 95 to 180 minutes,with a mean operation time of 105±23.7 minutes. Many authors[11,12,40] reported a shorter operation time; this may be explained by a longer learninng curve period as their study was conducted on large number of patients in comparison to 20 patients in the present study. However the operation time in our study dropped from 180 minutes in the first case to 95 minutes in the last case. In general LSG in any center takes a shorter operative time than malabsorpitive procedures reducing the anesthesia related complications[13].

The length of postoperative hospital stay showed marked variations among studies according to the severity of the obesity and comorbidities, perioperative complications and facilities among different centers. In our study the mean length of post operative hospital stay was 4.1±0.6 days that was longerer than that reported in other studies[14,15]. As a general role we prefer to keep our patients at hospital till assurance that they were no longer in need of inpatient medical care.

In our study, no intraoperative complications occurred; in line with Bellanger and Greenway[21]. On the other hand, in RYGB Luján et al reported 3 (6%) cases of splenic injuries that leaded to splenectomies[16], while in D.S Hess and Hess reported splenic injury and splenectomy in 4 (0.9%) patients[17].

Port wound infection occured

in 1 (5%) patient, wound infection after laparoscopic Roux-enY gastric bypass occurred in fewer than 5% of cases in most series and 6.6% in open cases. Laparoscopic port site infections are easy to manage with a short course of antibiotics and wound care and are less serious than open wound infections[18].

Our technique of SG does not include reinforcement or the use of buttressing material and, in our study, no early postoperative leakage was reported in keeping with results reported by Bellanger and Greenway[15]. Late postoperative leakage was reported in 1 (5%) patients. Although incidence of anastomotic leak is relatively low in SG, 2.2% (0% to 20%), its potential to increase morbidity, length of hospital stay, and mortality is great, and there is no agreed standard procedure for leak prevention[19]. Kasalicky et al. reported no leak at 18-month SG follow-up without a reinforced staple line; their technique incorporates waiting 30-60 seconds after stapler closure before firing, use of 1-2 interrupted stitches to control bleeding, and covering the staple line with 100% oxygenous cellulose to prevent residual bleeding[20]. The pathophysiology of staple-line leaks after LSG is unclear. Compromise of blood supply, especially at the angle of His near the crura, stapler device failure, poor technique, and postoperative gastroparesis with an intact pylorus causing increasing intragastric pressure have all been implicated. Although 3 of 5 bariatric surgeons surveyed at an international conference reported reinforcing the sleeve staple line, many still believe that the aforementioned pathophysiologic factors cannot be overcome with simple staple-line reinforcement[21,22].

Incisional hernia occured in 1 cases (5%) in our study. Surprisingly Hess and Hess recorded that 108 (24%) cases did repair of incisional hernias after open classical D.S[17] and Jose-Antonio Gracia et al also reported 75 (50%) cases after open BPD[23].

Postoperative follow up revealed 1 (5%) case that developed gallstones in the second year after surgery that required cholecystectomy. No puplished data defining

the incidence of gallstones development after sleeve gastrectomy. On the other hand, weight loss after gastric bypass surgery is accompanied by a rise in incidence of gallstones: 38% to 52% of patients develop stones within 1 year of surgery[24].

The procedure of SG involves resection of the gastric fundus, the predominant part of the stomach in the production of ghrelin, resulting in less stimulation of the hunger center and better weight loss than gastric banding[25].

In our study, LSG showed to be an effective procedure that has a sustained adequate EWL. EWL% (for the 20 patients) reached up to 65.87±7.54% at the end of the first year. Such EWL was maintained thereafter throughout the follow up period and at 24 months follow up the EWL% was 81.30±7.28%. Our EWL% results are in line with Leyba et al of 78.8% EWL, Gordejuela et al of 72.4% EWL and Kehagias et al of 73.2% EWL[26,28].

In our study 11 (55%) of the 20 patients were super-superobese, with a mean BMI of 64.85±3.93 kg/m^2 (range from 60 to 73 kg/m^2). The average preoperative weight was 166kg (range from 150-186 kg). In this group of patients sleeve gastrectomy allowed average loss of excess weight by 63.69% at 12 months and 79.58% at 24 months follow up. Weight loss was satisfactory in all patients of this group so, we also agree Catheline et al, that the sleeve gastrectomy in the super superobese can be a definitive treatment. However, there remains a wide range of reported weight loss in the literature; one review of 15 laparoscopic sleeve gastrectomy studies stated a %EWL between 33-90%[29,30]. This high variability in reported weight loss could in part be due to the poor standardization of SG with differing inter-institutional agreement on operational technique[31]. Nonetheless, this impressive weight loss has been shown to be sustainable in the long-term; Bohdjalian et al; demonstrated a mean %EWL of 55.0% at 5 years[32].

In our study 6 of the 20 patients (30%) were type II diabetic

on medications. After surgery and with weight reduction 3 (50%) patients had resolution and stop taking drugs to control the blood glucose. The blood glucose of the other 3 (50%) cases was controlled by much smaller doses. The operative glucose was 147±56.51; the greatest reduction in concentration of glucose in serum was observed (104.8±12.7 mg/dl) 1 month after the surgery; however, the results were statistically significant in every stage ($p<0.05$). Beneficial correlations between glucose and BMI reduction were observed. Resolution/improvement of DM in our study were 100% in line with that reported by Boza et al (100%), Ramalingam and KuiSeng (75%) and Bassiony et al (86.5%)[11,33,7] our results do not match with that reported by Magee et al; they reported improvement only in 23%[34].

Considering the desirable total cholesterol, <200 mg/dL and the normal triglycerides <150 mg/dL [35]. Dyslipidemia was recorded in 9 (45%) of our patients, in the form of elevated total serum cholesterol and triglycerides. Normalization of the serum levels occur in 5 cases (55.5%) and improvement in 2 cases (22.2%) and in 2 cases (22.2%) unchanged. In this study resolution/improvement of dyslipidemia was reported in 77.7%, in keeping with findings reported by Ramalingam and KuiSeng they recorded 77.7%, Boza et al recorded 94.6% and Al Khalifa et al recorded 86.7% and Benaiges et al recorded 75%[33,11,10,14]. Our results did not match with those reported by Berende et al; they reported only 50% improvements[36].

In our study 11 patients (55%) were hypertensive on antihypertension medications. Remission of hypertension was defined as blood pressure below 130/90 mmHg in the absence of anti-hypertensive treatment. During postoperative follow-up; 4 patients (36.36%) of them had become normotensive and stop medications, 5 cases (45.45%) were controlled by much smaller doses. Resolution/improvement of hypertension were 81.81%, this finding was in keeping with findings reported by Menenakos et al (88.5%), Bassiony et al (86%), Boza et al (98.2%) and Sarkhosh et al (75%)[25,7,11,37]. Hypertension is yet another component of the

metabolic syndrome, affecting nearly half of patients presenting for bariatric surgery. In a recent review of SG, 58% of patients reported resolutions of their hypertension at 1-year follow up, with 75% of patients experiencing at least some improvement[37]. This is in keeping with other bariatric surgeries, as Buchwald et al; reported that 78.5% of all surgical patients had resolution or improvement in their hypertension[38].

Morbid obesity is an important risk factor for OSA. In our study the incidence of OSA was recorded in 5 patients (25%). Resolution has recorded in 4 patients (80%) confirmed by continuousnight sleep and intermittent weaning from CPAP mask, but no improvement occurred in 1 case (20%). Resolution/improvement of OSA were 80% in keeping with that recorded by Berende et al who recorded improvement in 84.6%, Gluck et al recorded 97.3%, and Chopra et al recorded 90.7%[36,9,39].

All Malabsorptive procedures as duodenal switch usually require strict nutritional supplementation especially postoperatively as most of the intestinal tract is bypassed. Patients may suffer malnutrition due to incompliance and/or operation complications as indicated by Aasheim et al, Protein malnutrition is a major concern; it is manifested by clinical hypoalbuminemia, edema, asthenia, and alopecia. Regarding the nutritional status, all the patients in our study were given an iron supplementation in a multivitamin formula in amount of 5 mg per day only for the first six months, and patients did not develop an iron deficiency during all the study period. Two of our patients developed symptoms of hair falling and dry skin 12 months postoperatively and found to have Zinc deficiency they controlled well with oral Zinc suppleme-ntation[41].

Conclusion

In conclusion, the LSG procedure is a safe and effective primary operation for the treatment of morbid obesity that had excellent weight loss and resolution or improvement of co-morbidities without significant side effects; even in the super obese patients. The laparoscopic sleeve gastrecto-

my (LSG) is quickly becoming a preferred bariatric operation. It is supplanting the lap band and is widely performed now worldwide. LSG is an appealing option for patients with anemia, those requiring anti-inflammatory medications, and inflammatory bowel disease which make the intestinal bypass of the DS too risky.

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BENHA MEDICAL JOURNAL

EVALUATION OF LAPAROSCOPIC SLEEVE GASTRECTOMY AS A ONE-STAGE PROCEDURE FOR MORBIDLY OBESE PATIENTS

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IMPACT OF GENDER AND SMOKING ON PATTERN VISUAL EVOKED POTENTIAL (P-VEP) IN PATIENTS WITH GRAVES' DISEASE

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Abstract

Objective: *Among clinical aspects of Graves disease (GD), the ocular changes, Graves' orbitopathy (GO), is difficult to manage and may severely compromise patient's vision. This study was conducted to assess the effect of gender and smoking on pattern visual evoked potential (P-VEP) which detect optic neuropathy(ON).*

Subjects and Methods: *33 patients diagnosed with Graves disease and 18 volunteers with matching age, gender and smoking habit were subjected to optic nerve conduction study (P-VEP) and full medical and ophthalmological examination .The two groups, control group and Graves group were compared with each other as regard P-VEP. Also in Graves group males and females, smokers and non-smokers were compared with each other.*

Results: *Pattern visual evoked potential (P-VEP) is affected by gender; males show longer P-VEP than females. However, smoking did not affect P-VEP.*

Conclusion: *Males are more prone to have severe GO than females. In our study, smoking has no effect on P-VEP. Further research is needed to evaluate effect of gender and smoking on activity and severity of GO.*

Introduction

Optic neuropathy (ON) occurs in approximately 5% of patients with GO. Early manifestations are defective color vision and a usually normal disc appearance; advanced manifestations are optic nerve swelling and retina striae. ON is caused by optic compression at the orbital apex from enlarged extraocular muscles[1].

ON occurs in the presence or absence of significant proptosis. Electrophysiological abnormalities have proven to be the most sensitive indicator of incipient ON[2].

Visual Evoked Potential (VEP) is useful in the early detection of optic nerve abnormalities. In patients with GO affected by ON, vision loss occurs insidiously in the context of a congestive inflammatory orbitopathy[3].

In the absence of visual loss, other signs have been suggested as possible indicators of ON development, such as changes in color vision and optic nerve head-on ophthalmoscope and abnormal visual field examination[4].

Prolonged latency of VEP is associated with hypothyroidism, with reversion to normal values seen by achieving euthyroid state with levothyroxine administration[5].

Studies have shown that VEP in patients with GO could detect asymptomatic optic nerve dysfunction in the absence of deterioration of visual acuity. As a complimentary tool to visual field examination in the ophthalmological assessment, GO VEP could improve detection of the disease at an early stage[6].

Positive VEP testing should trigger intra-orbital imaging and close follow-up of patients.

Aim of The Work

The aim of this study was to assess the effect of gender and sex on P-VEP in cases of Graves disease.

Methods

The present study was conducted on 33 patients with Graves orbitopathy were chosen randomly from patients suffering from graves disease attending the En-

docrine Outpatient Clinic, Specialized Medical Hospital. The control group was eighteen healthy volunteers with age and sex matching with the patients groups, with negative results of any thyroid abnormality.

The two groups underwent full medical and ophthalmological examination and pattern visual evoked potential (P-VEP) for optic nerve.

Electrophysiological study of optic nerve:

Visual Evoked Potential:

Conventional pattern visual evoked potential (P-VEP) was recorded using (Roland Consult, Brandenburg, Germany).

P-VEP:

Stimulus: was reversing checkerboard display on a monitor 480 in diameter. Stimulus had a mean luminance of 80 cd /m2 and contrast close to 100%. Two check broad stimuli with check sizes of 15 and 60 minutes were used. Each reversed at two reversal per second. A small red spot was placed at the center of the stimulus to aid in fixation.

Subjects were refracted for viewing distance and wore the appropriate refractive correction. Recordings were obtained for each eye separately; the non tested eye was occluded. The stimuli were viewed through natural pupil. Subjects were seated at a distance of 1 meter. Three types of electrodes are connected to subjects. Positive electrode is connected to the midline of the head 2 finger breadth above inions. Ground electrode is connected to midline of head at level of ear lobule, Negative electrode is connected to middle of forehead.

The sites of electrodes are cleaning with cleaning with cleaning cream before putting the electrodes. The electrodes (gold, cup-shaped) are filled with gel before putting in their sites. For each eye and each check size, two recordings were obtained. The values of positive peak (P100 wave) amplitude and latency were recorded.

Statistical analysis was conducted by SPSS program. Shapiro-Wilk test was used as a test for normality. Mann-Whitney test was used to compare the two groups.

Results

There was no significant demographic variation between both groups of patients with graves orbitopathy (GO) and control subjects as regard age, gender or smoking.

On comparing between male and female patients with GO, male patients showed significantly higher levels of P-VEP than female patients (P value 0.003).

On comparing between smoker and non smoker patients with GO, smoker patients showed no significant statistical difference as regard P-VEP between both groups.

Table (1): Demographic Data of patient and control subject.

Demographic Data	Patient (n=33)	Control (n=18)	P. Value
age	36±14.2 yr	31.5±1.08 yr	.14
Gender:			
Males ♂	16 (48.4%)	8 (44.4%)	
Females ♀	17 (51.5%)	10 (55.6%)	
Smoking:			
smoker	11 (33.3%)	5 (27.8%)	
Non smoker	12 (66.7%)	13 (72.2%)	

Table (2): Comparison between males and females patients of graves orbitopathy as regard P-VEP:

Parameters	Male (n=16)	Female (n=17)	P value
P-VEP	128.4 ± 1.76	117 ± 1.25	0.003

Table (3): Comparison between smoker and non smoker patients of Graves orbitopathy as regard P-VEP:

Parameters	smokers (n=16)	Non-smokers (n=17)	P value
P-VEP	128 ± 1.57	121.1 ± 1.7	0.3

Discussion

As an autoimmune disorder, Graves orbitopathy (GO) represents the most common and most important extra-thyroidal manifestation of Graves disease, which at times can be associated with other autoimmune disorders and thyroid cancer[7].

Most hyperthyroid patients with Graves disease do not have GO, although approximately 20% have mild and inactive GO, 6% have moderate-to-severe and active GO, and fewer than 1% have sight-threatening GO with dysthyroid optic neuropathy (ON)[8].

In a few patients (initially euthyroid or hypothyroid), GO precedes hyperthyroidism[9].

Rather than being a complication of Graves disease, GO seems to be a concomitant expression of the same underlying pathological autoimmune process directed against cross-reactive autoantigens in the thyroid and retrobulbar tissues[10].

The prevalence of GO is 2.9 cases per 100,000 population per year in men and 16.0 cases per 100,000 population per year in women, with a peak in the fifth and seventh decades[11].

The influence of sex on the occurrence and progression of graves orbitopathy has been evaluated in several studies. Among them, Kendler et al., found that graves orbitopathy to be a disease most common in younger women, but more severe, by most indexes, in men and patients older than 50 years[12].

Thus men tend to have increased prevalence of graves orbitopathy and to have more sever disease than women do. The reasons for these sex - related differences are not clear[13].

Sawicka-Gutaj et al., (2014) have confirmed a significant influence of smoking on Graves' hyperthyroidism and particularly on Graves' orbitopathy. Smoking may increase the risk of disease development, may reduce the effectiveness of treatment, and eventually induce relapse[14].

We recommend that the man-

agement of GO to be approached by an interdisciplinary team involving endocrinologist, ophthalmologist and radiologist.

We recommend that cessation of smoking is very important to be advised to any patient with GO to decrease risk of activity and optic neuropathy.

We recommend that P-VEP should be done serially to any patient diagnosed with GD for early detection and management of optic neuropathy.

Further large scale studies should be done to formulate guidelines to diagnose and treat optic neuropathy.

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THE VALUE OF Ki67 AS A PROGNOSTIC MARKER FOR NON HODGKIN'S LYMPHOMA AND ITS CORRELATION WITH THE INTERNATIONAL PROGNOSTIC INDEX

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Abstract

NHL is a malignant heterogeneous group of disease with different clinical presentations, histologic subtypes, immunophenotypic profiles, molecular features and suitable mode of treatment. So, the identification of 'biological' prognostic factors is therefore very important in NHL to define the risk groups in clinical trials. This will guide to appropriate protocol of therapy and identify potential new therapeutic targets in this disease.

In this work, the value of Ki67 (example of proliferation marker) was studied as prognostic markers for NHL.

The current study was done on 150 cases of NHL previously diagnosed at pathology laboratory of oncology center of Mansoura University from September 2009 to November 2011. The patients are followed from September 2009 to the end of February 2014. Paraffin blocks of excision lymph node biopsy were yield to immunohistochemical staining for Ki67.

Despite of there was statistically significant difference between different types of NHL in relation to Ki67 Proliferation Index (PI), the current work reported no relation between Ki67 PI and the following prognostic parameters {rate of Complete Remission (CR), risk for death, potentiality of relapse, Progression Free Survival (PFS) and Overall Survival (OS)}.

Also, there was no correlation between Ki67 and International Prognostic Index (IPI).

Key words: *NHL, Ki67, Prognosis, IPI.*

Introduction

Non-Hodgkin's lymphomas are heterogeneous group of lymphoproliferative malignancies with different patterns of behavior and responses to treatment. The International Prognostic Index (IPI) describes clinical risk factors that are useful in risk stratification for clinical trials in NHL and for planning initial therapy for patients with this disease. Although this model has prognostic value in NHL, there is marked variability in outcome for patients within each IPI risk group, indicating the underlying biological heterogeneity of this disease[3].

The identification of 'biological' prognostic factors is therefore essential in NHL to allow risk stratification in clinical trials and to identify potential new therapeutic targets in this disease. So, many biological factors have been previously studied to predict the prognosis of NHL cases[1].

Any tumor bulk, including NHL, is the end result of rate of proliferation of its cells. So, Ki67 a proliferation marker is chosen in this study as prognostic markers for NHL. Several Previous studies have used immunohistochemical techniques to investigate the potential prognostic significance of Ki67 in NHL.Results from these studies have been variable; Broyde et al.[1] reported a good significance of Ki67 in risk stratification of NHL. However earlier study of Jerkman et al.[4] revealed that the reliability of Ki-67 as a marker of prognosis in this setting remains unclear.

Methods

This work was done on 150 diagnosed cases of Non Hodgkin's lymphoma with paraffin blocks of excision lymph node biopsy from September 2009 to November 2011. The blocks were consecutively retrieved from the surgical pathology laboratory archive files of the oncology center of Mansoura University.

Immunohistochemistry: All ar-

chival specimens had been fixed in neutral-buffered formalin and embedded in paraffin by routine methods.

For the study, the specimens were cut in 4μ thick sections on adhesive-coated glass slides. Deparaffinization with xylene and hydration through graded alcohol series were performed. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxidase in methanol for 5 min.

Epitope retrieval was carried out by boiling in a pressure cooker with HIER Citrate Buffer pH 6.5 for Ki67. Sections were then incubated with proteinase K 0.04% for 5 min. After washing with phosphate-buffered saline, the sections were incubated with the anti-human monoclonal antibodies.

The used Monoclonal antibody in this study was:

• Rabbit monoclonal anti Ki67 (Clone: SP6). GENNOVA, code No AP10244, 7 ml prediluted.

The primary antibodies were detected with the streptavidin-biotin for 30 min at room temperature. Diaminobenzidine was used as the chromogen, and incubation was carried out for 5 min at room temperature. Sections were counterstained in hematoxylin.

For the positive control, a tonsillar tissue was used, for the negative control; the primary antibody was omitted, and phosphate-buffered saline was used in each experiment.

Immunohistological scoring:

The Ki-67 PI was estimated by counting the number of positive lymphoma cell nuclei showing nuclear Ki-67 as brown color among the total number of malignant lymphoid cells irrespective of intensity. The hot spot with fields showing the most proliferative area of the lymphoma were chosen in low-power microscopy. A semi-quantitative assessment of the average percentage of Ki-67 positive cells in several fields counting altogether more than 1,000 cells was performed

Statistical analysis:

Statistical analysis was performed using the PRISM 6 (GraphPad Software Inc., San Diego, CA).

Descriptive data were in the form of mean±standard deviation, range and percentage. The Mann-Whitney test was used to compare two groups. To compare more than 2 groups; the Kruskal-Wallis test was used.

ROC curve statistical testing was used to estimate specificity, sensitivity, positive and negative predictive values and likelihood ratios for Ki-67 cut-off values.

Overall survival was computed with the product-limit method of Kaplan-Meier from date of diagnosis to date of last follow-up or death. Survival curves by two methods: the Mantel-Cox test and the Gehan-Breslow-Wilcoxon test. Correlation between different variables was calculated by Spearman rank correlation test. The P value was significant if ≤0.05.

Results

• Relation between Ki67 and histopathological types of NHL:

There was statistically significant difference between indolent and aggressive lymphoma in relation KI 67 PI. P value was <0.0001. A cut-off 40% for Ki67 can differentiate between indolent and aggressive NHL cases (P value=<0.0001) with sensitivity of 74% and specificity of 64% and likelihood ratio of two times.

There was also, statistically significant difference between various types of NHL in relation to Ki67 PI; the most prominent difference was between T cell Lymphoma type (photo 1, 2) and Small Lymphocytic B cell Lymphoma type (photo 3, 4) (P value: <0.0001) (table 1).

• Relation between Ki67 and clinicopathological characteristics of NHL patients:

Ki67 revealed statistically significant difference as regard to performance status, LDH blood level and bulky disease. While the difference was statistically non significant with other parameters (table 2).

• Ki67 PI in relation to clinical response with therapy, relapse and PFS:

There was no significant difference between Ki67 PI values and clinical responses of patients {complete remission (CR), partial

remission (PR), progressive disease (PD), stable disease (SD) and death} (P value=0.3004). Also, there was no significant difference between Ki67 PI and potentiality of relapse (P value=0.4408) and PFS<1 year (P value=0.7475).

• Ki67 PI in relation to Overall Survival (OS):

There was no significant difference between groups of patients with Ki 67 PI (>30%, ≤30%) in relation to OS (P values= 0.5285) (figure 1).

• Correlation between Ki67 PI and International Prognostic Index (IPI):

With sperman rank test Ki67 PI showed no significant correlation with IPI (P value=0.8304).

Table (1): Relation between Ki67 and histopathological types of the studied NHL cases.

Types	No of cases	Ki-67		P value
		Mean (SD)	Range (%)	
1) B Cell lymphomas	141	29.15% (14.3)	2% - 99%	<0.0001****
DLBCL	102	66% (24.9)	2% - 99%	
FL	25	26.7% (19)	3% - 70%	
SLL	8	11.6% (7)	3% - 25%	
MCL	6	12.3% (6.3)	7% - 23%	
2) T cell lymphoma	9	65.6% (28.9)	8% - 98%	

Table (2): Relation between Ki67 PI and clinical characteristics of the studied 150 NHL cases.

Variable	No of cases	Ki-67		P value
		Mean (SD)	Range (%)	
Age				0.1476
>60y	52	48.7% (29.6)	2% - 95%	
≤60y	98	56.5% (30.7)	3% - 99%	
Staging				0.0814
Stage1&2	16	57.8% (27)	3% - 98%	
Stage3&4	82	49.7% (30.5)	2% - 99%	
Stage 3+S	26	52% (32.1)	2% - 99%	
Stage 4+S	21	70.3% (29)	7% - 99%	
Stage 2+S	2	60% (21.2)	45% - 75%	
Stage 1+S	3	72.3% (19.6)	50% - 87%	
Extranodalinvolvement				0.1575
1 Site	24	56.7% (31)	7% - 99%	
>1 site	7	73.1% (23.3)	40% - 95%	
No extranodal sites	119	51.9% (30.5)	2% - 99%	
Performance levels				<0.0001****
Level 1	117	48% (30)	2% - 98%	
Level 2	33	73% (24.1)	10 - 99	
LDH				0.0112*
Normal	15	34.4% (26.6)	3% - 80%	
High	135	55.7% (30.3)	2% - 99%	
IPI				0.8350
1and2	85	52.4% (30.4)	3% - 98%	
3and4	65	55.6% (30.6)	2% - 99%	
Sex				0.1280
Male	83	49.6% (31.8)	2% - 99%	
Female	67	58.6% (28.2)	3% - 99%	
Anemia				0.1812
Yes	50	48.2% (31.1)	2% - 98%	
NO	100	56.7% (29.8)	3% - 99%	
B symptoms				0.6733
Yes	44	55.6% (28.7)	3% - 99%	
NO	106	51.8% (31.3)	2% - 99%	
Bulky disease				0.0018**
Yes	23	70.8% (32.3)	5% - 99%	
No	127	50.8% (29.3)	2% - 98%	
BM/CNS involvement				0.1815
Yes	27	45% (34)	7% - 98%	
No	123	55% (29.7)	2% - 99%	

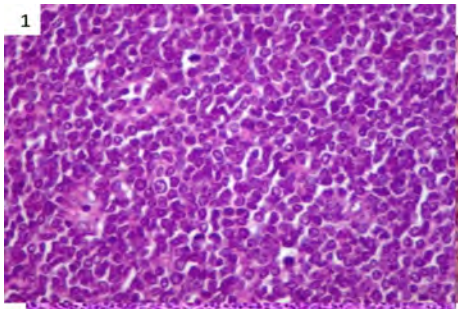

Photo (1): H&E of T Cell Lymphoma (lymphoblastic type) consist of medium sized lymphocytes with frequent mitotic figures x400.

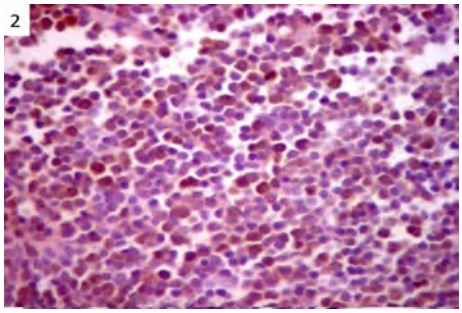

Photo (2): High nuclear proliferation index for Ki67 (75%) of T Cell Lymphoma case x400.

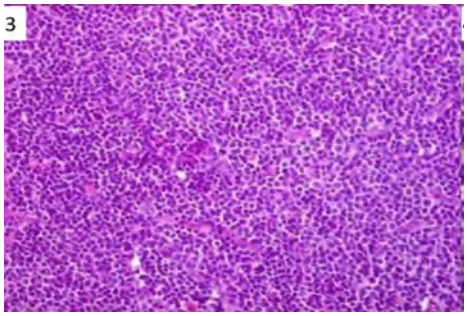

Photo (3): H&E of Small Lymphocytic Lymphoma consist of small lymphocytes with regular nuclear membrane x200.

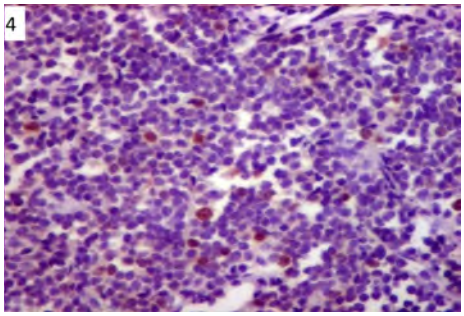

Photo (4): Low nuclear proliferation index for Ki67 (5%) of Small Lymphoctic Lymphoma case x400.

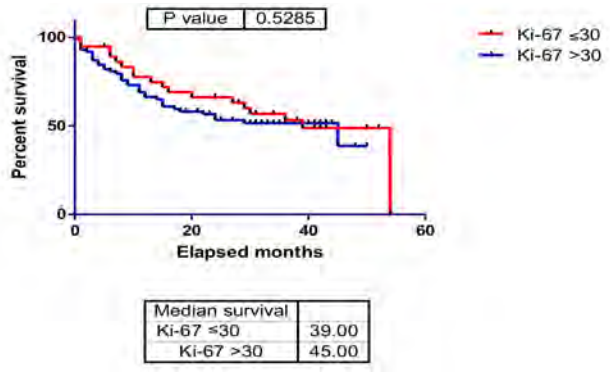

Fig. (1): Ki67 value in relation to OS.

Discussion

In the current study, Ki67 PI can discriminate between indolent and aggressive NHL cases by using a cut-off 40%. This cut-off value was near to those described by Naz et al.[6]. They used 45% to discriminate between indolent and aggressive NHL cases. On the other hand Li et al.[5] used 50% as a cut-off value distinguishing indolent from aggressive NHL.

In this study it was found that the mean Ki67 PI for T call lymphoma cases was relatively high (66%) with range between 8-98%. This result is inagreement with Broyde et al.[1]. On contrary, Naz et al.[6] revealed much lower mean of about 10%.

Also, this work expressed low mean Ki67 PI for SLL cases. It was 11.6% with range between 3-25%. This finding was inagreement with Broyde et al.[1]. However, Naz et al.[6] revealed mean Ki67 PI of SLL cases 6% with range between 1-10%.

In the present study, among all of these clinical parameters; Statistical analysis revealed significant relation between Ki67 PI and patient performance status, LDH blood level and bulky disease. But, there was no relation between Ki67 and IPI and other parameters.

Broyde et al.[1] found significant association between the Ki-67 PI and patient age and patient performance status. On the other hand, they found no association of Ki67 and other parameters including IPI as the result of the current study.

Naz et al.[6] reported significant association between Ki67 PI and extranodal involvement and with B symptoms. Also, Szczuraszek et al.[8] and Gaudio et al.[2] showed no significant relation between Ki67 PI and all clinical parameters including the IPI score. On the other hand, Salles et al.[7] reported significant relation between Ki67 PI and IPI.

The current study revealed that there was no significant relation between Ki67 PI and clinical response of NHL cases to chemotherapy. Also, there was no significant difference between Ki67 PI

and potentiality of relapse, PFS<1 year and OS. These findings were inagreement with Jerkman et al. [4] who reported that the reliability of Ki-67 as a marker of prognosis remains unclear. However, Broyde et al.[1] and Naz et al.[6] reported that the Ki-67 PI was found to be a statistically significant prognostic factor for overall survival for all NHL cases.

These results in the present study can be explained by the use of chemotherapy only as treatment for patients. This chemotherapy is highly effective on high proliferating cells which show high Ki67 expression. So, high Ki67 or low Ki67 has no effect on the outcome of patients.

Finally, the present work revealed that there was no significant relation between Ki67 and IPI. This finding goes hand in hand with Broyde et al.[1], Szczuraszek et al.[8] and Gaudio et al. [2]. However, Salles et al.[7] reported significant relation between Ki67 PI and IPI.

Conclusion

Ki67 PI has no significant prognostic value in NHL cases treated with chemotherapy alone. It has no relation with each of the following prognostic parameters {rate of Complete Remission (CR), risk for death, potentiality of relapse, Progression Free Survival (PFS) and Overall Survival (OS)}. Also, there was no correlation between Ki67 and International Prognostic Index (IPI). But it can discriminate between indolent and aggressive NHL by using a cut-off 40%.

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THE VALUE OF Ki67 AS A PROGNOSTIC MARKER FOR NON HODGKIN'S LYMPHOMA AND ITS CORRELATION WITH THE INTERNATIONAL PROGNOSTIC INDEX

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THE VALUE OF OLIG2 IN THE DIAGNOSIS OF OLIGODENDROGLIOMA AND OTHER GLIAL TUMORS OF THE CNS

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Abstract

Gliomas account for 78% of central nervous system (CNS) tumors. The majority of gliomas are classified as either astrocytomas (70% to 75%) or oligodendrogliomas (12% to 20%), or less frequently as oligoastrocytomas (5% to 10%).

Among gliomas, oligodendrogliomas have the best prognosis and cases which are cytogenetically characterized by loss of chromosomes 1p and 19q show a prolonged progression-free interval after combined radiotherapy and chemotherapy.

Differentiation between oligodendroglial and astrocytic tumors can be challenging and in some instances morphologic criteria alone are not sufficient to establish an unequivocal diagnosis. For this purpose, a specific oligodendroglial marker would be more helpful.

One new marker, oligodendrocyte transcription factor 2 (Olig-2) has been proposed as a useful marker for oligodendroglial differentiation. In this work, the value of Olig2 was studied as a diagnostic marker for oligodendroglioma and other glioma case.

The current study was done on 134 cases of glioma previously diagnosed at pathology laboratory of Pathology Department, Mansoura University from December 2009 to July 2014 and some private labs. Tissue microarray blocks were prepared and then stained immunohistochemically for Olig2.

Olig2 has shown no statistically significant differences in differentiation between oligodendrogliomas and other glial tumors.

Key words: *Glioma, Oligodendroglioma, Olig2.*

Introduction

Many years ago, numerous studies have been carried out to find immunohistochemical markers useful for evaluating oligodendroglial differentiation. A series of markers, such as S100 protein, Leu7, CD57, have been proposed, however, their real diagnostic value is controversial[1]. Preusser et al, 2007 has shown that neuronal markers such as NeuN and synaptophysin are expressed in oligodendroglial tumors and are unrelated to neurocytic differentiation[2].

Other reports about the diagnostic value of synaptophysin vary from 9.3% to 100% of cases of oligodendrogliomas studied, thus making this marker is not reliable.

One new marker, oligodendrocyte transcription factor 2 (Olig-2) has been proposed as a useful marker for oligodendroglial differentiation. Olig-2 encodes a basic helix-loop-helix transcription factor, which plays a critical role in oligodendrocyte differentiation during development[3].

There is much controversy about the usefulness of Olig2 immunohistochemistry in diagnosing oligodendroglioma

Ohnishi et al.[4] reported that Olig2 was intensely expressed in oligodendrogliomas, oligoastrocytomas, but quite weak in glioblastoma multiforme. Others reported that Olig2 expression is restricted to all glial tumours[5].

Methods

The cases of gliomas have been obtained retrospectively from the Pathology Department, Faculty of Medicine, Mansoura University, Egypt and some private pathology laboratories between December/2009 to July/2014.

Records of patients were studied and their archival paraffin blocks were retrieved. Using a tissue microarray (TMA) based approach described by Shebl et al., [6] and modified by Foda[7], 6 TMA blocks using mechanical pencil tip technique were prepared. Hematoxylin and eosin stained sections were cut and evaluated independently by two pathologists. They were graded according to 2007 WHO grading system for CNS tumors.

A total of 134 biopsies were included in this study. These included (21) pilocytic astrocytomas, (7) subependymal giant cell astrocytomas, (5) pleomorphic xanthoastrocytoma, (10) grade II diffuse astrocytomas, (4) grade III anaplastic astrocytomas, (35) GBMs, (14) grade II oligodendrogliomas, (15) grade III anaplastic oligodendrogliomas, (1) subependymoma, (1) myxopapillary ependymoma, (1) tanycytic ependymoma, (1) clear cell ependymoma (18) grade II cellular ependymomas and (1) anaplastic ependymoma.

Immunohistochemistry: All archival paraffin blocks had been fixed in neutral-buffered formalin.

For the study, the specimens were cut in 4µ thick sections on adhesive-coated glass slides. Deparaffinization with xylene and hydration through graded alcohol series were performed. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxidase in methanol for 5 min.

Epitope retrieval was carried out by boiling in a pressure cooker with HIER Citrate Buffer pH 6.5 for Olig2. Sections were then incubated with proteinase K 0.04% for 5 min. After washing with phosphate-buffered saline, the sections were incubated with the anti-human monoclonal antibodies.

The used Monoclonal antibody in this study was: Rabbit Anti-Olig2 Polyclonal Antibody diluted 1/400 (catalog # bs-11194R, Bioss, USA).

The primary antibodies were detected with the streptavidin-biotin for 30 min at room temperature. Diaminobenzidine was used as the chromogen, and incubation was carried out over night at room temperature. Sections were counterstained in hematoxylin. For the positive control, normal brain oligodendrocytes were used.

Immunohistological scoring: Olig2 staining pattern is nuclear and scoring was estimated semi quantitatively and graded as follows according to Hirose et al.[8]:

1. Negative (-), 0-10% positive cells.
2. Moderate (++), 25-50%.
3. Slight (+), 10-25%.
4. Extensive (+++), >50%.

Statistical analysis: All statistical analysis was done by Prism 5 (GraphPad Software Inc., San Diego, CA). Differences in immunohistochemical expression were analyzed using the ?2 test (Chi-square) and fisher exact test. The correlation between markers was analyzed using the non-parametric Spearman rank test. Sensitivity and specificity of different markers were calculated by Fisher exact test. P-value <0.05 was considered to be statistically significant.

Results

(1) Clinicopathological Data:

The age of the studied cases ranged from 1years to 73 years with a mean age 35.9±19.5 SD. Astrocystic tumours mean age was 37.6±21.3 (Pilocytic astrocytoma mean age 13.2±7.7SD, Subependymal giant cell astrocytoma mean age 17.5±4.7 SD, Pleomorphic xanthoastrocytoma mean age 4.5±12.8 SD, GII and GIII astrocytoma mean age 43±11.3SD, and Glioblastoma multiforme mean age 56.4±7.9SD). Oligodendroglial tumors mean age was 40.4±12.5SD. Ependymal tumours mean age was 25.2±16.3SD.

There was a significant difference in ages distribution among the different histopathological types of gliomas with p-value= 0.0012 (Chi-square test) (Fig 2).

Majority of the studied cases were males (62.7%) with male predominanace in the 3 histopathological types of gliomas. There was no significant difference in gender distribution among the different histopathological types of gliomas with p-value= 0.1073 (Chi-square test).

Cortical tumors were the majority of cases representing 65% (87 cases). Majority of ependymal cases was in the spinal intramedullary site representing 10.4% (13 cases). The majority of cortical giomas, was seen in the frontal lobe representing 26.7% (23 cases) followed by the temproparietal gliomas representing representing 25.5% (22 cases).

The histopathological diagnosis of the studied cases includes 3 pathological types of glioma. Astrocytoma was representing the majority of the studied cases [82 cases (61%)], as shown in (figure 2).

(2) Olig2 staining analysis:

Regarding astrocytomas, as shown in table (1) only 28% of cases gave negative expression for Olig2 marker. 71% of PA gave moderate (photomicrograph 1) to strong positive expression of Olig2 marker (15/21 case). Also 63% GBM cases gave moderate (photomicrograph 1) to strong positive expression of Olig2 marker (17/27 case). 50% of GII diffuse fibrillary astrocytoma cases gave strong expression for Olig2 (photomicrograph 2).

Oligodendroglial cases scoring of Olig2 marker was shown in table (2). 31% gave negative expression and at the same time, 31% of studied cases gave moderate to strong expression of the marker (photomicrogragh 3).

For ependymal tumors studied cases, the results were shown in table (3). 47.8% of cases (11 cases) gave negative expression, while 30.4% (11 cases) of cases gave strong positive Olig2 marker expression (photomicrograph 4).

Olig2 has shown no statistically significant differences between the 3 different histopathological types of gliomas. Olig-2 expressions did not reach statistical significance when oligodendroglial and astrocytic tumors were compared. Olig2 had low sesnsitivity and specificity (69% and 28% respectively) as shown in table (4).

Olig2 expressions did not reach statistical significance when oligodendroglial and ependymal tumors were compared. Regarding sensitivity and specificity, Olig2 marker has showed the sensitivity and specificity (69% and 49% respectively) in diffrentaition of oligodendroglial from astrocystic tumors as sown in table (5).

Table (1): Oligo2 scoring of studied astrocytoma cases:

Histopathological type	0	1	2	3	Total
PA	3	3	6	**9**	21(25.6%)
SEGA	**5**	1	1	0	7 (8.5%)
PXA	**4**	1	0	0	5 (6%)
Diffuse fibrillary astrocytoma	2	1	1	**4**	8 (9.75%)
Gemistocytic Astrocytoma	1	0	1	0	2 (2.4%)
Anaplastic astrocytoma	0	2	2	0	4 (5%)
Classic GBM	4	6	**11**	6	27 (32.9%)
Gliosarcoma	2	0	1	1	4 (5%)
Giant cell GBM	1	0	0	1	2(2.4%)
GBM with gemistocytic differeniation	0	0	0	1	1 (1.2%)
GBM with oligodendroglial differeniation	1	0	0	0	1 (1.2%)
Total	**23 (28%)**	**14 (17%)**	**23 (28%)**	**22 (27%)**	**82 (100%)**

Table (2): Olig2 scoring of studied oligodendroglial tumors cases:

Histopathological type	0	1	2	3	Total
Oligodendroglioma	**6**	3	3	4	16(55%)
Anaplastic oligodendroglioma	3	2	3	**5**	13(45%)
Total	**9 (31%)**	5 (17.2)	6 (20.8%)	**9 (31%)**	29 (100%)

Table (3): Olig2 scoring of studied ependymal tumors cases:

Histopathological type	0	1	2	3	Total
Subependymoma	**1**	0	0	0	1(3.4%)
Myxopapillary EPN	**1**	0	0	0	1(3.4%)
Classic cellular EPN	**7**	3	2	6	18 (78.2%)
Clear cell EPN	0	0	0	**1**	1(3.4%)
Tanycytic EPN	**1**	0	0	0	1(3.4%)
Anaplastic EPN	**1**	0	0	0	1 (3.4%)
Total	11 (47.8%)	3 (13%)	2 (8.6%)	7 (30.4%)	23 (3.4%)

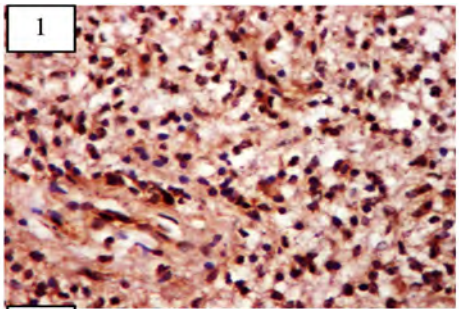
EPN: Ependymoma

Table (4): Comparison of Olig2 expression in oligodendroglial and astrocytic tumors.

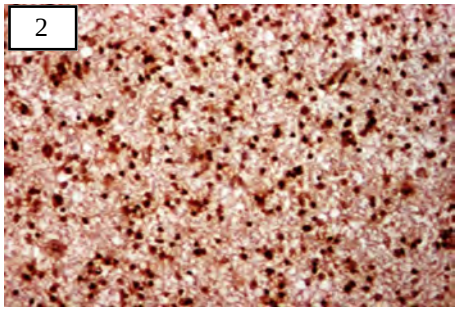
Markers	Scores	Oligodendroglial Tumors	Astrocytic Tumors	Fisher exact		
				P-value	Sensitivity	Specificity
Olig2	(+ve)	20	59	0.8132	69%	28%
	(-ve)	9	23			

Table (5): Comparison of Olig2 expression in oligodendroglial and ependymal tumors.

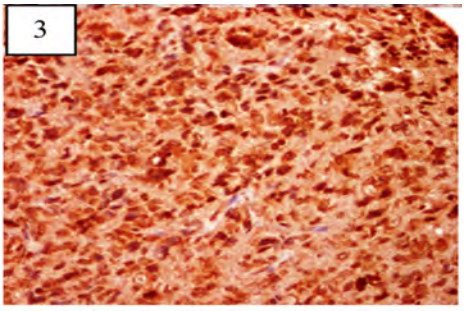
Markers	Scores	Oligodendroglial Tumors	Ependymal Tumors	Fisher exact		
				P-value	Sensitivity	Specificity
Olig2	(+ve)	20	12	0.2597	69%	49%
	(-ve)	9	11			



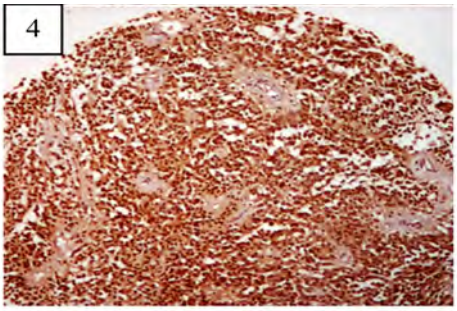
Photomicrograph (1): Pilocytic astrocytoma (20x) showing moderate nuclear staining for Olig2 (+2).



Photomicrograph (2): GII astrocytoma (20x) showing moderate nuclear staining for Olig2 (+2).



Photomicrograph (3): GIII oligodendroglioma (20x) showing extensive nuclear staining for Olig2 (+3).



Photomicrograph (4): Ependymoma (10x) showing extensive nuclear staining of Olig2 (+3).

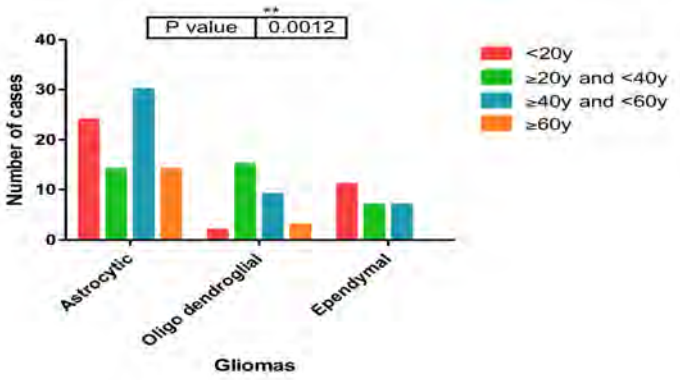


Fig. (1): Age distribution of the studied cases in relation to their histopathological diagnosis. **=moderate significance.

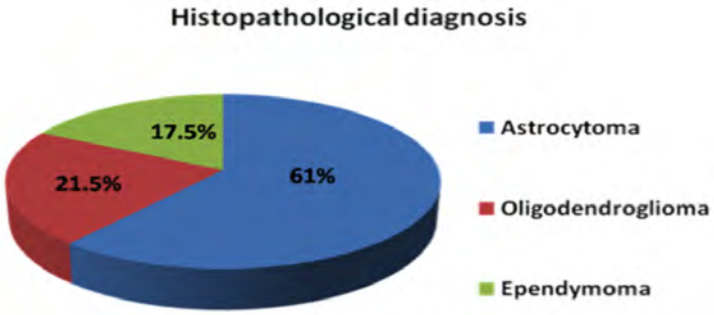


Fig. (2): Histopathological types of studied cases.

Discussion

Majority of the studied cases were males (62.7%) with male predominanace in the 3 histopathological types of gliomas. There was no significant difference in gender distribution among the different histopathological types of gliomas with p-value= 0.1073 (Chi-square test). These results are not consistent with other results that stated that there is no male or female predominance in most of the cases.

The site of origin of the studied cases was identified. The cerebral cortex was the most common (65%). Frontal lobe was the most common (26.7%) cortical site followed by the tempro parietal gliomas representing (25.5%) of cases.

Spinal intramedullary site was the most common site of origin re-

garding ependymomas representing about (56.6%). Intracranial ependymomas represent only 43.4%. In contrast to previous studies reported that the vast majority of pediatric ependymomas arise intracranial, but more than 60% of adult ependymomas are centered in the spinal cord[9].

Frontal lobe was the most common site of origin of oligodendrogliomas representing 44.8% of oligodendroglioma cases. This is consistant with the previous reported stated that the majority of oligodendrogliomas involve cerebral hemispheres, most frequently the frontal lobe and followed in frequency by parietal and temporal lobe locations[10].

Olig-2 stained 20 (69%) of 29 oligodendrogliomas and 59 (72%) of 82 astrocytomas and 12 (52%) of 23 ependymomas. Regarding Olig2 in diagnosing oligodendroglial tumors its sensitivity was 70% and its specificity was 32%.

The results are consistent with the other previously presented studies. Comparing these results to Marucci et al, they found that Olig-2 antibody stained most of the oligodendroglial tumors, thus showing high sensitivity (89.4%), but it also stained a large number of astrocytic tumors. Therefore, the specificity of Olig-2 was very low (25%)[11].

Hirose et al.[8] reported extensive expression (>50%) for Olig2 and for 100% of studied cases of oligodendrogliomas whether grade II or III in contrast to our result as we have 31% of cases stained extensively for Olig2 and while 24% of cases stained weakly (<25%) for Olig2.

Conclusion

Olig2 may be a sensitive but not specific marker in diagnosing oligodendrogliomas and other glial CNS tumors. Further studies are needed to find a more specific marker.

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THE VALUE OF OLIG2 IN THE DIAGNOSIS OF OLIGODENDROGLIOMA AND OTHER GLIAL TUMORS OF THE CNS

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EFFECT OF EXERCISE TRAINING ON SPIROMETRIC MEASUREMENTS AND ARTERIAL BLOOD GASES IN COPD PATIENTS

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Abstract

Background and objectives: *Chronic obstructive pulmonary disease (COPD) is characterized by persistent expiratory flow limitation which is usually progressive. The effect of exercise training on spirometric measures and blood gas is controversy. The aim of this study was to evaluate the effect of different exercise training modalities on spirometric measures, arterial partial pressure of oxygen (PaO_2), and O_2 saturation in the arterial blood.*

Methods: *Sixty male patients were assigned to 3 groups; twenty in each group. In addition to medical treatment given for all patients; patients in group A received peripheral muscles exercise training plus inspiratory muscle training (IMT) at intensity increased from 30% to 60% of their maximal inspiratory pressure (P_{Imax}). Patients in group B received peripheral muscles exercise training alone, and patients in group C did not receive training. All patients underwent history taking, clinical examination, chest X-ray, electrocardiogram, body mass index, and spirometry. Outcome measures in form of forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), maximum voluntary ventilation (MVV), PaO_2, and O_2 saturation in the arterial blood were carried out at study start, after 4 and 8 weeks.*

Results: *FEV_1 significantly increased in group B only. FVC, MVV, PaO_2, and O_2 saturation significantly increased in both group A and group B but not in group C with non-significant difference between*

group A and group B.

Conclusion: *Exercise training in patients with COPD can lead to improvement in spirometric measures, PaO_2, and O_2 saturation in the arterial blood.*

Keywords: *COPD; exercise training; spirometry; arterial blood gases.*

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality worldwide[1]. It is characterized by persistent expiratory flow limitation which is usually progressive [2]. The decline in forced expiratory volume in one second (FEV_1) is estimated to range from 47 to 79 ml/year in COPD patients as compared to 30 ml/year in healthy subjects[3]. Smoking cessation and pharmacotherapy are the only effective intervention to slow down FEV_1 decline[4,5].

Pulmonary rehabilitation program (PRP) is a comprehensive program provided by members of an integrated care team[6]. It provides the greatest benefits in patient-centered outcomes[7]. PRP leads to improvement in exercise capacity, health-related quality of life, and survival. It also reduces dyspnea, the number of hospitalizations and days in the hospital [2]. However, its effect on pulmonary function and arterial blood gases is controversy. The aim of this study was to evaluate the effect of different exercise training modalities on spirometric measures, arterial partial pressure of oxygen (PaO_2), and O_2 saturation in the arterial blood.

Patients and Methods

This prospective comparative interventional study was carried out at Chest Medicine Department and Rheumatology and Rehabilitation Department, Mansoura University Hospital; Egypt. All subjects were enrolled from October, 2011 to April, 2014. Ethics approval has been obtained from Medical Research Ethics Committee in July, 19th, 2011; Mansoura University.

Patients:

Sixty male patients with moderate to very severe COPD were recruited in this study. The severity

was classified according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) into stage II, III, and IV[2]. All patients were ex-smokers and had a stable clinical condition at the time of study. Patients with significant reversibility following bronchodilation, unstable cardiac diseases, uncontrolled hypertension, recent pneumothorax, recent abdominal or thoracic surgery, or known progressive neuromuscular disorders were excluded from the study.

Sixty patients completed the study and were classified into 3 groups:

1. Group A (n=20); patients treated with pharmacological therapy, peripheral muscle exercise training, and inspiratory muscle training (IMT).

2. Group B (n=20); patients treated with pharmacological therapy, peripheral muscle exercise training without IMT.

3. Group C (n=20); patients treated only with pharmacological therapy without any kind of pulmonary rehabilitation.

As regard pharmacological therapy; all patients received theophylline and inhaled long acting bronchodilators. In addition, patients with FEV_1 <60% predicted received also inhaled steroids.

Methods:

All patients were subjected to the following:

A. Thorough history taking and clinical examination including body mass index (BMI) (the weight in kilograms divided into the square of the height in meters) as described by Nici et al. (2006)[8].

B. Plain chest X-ray, electrocardiogram

C. Patients were evaluated at the start of the study, after 4 weeks and after 8 weeks (except for reversibility after bronchodilators) using the following:

1. Spirometry (using smart *pft* lab, Medical Equipment Europe GmbH, Germany) with measurement of FEV_1, forced vital capacity (FVC), FEV_1/FVC ratio, reversibility after bronchodilators, and maximum voluntary ventilation (MVV) as described by Miller et al. (2005)[9].

2. Arterial blood gas analysis: Arterial blood was obtained from the radial artery as described by Ruppel (2009)[10]. The sample was analyzed as soon as possible

(within 10 minutes) using blood gas analyzer with special attention to pH of the blood, arterial partial pressure of oxygen (PaO_2) (mmHg), arterial partial pressure of carbon dioxide ($PaCO_2$) (mmHg), bicarbonate level (HCO_3) (mmol/L), and O_2 saturation in arterial blood (%).

Training protocol:

1. Peripheral Exercise Training:

It was conducted to patients in group A and group B. Patients were subjected to the following types of training:

A. Lower limb endurance training using treadmill walking.

B. Upper limb endurance training with combination of raising both arms and arms together.

C. Upper limb strength training using hand weights for biceps and triceps.

D. Lower limb strength training using straight leg raise.

We followed The Pulmonary Rehabilitation Toolkit on behalf of The Australian Lung Foundation[11].

2. Inspiratory muscle training (IMT):

All subjects in group A only were trained daily, six times a week; each session consisted of 30 min, for 2 months using a threshold inspiratory muscle trainer (Threshold® Inspiratory Muscle Trainer, Healthscan, New Jersey, NJ, USA). Patients started breathing at a resistance that required generation of 30% of their P_{Imax} for one week. The load was then incrementally increased, 5-10%, to reach generation of 60% of their P_{Imax} at the end of the first month. Specific IMT was then continued at 60% of their P_{Imax} adjusted weekly to the new P_{Imax} achieved.

Statistical methods:

The statistical analysis of data was done using SPSS program version 16.0. Categorical data were presented as numbers (percentage). For data with normal distribution; descriptive statistics were used to calculate mean ± standard deviation (SD); paired sample t-test was used to compare the results in the same group. One way anova test was used to compare the results between the three groups; and post hoc Bonferroni test was used to compare the results between two groups. Statistical significance was de-

fined as p value less than 0.05.

Results

Sixty patients completed the study; twenty in each group.

Patients' baseline characteristics and spirometry (Table 1):

The demographics and spirometry of the three groups were well-matched with non-significant difference for any variable among the three groups.

Outcome Measures

Spirometry:

FEV_1 significantly increased after 4 weeks (p=0.004) and 8 weeks (p<0.001) in group B only. However, FVC significantly increased at the end of the study in both group A (p=0.001) and group B (p=0.005) but not in group C. Meanwhile; MVV significantly increased after 4 and 8 weeks in group A (p=0.009 and <0.001 respectively) and in group B (p=0.021 and <0.001 respectively) but not in group C (Table 2).

There was no significant difference as regard FEV_1 and FVC values among the three groups. After 8 weeks of training; MVV was significantly improved in group A in comparison to group C (p=0.045). No significant difference was detected between group A and group B in MVV after 8 weeks. Also, no significant difference between group B and group C in MVV after 8 weeks (Table 2).

Arterial oxygen tension and saturation:

PaO_2 and O_2 saturation significantly increased after 4 and 8 weeks of training in both of group A (p=0.010 and <0.001 respectively for PaO_2 and p=0.027 and <0.001 respectively for O_2 saturation) and group B (p=0.006 for O_2 saturation at 4 weeks and p <0.001 for PaO_2 at 4 and 8 weeks and for O_2 saturation at 8 weeks) but not in group C. There was no significant difference between the three groups in PaO_2 and O_2 saturation values (Table 3).

Table (1): Patients' characteristics, and baseline spirometry:

	Group A (n=20)	Group B (n=20)	Group C (n=20)	*P* value
Age (Years) (mean±SD)	57.20±5.08	55.70±6.12	56.80±4.95	0.664
Smoking Index (P-Y) (mean±SD)	55.60±19.63	58.30±28.79	44.95±15.38	0.137
BMI (Kg/m^2) (mean±SD)	24.98 ± 5.16	25.47 ± 4.90	27.61 ± 4.69	0.207
COPD group (N (%))				
GOLD II	7 (35%)	5 (25%)	5 (25%)	0.773
GOLD III	7 (35 %)	10 (50%)	11(55%)	
GOLD IV	6 (30%)	5 (25%)	4 (20%)	
MVV (% Predicted) (mean±SD)	40.17±10.96	39.42±10.43	38.16 ± 8.86	0.817
Base line spirometry: Pre-bronchodilator (mean±SD)				
FEV_1 (% Predicted)	41.77±17.76	38.04±14.05	41.01±12.17	0.704
FVC (% Predicted)	65.22±22.16	63.65±17.98	60.51±13.78	0.711
FEV_1/FVC (%)	50.40 ± 9.85	48.35 ± 9.68	53.25 ± 8.60	0.262
Base line spirometry: Post-bronchodilator (mean±SD)				
FEV_1 (% Predicted)	43.40±18.08	40.27±14.51	43.36±12.92	0.761
FVC (% Predicted)	68.19±20.98	64.85±17.36	62.60±13.37	0.600
FEV_1/FVC (%)	49.80 ± 9.09	50.02 ± 9.70	54.23 ± 8.01	0.220

P-Y: pack-year index; BMI: body mass index; COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; MVV: maximal voluntary ventilation.

Table (2): Changes in spirometry at 4 and 8 weeks

	Week 0	Week 4	Week 8	*P** value	*P*$^{\#}$ value
FEV_1 (% Predicted)					
Group A	41.77±17.76	41.19±17.27	41.58±16.96	0.535	0.813
Group B	38.04±14.05	39.12±13.59	39.73±13.59	0.004	0.000
Group C	41.01±12.17	40.80±12.09	41.25±12.20	0.675	0.461
p value	0.704	0.891	0.910		
FVC (% Predicted)					
Group A	65.22±22.16	67.97±21.02	69.34±20.13	0.064	0.001
Group B	63.65±17.98	64.84±16.38	66.37±16.52	0.245	0.005
Group C	60.51±13.78	60.98±15.02	62.15±14.08	0.698	0.134
p value	0.711	0.459	0.416		
MVV (% Predicted)					
Group A	40.17±10.96	41.61±11.45	45.07±10.31$^{\bullet}$	0.009	0.000
Group B	39.42±10.43	40.67±10.30	44.12±9.78	0.021	0.000
Group C	38.16±8.86	37.30±8.86	37.94±6.41	0.352	0.806
p value	0.817	0.383	0.031		

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; MVV: maximal voluntary ventilation; *p* value: *p* value between the groups; *p** value: *p* value within the group at 4 weeks in comparison to 0 week; *p*$^{\#}$ value: *p* value within the group at 8 weeks in comparison to 0 week; $^{\bullet}$: significant from group C. Values are expressed in mean±SD; number of patients in each group =20

Table (3): Changes in arterial oxygen tension and saturation at 4 and 8 weeks:

	Week 0	Week 4	Week 8	*P** value	*P*# value
PaO_2 (mm Hg)					
Group A	66.96±11.30	68.35±10.69	71.16±10.00	0.010	0.000
Group B	65.29±8.01	66.98±8.16	69.29±7.45	0.000	0.000
Group C	69.32±7.69	69.31±7.92	69.07±8.46	0.995	0.764
p value	0.381	0.714	0.707		
O_2 saturation (%)					
Group A	90.97±4.14	91.76±3.57	93.10±2.75	0.027	0.000
Group B	91.13±3.70	92.28±3.02	93.24±2.63	0.006	0.000
Group C	92.46±2.94	92.48±3.32	92.63±3.02	0.982	0.521
p value	0.365	0.787	0.777		

PaO_2: arterial partial pressure of oxygen; *p* value: *p* value between the groups; *p** value: *p* value within the group at 4 weeks in comparison to 0 week; *p*# value: *p* value within the group at 8 weeks in comparison to 0 week; Values are expressed in mean±SD; number of patients in each group =20

Discussion

COPD is characterized by airflow obstruction, as determined by the ratio of FEV_1 and FVC. Disease progression has been assessed using the rate of FEV_1 decline, which is greater than normal in COPD[12]. The aim of this study was to evaluate the effect of different exercise training modalities on spirometric measures, PaO_2, and O_2 saturation in the arterial blood.

In our study; FEV_1 improved in group B, while FVC and MVV statistically significantly improved in group A and group B but not in group C with no statistically significant difference between group A and group B (Table 2).

Our results were in agreement to Weiner and colleagues (1992) [13] who studied 36 COPD patients; of whom 12 patients were trained progressively from 15% to 80% of their P_{Imax} for 6 months besides general exercise training. They found small but significant ($p<0.05$) increase in FVC after six months in the group received additional IMT besides general exercise training. Casaburi et al. (1997)[14] studied 25 patients with severe COPD, and after 6 weeks of PRP including three per week 45-min sessions of cycle ergometer training. They found that an average FEV_1 increased by 9% ($p<0.05$). They speculated that these modest changes were due to improvements in bronchodilator

effectiveness. Mercken et al. (2005)[15] studied eleven patients with COPD and 11 healthy age-matched control subjects performed a maximal and submaximal exercise cycle ergometry test at 60% of peak workload for 8 weeks. FVC was significantly ($p<0.05$) improved after rehabilitation in COPD patients. Cote and Celli (2005)[16] studied 246 COPD patients. 130 patients received no PRP and 116 received PRP for 8 weeks. No PR patients showed a 160 mL decline in FEV_1, higher than PR participants who showed a 20 mL decline.

Also, Takigawa et al. (2007)[17] assessed 225 patients with COPD before and after comprehensive 4 to 8 weeks inpatient PRP between 1992 and 2003. There were significant differences of FEV_1 and FEV_1% predicted in stages III and IV, and FVC in stages II, III, and IV, when comparing the changes between pre- and post-PRP. In A multi-center study; Carone et al. (2007)[18] collected data from 1130 consecutive inpatients with stable COPD who underwent an inpatient PRP for an average duration of 24±4 days. FEV_1 improved. Incorvaia and colleagues (2014)[3] recently studied 257 COPD patients. They conducted PRP for 6 weeks. Then it was repeated every 6 months for 3 years. After 3 years, FEV_1 changed from 1240.0 ml (57.3% of predicted value) at baseline to 1252.4 ml (60.8%) in the group received PRP (n=190).

Although it is generally thought that PRP should not change the lung function of COPD patients, it has been reported that an individualized, graded PRP can determine an improvement in spirometry[15,19]. Individualization and gradation of the PRP could be a factor accounting for the improvement in lung function. Another possible explanation is based on treatment compliance during rehabilitation[18].

Our results were in disagreement with Goldstein et al. (1989) [20] who found no changes in MVV. Wanke et al. (1994)[21] also found no significant changes in neither FEV_1 nor FVC. Berry and colleagues (1996)[22] also found no significant changes in FEV1, FVE, FEV_1/FVC, or MVV. Also,

other authors[23-25] did not demonstrate changes in FEV_1. This may be explained by different patients' characteristics, training intensity, duration or training protocol.

In our study, PaO_2 and O_2 saturation significantly increased in group A and group B but not in group C (Table 3). This was in agreement with Takigawa et al. (2007)[17] who found significant differences of PaO_2 in patients with stages III and IV when comparing the changes between pre- and post-PRP. Also, Carone et al. (2007)[18] demonstrated significant improvement in PaO_2 for both patients with chronic respiratory failure (3.0 mmHg, p=0.01) and those without (2.2 mmHg, p=0.05).

On the other side, Zanchet and colleagues (2005)[26] found no statistically significant differences between pre-PRP and post-PRP blood gas analysis. Also Wanke et al. (1994)[21] did not find significant improvement in PaO_2 after 8 weeks of training. So the difference in the results may be due to different intensity of training and different protocol.

Conclusion

Exercise training in patients with COPD can lead to improvement in spirometric measures, PaO_2, and O_2 saturation in the arterial blood.

Conflict of Interest:

The authors have no conflict of interest to declare.

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EFFECT OF EXERCISE TRAINING ON SPIROMETRIC MEASUREMENTS AND ARTERIAL BLOOD GASES IN COPD PATIENTS

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DIAGNOSIS AND PHARMACOLOGICAL INTERVENTIONS FOR FETAL CARDIAC DISEASES: RESULTS OF FETAL CARDIOLOGY PROGRAM IN MANSOURA UNIVERSITY CHILDREN HOSPITAL-EGYPT

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Abstract

Background and Objectives: *Prenatal detection of congenital heart diseases (CHD) is important for fetal and perinatal management; however, there is little in literature regarding outcome of pharmacologic intervention for fetal CHD in Egypt. Therefore, we attempted to detect fetuses with cardiac structural or functional abnormalities using detailed fetal echocardiography and to plan pharmacologic intervention without endangering mother's life.*

Methods: *cases fulfilled inclusion criteria were diagnosed using detailed fetal Echocardiographic examination and antenatal treatment was described whenever indicated after a written consent. Postnatal Echo was done to confirm diagnosis and cases were followed throughout the neonatal period to determine outcome*

Results: *Seventy three fetuses fulfilled inclusion criteria with mean gestational age at diagnosis 27.97±5.679weeks. Mean maternal age at diagnosis was 26.74±5.18 years. The commonest referral indication was family history of CHD (31.5%). Ten fetuses (13.7%) received antenatal therapy: 2 with structural heart failure, and 8 had functional heart failure. Success in functional group was limited to tachyarrhythmia only.*

Conclusion: *Antenatal pharmacologic intervention is possible with impressive success in fetal arrhythmias and limited success in fetal structural and non-arrhythmic functional heart failure. Awareness should be raised among obstetricians about the need for referral to fetal echocardiography and fetal cardiac therapy*

Introduction

Antenatal detection of congenital heart diseases by echocardiography has been possible since 1980.[1] CHD represents by far the most common major congenital malformation. Most studies suggest an incidence of approximately 8 of 1000 live births.[2-4]

Fetal echocardiography was initially used to diagnose structural heart disease, but recent interest has focused on functional assessment. Effects of extracardiac conditions on the cardiac function such as volume overload, cardiac compression, increased placental resistance can be studied by ultrasound and may guide decisions for intervention or delivery.[5]

Cardiac embryogenesis occurs in the first 6 or 7 weeks of development but Lesions that develop during the first 6 weeks continue to evolve in the 2nd and 3rd trimesters; other lesions may develop or become apparent only in the second and third trimester after embryogenesis is complete. Given the potential for progression, appropriate prenatal counseling, planning of prenatal intervention and perinatal management may be needed.[6]

Little is found in literature regarding the pharmacologic intervention for fetal CHD in Egypt. Therefore, we attempted to detect fetuses with cardiac structural or functional diseases using detailed fetal echocardiography and to plan medical treatment intervention during the intrauterine life without endangering the mother's life.

Subjects and Methods

The study is a prospective single center study included all the referred cases to cardiology unit Mansoura University Children´ Hospital (MUCH) for fetal echocardiography which met the criteria of our study subjects in a 2-years period between July 2011 and July 2013.

1) Inclusion criteria: study included 73 pregnant females referred for fetal echocardiography according to American Society of Echocardiography indications.[7]

2) Exclusion criteria: Cases not fulfilling the Inclusion criteria: as cases referred due to recurrent

abortions (2 cases), immune hydrops fetalis or RH sensitized mothers (1 cases) and Oligohydramnios (1 cases).

3) Concent: All subjects included in the study had to write consent of acceptance to be enrolled in the study and for antenatal therapy.

4) Methods:

i. Gestational age estimation: it was determined at the first fetal echocardiographic examination according to biparietal diameter.

ii. Detailed fetal echocardiographic examination to assess cardiovascular structural anatomy and function according to American institute of ultrasound.[8] Fetal echocardiogram was done using the following machines: (1) Philips Sonos 5500 echocardiography machine (Philips Medical Systems, Andover, Massachusetts 2004) equipped with C3540 or C5 MHz transabdominal curvilinear Transducer or (2) Philips iE33 X matrix echocardiography machine (Philips Medical Systems, Bothell, WA, USA 2012) equipped with C5-1 MHz transabdominal curvilinear transducers.

iii. Assessment of Cardiovascular profile score (CVPS) for each case: Before treatment and with each follow up (see table 1).

iv. Whenever there was an indication for fetal therapy, mother was assessed by: baseline electrolytes level and full cardiologic assessment including basal ECG to exclude any contraindication for the drugs and in cases of congenital heart block, anti RO/ anti LA antibodies titers were measured before starting corticosteroids.

v. Pharmacologic antenatal treatments: For fetal tachycardia: digoxin, sotalol, flecainide or combinations of them (if there was a failure to single drug) were given to the mothers with affected fetuses. Digoxin oral dose was a loading dose 2x0.25 mg then maintenance 0.25mg/12hr,) while sotalol oral tablets were given in a dose 2x80-160 mg /day in 2-3 divided doses and flecainide in a dose 150-300mg /day in 3 divided doses. For atrioventricular block: Dexamethasone was given at a dose of 4mg daily either oral or intramuscular. For fetal congestive

heart failure: Digoxin tablets were given orally to the mother with the same mentioned dose for fetal tachycardia.

vi. The Follow up: whenever indicated by fetal echocardiography, ECG, maternal serum level of drugs or electrolytes and postnatal Echo.

5) Study groups: The study population was divided into three groups depending on the presence and etiology of the heart disease.

Group I: Fetuses with normal fetal echo.

Group II: Fetuses with structural heart disease.

Group III: Fetuses with functional heart disease which subdivided to:

a) Cardiac arrhythmias: including irregular rhythm, tacchyarrhythmias and bradyarrhythmias. Fetal tachycardia is defined as fetal heart rate >180 beats per minute (bpm).[10] Fetal bradycardia is fetal heart rate <110 bpm.[11]

b) Functional heart diseases other than arrhythmias: Which included: Hypertrophic cardiomyopathy, Dilated cardiomyopathy, High output failure: as in cases of twin-twin transfusion syndrome (TTTS), Cardiac compression: as in congenital cystic adenomatoid malformation (CCAM) or Diaphragmatic hernia, and idiopathic hydrops

6) Statistical Analysis: Statistical analysis was done by using the Statistical Package for the Social Sciences (SPSS), version 17 (SPSS, Inc, an IBM Company, Chicago, IL, USA) for analysis of data. Microsoft office Excel 2010 (Microsoft, Inc., Redmond, WA, USA) was used for formulating tables and graphs.

Results

During the 2-year study period from July 2011 to July 2013, 73 pregnant ladies were referred for fetal echocardiography. Out of the cases included in the study, 10 cases (13.7%) received antenatal pharmacologic intervention. The mean age of ladies included in the study at prenatal diagnosis is 26.74±5.18 years with range (17-40 years).

The mean gestational age at first referral is 27.97±5.679 weeks. ranging (16-39 weeks). The mean

number of fetal echocardiographic examinations per pregnant lady is 1.74±1.08 and range (1-6 times).

All fetuses had detailed echocardiographic examinations and were divided into three groups: normal (n=33), structural diseases (n=16) and functional heart disorders (n=24) as shown in table 2.

Figure 1 shows Indications of referral for fetal echocardiography. The most frequent cause of referral was family history of CHD (31.5%) followed by hydrops fetalis (23.3%). On the other hand, the least frequent cause of referral was familial inherited disease (1.4%). Interestingly, no cases were referred for increased nuchal translucency, chromosomal abnormality, teratogenic factors, exposure to prostaglandin synthetase inhibitors, and in vitro fertilization (IVF).

Table 3 shows the structural defects that were diagnosed. The most prevalent was VSD (31.3%) followed by HLHS (18.8%). Five cases had fetal heart failure and 3 cases (60%) were untreated. The untreated cases were HLHS, severe Epstein anomaly and restrictive foramen ovale. All the untreated cases had hydrops and were stillborn. The 2 cases received antenatal digoxin therapy were AVSD (n=1), and HLHS (n=1) and both were stillborn. We compared the treated functional cases before and after treatment using CVPS but there was no statistically significant difference regarding CVPS (p=0.317).

The group of functional heart diseases included 24 cases which further divided into: fetuses with arrhythmia f (n=6) 25% and fetuses with non- arrhythmic functional heart diseases (n=18) 75%. (See figure 2).

Table 4 shows the cases of the functional heart diseases and outcome. The treated cases with functional heart failure (n=8): SVT (n=2), AF (n=1), heart block (n=1), Dilated cardiomyopathy (n=2) and idiopathic hydrops (n=1). We compared the treated functional cases before and after treatment using CVPS but there was no statistically significant difference regarding CVPS (p=0.276).

Complication to treatment was

noticed on 1 mother (10%) of the 10 cases received antenatal pharmacologic therapy. She received digoxin for fetal heart failure due to fetal dilated cardiomyopathy. A period of bradycardia after labor was noticed which was managed by stopping digoxin and close observation for few days although drug was within therapeutic levels and potassium level was within average.

Table (1): cardiovascular profile score[9].

Category	2 points	1 point	0 points
Hydrops	None	Ascites or pericardial or pleural effusion	Skin edema
Heart size (HA/CA)	>0.2 and ≤0.35	0.35-0.5	<0.2 or >0.5
Cardiac function	Normal MV and TV, biphasic diastolic filling, LV or RV SFs >0.28	Holosystolic TR or LV or RV SFs <0.28	Holosystolic MR or TR dP/dt <400, monophasic diastolic filling
Arterial umbilical Doppler	Normal	AEDV	REDV
Venous Doppler	Normal	DV atrial reversal	UV pulsations

Score is 10 if no abnormal signs (2 points for each of 5 categories). **AEDV**, absent end-diastolic velocity; **dP/dt,** change in pressure over time of TR jet; **DV**, ductus venosus; **HA/CA,** Heart area/chest area; **LV**, left ventricle; **MR**, mitral regurgitation; **MV**, mitral valve; **S.F.,** shortening fraction; **TR**, tricuspid regurgitation; **TV**, tricuspid valve; **REDV,** reversed end-diastolic velocity; **RV**, right ventricle.

Table (2): The fetuses included in the study groups*.

Diagnosis	Number	Percent
Normal heart	33	45.2%
Structural heart disease	16	21.9%
Functional heart disease	24	32.9%
Total	73	100%

*Data are expressed as number and percentage.

Table (3): Structural heart diseases expressed as number, percent and outcome

Diagnosis	No.	Percent	Outcome
VSD	5	31.3%	FT, alive
moderate PS	2	12.5%	FT, alive Needed PBV
TOF	1	6.3%	Lost follow up
AVSD	1	6.3%	Antenatal ttt, SB
HLHS	3	18.8%	Antenatal ttt, SB
TA/PA	1	6.3%	NICU admission, early neonatal death
Severe Ebstein's Anomaly	1	6.3%	SB
restrictive foramen ovale	1	6.3%	SB
prematue ductus arteriosus constriction	1	6.3%	alive
Total	16	100%	

AVSD: atrioventricular septal defect, **FT:** fullterm**, HLHS:** hypoplastic left heart syndrome**, NICU**: neonatal intensive care unit, **PBV**: pulmonary balloon valvuloplasty, **PS:** pulmonary stenosis, **SB**: stillborn**, TA/PA:** tricuspid atresia/ pulmonary atresia**. TOF:** tetralogy of fallout**, ttt**: treatment, **VSD:** ventricular septal defect, **w**: week.

Table (4): fetuses with functional heart diseases

Diagnosis	Frequency	Percent	Outcome
atrial flutter	1	4.2%	FT, alive
SVT	2	8.3%	AT, FT, alive
Non-conducted PAC	2	8.3%	FT, alive
heart block(3^{rd} degree)	1	4.2%	AT, PT, pacemaker after birth surgically
Dilated Cardiomyopathy	2	8.3%	AT, SB
Hypertrophic Cardiomyopathy	4	16.7%	FT, alive
Cardiac compression: CCAM	1	4.2%	PT, early neonatal death
Cardiac compression: Diaphragmatic hernia	1	4.2%	terminated
High output failure: TTTS recipient twin	2	8.3%	1AT, SB 1 SB
Idiopathic hydrops	8	33.3%	1AT, SB 2 terminated 5 SB
Total	24	100%	

AT: antenatal treatment, **CCAM**: congenital cystic adenomatoid malformation, **FT**: full term, **PAC**: premature atrial contractions, **PT:** preterm, **SB**: stillborn, **SVT:** supraventricular tachycardia, **TTTS**: twin- twin transfusion syndrome.

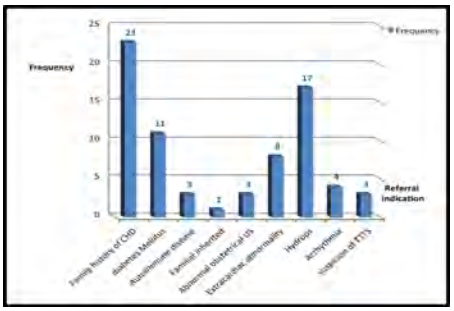

Fig. 1: Column Chart for the frequency of referral Indications for fetal echocardiography. **CHD:** congenital heart disease, **US:** ultrasound, **TTTS:** twin-twin transfusion syndrome.

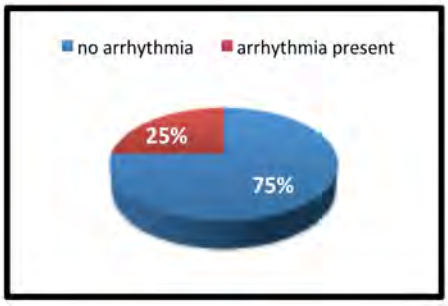

Fig. 2: Pie chart show the percentage of cases of arrhythmia and non-arrhythmic functional heart diseases

Discussion:

Congenital heart defects are the most common congenital malformations. It is six times more common than chromosomal abnormalities and four times more common than neural tube defects.[12] These defects significant morbidity and mortality result in an economic burden on the health care system and a financial and emotional burden on the involved families.[13] Prenatal detection of congenital heart diseases is important for fetal and perinatal management; however, there is little research regarding the pharmacologic intervention for fetal CHD in Egypt. Therefore, it was vital to detect fetuses with cardiac structural or functional diseases using detailed fetal echocardiography and to plan antenatal medical treatment intervention whenever indicated without endangering the mother's life.

Indications of referral:

The most common indication for fetal echo referral in our study is family history of CHD (31.5%). In concordance to our results, Meyer-Wittkopf et al., reported that the most common indication for fetal echo was family history of CHD (44.5%).[14] While more recent studies reported the commonest referral indication to be the abnormal cardiac shadow on obstetric ultrasound screening.[15]

This difference may be due to the advanced obstetric screening programs recently that extend ended from the four chamber view to take the outflow of great arteries. But in Egypt this is not fully applied. In addition, some obstetricians are not completely oriented about the benefits of intrauterine diagnosis, or management of CHD and their impact on fetal and neonatal outcomes.

It is noticed that screening tests performed at the first trimester as nuchal translucency measurement, lead to an earlier diagnosis of all major types of congenital heart defects.[16] But in our study there was no cases at all referred for the increase in nuchal translucency or abnormalities of ductus venosus.

No cases referred due to other well-known indications like maternal teratogens and IVF although these are circumstances that obstetricians frequently encounter.

Structural heart diseases:

The most commonly detected CHD prenatal in our study was VSD (31.3% of structural defects). Similar results were obtained by Yu et al. and Trivedi et al.[17,18] Other studies found the commonest structural CHD diagnosed with fetal echo is HLHS.[19,20] While Cha et al., reported that TOF (15.9%) was the commonest CHD, and isolated VSD (13.1%) was the second most common.[21]

Regarding pharmacologic intervention to structural lesions in our study, 2 cases received digoxin to manage fetal heart failure and were stillborn. These results are against data published by Patel et al., in which 19 cases with structural lesions received digoxin with 2 stillbirths and 3 neonatal deaths (survival rate till the end of neonatal period 74%).[22]

The outcome for fetuses with major CHD (e.g HLHS) in our study was poor despite antenatal diagnosis and treatment. This finding is concordant with the findings in previous study as intrauterine heart failure was identified as an unfavorable prognostic sign despite intrauterine treatment.[23]

Functional heart diseases:

The most common functional

heart disorders are the fetal arrhythmias and occur in 1 to 3% of all pregnancies.[24] Concerning fetal tachycardia, the most common fetal tachyarrhythmias are SVT and atrial flutter, which account for 66-90% and 10-30% of cases, respectively.[25]

In the current work, we diagnosed 3 cases with fetal tachyarrhythmia. We started Digoxin alone (1 cases) or in combination (2 cases) with either sotalol or flecainide. In a non-randomized multicenter study, use of digoxin or flecainide as a first-line therapy was associated with a higher arrhythmia termination compared with sotalol.[26] On the contrary, other centers start with sotalol as first line drug followed by addition of digoxin or flecainide if needed.[27,28]

In our study, fetuses with fetal tachyarrhythmias had fetal hydrops with success to restore sinus rhythm intrauterine in 2 cases (66.67%), decreasing the ventricular rate to average in one case of atrial flutter (33.33%). No fetal demise or neonatal complications or mortality in the tachyarrhythmia cases.

In accordance to our results, other studies on fetal SVT and AF reported no mortalities even in hydropic cases.[27,29] On the contrary, mortality in fetal tachycardia with hydrops reached 17-27% of cases despite antenatal therapy.(25,26) Another study reported death rate of 10% of cases in spite of antenatal treatment and about 33% of cases did not convert to sinus rhythm intrauterine.[30]

Another rhythm related disorder is congenital AVB. In our study, one case of complete AVB was given dexamethasone antenatal but with no response and surgical epicardial pacemaker implantation.

Our results are consistent with a multi-institutional study proved that third-degree AVB was considered irreversible.[31]

Regarding digoxin use in functional heart failure other than tachyarrhythmia, it was reported in with hydrops resolution and fetuses born alive TTTS.[32,33] A single study in USA used digoxin on 9 cases of fetal functional heart failure. Two cases died in utero (cystic hygroma and cardiomyopathy)

and 7 cases born alive.[22]

In the present study, we used digoxin in treating fetal functional heart failure in dilated cardiomyopathy (n=2), TTTS (n=1), idiopathic hydrops fetalis (n=1). But results were not satisfactory with the fetuses died intrauterine or shortly after birth.

We took every effort to prevent maternal complications by proper maternal cardiac assessment prior to therapy initiation to exclude contraindications to therapy. Regarding the present study, we had one case only of maternal bradycardia after labor that was attributed to digoxin although serum level was within therapeutic levels; she needed only close monitoring for couple of days. No documented complications correlated with other drugs used in the study.

Results of fetal pharmacologic therapy were statistically insignificant may be due to small sample size. Long term study with larger sample is needed

Conclusion

Fetal cardiac diagnosis could be achieved using fetal echocardiography. Antenatal pharmacologic intervention is feasible with success in fetal arrhythmias and limited success in structural and non-arrhythmic functional heart failure. Awareness should be raised among obstetricians about the need for referral to fetal echocardiography and fetal cardiac therapy.

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BENHA MEDICAL JOURNAL

DIAGNOSIS AND PHARMACOLOGICAL INTERVENTIONS FOR FETAL CARDIAC DISEASES: RESULTS OF FETAL CARDIOLOGY PROGRAM IN MANSOURA UNIVERSITY CHILDREN HOSPITAL-EGYPT

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PRELIMINARY STUDY ON THE EFFICACY AND DURATION OF HEPATITIS-B VIRUS VACCINE

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Abstract

The aim of this study was to evaluate the long term maintenance of antibody levels in children vaccinated with a three dose regimen of hepatitis B (HB) vaccine beginning at birth. The study was conducted on 98 children. In these children, the serum levels of anti-HB titres were measured at different times after vaccination up to 6 years after vaccination. The results showed that the longer the time after the last dose of HB vaccine, the lower the protection rate (89% in children aged 1-2 years, compared to 61% at 5-6 years).

Introduction

Hepatitis B virus (HBV) infection is a serious health problem and is a major cause of acute and chronic liver disease; hepatitis, cirrhosis, or cancer of the liver (hepato-cellular carcinoma; HCC) worldwide[1,2,3]. About 2 billion people around the world have been infected with hepatitis B with >300 million people chronically infected[4,5]. According to World Health Organisation (WHO), about one million people die each year from acute and chronic sequelae secondary to HBV infection[6]. Approximately, 4.5 million new cases of HBV infection occur worldwide each year, and 25% of these cases progress to liver disease[7]. The virus infects people of all ages. Before the introduction of the hepatitis B vaccine, over 30,000 children were infected annually, and it is estimated that about a third of the chronic HBV infections are persons infected either as children or infants prior to the introduction of the hepatitis B vaccine and also serve as the main reservoir for continued HBV transmission[3,8]. The risk of death from HBV-

related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood[9-11].

The strategy for the control of HBV infection, as outlined by the World Health Organization and endorsed by the Advisory Committee on Immunization Practices (ACIP), is the introduction of hepatitis B immunization at birth[12-14]. This strategy is designed to reduce the risk of early childhood acquisition of HBV and reduce the number of chronic carriers in endemic populations. Also, when the hepatitis B vaccination is initiated at birth, there is an increased likelihood that the child will complete the series[15,16].

In 1991, the World Health Organization[17] called for all children to receive the hepatitis B vaccine, and by 2011, 179 countries have universal infant hepatitis B vaccination programs and 93 member states have introduced the hepatitis B birth dose[18]. This program has reduced not only the rate of persistent infection and the total prevalence of HBV in the younger generation, but also the occurrence of childhood HCC and fulminant hepatitis[4,19,20]. The series of immunizations consists of three IM injections of the hepatitis B antigen over a period of 6 months[21,22]. Immunity is conferred after vaccination in 85 to 99% of people and is proven by finding a titer greater than 10 milli-International Units per milliliter (mIU/mL) of surface antibody in the vaccine recipient[14,23].

Information on long-term efficacy of the vaccines and the duration of protection after hepatitis B vaccination of infants and the need for booster doses of the vaccine are still debated. In endemic regions, the duration of protection induced by vaccination has been shown to be from 10 to 15 years in most vaccines[24-27]. Other studies have shown that the protective efficacy against HBV events declined somewhat over time. They had shown that the protection against chronic carriage persisted at least 5-6 years[13,28-30] and others had reported the loss of protective antibody levels at 10 years of age[24,26]. The Taiwanese experience in initiating the first universal hepatitis B vaccination

program for infants, which is now 20 years old, has shown dramatic reductions in HBV infection rates in children and those now <20 years of age, and importantly, has indicated that a booster dose is not required as protection lasts at least 20 years[31].

Since 1993, all Libyan children have received hepatitis vaccine as part of state wide vaccination program. Hepatitis B vaccination is initiated within first 7 days of life, with second dose given at 4-6 weeks and the third at 6 months of age. We examined the long term maintenance of antibody to hepatitis B surface antigen (anti-HBs) in children at ages from 1 to 6 years whose mothers were HBsAg-negative and who originally received a course of hepatitis B vaccine starting at birth. Children with serum anti-HBs ≥10 mIU/ml, the accepted protective concentration, are considered protected[32].

Materials and Methods

Study Population:

A group of children consisting of 98 children visiting the outpatient clinic in Tajoura Hospital were included in the study. The children have received the 3 doses of the hepatitis B vaccine. The Engerix-B, 10 µg (SmithKline Beecham) vaccine have been used. The children were divided into 4 groups according to their age at the time of testing. Group I aged from 1 to 2 years, group II aged from 2-3 years, group III aged from 3-4 years and group IV aged 5-6 years. Sera were obtained from all children and tested for anti-HBs.

Laboratory Testing:

All serologic specimens were tested quantitatively by electrochemilumiesscence immunoassay (ECLIA) intended for use on Elecsys and Cobase immunoassay analyzers. The anti-HBs titers were reported in milli-International Units (mIU) per ml using the WHO international reference standard[33].

Data Analysis:

Anti-HBs >/=10 mIU/ml in a child was considered protected against hepatitis B infection[32]. Anti-HBs titres were log-transformed for calculating geometric mean titer (GMT), and com-

parisons of GMT over time were made with a paired t-test with the log-transformed values.

Results

Of all the 98 children (group I to group IV) who had received 3 doses of the hepatitis B vaccine as infants, 78 (79%) had anti-HBs titers >/=10 mIU/ml at the time of testing for each group, between ages of 1 to 6 years after vaccination.

The highest rate of protection against the virus was seen in group I (1 to 2 years) with 89% of the children with anti-HBs ≥10 mlU/ml. The protection rate was reduced in group II to 85%, group III to 76% and was the lowest in group IV (61%) at 5-6 years of age (Figure 1).

Comparison of the GMT of the different age groups to GMT of group I have shown a significant reduction in GMT at 2-3 year, 3-4 years and at 5-6 years of age (Figure 2). The data showed that at the age of 5-6 years about 39% of the children lost their protective antibody levels.

Comparison of the GMT between Males and females in each group showed significant difference only in the first group; at age of 1-2 years. GMT values were 2.75 compared to 1.71 ($p<0.001$). In other age groups, there was no significant difference in GMT values between the females and males groups.

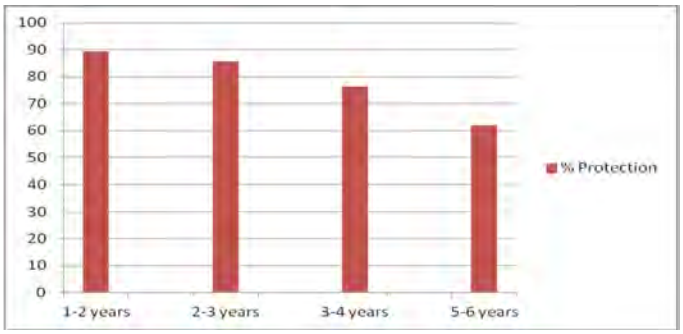

Fig. 1: The % of protection following hepatitis B vaccination.

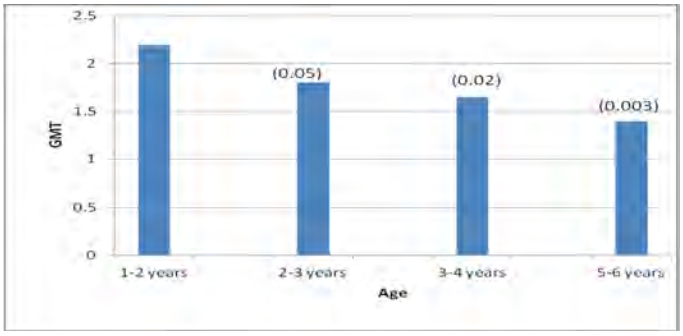

Fig. 2: Log GMT of the different age groups compared to the group I.

Discussion

Hepatitis B vaccination for neonates was introduced as a part of the national immunization program as recommended by WHO in 1993 in Libya. However, no data is available on the efficacy and duration of hepatitis B vaccine in Libya. This is the first study that examines the efficacy and duration of protection in children following vaccination with the 3 doses of the vaccine starting at birth. Intramuscular Hep-B(Eng) [0-, 1-, 6-month schedule] has been shown to has an excellent immunogenicity in healthy neonates, infants, children, adolescents and adults, with seroprotection rates of 85-100% seen approximate, 1 month after the final dose of vaccine [34,35]. In all the children who received the hepatitis B vaccine in infancy, 89% had anti-HBs ≥10 mIU/ml approximately one year after vaccination. These results are similar to those reported previously (85-100%) with the Hep-B (Eng) vaccine[34,35]. The results also showed that the anti-HBs titer began to decline from about one year after vaccination with the greatest decline occurring at 5 to 6 years of age and that at this age; about 39% of the children have an anti-HBs antibody titer of <10 mIU/ml.

Several studies have shown that most low-risk children vaccinated at birth did not have antibodies against hepatitis B in their blood by the time they reached the age of five[36] and about 10 years after vaccination[37]. Zanetti et al.[38] have shown that 10 years

after vaccination, protective anti-HBs concentrations were retained in 64% of the children and that antibody levels <10 mIU/ml were recorded in 36% of the children. Others have shown a precipitous decline in antibodies at 15 years in individuals vaccinated at a very young age[27,39,40]. Therefore, children vaccinated in early childhood may be more likely to have undetectable levels of anti-HBs in late adolescence or early adulthood. Recent studies showed that 2 decades after universal vaccination, this program provides long-term protection for more than 20 years[41,42]. They also indicated that despite the decline in anti-HB antibody levels with time, there is no need for a booster dose of the vaccine as immune memory (Anamnestic anti-HBs response) will protect the immunized individuals if exposed to HBV. Long-term protection had been demonstrated by the rapid, 5-7 days, development of anamnestic antibody responses among vaccinees who no longer had detectable anti-HBs. Following challenge with hepatitis B vaccine, anamnestic responses correlate with lymphoproliferative T-cell responses and the ability of circulating B-cells to produce anti-HBs in vaccinees that lost their detectable antibodies. These studies indicated that despite the antibody decline or loss, immune memory exhibits long-term persistence [5,37,38,42].

According the WHO recommendations, the Libyan Ministry of health had instituted a fourth booster dose, added to the schedule, to be administered at 6 years of age (just before starting school). This dose will ensure seroprotection for the vaccinee's during adolescence and adulthood.

In conclusion, hepatitis B vaccine could be highly protective against HBV infection as evidenced by the presence of protective level of anti-HBs in the vaccinated groups and that the vaccine efficacy against infection waned with time. The longer the time lapse since vaccination, the lower was the seroprotection rate and the lower the anti-HBs levels. However, the duration of protection afforded by hepatitis B vaccination beyond ten years and the possible need for booster doses of this vaccine are still debated. Hep-

atitis B vaccine boosting could be advised for high risk subjects including adult health care workers and diabetic children at five years intervals.

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PRELIMINARY STUDY ON THE EFFICACY AND DURATION OF HEPATITIS-B VIRUS VACCINE

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EFFECT OF GHRELIN ON ISOPRENALINE INDUCED MYOCARDIAL INFARCTION IN RATS

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Abstract

Aim: *To investigate the cardiac effects of ghrelin in rats with myocardial injury induced by isoprenaline (ISO).*

Methods: *Forty healthy adult male albino rats weighting 180-200 gm were included in this study. The rats were randomly classified into 4 equal experimental groups; Group I (Control group): Received no medication and given free access to food and water. Group II (ghrelin group): Received Ghrelin (Gh) only in a dose of 10nmol/kg subcutaneous (S.C) for 2 days. Group III (isoprenaline group): Received single dose of isoprenaline (ISO) 75mg/kg intraperitoneal (i.p.) for induction of myocardial infarction (M.I). Group IV (ghrelin treated group): Received ghrelin in adose of 10 nmol/kg/day SC for 2 days before induction of myocardial infarction with ISO (as in ISO group). Thirty minutes after induction of M.I., a blood sample was collected from the right ventricle for estimation of plasma CK-MB, ghrelin and endothelin-1 levels and heart was excised for measurement of infarction size as percentage of left ventricle.*

Results: *About 20 % of rats after treatment with ISO alone died during experimental periods. However, no rats died after administration with ghrelin 10 nmol/Kg/d. Endothelin-1 and CK-MB level in plasma was decreased by ghrelin (10 nmol/kg) with significant reduction of the infarction size. Plasma ghrelin level was increased in the rats after ISO. The plasma ghrelin level was further increased after ghrelin administration.*

Conclusion: *Ghrelin has a protective effect against ISO-induced myocardial injury.*

Introduction

Ghrelin, a peptide of 28 amino acids, was first reported by Kojima in rats' and human stomachs in 1999[1]. Two thirds of the circulating ghrelin originates in the X/A-like cells in the oxyntic mucosa of the stomach and the rest is produced by cells of the small intestine[2]. In addition, a smaller amount of ghrelin is also produced by other organs such as heart, lung, kidney, pancreas, placenta, lymphatic tissue, gonads, thyroid, adrenal, pituitary, hypothalamus and some neoplastic tissues and cancer-cell lines[3,4,5,6]. Ghrelin is also detected in cerebrospinal fluid but at much lower concentration (about 1/30-1/40 of that of circulating ghrelin)[7]. Ghrelin was initially identified to be a strong stimulant for the release of GH that has been identified as an endogenous ligand for the GH secretagogues receptor (GHS-R), GHSR-1a, a G protein-coupled receptor mainly expressed in the pituitary and the hypothalamus[6,8]. In subsequent research, ghrelin and its various receptors were found everywhere in many organs and tissues. Moreover, they participate in the regulation of appetite, energy, bodyweight, metabolism of glucose and fat, as well as modulation of gastrointestinal, cardiovascular, pulmonary, immune functions, cell proliferation/apoptosis, anti-inflammtory, reproductive and bone formation [9,10]. Cardiovascular tissue is rich in ghrelin receptors[11]. The best investigated ghrelin receptor, GHS-R1a, GHS-R1b are highly expressed in human myocardium and aorta[6]. An unknown subtype of ghrelin receptor, distinct from GHS-R1a and GHS-R1b, is found in cardiomyocytes and endothelial cells. In addition, CD36 is expressed in rat and human cardiovascular tissues[12]. Recent studies showed that ghrelin possessed a variety of cardiovascular effects independent of GH secretion, including increased myocardial contractility and vasodilatation, protection from heart failure induced by myocardial infarction or septic shock, decrease cardiac injury induced by ischemia/reperfusion (I/R) or isoprenaline and reduce infarction size[13,14]. Based on these observations, we raised the hypothesis that ghrelin might have a protective effect on heart injured by ischemia.

Materials and Methods

I- Chemicals used:

1. Synthetic rat ghrelin and ghrelin RIA assay kit were produced by Phoenix Pharmaceuticals (CA, USA).

2. ISO was provided as white powder from (Sigma Company, USA) and dissolved in distilled water.

3. Urethane: provided as white powder (Sigma, U.S.A.). It was dissolved in saline before use.

4. Creatine Kinase - MB kits: (Spinreact, Spain).

5. Triphenyltetrazolium chloride: provided as white powder (MP biomedical, France).

II- Animals used:

Experimental protocol for the study was approved by the ethics committee on animal experiments in Benha University. Forty healthy adult male albino rats weighting 180-200 gm. averaging 16 weeks old were brought from Experimental Animal Breeding Farm, Helwan - Cairo to be utilized in this study. Rats were grown on normal diet and water. At the time of experiment, the rats were randomly classified into 4 equal experimental groups. Group I (Control group): Received no medication and given free access to food and water. Group II (Ghrelin group): Received Ghrelin only in adose of 10nmol/kg SC for 2 days[15]. Group III (ISO group): Received single dose of isoprenaline 75mg/kgm injected intraperitoneal for induction of M.I.[16]. Group IV (Ghrelin treated group): Received ghrelin in adose of 10 nmol /kg SC for 2 days before induction of myocardial infarction with ISO (as in ISO group). The rats were anesthetized by urethane in a dose 1.25g/kg body weight dissolved in saline, half the dose was injected i.p. for a rapid action and the other half was injected S.C for a slow sustained action[17].

- **ET-1 and ghrelin level in plasma:** Were assayed using radioimmunoassay methods[18].

- **CK-MB estimation:** After separation of sera, CK- MB was determined using CK- MB reagent provided by Spinreact Company, Spain[19].

- **Measurement of infarction size:** At the end of the experiment, intracardiac blood sample with-

drawaled then the heart was excised and the excised beating heart was submerged in cold (8°C) 30 mmol KCl to achieve diastolic arrest. The right ventricle and both atria were excised to isolate the left ventricle (the septum and free wall). The left ventricle was then sectioned by a sharp surgical scissor into transverse slices, each of about 1.5mm thick. The slices were then stained in a 1.5% triphenyltetrazolium chloride (TTC) in phosphate buffer, PH 7.4, for 10-15 minutes at 37°C. The TTC stain form red color precipitates in the presence of intact dehydrogenase enzyme system. Areas of necrosis lost dehydrogenase activity and therefore fail to stain[20,21]. The slices were washed with saline and then clear glass plates were placed over both sides of each slice. Epicardial and endocardial outlines as well as the TTC stained and non- stained areas were traced on clear plastic sheets. The plastic sheet was then fixed on an E.C.G paper and the small squares occupying the stained and non-stained areas were counted giving each in mm2. The sum of the stained and the non-stained areas give the surface area of the whole heart slices. The surface area of the whole left ventricle was calculated by adding the surface areas of all cardiac section measured on E.C.G paper. The surface area of infract tissue in the whole heart was obtained by adding the surface area of infract tissue in all cardiac slices and the infraction size was calculated as percentage of the sum of infract areas to the sum of surface areas of all the slices[22].

Results

Ghrelin reduced the mortality rate in the ISO treated rats. All rats in the control and the Gh group remained alive. During the experimental period, about 20% rats in the ISO group died, whereas the mortality rate was 0% in Gh+ISO group ($P<0.05$) as compared with ISO group.

**** Effect of Ghrelin on plasma CK-MB level (Table 1 & Fig. a):** In this study; the plasma CK-MB level was significantly increased in rats of ISO group compared with that of control group (Control group 234.38±33.43, in ISO group 1497.5±111.45; $P<0.001$). Ghrelin alone did not affect the plasma

CK-MB level, in comparison to those of control group (P>0.05). Interestingly, administration of ghrelin before ISO significantly attenuated myocardial injury, that CK-MB level in plasma decreased from 1497.5±111.45 in ISO group to 597.5±138 in ISO + Gh group (P<0.001).

**** Effect of Ghrelin on plasma ET-1 level (Table 1 & Fig. b):** The plasma ET-1 level was significantly increased in ISO group compared to control group (Control group 143.75±27.87, ISO group 239±19.54; P<0.001). Exogenous administration of ghrelin before ISO induced myocardial injury significantly reduced plasma ET-1 level (ISO group 239±19.54, Gh+ ISO group 146.0±15.03; P<0.001), while ghrelin alone did not affect the plasma ET-1 level in comparison to those of control group (P>0.05).

**** Effects of ISO on plasma ghrelin level (Table 1 & Fig. c):** Exogenous administration with ghrelin alone significantly increased plasma ghrelin level from 197±17.48 in control group to 458.75±23.72 in Gh group (P<0.001). Treatment with ISO significantly increased the plasma ghrelin level when compared with control group (P<0.001). Administration of ghrelin before ISO significantly increased the plasma ghrelin level when compared with ISO group (ISO group 266.25±24.16, Gh + ISO 485.0±23.75 P<0.001).

**** Effects of Ghrelin on infarction size (Table 1 & Fig. d):** Administration of ghrelin before ISO induced myocardial injury significantly decreased infarction size from 34.88±4.75 in ISO group to 17.38±3.11in Gh + ISO group (P<0.001).

Table (1): Shows the values as mean ± standard deviation. Then P values were calculated using Student t- test.

	Control group	Gh group	ISO group	Ghtreatedgroup (Gh + ISO)
CK-MB (U/L)	234.38 ±33.53	246.25±29.12	1497.5±111.45	597.5±138.95
ET-1 (ng/L)	143.75±27.87	131.88±27.64	239.38±19.54	146.0±15.03
Gh (mmol/L)	197.75±17.98	458.75±23.72	266.25±24.16	485.0±23.75
Infarction size (% LV)	0.0	0.0	34.88±473	17.38±3.11

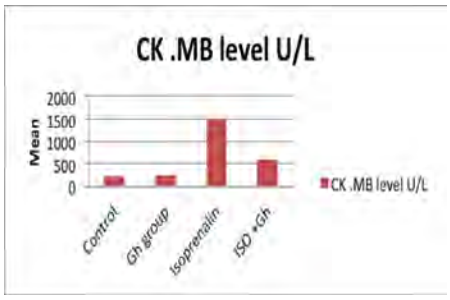

Fig. (a)

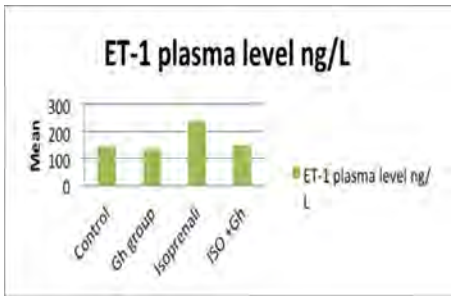

Fig. (b)

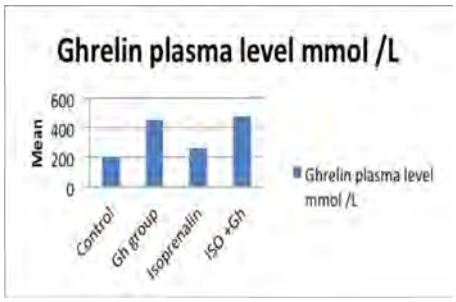

Fig. (c)

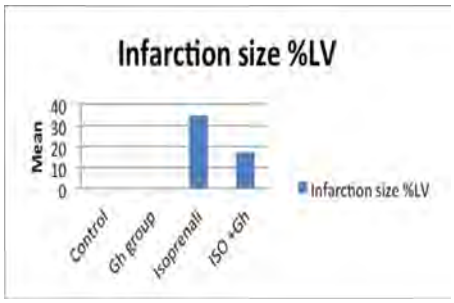

Fig. (d)

Discussion

Ghrelin, is synthesized as a preprohormone and then proteolytically processed to yield a 28-amino-acid peptide. This peptide predominantly produced by the stomach, strongly stimulates growth hormone release when they activates ghrelin receptor known as a G-protein coupled receptor mainly expressed in the pituitary and hypothalamus. The ghrelin receptor was widely distributed in the body. In addition to pituitary and hypothalamus, cardiovascular tissues such as myocardium, aorta, coronary artery and vein were rich in ghrelin receptors[7,23] suggesting that ghrelin could directly exert cardiovascular effects by growth hormone independent mechanisms and likely involve binding to cardiovascular receptors, a process that is up regulated during myocardial injury[24].

In the present study, intraperit-

oneal injection of ISO resulted in a significant myocardial Injury. ISO group showed increase CK-MB level and infarction size. Overdose of ISO was a commonly used agent to induce myocardial injury through increase intracellular Ca^{++}, cAMP, increase heart rate, myocardial contractility and oxidative stress[5,25]. Plasma ghrelin level was increased in rats after ISO-induced myocardial injury, perhaps due to a compensatory, self-protective mechanism. Moreover administration of exogenous ghrelin can protect against ISO induced myocardial injury[15] that administration of ghrelin resulted in obviously improved myocardial damage induced by ISO by reducing CK-MB level and infarction size and decreased mortality [15,26]. These findings suggest that ghrelin could be an endogenous protective factor against myocardium damage.

There are several mechanisms by which ghrelin improved myocardial function and metabolism following to myocardial damage. Ghrelin has been found to improve cardiac contractility to reduce the infarct size and to attenuate the reduction in left ventricular function induced by ischemia-reperfusion[23] Also Ghrelin exerted its cardioprotective effects by prevent cardiaomyocyte apoptosis, through GHSR-1a[12] that activates of extracellular signal regulated kinase (ERK) and Akt serine kinases in cardiomyocytes and endothelial cells[23,27,28,29], in addition to its anti-inflammatory effect, that decrease inerleukin- 6 (IL6) and tumour necrosis factor-α (TNF-α) through activation of GHSR-1a and AKT dependant pathway[29,30] and antioxidative effect, through attenuation in vascular superoxide production and NADPH oxidase activity[31] and decrease the content of lipid peroxidation product malondialdehyde in myocardium of rat hearts [24,32].

The results in this study showed that the plasma ET-1 level was significantly increased in the ISO-treated rats, peptide ET-1 was a potent and long-lasting vasoconstrictor and mytogen[33] and evidenced as an important pathogenic factor of cardiovascular diseases, including ISO-induced myocardial injury[34]. Interesting-

ly, exogenous supplement with ghrelin significantly reduced plasma ET-1 level , indicating that the protective effects of ghrelin was at least in part mediated through interfering ET-1 synthesis and release[15,23]. It was found that ghrelin might act on the cardiac vagal afferent nerve, which sends projection to the nucleus of the solitary tract, resulting in a suppression of cardiac sympathetic activity and heart rate in rats with MI leading to improved left ventricular (LV) dysfunction and early cardiac remodeling, which is a major cause of subsequent heart failure and death following myocardial infarction (MI)[35,36,37]. Also it was found that ghrelin can markedly increase blood flow in mice with ischemia and reperfusion injury, that ghrelin improved endothelial function by increasing the nitric oxide production using a signaling pathway involving GHS-R 1a, phosphatidylinositol-3-kinase (PI 3 kinase), AKT, and eNOS[38,39].

Conclusion

Ghrelin had potent cardiovascular protective effects. Considering that there are amount of ghrelin receptor in cardiovascular system, ghrelin may has cardioprotective significance in physiological and pathophysiological conditions. Exogenous administration with ghrelin may serve as a novel therapeutic strategy for ischemic cardiovascular diseases.

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EFFECT OF GHRELIN ON ISOPRENALINE INDUCED MYOCARDIAL INFARCTION IN RATS

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CARDIOPROTECTIVE EFFECT OF ERYTHROPIOTIN ON ISOPRENALINE INDUCED MYOCARDIAL INFARCTION IN RATS

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Abstract

Background: *Erythropoietin (Epo) has been shown to have important cytoprotective properties against ischemic damage of the myocardium besides its important hematopoietic effect.*

Aim: *This study was designed to find out the possible effects of treatment with recombinant human erythropoietin in rat model of myocardial infarction induced by isoproterenol.*

Materials and Methods: *Adult male albino rats weighing 200-220 gm were divided into 4 groups: group I (Control group) Received no medications and given free access to food and water. Group II (ISO group): Injected intraperitoneal "i.p." with a single dose of isoprenaline in a dose of 75 mg/kg body weight for induction of myocardial infarction. Group III (ISO + rhEPO): Injected i.p. by single dose recombinant human EPO (rhEPO) in a dose of 5000 I.U/kg body weight, 2 hours after induction of myocardial infarction with isoprotrenol. Group IV (rhEPO 10 days + ISO): Injected i.p. with rhEPO in a dose of 5000 I.U/kg body weight/day for 10 days before induction of myocardial infarction with isoprenaline.*

Results: *A significant reduction in the infarction size, serum cardiac enzymes (CPK and LDH), significant increases in plasma NO and non-significant change in both mean systemic arterial blood pressure (MSABP) and hematocrit (Hct) value were observed in rats injected with a single dose of rhEPO (5000 I.U/ kg body weight), 2 hours after induction of myocardial infarction when compared with rat model of myocardial infarction (ISO group). Pretreatment of rats with rhEPO (5000 I.U/ kg body weight/day) for 10 days before induction of myocardial infarction causes significant decrease in cardiac enzymes and infarction size,*

non-significant change in plasma NO, significant increase in Hct value and MSABP when compared with rat model of myocardial infarction. While on comparing these results with those rats treated with single dose of rhEPO, 2 hours after induction of myocardial infarction, there was significant decrease in cardiac enzyme, plasma NO, Significant increase in MSABP, Hct value and non- significant change in the infarction size.

Conclusion: *rhEPO has a Cardioprotective effect and this effect conferred by rhEPO has many mechanisms. One of them is by modulating the hemodynamic functions through increasing the plasma NO concentration. Although, treatment with rhEPO for longer periods may result in increased hematocrit associated side effects such as hypertension or thromboembolism.*

Introduction

Erythropoietin (EPO) is a 165 amino acid glycoprotein hormone and a member of the large and diverse cytokine superfamily produced by the fetal liver and adult kidney. It is mainly synthesized in the peritubular cells in the corticomedullary border of the kidney in response to hypoxia to increases erythropoiesis by stimulating erythroid progenitor (precursor) cells proliferation and differentiation thus increases the number of circulating mature red blood cells, thereby increasing O_2 carrying capacity and protecting red blood cells from apoptosis[1]. Thus, it also plays a major role in regulating plasma haemoglobin (Hb) concentration[2]. So it became widely used in treating anaemias resulting from chronic kidney disease and myelodysplasia occurring after chemotherapy or radiation[3].

The effects of EPO are mediated by a specific trans¬membrane EPO receptor (EPO-R) present in erythroid precursors[4], and in various non haematopoietic cells such as neuronal cells[5], vascular smooth muscle cells, endo¬thelial cells, cardiac myocytes, megakaryocytes, skeletal myoblasts[6] and nephrons[7]. EPO was observed to interact with 2 different receptors. The classic EPO receptor is responsible for the red blood cell response, whereas the interaction

with the β- receptor is responsible for the cardiac tissue protective effects[8].

Recent studies have identified multiple paracrine and autocrine functions of EPO such as coordinating local responses to injury, maintaining vascular autoregulation, stimulating endothelial progenitor cells (EPCs) formation and mobilization, enhancing new vessel formation and attenuating both primary (apoptotic) and secondary (inflammatory) causes of cell death[9] so multiple researches are now focusing on the non-hematopoietic effects of erythropoietin and its potential use against tissue ischemia and its role in tissue repair and regeneration after brain and spinal cord injuries[10].

Cardiovascular disease is fast becoming the number one health concern worldwide. The mortality and morbidity from ischemic cardiovascular diseases remain the greatest unsolved public health challenge throughout the industrialized world due to their high mortality rate. Although the technical advances of thrombolysis and angioplasty have resulted in a remarkable increase over the last 20 years in both the short and long term survival of patients reaching advanced medical care but a significant percentage of surviving patients remain severely disabled. Novel approaches that protect against injured heart tissue would constitute important advances in the therapy of this disease[9,11].

Materials and Methods

I- Chemicals used:

1. Recombinant human erythropoietin: Provided in vials (Erypoietin). Each vial containing 4000 I.U/ml. (by Sigma CO., USA).

2. Isoprenaline: provided as white powder (Sigma CO., USA). It was dissolved in distilled water before use.

3. Urethane: Provided as white powder (by Sigma, U.S.A.). It was dissolved in saline before use.

4. Triphenyl tetrazolium chloride: Provided as white powder (by MP biomedicals, France).

5. Creatine phosphokinase (CPK) kits (Spinreact Co., Spain).

6. Lactate dehydrogenase (LDH) kits (Spinreact Co., Spain).

7. Plasma Nitric Oxide commer-

cial kit (Roche Co., Germany).

II- Animals used:

Experimental protocol for the study was approved by the ethics committee on animal experiments in Benha University. Twenty eight adult male albino rats weighting 200-220 gm. averaging 18 weeks old were brought from Experimental Animal Breeding Farm, Helwan - Cairo to be used in this study. They were housed in cages (7 rats/cage) under standard laboratory conditions (12h light/dark cycle, 20-25°C, relative humidity 55%). The animals were given commercial standard caloric diet and tap water ad libitum. All animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences. After acclimatization for 1 week, the rats were randomly classified into 4 equal experimental groups: Group I (Control group): Rats of this group received no medication and given free access to food and water. Group II (ISO group): In which, rats were injected by a single dose of isoprenaline (75 mg/kg body weight) i.p. for induction of myocardial infarction. Group III (ISO + rhEPO): In which, rats were injected by a single dose of rhEPO (5000 I.U/kg body weight) i.p., 2 hours after induction of myocardial infarction with isoprenaline. Group IV (rhEPO for 10 days + ISO): Rats of this group were injected by rhEPO i.p. in a dose of 5000 I.U/kg body weight/day for 10 days before induction of myocardial infarction with isoprenaline.

IV- Procedure of the experiment:

No rats were died during the experimental periods. The rats were anaesthized by urethane and three hours after induction of myocardial infarction by isoprenaline, a longitudinal incision in the anterior aspect of the neck was done for measurement of arterial blood pressure then the chest was opened and intracardiac blood samples was collected and Hct value was determined. The plasma was taken for measurement of plasma nitric oxide (NO) and cardiac enzymes (CPK and LDH). Then the still beating heart was excised for measurement of the infarction size.

1- Measurement of the mean systemic arterial blood pressure:

The rats were anesthetized by urethane in a dose 1.25g/kg body weight dissolved in saline, half the dose was injected i.p. for a rapid action and the other half was injected S.C for a slow sustained action[12] then a longitudinal incision was done in the anterior aspect of the neck through which the trachea was detected and elevated by a lever for exploration of carotid artery in which the carotid cannula connected to the oscillograph (washing Ton, 400 MD4c oscillograph) was inserted for measurement of both systolic and diastolic arterial blood pressure [13] then mean systemic arterial blood pressure was calculated.

2- Measurement of Hematocrite value:

Blood sample was collected from the right ventricle and rapidly placed in wintrobe tube to be centrifugated at 5000 rpm for 15 minutes. The R.B.Cs will be separated at the bottom of the tube leaving clear plasma above. Hct value was calculated by measuring the percentage ratio of R.B.Cs column on the gradation to the total blood column[14].

3- Measurement of plasma cardiac enzymes and Nitric oxide:

The clear supernatant was used for the estimation of the following biochemical parameters; plasma CPK[15], LDH[16] and NO using standard commercial kits[17].

4- Measurement of the infarction size:

After withdrawal of intracardiac blood sample, The excised beating heart was submerged in cold (8°C) 30 mmol Kcl to achieve diastolic arrest[18]. The right ventricle and both atria were excised to isolate the left ventricle (the septum and free wall) which is then sectioned by a sharp surgical blade into transverse slices, each of about 1.5mm thick which was then submerged in a 1.5% triphenyl tetrazolium chloride (TTC) stain in phosphate buffer, PH 7.4, for 10-15 minutes at 37°C. This stain form red colour precipitates in the presence of intact dehydrogenase enzyme system thus the necrosed areas fail to stain[19]. The slices were washed with saline and then clear glass plates were

placed over both sides of each slice. Epicardial and endocardial outlines as well as the TTC stained and non- stained areas were traced on clear plastic sheets. The plastic sheet was then fixed on an E.C.G paper and the small squares occupying the stained and non-stained areas were counted giving each in mm2. The sum of the stained and non-stained areas give the surface area of the whole heart slices and the infraction size was calculated as percentage of the sum of infract areas to the sum of surface areas of all the slices[20].

Results

Table (1) & Fig. (1): Show that isoprenaline injection significantly increases the infarction size from 0 in control group to 49.71±5.47 in ISO group (group II) (P<0.001). Injection with a single dose of rhEPO (5000 U/kg body weight), 2 hours after induction of myocardial infarction significantly decrease it from 49.71±5.47 in ISO group to 16.29±3.77 in group III (P<0.001). Also daily injection with rhEPO (5000 U/kg body weight/ day for 10 days before induction of myocardial infarction) significantly decreases it from 49.71±5.47 in ISO group to 14.57±3.21in group IV (P<0.001) but there was non-significant change in infarction size (P>0.05) in rats injected daily with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III).

Table (1) and Fig. (2 & 3): Show that isoprenaline injection significantly increases the cardiac enzymes (CPK and LDH) from 107.71±4.64 and 148.57±3.99 in control group to 437.43±8.87 and 524.71±26.98 respectively in ISO group (P<0.001). Injection with a single dose of rhEPO 2 hours after induction of myocardial infarction significantly decrease them from 437.43±8.87 and 524.71±26.98 in ISO group to 142.14±3.24 and 155.43±4.24 respectively in group III (P<0.001). Also daily injection with rhEPO for 10 days before induction of myocardial infarction significantly decrease them from 437.43±8.87 and 524.71±26.98 in ISO group to 125.71±8.79 and 149.14±6.23 in group IV

(P<0.001). There was significant decrease in plasma CPK and LDH in rats injected daily with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III) as they were decreased from 142.14±3.24 and 155.43±4.24 to 125.71±8.79 and 149.14±6.2 (P<0.001 and <0.05) respectively.

Table (1) & Fig. (4): Show that isoprenaline injection significantly decreases plasma nitric oxide (NO) from 29.43±5.74 in control group to 9.86±3.98 in ISO group (P<0.001). Injection with a single dose of rhEPO, 2 hours after induction of myocardial infarction significantly increases it from 9.86±3.98 in ISO group to 46.14±7.34 in group III (P<0.001) while daily injection with rhEPO for 10 days before induction of myocardial infarction causes non-significant change in plasma NO when compared with ISO group as it was increased from 9.86±3.98 in ISO group to 11.14±2.41in group IV (P>0.05). There was significant decrease in plasma NO in rats injected with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III) as it was decreased from 46.14±7.34 in group III to 11.14±2.41in group IV (P<0.001).

Table (1) & Fig. (5): Show that isoprenaline injection significantly decreases the mean systemic arterial blood pressure (MSABP) from 95.86±4.22 in control group to 91±3.74 in ISO group (P<0.05). Injection with a single dose of rhEPO, 2 hours after induction of myocardial infarction caused non-significant change in MSABP as it was changed from 91±3.74 in ISO group to 91.14±5.01in group III (P>0.05) while daily injection with rhEPO for 10 days before induction of myocardial infarction caused significant increase in MSABP from 91±3.74 in ISO group to 117.29±10.03 in group IV (P<0.001). Also there was significant increase in the MSABP in rats injected daily with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats Injected with a

single dose of rhEPO, 2 hours after induction of myocardial infarction (group III) as it was increased from 91.14±5.0 in group III to 117.29±10.03 in group IV (P<0.001).

Table (1) & Fig. (6): Show that isoprenaline injection caused non-significant change in hematocrit (Hct) value as it was changed from 47.43±3.35 in control group to 48.57±2.88 in ISO group (P>0.05). Injection with a single dose of rhEPO, 2 hours after induction of myocardial infarction had no significant change on Hct value as it was changed from 48.57±2.88 in ISO group to 49.71±2.82 in group III (P>0.05) while daily injection with rhEPO for 10 days before induction of myocardial infarction caused significant increase in Hct value when compared with ISO group as it was increased from 48.57±2.88 in ISO group to 76.29±7.95 in group IV (P<0.001). There was significant increase in Hct value in rats injected daily with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III) as it was increased from 49.71±2.82 in group III to 76.29±7.95 in group IV (P<0.001).

Table (1): Shows the values of infarction size (% LV), plasma CPK (U/L), plasma LDH (U/L), plasma NO (µmol/ml), MSABP (mmHg) and hematocrite value (%) as mean ± standard deviation. Then P values are calculated using Student t- test.

	Group I (Control)	Group II (ISO)	Group III (ISO + rhEPO Single dose)	Group IV (rhEPO 10 days + ISO)
Infarction size	0	49.71 ± 5.47	16.29 ± 3.77	14.57 ± 3.21
Plasma CPK	107.71 ± 4.64	437.43 ± 8.87	142.14 ± 3.24	125.71 ± 8.79
Plasma LDH	148.57 ± 3.99	524.71 ± 26.98	155.43 ± 4.24	149.14 ± 6.23
Plasma NO	29.43 ± 5.74	9.86 ± 3.98	46.14 ± 7.34	11.14 ± 2.41
MSABP	95.86 ± 4.22	91 ± 3.74	91.14 ± 5.01	117.29 ± 10.03
Hct	47.43 ± 3.35	48.57 ± 2.88	49.71 ± 2.82	76.29 ± 7.95

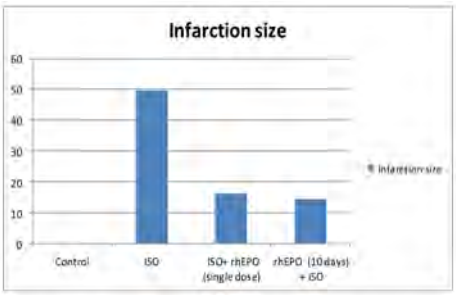


Fig. 1

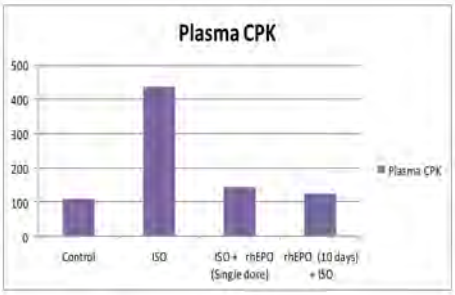
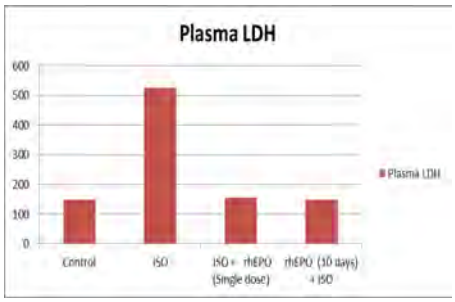


Fig. 2



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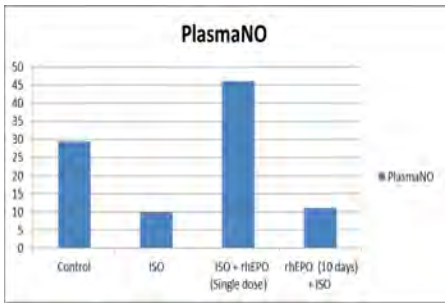

Fig. 4

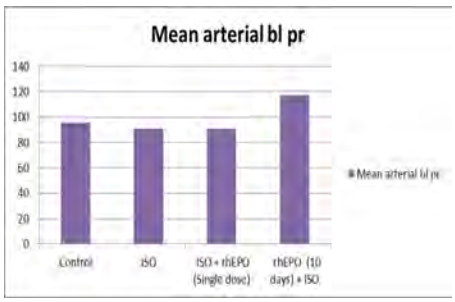

Fig. 5

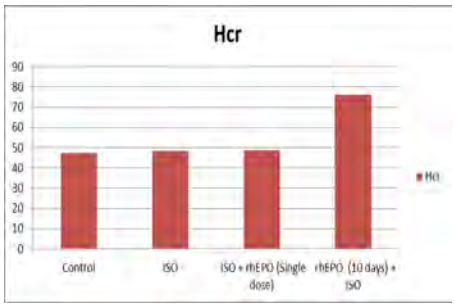

Fig. 6

Discussion

Myocardial infarction (MI) continues to be a major public health problem and the leading cause of morbidity and mortality worldwide[21]. The hormone erythropoietin (EPO), produced by adult kidney and the fetal liver, is well known in regulating mammalian erythropoiesis. Exogenous EPO, the recombinant human EPO (rhEPO), was introduced approximately two decades ago for the treatment of anemia resulting from variety of conditions such as chronic renal failure and chemotherapy. However during the last decade, EPO and its receptor (EPOR) were found to present outside the liver and the kidney as in the brain and heart. At the same time, several experimental studies using rhEPO have shown its potential neuroprotective and cardioprotective role against ischemia occurring independently on its hematopoietic action[22].

So we aimed in our study to find out the efficacy of treatment with rhEPO against isoprenaline induced myocardial infarction, its possible mechanism of action and the dependence of this efficacy and safety on the time and duration of treatment. This was assessed by measuring plasma levels of cardiac enzymes (CPK and LDH) and Nitric oxide, infarction size, mean systemic arterial blood pressure and hematocrit value.

Our study revealed that isoprenaline, a non-selective β- adrenergic agonist induces myocardial infarction in rats which was confirmed by significant increased myocardial infarction size (% LV) and elevated plasma levels of cardiac enzymes (CPK and LDH). This can be explained by its effect on increasing intracellular Ca^{++} and cAMP, increasing heart rate and cardiac contractility[23,24], enhances free radical formation and endothelial dysfunction and down-regulates the expression of endothelial nitric oxide synthase enzyme[25,26].

In our study, Injection with a single dose of rhEPO, 2 hours after induction of myocardial infarction caused significant decrease in infarction size and plasma level of cardiac enzymes, significant increase in plasma NO but non-significant change in mean sys-

temic arterial blood pressure and Hct value was noticed. On injecting the rats with a daily dose of rhEPO for 10 days before induction of myocardial infarction, there was, significant decrease in infarction size and plasma level of cardiac enzymes, significant increase in mean systemic arterial blood pressure and hematocrite value but non-significant change in plasma NO were noticed.

When we compared the results of the rats injected daily with rhEPO for 10 days before induction of infarction with those which were injected by a single dose of rhEPO, 2 hours after induction of infarction, there was significant decrease in cardiac enzyme, plasma NO, Significant increase in MSABP, Hct value and non- significant change in the infarction size.

In agreement with our results, EPO treatment lead to reduction in the infarction size and promote endothelial progenitor cell growth which increases angiogenesis whether it is injected before ischemia, during ischemia or during reperfusion[27,28,29]. However, its beneficial effect when given after reperfusion were better than when given before or during ischemia [29]. Exogenous rhEPO administration stimulates vascular NO production either directly by increasing the activity of nitric oxide synthase enzyme[30,31,32,33] or indirectly through increased shear stress in endothelial cells[33]. This may explain the cardioprotective role of EPO in myocardial infarction via increasing vasodilatation and facilitating effective collateral circulation[34,35]. Other potential mechanisms that may contribute to the cytoprotective effect of Epo include inhibition of apoptosis by the activation of Janus kinase/ signal transducer and activator of transcription (JAK/STAT), phosphatidylinositol-3-kinase (PI-3Kinase) and protein kinase-C (PKC) and improved cardiomyocyte survival[1,36,37] in additional to attenuation of inflammatory responses[1,38], oxidative stress, modulation the cardiac Na^+/K^+ pump and stimulation of atrial natriuretic peptide release[1]. Epo administration in patients with an acute coronary syndrome is safe and feasible[29]. However, the main side effect and limiting factor of high and prolonged dose of rhE-

PO therapy resulted from increased hematocrit associated side effects such as increased blood viscosity, systemic hypertension, down regulation of nitric oxide synthesis and impaired tissue blood flow that results in thromboembolism[39,40,41].

In contrast to our results, showed that increase hematocrit which resulted from prolonged injection of 5.000 I.U/kg body weight of rhEPO is not harmful but it might improve cardiac function by improving delivery of oxygen to a hypoxic myocardium and that its protective effect against ischemic damage of myocardium is not associated with NO activation or NO mediated hemodynamic responses[42].

Conclusion

Administration of EPO was not only beneficial when it is used in a single dose after induction of myocardial infarction but also when used as pretreatment before induction of myocardial infarction. Although, its beneficial effect when given after infarction was better than when given before or during infarction.

The significant effect of rhEPO within the injured heart also strongly supports the concept that EPO is possibly a tissue-protective cytokine for other organs that express the EPO receptor. We hope that acute rhEPO administration will represent a pharmacological approach to cytoprotection in the upcoming years.

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