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MANAGEMENT OF DUODENAL INJURIES: MANSOURA EXPERIENCE

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Abstract

Hypothesis: *Duodenal injuries are uncommon but continue to pose a diagnostic and therapeutic challenge. This study reviews the results of Management of duodenal injuries at our Emergency surgery unit to re-evaluate our experience.*

Patients and Methods: *This is a retrospective study of patients sustaining duodenal injury and was admitted to Emergency Hospital Mansoura University, during a 15-years period from January 1995 to December 2009. Reviewing all data of management, during the study period comparing to the literatures to identify any statistical significance.*

Results: *75 patients were entered the study, 66 men (88%) and 9 women (12%), mean of age was 34 years ± 3.7 SD , 56 patients (74.7%) sustained penetrating injuries, while blunt trauma caused 19 injuries (25.3%). 36 (48%) of patients were admitted in shock, diagnostic delay was encountered in 2 patients, 59 (78.7%) of the patients had two or more associated injuries, Suture closure was the most common reparative technique used in 59 patients (78,7%). Dehiscence was encountered in 7 patients. 14 patients died with overall mortality of 18.7%.*

Conclusions: *Management of duodenal injuries at our unit has acceptable results. Improved outcome for those patients awaits advances in injury prevention, diagnosis and reparative techniques.*

Key words: *Duodenal injuries, associated trauma, delay, drainage.*

Introduction

Duodenal injuries are relatively rare, but with significant morbidity and mortality[1,2]. Injuries to the duodenum present three major challenges to the trauma surgeon: establishing the diagnosis, intra-operative and post-operative management of these critically ill patients [3].

The anatomy of the duodenum is unique and complex because of its close relationship with vital structures [4], retro-peritoneal location may produce minimal, vague and nonspecific symptoms, sometimes even nonexistent until severe, life-threatening peritonitis develops!!,[3&5], so diagnosis of duodenal injuries is usually delayed[6&7].

Lacks complete serosal covering so have a tendency to leak[6&7]. Also injuries result in leakage of bowel content which when combined with bacterial contamination in retro-peritoneum makes repair difficult. Associated pancreatic or biliary injury adds chemical peritonitis. Significant delay greatly enhances the likelihood of septic complications and anastomotic disruption[8&9].

Extra-luminal air is a reliable sign of duodenal perforation and is highly predictive of such perforation so radiological studies and computed tomography may lead to the diagnosis [10&11].

Exploratory laparotomy remains as the ultimate diagnostic test if a high suspicion of duodenal injury continues even in the face of absent or equivocal radiographic signs[8]. Intra-operative diagnosis was also challenging, Complete mobilization of the duodenum was necessary [2].

Surgeon must choose an appropriate method of repair, wide variety of treatment options indicates a lack of satisfaction with any of them [12&13].

This study reviews the results of Management of duodenal injuries at our emergency surgery unit to reevaluate our experience.

Patients and Methods

This is a retrospective study of patients sustaining duodenal injury and was admitted to Emergency

Hospital Mansoura University, during a 15-years period from January 1995 to December 2009.

We review data of all injured patients whom were admitted with abdominal trauma then we identify Patients with Duodenal Injury.

Reviewing all data as regard incidence of duodenal injuries, sex and age of patients, mechanism of trauma, diagnostic signs and tools, timing of management,

Site and severity of injuries, methods of repair, post operative morbidity, mortality and hospital stay. Statistical analysis was performed by using Student t-test. A P value of < 0.05 was considered significant.

Results

75 patients were entered the study, 66 (88%) men and 9 (12%) women, age of The youngest was 13 years and of the oldest was 73 years with mean of age was (34 ± 3.7 SD) years.

During the study period, the number of patients with significant abdominal trauma was 2348 but laparotomy was done only for 1756 patients, duodenal Injuries were documented in those 75 patients whom were enrolled in the study, about (4.3%).

Mode of Injury :

56 patients (74.7%) were sustained penetrating injuries, while blunt trauma caused 19 injuries (25.3%) with significant P value (< 0.05). Blunt injuries resulted in two patients' deaths with (10.5%) mortality rate, while 12 patients with penetrating injuries died with mortality rate of 18.5%. Modes of injury are listed in Table 1.

Diagnosis of Injury :

Gunshots indicates exploratory laparotomy in 13 patients while Stab wounds, suspected to enter the peritoneal cavity but without physical signs of peritoneal irritation were locally explored first for, documentation of deep penetration in 4 of 39 patients.

In blunt trauma, the decision to undertake exploratory laparotomy was made on the basis of physical findings in alert and cooperated patients (12 of 19 patients). When the physical exami-

nation is un-reliable, or equivocal peritoneal lavage is performed using standard techniques. Positive test dictates exploratory laparotomy (2 of 19 patients).

28 patients had abdominal roentgenograms with upright views; in only 4 was air visible. Contrast gastro-duodenography was employed in three patients; one study was positive for injury. Serial observations of serum amylase levels were done in 26 patients with proven injuries and only 2 were elevated .abdominal CT was positive in 2 of 5 patients (Fig. 1 & 2).

Timing of management :

Delay in diagnosis was encountered in two patients of blunt trauma. In one of them, peritoneal lavage was not performed until 22 hours after admission, but was positive at that point. The second patient was admitted after 34 hours of trauma with positive physical findings, both patients died. Diagnostic delay was not encountered in patients with penetrating injuries because, all patients were explored.

The time interval from injury to time of operative repair in all patients ranged from 3 to 37 hours, (mean 5 ± 8 SD hours).

Exploration

A long, midline laparotomy incision was routinely used. A thorough search for intraperitoneal injuries is followed by complete exposure of the duodenum periduodenal hematoma, bile staining, or retroperitoneal gas were absent in 6 of 19 patients explored for blunt injury, and trans-luminal lacerations requiring repair appear only after complete exposure (Fig. 3).

Location of Injury :

The distribution of injuries is listed in Table 2. Penetrating injuries were distributed throughout all duodenum, without Statistical significant ($P>0.05$) However, blunt trauma caused injury to the second portion of the duodenum in the majority of patients (8 of 19) with Statistical significant ($P<0.05$), the posterior surface being the most frequent site of perforation.

Severity of Injury :

Injuries were classified as follows : 1) hematomas and contusions not requiring repair, 2) lacerations involving less than 20% of the luminal circumference; 3) lacerations involving more than 20% but less than 70% of luminal circumference; and 4) lacerations involving more than 70% of luminal circumference. Severity of injury in Table 3.

Associated Injuries :

Isolated duodenal injury was uncommon; The associated injuries are listed in Table 4. 75% of our patients had two or more associated intra-abdominal injuries. 48% of the patients were admitted in shock.

Associated injury influenced mortality significantly ($P < 0.05$). 10 of 27 patients (37%) having four or more additional organ injuries died while only 4 of 48 (8.3%) with fewer than four organs involved died.

Operative techniques :

The injury is repaired according to the surgeon's assessment of site and severity of injury, the associated injuries, and the general condition of the patient.

Suture closure of trans-mural injuries, using two layer technique is preferred. Obviously devitalized tissue is debrided and closure affected transversely, Suture repair was possible in 53 (70.7%) patients. 6 (8%) patients had suture-line dehiscence and resulting sepsis. 4 of these patients died. Suture lines were buttressed with jejunal serosal patches in 6 additional patients. One nonfatal anastomotic leak occurred.

A Roux-en-Y Duodeno-jejunostomy was performed after segmental duodenectomy when direct suture is not feasible this occurred in 8 patients. No suture line leaks were observed in these patients. But one death resulted from renal failure. 3 patients with injuries of fourth portion of the duodenum were managed by conservative duodenectomy and direct duodeno-jejunostomy.

Duodenal resection, Isolation-drainage (diverticulization) and pancreaticoduodenectomy (Whipple) were not used.

Do the least possible to control the life-threatening injuries and "Come back another day as a damage control concept have recently been added in 2 cases.

Suction drainage of the periduodenal area was routinely used.

Duodenal decompression was used only in 10 patients, and was obtained by long tubes passed trans-nasally through the pylorus (6 patients). Foley catheter duodenostomy and decompressing jejunostomy were used in two patients and pyloric exclusion in two patients. No suture-line leaks were encountered in these patients.

The specific measures to repair and related outcome in (Table 5).

Mortality :

14 of 75 patients died for an overall mortality of 18.7 %. 5 patients died less than 24 hours after admission (6.7%) due to hemorrhagic shock or massive associated injuries. Overall mortality for patients surviving more than 24 hours was 12%. Causes of death are listed in Table 6.

Hospital stay :

The hospital stay of patients ranged from 7 to 87days, mean 26.6 ± 9,7days.

Table1. Mode of injury.

Mode of Injury	Number of Patients
Penetrating	**56**
Stab	39
Gunshot	13
Miscellaneous	4
Blunt	**19**
Motor vehicle	17
Direct blow	2
Miscellaneous	0

Table 2. Location of Injury.

	Portion				
	1st	2nd	3rd	4th	Multiple
Penetrating	7 (12.5%)	12 (21.4%)	7 12.5%	13 23.2%	17 (30.4%)
Blunt	2 (10.5%)	8 (42.1%)	1 (5.3%)	3 15.8%	5 (26.3%)

Table 3 : Severity of Injury.

Grads	Blunt	Penetrating
I	4 (%)	1(%)
II	10(%)	29 (%)
III	3(%)	21 (%)
IV	2 (%)	5 (%)

Table 4. Associated Injuries*

Intraabdominal		Extraabdominal	
Liver	31	Long bone fractures	5
Colon	29	Rib fractures	2
Small intestine	25	Maxillofacial injuries	2
Stomach	24	Soft tissue injuries	2
Pancreas	5	Epidural hematoma	1
Vena cava	13		
Kidney	12		
Biliary tract	11		
Gall bladder			

* Some patients had more than 1 associated injury

Table 5. Management of duodenal Injury.

Types of Repair	No	Anastomotic Leaks
Suture	53	6
Serosal patch	6	1
Duodenojejunostomy	11	0
• Direct	3	0
• Roux-en-Y	8	0

Table 5. Management of duodenal Injury.

Types of Repair	No	Anastomotic Leaks
Suture	53	6
Serosal patch	6	1
Duodenojejunostomy	11	0
• Direct	3	0
• Roux-en-Y	8	0

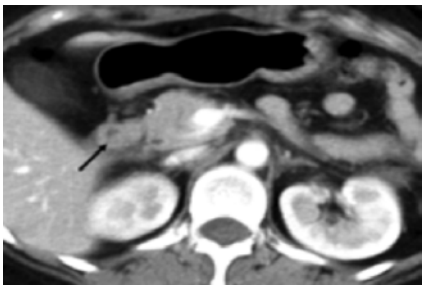

Fig 1 : Grade I duodenal injury. Axial CT shows thickening of the descending duodenal wall (arrow) without evidence of free air.

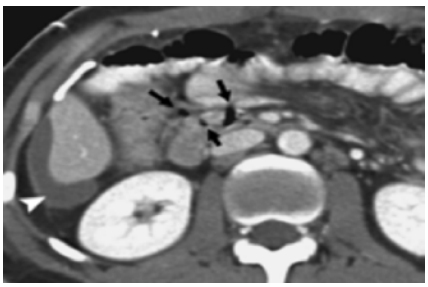

Fig 2 : Grade II duodenal injury. Injury of the horizontal part of the duodenum with extra-luminal air (arrows). A sub-capsular hematoma is present at right liver lobe (arrowhead).

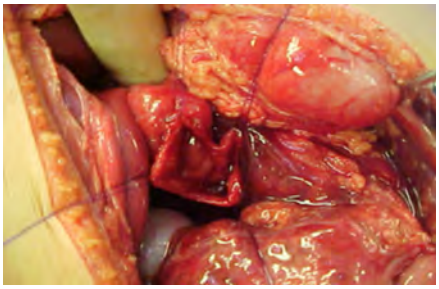

Fig 3 : Operative finding of second part injury.

Discussion

Duodenal injury following blunt or penetrating trauma remains one of the most challenging problems confronting the trauma surgeon [6&14].

Duodenal injuries are uncommon because Lying deep within the abdomen, so is well protected in the retro-peritoneal space. The reported incidence of duodenal injury ranged from 3.7% to 5% of all laparotomies for trauma[10]. Asensio and associates reported 17 duodenal injuries in 402 cases of abdominal trauma (4.2%) during Korean war[9&15]. In 1968, Morten and Jordan reported an incidence of 5% among 280 patients with abdominal trauma[16].

Owing to the increased incidence of automobile accidents and the greater devastation of the modern weapons being used in violent assault, duodenal injuries are seen with much greater frequency than 40 years ago[3&17] we document 75 cases among 1756 patients (4.3 %).

Although penetrating trauma is the most common cause of duodenal injuries, blunt trauma continues to predominate in rural areas[9]. Penetrating injuries to the duodenum are most often the result of gunshot wounds to the abdomen,[17]. Blunt injuries are commonly the result of direct compression of the upper abdomen. In a review of 17 series with 1513 cases of duodenal injuries, Asensio and co-workers reported an incidence of 77.7% occurring as the result of penetrating trauma and 22.3% from blunt trauma. In this report, among the 1096 penetrating injuries, 74.6% were caused by gunshot wounds, 19.5 were due to stabbing and 5.9% were the result of gunshot blasts, among the 230 blunt injuries, 77.3% were caused by motor vehicle accidents, 9.6% by falls, 9.6% by assaults and 3.5% by miscellaneous mechanisms [2,3&9].

Our data are similar but stab are much more than gunshots, 6 of 13 automobile accident victims in our series were drivers. None was wearing a seat belt.

Corley and associates have suggested that frequent physical examinations will lead to early

diagnosis[18]. Serum amylase levels rarely indicated duodenal injury, Likewise, peritoneal lavage in cases of retroperitoneal injuries [19].

In our patients, peritoneal lavage was particularly helpful in two patients. In retrospect, an altered state of consciousness on admission should have led to an early peritoneal lavage.

Free intra-peritoneal or retroperitoneal air was rarely observed. Repeated CT should be considered for patients in stable condition when there is a strong suspicion of pancreatic injury despite normal findings at admission CT[18&20].

Our observations indicate that roentgenographic examinations searching for free air and blood chemistry studies, particularly serum amylase level, are infrequently positive that they are useless. These data confirm the findings of Talbott and Shuck and Davis and associates[20&21].

Delayed diagnosis was the principal factors determining the morbidity and mortality[22&23]. Since exploratory laparotomy is routinely employed in penetrating trauma, delay in diagnosis is not usually a factor influencing subsequent complications[13]. In this series, all 17 patients operated on within 12 hours after blunt injury survived whereas two patients who underwent delayed operation died.

The time interval from injury to time of operative repair in all patients ranged from 2 to 72 hours, mean + SD 19.5 + 21.61 hours.[22] We are near.

Intra-operative diagnosis was facilitated through the use of extended exposure of the duodenum. The wide Kocher maneuver and exposure of the fourth portion of the duodenum are essential elements of operative management [5,24&25].

Intra-operative diagnostic findings were frequently equivocal. Obvious peri-duodenal hematoma, bile staining, or retroperitoneal gas were absent in 6 of 19 patients explored for blunt injury, and trans-luminal lacerations

requiring repair would have been missed in each of these patients if our policy not been followed.

Severity of the injured duodenum was classified according to many systems as the American Association for the Surgery of Trauma-Organ Injury Scale (AAS-TOIS)[7&26] and, according to Snyder et al., criteria[3&22]. We use more practical easy scale.

The frequency of associated injuries, particularly to other portions of the gastrointestinal tract and to the pancreas, insures that heavy bacterial contamination and prolonged intra-peritoneal and retroperitoneal inflammation will be encountered[16,21,27,2,4]. So Preoperative broad-spectrum intravenous antibiotics are begun and continued for 72 hours[18&28].

Duodenal trauma, with early diagnosis[29&30], and Uncomplicated duodenal wound can be safely treated by simple suture repair. This may account for approximately 80% of all duodenal wounds[6&31]. For more complicated duodenal injuries, more complex procedures may be required to protect the suture line [32,33,10,34].

Here, we report a case of multi-level blunt duodenal injury successfully managed with, Roux-en-Y gastro-jejunostomy and catheter duodenostomy We advocate the use of pyloric exclusion and gastro-jejunostomy without vagotomy in patients with severe pancreato-duodenal injuries, In our experience, this has functioned well in patients with large defects in the 2nd portion of the duodenum in close proximity to the ampulla, where 1ry repair or anastomosis may compromise the lumen.

Our data support the addition of duodenal decompression to the suture repair of duodenal lacerations as suggested by Stone and Fabian[14]. A double jejunostomy tube arranged to provide duodenal compression as well as enteral feedings has merit. Foley catheter duodenostomy as described by Jones and associates[30] also provides excellent duodenal decompression.

We encountered 5 patients with combined pancreatico-duodenal

injury and found pancreaticoduodenectomy not required in these patients.

Signs of sepsis and organ failure in the postoperative period indicate the need for reoperation, debridement and drainage[35]. The most serious complication following the treatment of duodenal injury is the development of a duodenal fistula from suture line dehiscence. In a collective review of 15 series with 1408 patients with duodenal injuries, Asensio and collegues noted a 0% to 17% incidence of duodenal fistula with an average rate of 6.6%[9&10]. We found this in 7 cases.

The overall mortality rate of duodenal injuries continues to be significant. Miller, in 1916, cited a mortality rate of 90% Although recent reviews document mortality reduced to 15% or less, Several series reported a range of 5.3% to 30%, with an average of 17%[25]. Delayed treatment and technical failures continue to contribute to death and disability for these patients[25]. The incidence is still relatively high[7] our results are similar.

The hospital stay in patients who had duodenum injuries ranged from 5 to 90 days, mean 26.6 + 35.67 days. Our time is similar but there're many factors affecting this not only duodenal injury Perce.

Improved outcome for patients with duodenal injury awaits advances in injury prevention, diagnosis and reparative techniques.

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PORTAL HEMODYNAMIC CHANGES IN PATIENTS WITH CHRONIC HEPATITIS C TREATED WITH COMBINED PEGYLATED INTERFERON ALFA AND RIBAVIRIN

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Abstract

Objective. *There are few studies that have described the use of pulse wave Doppler ultrasonography for assessing the effectiveness of IFN treatment and there is much controversy regarding the reliability and reproducibility of these techniques. We performed a prospective study to evaluate whether these methods can assess the effectiveness of and the response to IFN therapy in patients with hepatitis C virus (HCV)–related liver disease*

Subjects and Methods. *Fifty- three patients with biopsy-proven HCV-related liver disease were recruited, and all patients received treatment for chronic hepatitis C in the form of combined therapy of pegylated interferon plus ribavirin.*

Before and after treatment, pulse wave Doppler ultrasonography was performed and five Doppler parameters were measured: portal vein velocity (PVV), hepatic artery resistive index (HARI), hepatic artery pulsatility index (HAPI), splenic artery resistance index (SARI) and splenic artery Pulsatility index (SAPI).

Results. *Significant improvement in all portal hemodynamic parameters after treatment in comparison with that before treatment (in both responders and non responders groups) with non significant difference observed between both groups*

Conclusion. *All portal hemodynamic parameters improved after treatment whatever the response to therapy. Doppler ultrasonography*

is a less reliable test for evaluating the effectiveness and the response of IFN treatment in patients with chronic hepatitis c.

Abbreviations: ***IFN:*** *interferon,* ***PVV:*** *Portal vein velocity,* ***HARI:*** *Hepatic artery resistive index,* ***HAPI:*** *Hepatic artery pulsatility index,* ***SARI:*** *Splenic artery resistive index* ***SAPI:*** *Splenic artery pulsatility index*

Key words: *portal hemodynamics, chronic hepatitis, pegylated interferon alfa.*

Introduction

Hepatitis C virus (HCV) infection is a leading cause of liver disease worldwide. Infected patients usually develop chronic hepatitis, which may progress producing diffuse disorganization of normal hepatic structure, to liver cirrhosis over a long period. Approximately 10%-20% of patients with chronic HCV infection have cirrhosis at the first clinical presentation, and near 20%-30% of those who do not present liver cirrhosis will eventually develop this illness and its complications [(1)].

To date, the most effective treatment to prevent disease progression and eventually cure chronic hepatitis C (CHC) virus infection is the combined therapy of pegylated (PEG) interferon (IFN) alpha and ribavirin (RBV). The rate of sustained viral response (SVR) in immuno-competent patients undergoing this antiviral regimen ranges from 42% to approximately 80%, depending on the HCV genotype, with an acceptable safety profile [(2)].

A number of studies have reported that interferon (IFN) is effective for the treatment of CHC and have demonstrated improvements in hepatic function biochemically, histologically, and virologically. In addition, IFN has been found to improve the prognosis of patients because it has an anticarcinogenic effect on the progression of hepatic cirrhosis [(3)].

In assessing the effectiveness of IFN treatment on patients with CHC, there are unequivocal indices in clinical and laboratory tests, such as hepatitis C virus RNA (HCV-RNA) and alanine ami-

notransferase (ALT) levels, which can also be expected to serve as indicators of the complete elimination of HCV-RNA by IFN (sustained viral response: SVR). However, IFN is not effective in all patients with CHC. The effectiveness of IFN treatment has been reported to vary depending on a number of background factors such as the HCV-RNA value before IFN administration, the HCV genotype, and the histopathological stage [4]. These factors can be evaluated before the start of IFN administration in order to predict the effectiveness of IFN treatment.

In the meantime, ultrasonography has been widely recognized as a noninvasive, easy-to-perform diagnostic imaging modality that is indispensable for assessing the condition of the liver in patients with CHC. In addition, pulse wave Doppler ultrasonography, which is based on the Doppler effect, has been widely employed in clinical practice. However, there are only two studies that have described the use of pulse wave Doppler ultrasonography for assessing the effectiveness of IFN treatment ; there are the study of [5], which reported the 12-weeks follow-up results after treatment with interferon-alpha in patients with CHC, and[4] which reported the 24-weeks follow-up results after treatment with interferon-alpha in 14 patients with CHC.

Aim of the work

to study some portal hemodynamic parameters - by employing pulse wave Doppler ultrasonography - before and after treatment in patients with CHC in order to investigate the impact of this therapy on the progression of liver disease, and the possibility of using these parameters to assess the effectiveness of and the response to IFN therapy.

Subjects and Methods

This study was carried out on fifty three patients with chronic HCV infection (45 males and 8 females). Patients were studied from Mansoura University Hospital (MUH) and Mansoura Specialized Medical Hospital (SMH). The study was performed from January 2009 to November 2011, and their ages ranged from 35 to 49 years. In addition, 20 apparently healthy controls (16 males and 4 females), of

matched age with patients were included.

All patients had serum antibodies to HCV; positive polymerase chain reaction (PCR) tests to HCV RNA and with biopsy proven HCV-related liver disease.

Excluded from the study Patients with : decompensated cirrhosis; autoimmune hepatitis; hepatitis B infection; HIV infection; previously treated patientsby 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, Prothrombin time), serum creatinine, Alpha feto protein (AFP), Thyroid-Stimulating Hormone (TSH), Hepatitis C virus antibodies, polymerase chain reaction (PCR), (Before and after treatment of the patients).

Pulse wave Doppler ultrasonography was performed to both the patients (before and after treatment) and the control subjects by one operator using Hitachi-EUB-7500 ultrasound machine (Hitachi - medical corporation Tokyo- Japan). Five Doppler parameters were measured: portal vein velocity (PVV), hepatic artery resistive index (HARI), hepatic artery pulsatility index (HAPI), splenic artery resistance index (SARI) and splenic artery Pulsatility index (SAPI).

On the basis of the response to treatment 24 weeks after completion of treatment; the patients were classified into one of the following two groups: Responders (17 patients), included patients with sustained virological response (SVR) and Non responders: (36 patients), included patients not achieving the SVR).

All of patients received treatment for chronic hepatitis C in the form of: pegylated interferon (peg- IFN)-α2a at a dose of 180 mg/wk plus weight-based ribavirin (RBV) at a dose of 1000 to 1200 mg/d.

The studied HCV patients were

classified into two groups according to the response to treatment:

1) Responders: Included 17 patients, 15 males and 2 females; their ages ranged from 34 to 48 years. They included patients with sustained virological response (SVR). (SVR were defined by undetectable HCV RNA 24 weeks after completion of treatment).

2) Non responders: Included 36 patients, 30 males and 6 females; their ages ranged from 35 to 50 years. They included patients who did not achieve SVR.

Results

Portal hemodynamic parameters in all patients and control groups.

before treatment.

• A highly significant difference was observed as regard the portal hemodynamic parameters (PVV: Portal vein velocity, HARI: Hepatic artery resistive index, HAPI: Hepatic artery pulsatility index SARI: Splenic artery resistive index SAPI: Splenic artery pulsatility index) between all patients versus control groups before treatment (p=0.000) (table 1).

After treatment

A- Responders

• The more improvement after treatment occurred in PVV but not statistically significant (p= 0.382).

• Improved other parameters but still high significant difference observed as regard the other portal hemodynamic parameters(HARI, HAPI, SARI and SAPI) in responders versus control subjects after treatment as shown in table (2).

B- Non responders

• A highly significant difference was observed as regard the portal hemodynamic parameters (PVV: Portal vein velocity, HARI: Hepatic artery resistive index, HAPI: Hepatic artery pulsatility index SARI: Splenic artery resistive index SAPI: Splenic artery pulsatility index) in non responders versus control subjects after treatment (p= <0.0001) as shown in table (3).

Portal hemodynamic parameters among responders versus non Responders groups:

Before treatment

Non significant difference was observed as regard the portal he-

modynamic parameters (PVV: Portal vein velocity, HARI: Hepatic artery resistive index, HAPI: Hepatic artery pulsatility index SARI: Splenic artery resistive index SAPI: Splenic artery pulsatility index) in responders versus non responders groups before treatment as shown in table (4).

After treatment :

Non significant difference was observed as regard the portal hemodynamic parameters (PVV: Portal vein velocity, HARI: Hepatic artery resistive index, HAPI : Hepatic artery pulsatility index SARI : Splenic artery resistive index SAPI : Splenic artery pulsatility index) in responders versus non responders groups after treatment as shown in table (5).

Table (1): Comparative analysis of the portal hemodynamic parameters between all patients and control groups ***before treatment***.

Group / *paramerers*		**Patients (n = 53)**	**Control (n = 20)**	**t**	**P value**
PVV (cm./s.)	mean±SD	13.61 ± 5.53	17.99 ±1.85	5.067	0.000
HARI	mean±SD	1.04 ± .25	0.66 ± .05	10.354	0.000
HAPI	mean±SD	1.83 ± .37	1.26 ± .10	10.218	0.000
SARI	mean±SD	0.98 ± .23	0.55 ± .06	12.428	0.000
SAPI	mean±SD	1.56 ± .39	0.90 ± .13	10.776	0.000

Table (2): Comparative analysis of the portal hemodynamic changes between responders and control groups ***after treatment.***

Group / *parameters*		Responders (SVR) (n = 17)	Control (n = 20)	t	P value
PVV (cm./s.)	mean±SD	17.15±3.76	17.99± 1.85	0886	0.382
HARI	mean±SD	0.83± .12	0.66 ± .05	5.284	0.000
HAPI	mean±SD	1.55 ± .40	1.26 ± .10	2.918	0.009
SARI	mean±SD	0.77±.17	0.55±.06	5.107	0.000
SAPI	mean±SD	1.21 ± .38	0.90±.13	3.266	0.004

Table (3): Comparative analysis of the portal hemodynamic parameters between non responders and control groups ***after treatment.***

Group / *parameters*		Non responders (no SVR) (n = 36)	Control (n = 20)	t	P value
PVV (cm./s.)	mean±SD	17.97±4.26	17.99±1.85	0.020	0.984
HARI	mean±SD	0.89±0.23	0.66±0.05	5.784	0.000
HAPI	mean±SD	1.64±0.39	1.26± 0.10	5.460	0.000
SARI	mean±SD	0.73±0.17	0.55±0.06	5.775	0.000
SAPI	mean±SD	1.15±0.33	0.90±0.13	4.056	0.000

Table (4) : Comparative analysis of portal hemodynamic parameters between responders and Non Responders groups ***before treatment***.

Group / *parameters*		Responders (SVR) (n = 17)	Non responders (no SVR) (n = 36)	t	P value
PVV (cm./s.)	mean±SD	14.31±2.78	14.81±3.03	0.580	0.565
HARI	mean±SD	1.06±0.33	10.3±0.21	0.381	0.705
HAPI	mean±SD	1.84±0.48	1.83±0.32	0.118	0.907
SARI	mean±SD	1.04±0.20	0.95±0.24	1.294	0.201
SAPI	mean±SD	1.62±0.35	11.53±0.41	0.774	0.442

Table (5): Comparative analysis of portal hemodynamic parameters between responders and Non Responders groups ***after treatment***.

Group / *parameters*		Responders (SVR) (n = 17)	Non Responders (no SVR) (n = 36)	t	P value
PVV (cm./s.)	mean±SD	17.15±3.76	17.97±4.26	0.680	0.500
HARI	mean±SD	0.83±0.12	0.89±0.23	1.003	0.321
HAPI	mean±SD	1.55±0.40	1.64±0.39	0.710	0.481
SARI	mean±SD	0.77±0.17	0.73±0.17	0.648	0.520
SAPI	mean±SD	1.21±0.38	1.15±0.33	0.588	0.559

Discusion

Chronic HCV infection affect and deteriorate the portal hemodynamics significantly, as there is a highly significant difference was observed as regard the portal hemodynamic parameters between all patients versus control subject. The previous results support the results of Rochaetal., 2012. Who compared Doppler parameters of portal vein in patients with CHC with a healthy control group and correlate these parameters with fibrosis degree obtained by liver biopsy, he concluded that the portal venous index was useful in differentiating healthy subjects from patients with fibrosis due to chronic HCV. (6)

After interferon therapy portal hemodynamic parameters have been improved - in both responders and non responders - but not reaching the levels of the control subjects.

The previous results support the results of Poynard et al., 2003, who reported that PEG-interferon and ribavirin combination significantly reduces the rate of fibrosis progression in patients with hepatitis C.(7)

Other studies also explain this by : In addition to having antiviral and immunomodulatory effects, interferon- based therapies also have antiproliferative effects. The exact mechanism by which IFNa reverses the progression of hepatic fibrosis is not known, but may well be mediated by direct or indirect effects on hepatic stellate cells.(8)&(9)

But these results are not in agreement with Walsh et al., 1998, who reported that, patients with chronic hepatitis C have elevated hepatic artery blood flow. Hepatic blood flow indices have no relationship to the severity of histological liver injury in chronic hepatitis C, and these flow indices are not affected by a 12 week course of interferon, but this difference may be related to the short course of the therapy; as our protocol was applied for 48 weeks, and the different genotype of HCV in our patients.(5)

When we compared portal hemodynamic parameters among responders versus non responders

groups we found a non significant difference as regard the portal hemodynamic parameters before and after treatment. Indicating that all portal hemodynamic parameters improved after treatment whatever the response to therapy.

The previous results support the results of [10], who stated that in patients with chronic hepatitis C who were treated with interferon-based therapies, histologic benefits may be observed even in the absence of a sustained virological response.

This result also in agreement with[11], who reported that the main outcomes of antiviral therapy for chronic hepatitis C detected are the viral clearance and its lasting time. Actually, IFN therapy seems also be able to determine a decrease in the rate of functional disease progression in same patients, even in non-responders and relapsers. Also in agreement with[12], who stated that IFN-beta-1a therapy improves the liver histology of chronic HVC regardless of the viral response.

This is not in agreement with [4], who evaluate the changes in PVV in patients with CHC receiving IFN treatment and concluded that patients judged to have SVR showed a significantly higher PVV at the end of IFN treatment than before IFN treatment. In addition, no response to IFN was suggested when the portal blood flow velocity at the end of IFN treatment was not significantly higher than the pre-IFN value, and recommended that in the assessment of the effects of IFN treatment in patients with chronic hepatitis C, pulse wave Doppler ultrasonography permits the portal blood flow velocity to be easily measured and is therefore considered to be a clinically useful method for evaluating treatment effectiveness. And this differance may be explained by using only a single parameter (PVV), a small number of patients (14 patients), a short duration of therapy (24 weeks) and the difference in the genotypes of HCV.

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BENHA MEDICAL JOURNAL

PORTAL HEMODYNAMIC CHANGES IN PATIENTS WITH CHRONIC HEPATITIS C TREATED WITH COMBINED PEGYLATED INTERFERON ALFA AND RIBAVIRIN

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HISTOLOGICAL STUDY OF 5-FLUOROURACIL INDUCED SMALL INTESTINAL MUCOSITIS IN RATS AND THE POSSIBLE PROTECTIVE EFFECT OF OMEGA-3 FATTY ACIDS

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Abstract

Background: *Mucositis is a common debilitating side effect of chemotherapy, associated with severe small intestinal inflammation. Omega-3 fatty acids have reported as a therapeutic potential for treatment of many inflammatory conditions.*

Aim of the Work: *This study was performed to assess the light and electron microscopic aspects of 5-fluorouracil induced cytotoxicity on the jejunum and the possible protective effect of omega-3 fatty acids administration.*

Materials and Methods: *Twenty four adult male Wister albino rats were used. The rats were randomly assigned into 3 groups: control group, FU & Omega groups. FU group received a single intraperitoneal dose of 5-fluorouracil (5-FU) (150 mg/kg) at day 5 of the 8 day experiment, and was subdivided into FU1 and FU3 groups (rats sacrificed 1 and 3 days after 5-FU injection, respectively). Omega group received Omega-3 treatment (1ml / animal) daily by orogastric tube from day 1 till the day of sacrifice. At day 5, they received 5-Fluorouracil as FU group. They were subdivided into Omega1 and Omega 3 groups (rats sacrificed 1 and 3 days after 5-FU injection, respectively). Jejunal specimens were collected and processed for light and transmission electron microscopic examination. Histomorphometric and statistical analysis for villus height, crypt depth, apoptotic and mitotic indices were performed for each group.*

Results: *Signs of mucosal damage were observed in FU1 and FU3*

groups including detachment of epithelium from lamina propria; shortening, blunting and broadening of villi; cellular infiltration in lamina propria, reduction in number and depth of crypts, loss of surface epithelium and denuded villi. Statistically, there was significant decrease in the mean villus height and crypt depth for FU1and FU3 groups compared to control rats. A significant increase in the apoptotic index was observed in FU1 group compared to the control, accompanied by a significant decrease in the mitotic index. Omega-3 treated rats exhibited less damage compared to FU treated rats. The intestinal structure was more or less preserved. The mean villus height and crypt depth were significantly higher in Omega groups compared to FU groups. A significant decrease in the apoptotic index and a significant increase in the mitotic index were observed in Omega groups compared to FU groups. Electron microscopic examination of FU3 group revealed marked widening of the intercellular spaces and morphological alterations in intestinal epithelial cells. On the other hand, the Omega3 group showed preserved ultrastructure.

Conclusion: *Omega-3 fatty acids can protect the small intestine from the cytotoxicity associated with 5-FU chemotherapy and fasten the recovery. Therefore, it is recommended for cancer patients receiving chemotherapy.*

Introduction

Chemotherapy is an important primary and adjuvant treatment for cancer patients. 5- Fluorouracil (5-FU) is an anti-metabolite drug that is widely used as a chemotherapeutic agent in a wide range of cancers. It acts by blocking DNA synthesis via inhibition of thymi¬dylate synthase enzyme[1&2]. However, its indiscriminate mechanism of action targets not only cancer cells, but all rapidly dividing cells within the body. The high proliferative activity of the gastrointestinal tract renders it highly susceptible to the deleterious side-effects of chemotherapy, resulting in an inflammatory condition referred to as mucositis [3]. Mucositis occurs in approximately 40% of patients receiving chemotherapy. It may affect any part of the GIT, particularly the mouth

and the small intestine. The symptoms of mucositis are debilitating, and include severe inflammation and ulceration of the GIT, resulting in stomatitis, abdominal pain, nausea, vomiting, diarrhea and weight loss [4].

The intestinal epithelium is maintained by continuous proliferation of the stem cells located at the crypts [5]. Although the pathogenesis of mucositis is not completely understood, it has been reported that the primary mechanism of mucositis is reduced crypt cell proliferation and increased apoptosis that leads to loss of intestinal structure and function primarily via villi shortening and crypt ablation[6]. Furthermore, chemotherapy initiates the release of reactive oxygen species (ROS) and pro-inflammatory cytokines, such as tumour necrosis factor (TNF) α and interleukins (IL) 1 and 6 which play an indirect role in the amplification of intestinal damage [7].

Currently, there is a deficiency of effective prophylactic or curative treatments for intestinal mucositis [8]. A number of new strategies have been tested[9,10]. However, so far no effective treatment has been found and there is a clear need to develop new strategies.

Omega-3 fatty acids are polyunsaturated fatty acids. They are the main components of cell membranes. The major omega-3 Fatty acid, the alpha-linolenic acid (ALA) is metabolized in the body to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA can be found in larger quantities in fish oils and oily fish. Omega-3 fatty acids are considered as essential fatty acids as they cannot be manufactured by the body. For this reason, they must be obtained from diet or taken as supplements [11&12].

Omega-3 fatty acids are considered as strong antioxidants[13]. Fish oil omega-3 fatty acids have also been reported to have beneficial effects in the treatment or prevention of the risk factors associated with many acute and chronic inflammatory conditions such as inflammatory bowel diseases[14]. Omega-3 fatty acids can enhance the effectiveness of

chemotherapy toward tumor cells; while at the same time reduce toxicity to normal cells [15].

Few studies were carried out to study the protective effect of Omega-3 fatty acids in a rat model of mucositis caused by 5-fluorouracil administration. Therefore, the current study has been performed to assess the light and electron microscopic aspects of 5-fluorouracil induced intestinal cytotoxicity and the possible protective effect of omega-3 fatty acids administration.

Materials & Methods

Materials

1-Animals:

The present study was conducted on 24 adult male Wister albino rats weighing between 200 and 250 grams. The rats were housed in separate cages under constant environmental conditions and were allowed free access to food and water.

2- Drugs:

5-Fluorouracil: A product of Biosyn, Arzneimittel, Gmbh, Germany with a trade name; 5-fluorouracil biosyn. It is available in the form of ampoules, with a concentration of 1000 mg / 20 ml of 5-fluorouracil. Omega-3 : A product of Puritan's pride, INC, USA. It is available in the form of gelatinous capsules with a trade name; (natural omega-3), each capsule contains 1000 mg fish oil providing 300 mg of omega-3 fatty acids (DHA & EPA).

Experimental design:

The duration of the experiment was 8 days. The rats were randomly assigned into three groups and treated as follows:

I- Control group: Consisted of 8 rats. They were daily given water by orogastric tube from the first day of the experiment till the time of sacrifice. At day 5 of the experiment, they received saline (a single intraperitoneal injection), equivalent to the 5-fluorouracil dose.

II- FU group: Consisted of 8 rats. They were given water by orogastric tube from the first day of the experiment till the time of sacrifice. In addition; they received a single intraperitoneal dose of 5-fluorouracil (5-FU) (150 mg/kg) at day 5 [16]. This group was further

subdivided according to the time of sacrifice into 2 subgroups (4 rats each):

FU1 group: rats sacrificed one day after 5-FU injection.

FU3 group: rats sacrificed three days after 5-FU injection.

III- Omega group: Consisted of 8 rats. They were given Omega-3 treatment (1ml / animal) daily by orogastric tube from day 1 till the day of sacrifice. At day 5 of the experiment; they were injected intraperitoneally with 5-Fluorouracil as FU group (17). This group was further subdivided according to the time of sacrifice into 2 subgroups; (4 rats each):

Omega1 group: rats received Omega 3 for 5 days and sacrificed 1 day after 5-FU.

Omega 3 group: rats received Omega 3 for 8 days and sacrificed 3 days after 5-FU.

Methods:

At each duration, 4 animals from each group were sacrificed. The animals were anaesthetized intraperitoneally with thiopental sodium (10-15mg/kg), the abdomen was opened surgically by a midline incision and sections from the jejunum were obtained and flushed with isotonic saline.

I. HISTOLOGICAL STUDY

For Light Microscopy:

The specimens were fixed in 10% buffered neutral formalin for two days, dehydrated, cleared and embedded in paraffin wax. Paraffin sections (5um thick) were prepared and stained with haematoxylin and eosin (H & E) [18].

For transmission electron microscopy:

Small fragments (about $1mm^3$) of the jejunum from the following groups; control, FU3 and Omega3 were obtained, fixed in 2.5% buffered gluteraldehyde and processed to obtain ultrathin sections[18]. After the usual staining, sections were examined and photographed by JEOL-JEM-100 SX transmission electron microscope in electron microscopy unit, Faculty of Medicine, Tanta University.

II. Histomorphometric and Statistical studies:

1. Measurements of villus height (from villus base to tip) and longitudinal crypt depth (invagina-

tion depth between adjacent villi) were obtained from H &E stained sections. The slides were photographed using Olympus® digital camera installed on Olympus® microscope with 0.5 X photo adaptor, using 10 X objective lens and saved in tagged image format file (TIFF). The resulting images were analyzed on Intel® Core I3® based computer using videotest Morphology® software (Russia) with a specific built-in routine for calibrated distance measurement. 10 well oriented villi and 10 crypts were randomly measured from each animal of all groups.

2. Apoptotic index: Apoptotic bodies were counted in H &E stained sections in 20 randomly chosen crypts for each sample. This was done under light microscopy at high magnification (X1000).

3. Mitotic index: Well-defined mitotic figures were counted at crypt bases in H & E stained sections in 20 randomly chosen longitudinal crypts for each sample. This was done under light microscopy at X 400 magnification.

Statistical analysis of the data was done by using Statistical Package for Social Science (SPSS) version 15.0 The data were parametric by using Kolmogrov-Smirnov test. The data were expressed as Mean ± SD. Comparisons were carried out by analysis of variance (ANOVA) with the least significance (LSD) Post hoc analysis for inter group comparison. Significance was considered when P value < 0.05. All graphic representations of the data were performed with Microsoft® Excel® for windows®. (Microsoft Inc., USA).

Results

I- Light Microscopic Results:

1- Control Group: Examination of H&E stained sections of the jejunum of control rats showed that the mucosa was formed of villi, crypts, lamina propria and muscularis mucosa. The intestinal villi had connective tissue cores and were covered by columnar absorbing cells and goblet cells. The columnar absorbing cells had brush borders on their free surfaces and basal oval nuclei (Fig. 1). The lamina propria extended to form

the villi cores and surrounded the crypts. The crypts were lined with columnar absorbing cells and goblet cells. Mitotic figures were observed at the crypt cells. The muscularis mucosa separated the intestinal crypts from the submucosa (Figs. 1, 2).

2- FU Group : Histological examination of jejunal sections of FU1 group revealed that the villi were short; some were broad and blunt (Fig. 3) The surface epithelium of the villi was markedly detached from the lamina propria that contained cellular infiltration (Fig. 4). The columnar absorbing cells were apparently reduced in height (Fig. 4). There was an apparent reduction in the number and depth of the crypts (Figs. 3). Many Apopotic bodies were noticed at the lower part of crypts (Fig. 5). The jejunum of FU3 group showed short denuded villi (Fig 6 A). The columnar absorbing cells covering the villi were reduced in height, enlarged with pale vacuolated cytoplasm and pale nuclei or extensively sloughed into the lumen. Excessive cellular infiltration appeared in villi cores (Fig. 6 B). There were areas of complete loss of villi (Fig. 7). The crypts were few, short, distorted and were lined with enlarged cells with pale vacuolated cytoplasm and pale nuclei (Figs. 7, 8).

3- Omega Group: Compared to the FU1 group, the structure of the mucosa in Omega1 group was preserved. Intact villi and crypts were observed (Fig. 9). In Omega 3 group, the villi were covered with intact surface epithelium. However, some columnar absorbing cells were reduced in height. Slightly detached lamina propria and cellular infiltration were seen in the cores of some villi (Fig. 10 A). The lining cells of the crypts were intact; some cells were enlarged and pale (Fig. 10 B).

I- Electron Microscopic Results:

1- Control Group: The columnar absorbing cells possessed microvilli on their luminal surface and basal oval vesicular nuclei (Fig. 11). The cytoplasm was rich in mitochondria, rER cisternae, free ribosomes and few lysosomes. The neighboring cells were firmly attached near the luminal surfaces by junctional complexes

consisted of zonula occludens, zonula adherens and desmosomes. A prominent terminal web under the microvilli and lateral interdigitations between the cells were also observed (Fig. 12). The goblet cells had an apical part distended with mucous globules and a thin basal part containing the nucleus. Some of the mucous globules demonstrated electron dense cores. Intraepithelial lymphocytes were seen (Fig. 11).

Enteroendocrine cells were seen among the intestinal epithelium. The cell had a highly vesicular nucleus with a prominent nucleolus. The cytoplasm contained mitochondria, rER and small, round basal granules which were either electron dense or had electron lucent cores (Fig. 13). Paneth cell was seen at the base of the crypts. The cell had a vesicular nucleus with prominent nucleoli. The cytoplasm contained large spherical electron dense granules with clear halos indenting the nucleus. rER cisternae, mitochondria and lysosomes were also seen (Fig. 14).

2- FU Group: The FU3 group showed disturbed cell architecture with marked widening of the intercellular spaces; however the cells were still bound to each other by some cytoplasmic extensions (Fig. 15). The columnar absorbing cells showed complete loss of microvilli and cytoplasmic vacuolations (Fig. 16), extensive interdigitations, dilated vesiculated rER; which may be partially degranulated (Fig. 17), many lysosomes and swollen mitochondria with few short disintegrated cristae (Fig. 18). Pyknotic nuclei and widening of the perinuclear space were noticed in some cells (Fig. 16, 21). The goblet cells appeared depleted of mucous; only few mucous granules were seen in the cytoplasm. Dilated rER, vacuoles and many lysosomes were also observed (Fig. 19). Cytoplasmic vacuolations and autophagic vacuoles were seen in the enteroendocrine cells (Fig. 20). Paneth cells contained dilated and vesiculated rER, large lysosomes and swollen degenerated mitochondria (Fig. 21).

3- Omega Group: Compared to FU3 group, the Omega 3 group showed more or less intact struc-

ture (Fig. 22). Minimal widening of the intercellular space was seen (Figs. 24, 25). The columnar absorbing cells showed preserved microvilli, lateral interdigitations, mitochondria, rER cisternae and some lysosomes (Figs. 22-24). The goblet cells were distended with mucous globules and contained intact rER (Fig. 24). The enteroendocrine cells appeared normal except for few cytoplasmic vacuolations (Fig. 22). Paneth cells showed intact rER and mitochondria. Only few mitochondria exhibited partial cristolysis (Fig. 25).

I. Histomorphometric and Statistical Results:

1- Mean Villus Height: The mean villus height of the jejunum in FU1 and FU3 groups was significantly decreased compared to control groups. The maximum reduction was recorded in FU3 group. On the other hand, the mean villus height was significantly increased in Omega1 and Omega 3, compared to FU groups (Fig. 26).

2- Mean Crypt Depth: The mean crypt depth of the jejunum in both FU1 and FU3 groups was significantly decreased compared to the control groups. While the mean crypt depth in Omega 1 and Omega 3 groups was significantly increased compared to FU groups (Fig. 27).

3- Apoptotic Index: The apoptotic index in the jejunum of FU1 group was significantly increased compared to the control groups. However, this change was non-significant in FU3 group compared to the control. In Omega 1 group, the apoptotic index was significantly decreased compared to FU1 group, while in Omega 3 group the decrease was non-significant compared to FU3 group (Fig. 28).

4- Mitotic Index: There was a significant decrease in the mitotic index in the jejunum of both FU1 and FU3 groups compared to control groups, with maximum reduction in FU1 group. On the other hand, a significant increase in the mitotic index was recorded in both Omega1and Omega3 groups compared to FU groups (Fig. 29).

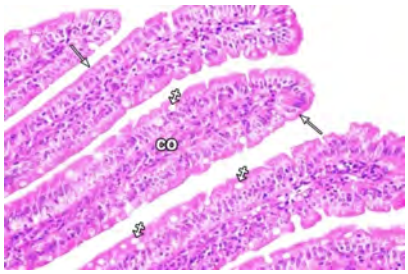


Fig. 1 : A photomicrograph of control rat jejunum. The villi have connective tissue cores (CO) and are covered with columnar absorbing cells with brush borders (arrows) and goblet cells (crossed arrows). (H&E X400)

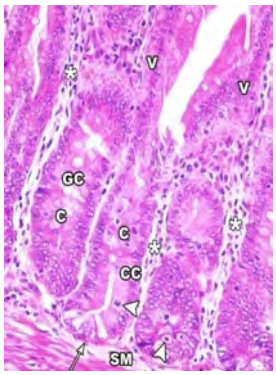
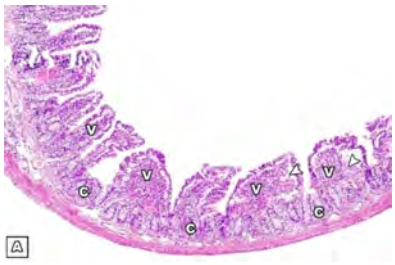


Fig. 2 : The mucosa consists of villi (V), crypts (C), lamina propria (asterisk) and muscularis mucosa (arrow). The lamina propria contains mononuclear cells, extends into the villi cores and surrounds the crypts. The crypts are lined with columnar absorbing cells (CC) and goblet cells (GC). Note the mitotic figures (arrow heads) at the crypt cells. The muscularis mucosa separates the crypts from the submucosa (SM). (H&E X400)



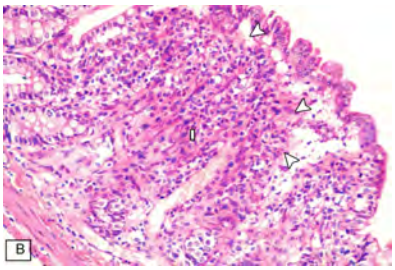


Fig. 3 : Photomicrographs of the jejunum of FU1 group). A: The villi (V) are short and some are broad. The epithelium is detached from the lamina propria (arrow heads) and the crypts (C) are apparently reduced in number and depth. B: Shows a short broad and blunt villus with heavy inflammatory cell infiltrate (I) in its core. The lamina propria appears detached in some areas (arrow heads). (H & E: A, X100; B, X400)

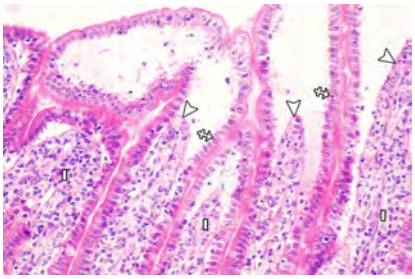


Fig. 4 : A photomicrograph of the jejunum of FU1 group. The surface epithelium is extensively detached from the lamina propria (arrow heads). There is an apparent decrease in the height of columnar absorbing cells (crossed arrows). Cellular infiltrate (I) is seen in the core of the villi. (H&E X400)

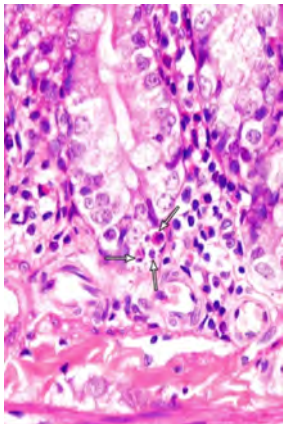


Fig. 5 : A photomicrograph of the jejunum of FU1 group showing apoptotic bodies (arrows) at the lower part of the crypt. (H&E X1000)

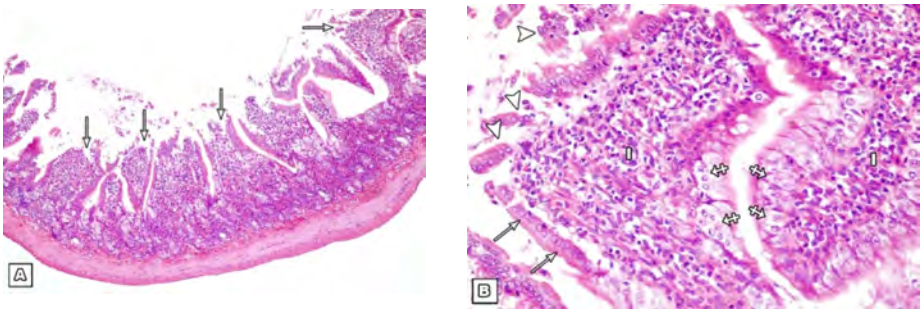


Fig. 6 : Photomicrographs of the jejunum of FU3 group. A: Shows villi shortening and extensive sloughing of the surface epithelium into the lumen (arrows) leaving denuded villi. B: Higher magnification of Fig. (A).The columnar absorbing cells are either reduced in height (arrows) or enlarged with pale vacuolated cytoplasm and pale nuclei (crossed arrows) or sloughed into the lumen (arrow heads). Note the excessive cellular infiltration (I) in the villi cores. (H&E: A, X100; B, X400)

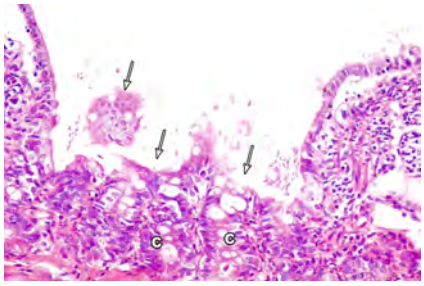


Fig. 7 : A photomicrograph of the jejunum of FU3 group showing an area with complete loss of the villi and sloughing of the epithelium (arrows). The crypts (C) are short and distorted. (H&E X400)

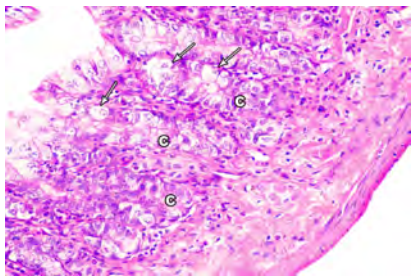


Fig. 8 : A photomicrograph of the jejunum of FU3 group. The crypts (C) are few and ill-defined. The crypt cells (arrows) are enlarged with pale vacuolated cytoplasm and pale stained nuclei.
(H&E X400)

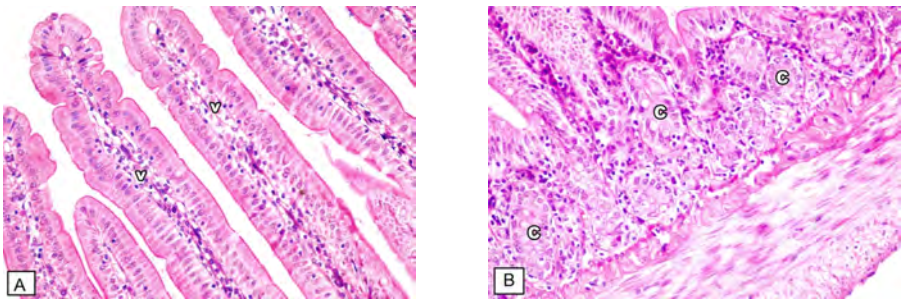


Fig. 9 : Photomicrographs of the jejunum of Omega1 group. A-B: The overall histological structure is preserved and shows nearly normal appearance. Intact shape and structure of the villi (V) and crypts (C)
(H&E: A&B, X400)

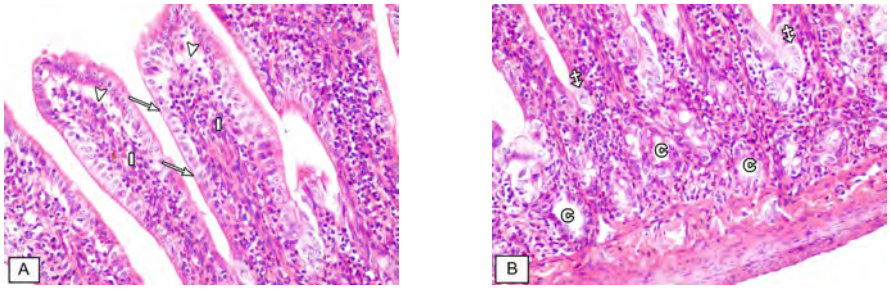


Fig. 10 : Photomicrographs of the jejunum of Omega 3 group. A: The villi show intact surface epithelium, slight reduction in height in some cells (arrows) and slightly detached lamina propria (arrowheads). The core of the villi shows cellular infiltration (I). B: The cells lining the crypts (C) are intact, few cells are large and pale (crossed arrows).
(H&E: B&C, X400)

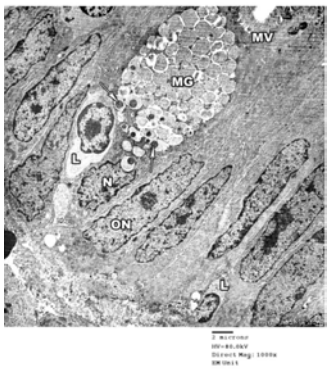


Fig. 11 : An electron micrograph of the control rat jejunal mucosa. The columnar absorbing cells have apical microvilli and basal oval nuclei (ON). The goblet cell has an apical part distended with mucous globules (MG) and a thin basal part containing the nucleus (N). Some mucous globules show electron dense cores (arrows). Intraepithelial lymphocytes (L) are seen
(TEM X1000)

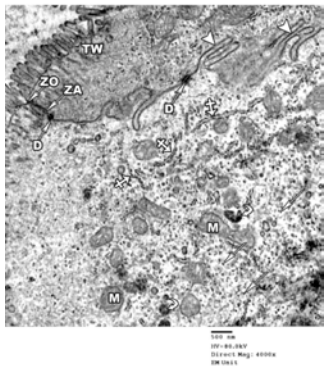


Fig. 12 : An electron micrograph of the control rat jejunum. The neighboring absorbing cells are firmly attached near the luminal surface by junctional complexes consisting of zonula occludens (ZO), zonula adherens (ZA) and desmosomes (D). Lateral interdigitations (arrow heads) are also seen between the cells. rER Cisternae (crossed arrows), mitochondria (M), few lysosomes (curved arrows) and free ribosomes (arrows) are present in the cytoplasm. Note the terminal web (TW) under the microvilli (TEM X 4000)

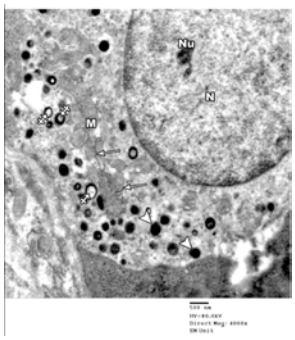


Fig. 13 : An electron micrograph of an enteroendocrine cell of control rat jejunum. The cell has a highly vesicular nucleus (N) with a prominent nucleolus (Nu). The cytoplasm contains mitochondria (M), rER (arrows) and small, round basal granules which are either electron dense (arrow heads) or have electron lucent cores (crossed arrows).

(TEM X4000)

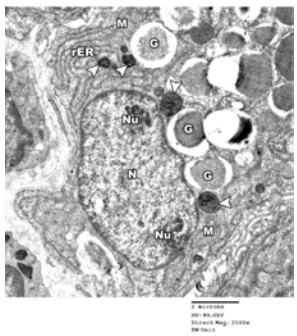


Fig. 14 : An electron micrograph showing a Paneth cell with vesicular nucleus (N) and prominent nucleoli (Nu). The cytoplasm contains Large spherical electron dense granules (G) with clear halos indentjng the nucleus. rER cisternae, mitochondria (M) and lysosomes (arrow heads) are also seen.

(TEMX 2500)

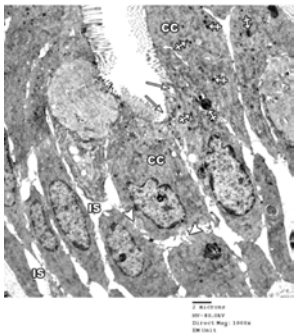


Fig. 15 : An electron micrograph of FU3 jejunum showing disturbed cell architecture. The intercellular spaces (IS) are markedly widened but the cells are still bound to each other by cytoplasmic extensions (arrow heads). Areas of partial or complete loss of microvilli (arrows) and many lysosomes (crossed arrows) are observed in the columnar absorbing cells (CC). (TEM X1000)

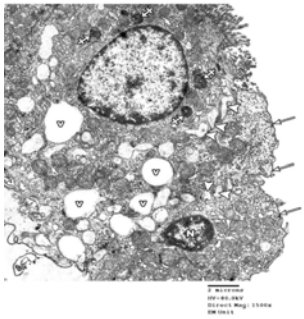


Fig. 16 : An electron micrograph of FU3 jejunum. Two absorbing cells completely devoid of microvilli are seen (Arrows). Cytoplasmic vacuolations (V), increased lysosomes (crossed arrows), pyknotic nucleus (N) and widened intercellular spaces (arrow heads) are observed. (TEM X1500).

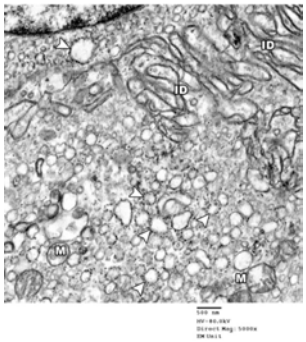


Fig. 17 : An electron micrograph of FU3 jejunum. The absorbing cells show extensive lateral interdigitations (ID), dilated vesiculated and partially degranulated rER (arrow heads) and mitochondria (M) with few and disintegrated cristae. (TEM X5000).

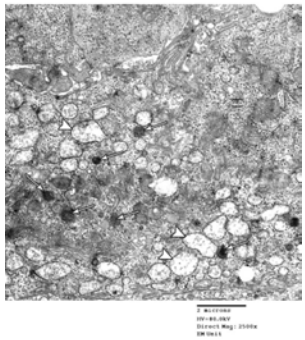


Fig. 18 : An electron micrograph of FU3 jejunum. Many lysosomes (arrows) and swollen mitochondria are observed in the columnar absorbing cell cytoplasm. (arrow heads). The swollen mitochondria have few, short and disintegrating cristae. (TEM X 2500).

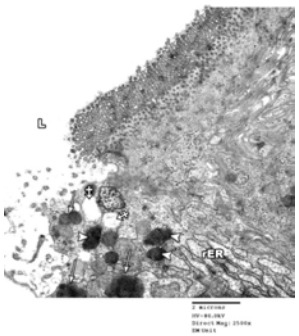


Fig. 19 : An electron micrograph of FU3 jejunum. A goblet cell is seen opening into the lumen (L). It appears depleted of mucous; only few electron dense mucous globules are seen (arrows). The cytoplasm contains dilated rER cisternae, vacuoles (crossed arrows) and many lysosomes (arrow heads). (TEM X2500).

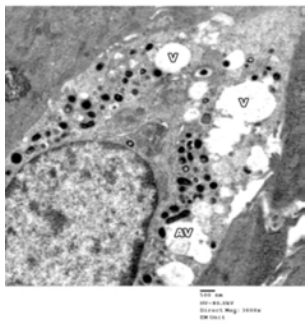


Fig. 20 : An electron micrograph of an enteroendocrine cell of FU3 group. Cytoplasmic vacuolations (V) and autophagic vacuoles (AV) containing degenerated mitochondria and other damaged organelles are seen. TEM X3000)

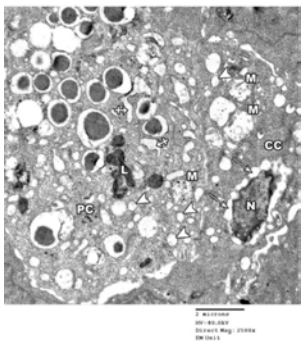


Fig. 21 : An electron micrograph of FU3 jejunum showing a Paneth cell (PC) and an adjacent columnar cell (CC). The Paneth cell shows dilated (crossed arrows) and vesiculated rER (arrow heads), large lysosomes (L) and swollen degenerated mitochondria (M). The columnar cell has a pyknotic nucleus (N) with a very wide perinuclear space (arrows), dilated vesiculated rER (arrow heads) and swollen mitochondria (M) with disintegrated cristae. (TEM X2500)

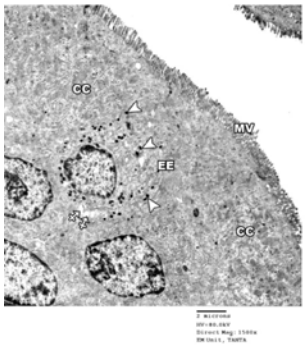


Fig. 22 : An electron micrograph of Omega3 jejunum showing more or less intact structure. The columnar absorbing cells (CC) contain intact organelles and intact microvilli (MV). An enteroendocrine cell (EE) shows small dense granules (arrow heads) and few vacuolation in the cytoplasm (crossed arrows).
(TEM X1500)

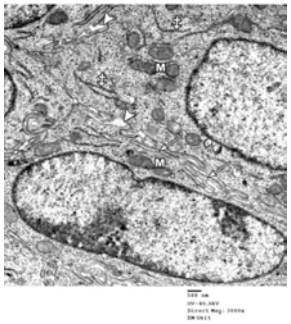


Fig. 23 : An electron micrograph showing the columnar absorbing cells of omega3 jejunum, normal Lateral interdigitations (arrows) and slight widening of intercellular spaces (arrow heads). Preserved intact mitochondria (M) and rER cisternae (crossed arrows) are seen. (TEM X3000)

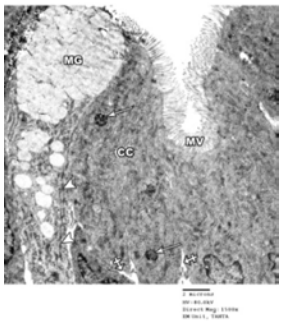


Fig. 24 : An electron micrograph of omega3 jejunum. The columnar absorbing cells (CC) show preserved microvilli (MV) and some lysosomes (arrows).The goblet cell is distended with mucous globules (MG) and contains intact rER cisternae (arrow heads). Note the minimal widening of the intercellular space (crossed arrows).
(TEM X1500)

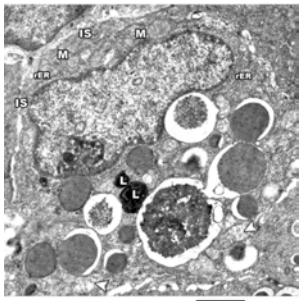


Fig. 25 : An electron micrograph of a jejunal Paneth cell of omega3 group. The cytoplasm contains intact mitochondria (M); some show partial cristolysis (arrow heads). Intact rER cisternae and lysosomes (L) are also seen. There is slight widening of intercellular space (IS).
(TEM X2500)

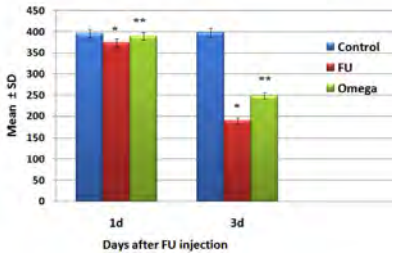


Fig. 26 : The mean villus height (in micrometer) of rat jejunum in different groups. (*) significant difference between FU and control groups. (**) significant difference between Omega and FU groups.

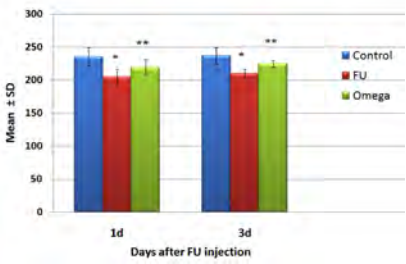


Fig. 27 : The mean crypt depth (in micrometer) of rat jejunum in different groups. (*) significant difference between FU and control groups. (**) significant difference between Omega and FU groups

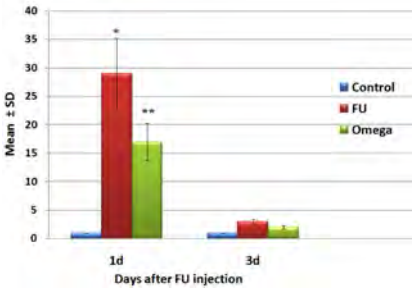


Fig. 28 : The apoptotic index of rat jejunum in different groups. (*) significant difference between FU and control groups. (**) significant difference between Omega and FU groups.

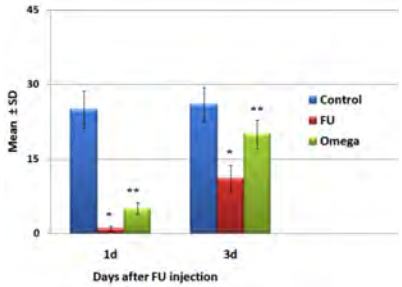


Fig. 29 : The mitotic index of rat jejunum in different groups. (*) significant difference between FU and control groups. (**) significant difference between Omega and FU groups.

Discussion

In the present work, the histological structure of the small intestine of the control rats examined with the light and electron microscopes revealed similar structures as those mentioned in literature [19].

The present study revealed marked histological changes in the rat jejunum following fluorouracil administration. In FU1 group, light microscopic examination showed marked detachment of the surface epithelium from the lamina propria. A similar finding has been reported in the rat small intestine after direct irradiation and was suggested to be due to the presence of oedema between the villus columnar absorbing cells and the stroma [20]. Shortening, blunting and broadening of the villi and apparent reduction in the number and depth of the crypts were also observed in FU1 and FU3 groups. Moreover, Loss of surface epithelium, marked exfoliation and denudation of the villi, and areas with complete villus loss were markedly observed in FU3 group. Statistically, a significant decrease in the mean villus height was recorded in both FU1 and FU3 groups compared to control rats. However, the maximum reduction was found in FU3 group. A significant decrease in the mean crypt depth was also recorded in both groups compared to the controls. These results are in accordance with those reported in previous studies[21,22], 5-FU has been found to cause villus shortening and blunting and to reduce crypt length in rat jejunum[21]. Destruction, exfoliation of the villi tips and degeneration of surface epithelium has been also reported, 12 and 24 hours following methotrexate chemotherapy in rats [22].

Light microscopic examination in the current study also revealed morphological alterations of the columnar absorbing cells in FU3 group. Some cells were reduced in height, others appeared pale and vacuolated with pale nuclei. Such shortened villi covered with flattened and vacuolated cells were previously described in 5-FU induced mucositis[16]. Moreover, heavy cellular infiltration was markedly seen in the lamina propria in both FU groups. This heavy cellular infiltration may be due to the increase in the proin-

flammatory cytokines TNFα and IL-1b concentrations after 5-FU administration [23].

One day after 5-fluorouracil injection (FU1 group), Haematoxylin and Eosin stained sections of the jejunum showed numerous apoptotic bodies at the lower parts of the crypts. This was confirmed statistically by a significant increase in the apoptotic index in this group compared to the control. Meanwhile, a significant decrease in the mitotic index of crypt cells has been recorded in FU1 group compared to the control, indicating mitotic inhibition. A significant decrease in the mitotic index was also observed three days after FU administration (FU3 group); however, the maximum reduction was at the first day. Intense apoptosis has been previously reported on the first day following 5-FU administration and was linked to mitosis reduction in the intestinal crypts of the rats [16].

The crypts of Lieberkuhn are the proliferative zones of the intestine. The actual stem cells are located in cell positions 4-6 up from the base of the crypts i.e. above Paneth cells[24]. The presence of apoptotic bodies at the lower part of the crypts, observed in the present study, most probably corresponds to the location of the stem cells. 5-FU has been found to act primarily at the level of the stem cells in the small intestinal crypts and the apoptosis was most prominent in the same zone [25].

Several mechanisms may be involved in 5-FU induced apoptosis. In the initial stages of mucositis; inhibition of DNA synthesis, DNA damage and production of reactive oxygen species by chemotherapy impair the metabolism within the rapidly dividing progenitor cells and cause inhibition of mitosis and increased apoptosis[8,26]. This might explain the increased apoptosis and mitotic inhibition observed in the current study. It was demonstrated that the early apoptotic response of 5-FU may be p53 mediated as it induces maximal p53 expression in the intestinal crypts after 24 h of treatment and declines thereafter[27]. Moreover, the role of nitric oxide (NO) in the pathogenesis of 5-FU induced mucositis and apoptosis has been

postulated[28]. NO can induce apoptosis in various cell types in association with p53 and expression changes in Bcl-2 family members[29].

The increased apoptotic index and reduced mitotic index with the consequent loss of cells from the villi and crypts may explain the observed signs of mucosal damage. The present results are in agreement with those of previous researchers who found that following 5-FU, the stem cells in the crypts were prevented from subsequent replication, due to loss by apoptosis as well as cytostasis resulting from mitotic inhibition. The end results were lowered villi and crypts cellularity which was accompanied with loss of villus and crypt area, disappearance of the crypts over the first 2-3 days post treatment and shortening of the villi and crypts[27]. It has been mentioned that, following chemothera¬py, the stem cells are damaged and no longer divide or differentiate into specific cell lineages. Cell renewal is affected by this process, and the villus mucosa is not replaced, leading to a rapid loss of structure and function [8].

Electron microscopic examination of FU3 group demonstrated disturbance of intestinal cell architecture with marked widening of intercellular spaces. Similar findings were previously described in the small intestine of patients receiving methotrexate chemotherapy[30] and also in rats exposed to irradiation[31]. It was explained that ionizing irradiation causes breakdown of tight junctions and loosens the contact of intestinal epithelial cells with each other [32].

Marked ultrastructural changes were also observed in FU3 group. The columnar absorbing cells showed areas of partial or even complete loss of microvilli, cytoplasmic vacuolations, increased lysosomes, dilated vesiculated and degranulated rER; and swollen mitochondria with few short disintegrated cristae. The nuclei were pyknotic in some cells and may show widened perinuclear spaces. The goblet cells appeared depleted of mucous with dilated rER, vacuoles and many lysosomes. The enteroendocrine

cells showed cytoplasmic vacuolations and autophagic vacuoles. Paneth cells contained dilated, vesiculated rER and swollen degenerated mitochondria. Similar ultrastructural changes have been described in the absorbing cells of patient's jejunal biopsy specimens 24-72 hours following methotrexate [30]. It was suggested that the resulting cystic spaces from the dilated organelles gave rise to the degenerative vacuolar appearance. This may explain the vacuolated appearance of the cells observed in the present study. Two mechanisms may be involved in such changes: an early direct toxic effect on the mature enterocyte, coupled with interference with crypt cell generation, possibly causing aging of cells [30].

The increase in reactive oxygen species which is known to be an early response following chemotherapy may explain the mitochondrial damage in the epithelial cells. Supporting this concept, it has been found that, following irradiation, the concentration of reactive oxygen species greatly increases and the products of lipid peroxidation accumulate, resulting in alterations in the structure and function of mitochondria in irradiated cells [33].

The jejunum of omega-3 treated rats exhibited less mucosal damage compared to the FU treated animals. The structure was more or less preserved with relatively intact appearance. However, minimal changes were observed in Omega 3 group with light microscopy. These changes included minimally detached lamina propria, little cellular infiltration and the presence of some enlarged pale cells in the crypts. Similarly, it has been found that lyprinol (a marine oil rich in omega-3 fatty acids) provided some protective effect on 5-FU induced mucositis by lowering the damage severity scores [17]. Moreover, the effect of fish oil in rats with experimentally induced colitis was assessed [34]. An improvement of the inflammatory changes in the colonic architecture and reduction in tissue damage scores were reported in these rats.

The present work revealed a significant decrease of apoptotic index in Omega1 compared to FU1

group, suggesting a protective effect of omega-3 fatty acids against apoptosis. This result comes in agreement with a previous study that reported a significant decrease in the number of apoptotic figures in the duodenal crypts following consumption of AAFA (an omega-3 product containing total of 55% EPA + DHA) prior to and during irinotecan chemotherapy in mice. It was suggested that omega-3 fatty acids have a role in protection of the intestine from genomic damage resulting in programmed cell death [35].

Comparing the other morphometric parameters of the Omega and FU groups revealed a significant increase in the mean villus height, crypt depth and mitotic index in Omega1 and Omega 3 groups, compared to the corresponding FU groups, suggesting a protective effect of omega-3 against the 5-FU induced damage. Moreover, the higher mitotic index and crypt depth in Omega-3 group indicate a sooner and faster recovery. Similarly, it was found that docosahexaenoic acid (DHA) has a protective effect and prevented the negative action of 5-FU on the ileal mucosal morphometry (villus and crypt length) [36]. In addition, lyprinol has been found to increase intestinal crypt depth and cell proliferation in rats receiving lyprinol with 5-FU. The increased cell proliferation was thought to be due to the high omega-3 fatty content of lyprinol[17]. The stimulatory effect of omega-3 on intestinal epithelial cell proliferation may explain the increased intestinal mitotic activity and improved intestinal histology observed in omega-3 treated rats in our study.

Ultrastructural examination of Omega 3 group in the current study confirmed the protective effect of omega-3 fatty acids observed by light microscopy. Minimal widening of intercellular spaces was found. Both absorbing cells and goblet cells showed intact structure. The enteroendocrine cells and Paneth cells appeared intact except for few vacuolations and some mitochondria with partial cristolysis. A previous study has shown that administration of fish oil omega-3 completely treated the colonic structure in a rat model of induced colitis. In omega-treated

rats, as in the present study, the enterocytes had preserved apical brush border, the nuclei appeared normal, the mitochondria and intercellular junctional complexes were intact and the goblet cells were distended with mucinogen granules [37].

The protective action exerted by omega-3 fatty acids on the intestinal structure can be explained by several mechanisms which may act together to ameliorate the damage induced by 5-FU. One of these mechanisms, is their anti-inflammatory properties. Omega-3 fatty acids compete with arachidonic acid and inhibit the pro-inflammatory eicosanoids PGE2 & LTB4 production. The eicosanoids alter the absorptive and secretory functions of the intestine and act as chemotactic factors to leucocytes and neutrophils into the mucosa with subsequent tissue damage. These fatty acids also produce eicosanoids with anti-inflammatory actions and inhibit the production of inflammatory cytokines TNF, IL1& IL6 (34&38). Omega-3 fatty acids are also origin of other anti-inflammatory lipid mediators known as resolvins and protectins that have an impact on resolution, by antagonizing the pro-inflammatory mediators and actively promoting the return to health [39]. Another mechanism is the antioxidant activity of omega-3 fatty acids through scavenging free radicals and inhibiting lipid peroxidation[13]. In addition, incorporation of omega-3 fatty acids into membrane phospholipids promote morphological mucosal integrity and contribute to the regulation of transduction signals through the effect of some phospholipids on the activation of enzymes involved in intracellular signaling such as protein kinase C [40].

In conclusion, the present study demonstrates that omega-3 fatty acids can protect the small intestine from 5-FU cytotoxicity, ameliorates the associated injury and fastens recovery. It is highly recommended for cancer patients receiving chemotherapy to take omega-3 fatty acids as a potential pharmacologic therapy for prevention and treatment of chemotherapy-induced intestinal mucositis and associated damage.

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HISTOLOGICAL STUDY OF 5-FLUOROURACIL INDUCED SMALL INTESTINAL MUCOSITIS IN RATS AND THE POSSIBLE PROTECTIVE EFFECT OF OMEGA-3 FATTY ACIDS

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HISTOLOGICAL AND ULTRASTUCTURAL STUDY OF THE PROTECTIVE EFFECT OF ASCORBIC ACID AND CURCUMIN ON METHOTREXATE INDUCED SMALL INTESTINAL MUCOSAL INJURY

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Abstract

Background: *One of the main toxic side effects of methotrexate (MTX) treatment is intestinal mucositis. MTX induced inestinal mucositis may lead to its dose reduction or withdrawal.*

Aim of work: *This study was designed to evaluate the protective effect of ascorbic acid and/or curcumin administraion in methrotrexate induced acute intestinal injury by histological, histochemical, and ultrastructural methods.*

Material & Methods: *Fifty male albino rats have been used in his study. They were divided into five groups. Group I: control group. Group II: given methotrexate for 3 days. Group III: given methotrexate for 3 days and ascorbic acid for 2 weeks before methotrexate treatment. Group IV: given methotrexate for 3 days and curcumin for 2 weeks before methotrexate treatment. Group V: given methotrexate for 3 days & ascorbic and curcumin for 2 weeks before methotrexate treatment. Sections from the jejunum of each rat was processed and used for light and electron microscopic examination to study the effects on the mucosa.*

Results: *The jejunal mucosa of methotrexate treated albino rats showed a severe form of mucositis. Broadening, fusion of the villi and oedema, inflammatory infiltration in both the corium and submucosa were clearly observed. The alkaline phosphatase reaction was weak or negative in the brush border of the columnar abrsorptive cells of the*

disrupted villi. Electron microscopic examination revealed severe form of cytopathy in these cells. This was in the form of loss of most of the microvilli which were abnormally short or long. Their cytosol showed abnormal shaped mitochondria and dilated sER. The histological architecture in the jejunum of group V was nearly unaffected (similar to control group) but group III and IV were partially affected.

Conclusion: *The structure of jejunal mucosa is affected by treatment with MTX. Pretreatment with both ascorbic acid and curcumin simultanously for 2 weeks before MTX could relieve MTX mucositis more effectively than when one of them is being used alone.*

Introduction

Fifty male albino rats ranging from 140 to 180 gm body weight have been used in this study. They were given laboratory food and water ad-libitum . They were divided into five groups (ten animals each).

I. Experimental animals

Group I: It represents the control group where the rats were given water by intra-gastric intubation.

Group II: Its rats were given methotrexate (2.5 mg/kg /d)[1] orally and for 3 days.

Group III: Its rats were given methotrexate (as in Group II) and ascorbic acid (150 mg/kg/d)[7], by intra-gastric intubation, daily for 2 weeks before methotrexate treatment.

Group IV: Its rats were given methotrexate (as in Group II) and curcumin (100 mg/kg/d), orally, by intra-gastric intubation daily for 2 weeks before methotrexate treatment[10].

Group V: The rats were given methotrexate (as in Group II) & ascorbic acid (as in Group III) and curcumin (as in Group IV).

Methotrexate was obtained from Sanofi Aventis Company in the form of Methotrexate 2.5 mg tablet & dissolved in distilled water and was given by intra-gasric intubation (2.5 mg/kg /d). Giving methotrexate at a higher dose or for a prolonged duration led to

death of the animals.

Ascorbic acid was obtained from Glaxo Company in the form of Vitamin C SR 500 mg capsules & dissolved in distilled water and was given by intra-gasric intubation (150 mg/kg/d).

Curcumin was obtained as a natural herbal powder. It was purified dissolved in distilled water and was given orally, by intragastric intubation (100 mg/kg/d).

At the time of sacrifice, the rats were anaesthetized by IP injection of 50mg/kg pentobarbital. In each rat the jujenum was dissected; from which, three slices were obtained; the first was used for the paraffin section preparation and the second for frozen section preparation while the third slice was used for preparation of sections for the electon miroscopic study.

II. The staining techniques:

1. Paraffin sections stained with Hematoxylin and eosin : for routine histological study [11&12].

2. Cryocut frozen sections will be incubated for assessment of alkaline phosphatase enzyme activity [13].

3. Fine fragments will be processed to prepare semithin and ultrathin sections for transmission electron microscopic examination (TEM) [14].

- Sections for transmission electron microscopy were examined in the Electron Microscope Unit in Alexandria University.

Results

1. Control group :

Hematoxyline and eosin stained sections of the jejunal mucosa of control albino rats revealed that the epithelium covered the villi and lined the crypts. Each villus was covered by columnar absorping cells and goblet cells and its core was formed of thin layer of loose connective tissue . The brush border of columnar cells was acidophilic and showed strong positive alkaline phosphatase reaction and the epithelium lining the crypts showed frequent mitotic figures and intraepithelial leucocytes (Fig.1&2&3).

By the electon microscope the columnar absroptive cells showed

cylindrical mitochondria, rough endoplasmic reticulum (rER) and less frequent smooth endoplasmic reticulum (sER) in the herteogenous area of supranuclear cytosol. Their nuclei were basal and vesicular. The top surface of the columnar absroping cells was covered by numerous microvilli. Goblet cells were detected with the characteristic honey comb appearance of the mucus globules in the expanded apical part, basal flattend nucleus in the bottom. Mucous secretion was evident at the free border of the goblet cells (Fig. 4).

2. Second group (rats treated with methotrexte per se):

The jejunal mucosa of methotrexate treated albino rats showed a severe form of mucositis. Broadening, fusion of the villi, oedema and inflammatory infiltrate in both the corium and submucosa were clearly observed. Other findings were seen as : crypt disruption & atophy of the villi with denudation of the epithelium covering the villi and lining the crypts (Fig. 5).

Fresh frozen section in the jejunal mucosa of methotrexate treated albino rats showed weak to negative alkaline phosphatase reaction in most of the brush border of the columnar abrsorping cells of the disrupted villi (Fig. 6).

Electron microscopic examination of ultrathin sections of the jejunal mucosa of methotrexate treated albino rats revealed severe form of cytopathy in the columnar absroping cells. This was in the form of loss of most of the microvilli, abnormally short or long microvilli. The sER was widely dilated and the cytosol showed heterogenous group of abnormal shaped mitochondria.In addition ,the cytosol revealed some profiles of abnormal shaped rER with few vacuoles. The lateral borders were disrupted (Fig. 7).

3. Third group (rats treated with methotrexte and ascorbic acid):

Hematoxyline and eosin stained sections in the jejunal mucosa of methotrexate and ascorbic acid treated animals showed mildly disrupted broad villi with elevation of the overlying epithelium in most fileds while others were nearly normal. A rela-

tive increase in intraepithelial leucocytes together with appearance of frequent mitotic figures in some fields were observed when compared to group II (Fig. 8).

Most of the columnar abrsorping cells covering the villi showed an alkaline phosphatase reaction that was nearly similar to the control group (Fig. 9).

Electron microscopic examination of ultrathin sections of the jejunal mucosa of methotrexate and ascorbic acid treated animals showed mild degree of cytopathy. Fewer mitochondria with abnormal shape were observed while many others were normal in most columnar absroping cells. Also mild distortion of the arrangement of mucous globules was obsereved. Some of the mucous globules were discharged while other were still stored (Fig. 10).

4. Fourth group (rats treated with methotrexte and curcumin):

Hematoxyline and eosin stained sections in the jejunal mucosa of methotrexate and curcumin treated albino rats revealed relatively normal villi apart from that they were short and broad . Mild dilatation of the subepithelial connective tissue space together with presence of some goblet cells were detected (Fig. 11).

The brush border of the surface of the columnar abrsorping cells showed moderate alkaline phosphatase reaction in most cells covering the villi and lining the crypts (Fig. 12).

Electron microscope examination of ultrathin sections of the jejunal mucosa of methotrexate and curcumin treated albino rats revealed that the columnar absroping cells had a mild degree of cytopaty.Their microvilli were slightly distorted in shape.The cytosol of them shows numerous rER and few mildly dilated sER (Fig. 13).

5. Fifth group (rats treated with methotrexte & ascorbic acid and curcumin):

Hematoxyline and eosin stained sections in the jejunal mucosa of methotrexate & ascorbic acid and curcumin treated albino rats showed very little affec-

tion of jejunal villi and crypts. They were covered by intact epithelium formed of columnar absorping cells with intact brush border in addition to nearly normal percentage of goblet cells (Fig. 14).

The brush border of the columnar abrsorping cells showed strong positive alkaline phosphatase reaction both in most cells covering the villi as well as in cells lining the crypts (Fig. 15).

Electron micrograph of the columnar absroping cells in the jejunal mucosa of methotrexate & ascorbic acid and curcumin treated albino rats revealed slight degree of the cytopathy. The columnar absroping cells had numerous intact well fromed microvilli at the top surface. The nuclei of these cells were vesicular with copious amount of euchromatin and nuclear sap. Frequent nondilated sER and rER were detected. Moreover, in the lateral borders of the cells the contact between them was intact and not disintegrated. Enclosed between columnar absroping cells, various forms of goblet cells were seen (Fig. 16).

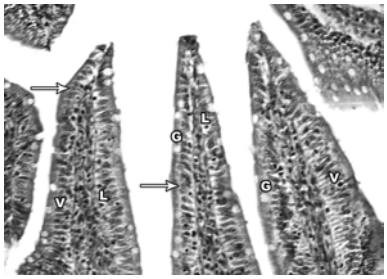

Fig. (1) : A photomicrograph of paraffin section of the jejunal mucosa of a control albino rat.The jejunal villi (V) are covered by columnar cells (arrows) and goblet cells(G) with occasional intraepithelial leucocytes(L). The core of the villi is formed of thin layer of loose connective tissue (CO). The brush border is more acidophilic and is visible at top of the covering epithelium. (Hx,Ex400)

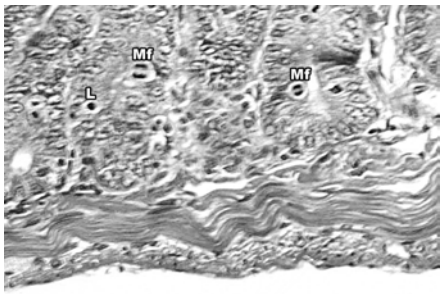

Fig. (2) : A photomicrograph of paraffin section in the jejunal mucosa of a control albino rat.The epithelium of the crypts of Lieberkuhn shows frequent mitotic figures(Mf) and frequent intraepithelial leucocytes (L). (Hx,E x 1000)

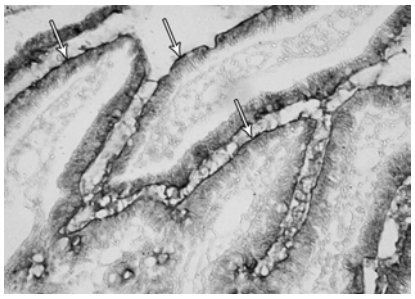

Fig. (3) : A photomicrograph of fresh frozen section in the jejunal mucosa of a control albino rat. The brush border of the columnar abrsorping cells shows strong positive alkaline phosphatase reaction (arrows). (Alkaline phosphatase x 400)

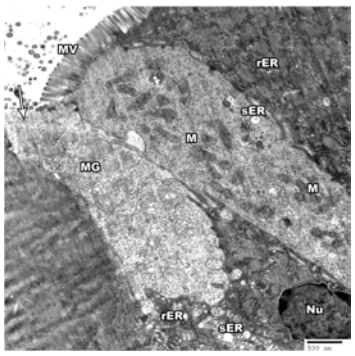

Fig. (4) : An electron micrograph of columnar absroping cells and an adjacent goblet cell in the jejunal mucosa of a control albino rat. The columnar absroping cell shows abundant cylindrical mitochondria (M) , rough endoplasmic reticulum (rER) and nondilated smooth endoplasmic reticulum (sER). The top surface is covered by numerous microvilli (MV). Goblet cell shows the characteristic honey comb appearance of the mucus globules (MG) in the expanded apical part and basal flattend nucleus in the bottom. Mucous secretion is evident at the free border of the goblet cells. (TEM x 3000)

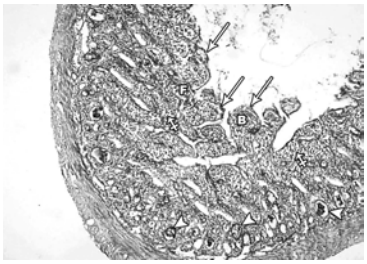

Fig. (5) : A photomicrograph of paraffin section in the jejunal mucosa of methotrexate treated albino rat .It shows broadening (B) , fusion(F) of the villi and oedema , inflammatory infiltrate (crossed heads) in both the corium and submucosa. The crypts show disruption (arrow heads). (Hx,E x 250)

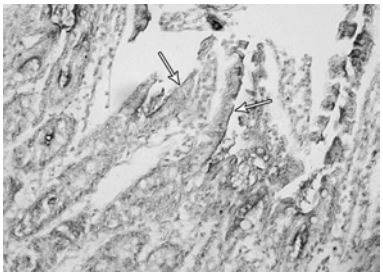

Fig. (6) : A photomicrograph of fresh frozen section in the jejunal mucosa of methotrexate treated albino rat.It shows weak to negative Alkaline phosphatase reaction in most of the brush border of the columnar abrsorping cells of the disrupted villi (arrows). (Alkaline phosphatase x 400)

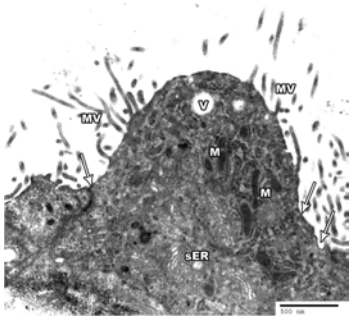

Fig. (7) : An electron micrograph of columnar absroping cell in the jejunal mucosa of a methotrexate treated albino rat. It shows loss of most of the microvilli (MV), The cytosol shows many abnormal shaped mitochondria (M). Widely dilated sER and few vacuoles (V) are seen. (TEM x 5000)

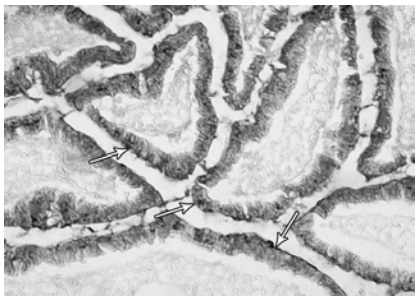

Fig. (9) : A photomicrograph of fresh frozen section in the jejunal mucosa of methotrexate and ascorbic acid treated albino rat. The brush border of most of the columnar abrsorping cells covering the villi show strong positive Alkaline phosphatase reaction.
(Alkaline phosphatase x 400)

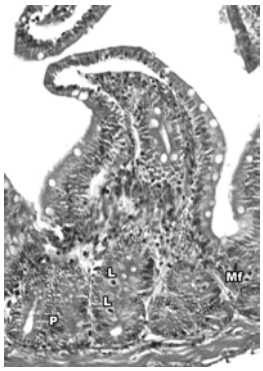

Fig. (8) : A photomicrograph of paraffin section in the jejunal mucosa of methotrexate and ascorbic acid treated albino rat. It shows a relative increase in intraepithelial leucocytes (L) together with appearance of frequent mitotic figures (Mf).Mildly disrupted broad villi with elevation of the overlying epithelium in most fileds are shown.
(Hx,E x 400)

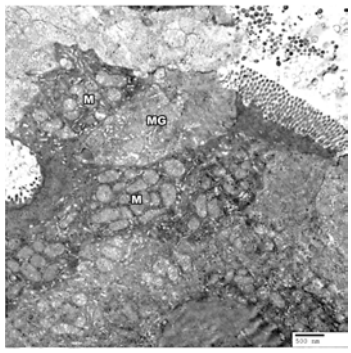

Fig. (10) : An electron micrograph of goblet cell in the jejunal mucosa of methotrexate and ascorbic acid treated albino rat.It shows mild distortion of the arrangement of mucous globules. Some of the these globules (MG) are dischrged while other are still stored . The adjacent columnar absroping cells show few mitochondria (M) with abnormal shape while many are normal. (TEM x 2500)

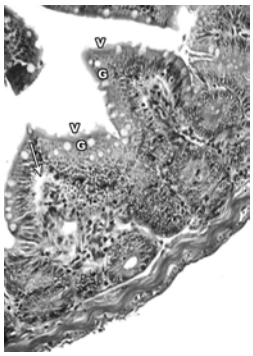

Fig. (11) : A photomicrograph of paraffin section in the jejunal mucosa of methotrexate and curcumin treated albino rat . It shows a relatively normal villi (V) apart from the dilated subepithelial space (arrows). (Hx,E x 400)

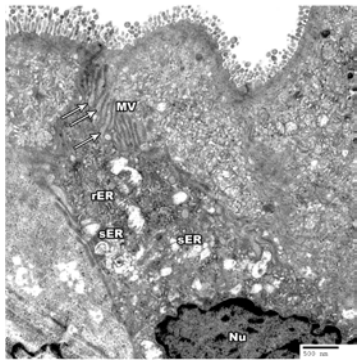

Fig. (13) : An electron micrograph of columnar absroping cells in the jejunal mucosa of methotrexate and curcumin treated albino rat . They show mild degree of cytopathy. Their cytoplasm are rich in both in rER and mildly dilated sER profiles. Their microvilli are slightly distorted in shape. (TEM x3000)

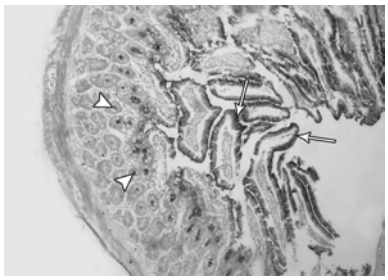

Fig. (12) : A photomicrograph of fresh frozen section in the jejunal mucosa of methotrexate and curcumin treated albino rat. The brush border of the surface of the columnar abrsorping cells shows moderate Alkaline phosphatase reaction. (Alkaline phosphatase x 250)

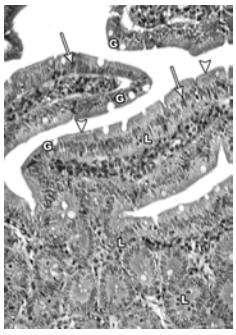

Fig. (14) : A photomicrograph of paraffin section in the jejunal mucosa of methotrexate & ascorbic acid and curcumin treated albino rat. The villi are covered by intact epithelium formed of columnar absorping cells (arrows) with intact brush border (arrow heads) in addition to goblet cells (G) and intraepithelial leucocytes (L). (Hx,E x 400)

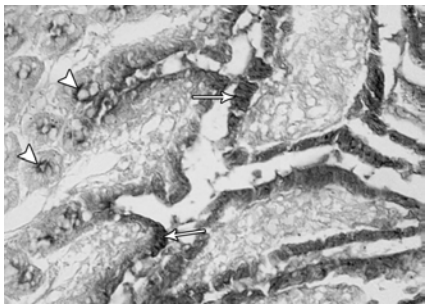

Fig. (15) : A photomicrograph of fresh frozen section in the jejunal mucosa of methotrexate & ascorbic acid and curcumin treated albino rat . The brush border of the columnar abrsorping cells show strong reaction in most cells covering the villi (arrows) and lining the crypts (arrow heads).
(Alkaline phosphatase x 400)

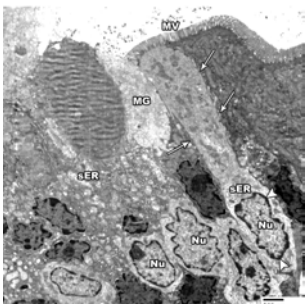

Fig. (16) : An electron micrograph of the columnar absroping cells in the jejunal mucosa of methotrexate & ascorbic acid and curcumin treated albino rat. The columnar absroping cells show numerous intact well fromed microvilli (MV) at the top surface. The nuclei of these cells are vesicular and nondilated sER is detected. Moreover, in the lateral borders (arrows) between cells are intact and not disintegrated. Enclosed between columnar absroping cells, various forms of goblet cells are seen; one of them is discharging its mucus secertion; other is expanded by a more electron dense mucus globules in the expanded apical part (MG). Connective tissue cells in the corium having vesicular central nuclei (Nu)are seen just below the epithelium. (TEM x2000)

Discussion

The jejunal mucosa of methotrexate treated albino rats showed a severe form of mucositis . Broadening, fusion of the villi & oedema and inflammatory infiltrate in both the corium and submucosa were clearly observed. Other findings were seen as : crypt disruption & atophy of the villi with denudation of the epithelium covering the villi and lining the crypts. Similar results were obtained by[1&15].

It is postulated that MTX alters intestinal mucosa and protein metabolism in a specific manner that lead to a decrease of protein synthesis, in particular mucin production, and an increase of proteolysis mediated by lysosomal pathway[1]. This might partially explain the changes occurred in the mucosa after MTX treatment. Other authors[16&17&18&19] attributed the main MTX effects to the free radicals produced from its oxidant effect. On the other

hand,[20&21] postulated that activation of the apoptotic pathways was the main mechanism involved in MTX induced mucositis.[15] claimed that MTX treatment for 3 day changed small bowel function by disrupting intestinal permeability and inducing electrolyte secretion in parallel with substantial histological damage.

The jejunal mucosa of methotrexate and ascorbic acid or methotrexate and curcumin treated animals showed mildly disrupted short broad villi with elevation of the overlying epithelium in most fileds while others were nearly normal. This was in agreement with[7] who reported that pretreatment with ascorbic acid significantly decrease DNA damage in the crypt cells and prevented denudation of intestinal mucosa. He added that the up-regulated expressions of apoptosis-related genes in the small intestine, are down-regulated in ascorbic acid-pretreated mice before induced enteritis.

The anti-inflammatory effect of curcumin were described by[5] who reported that a significantly decreased MTX induced disease activity, histological severity and lowered activities of lactate, ICAM-1 and MPO in comparison with the MTX treated animals . They added that curcumin may offer protective effects against MTX-induced rat enteritis by lowering the intestinal mucosal permeability.

Curcumin had been reported to relieve enteritis by inhibiting apoptosis as postulated by[10] who reported that a daily protective dose of 100 mg/kg of curcumin showed a few apoptotic bodies in radiation induced enteritis. Moreover, curcumin is said to have anti-inflammatory effect by which it reduced edema and the prominent infiltration of neutrophils and plasma cells. Thus, it is proposed that curcumin may be useful in the releave of organ dysfunction associated with local or systemic inflammation[22].

Many authors had attributed the chemoprevention of curcumin to the global gene expression profiles elicited by curcumin in mouse small intestine. They postulated that curcumin-

regulated nuclear factor E2-related factor 2 (Nrf2) - dependent genes regulate many detoxification / antioxidant enzyme[23]. On the other hand,[24] claimed that, the inhibition of NF-kappaB by using curcumin resulted in a partial amelioration of villous atrophy normally seen in the small intestine upon MTX treatment which causes MTX-induced mucosal barrier injury.

Goblet and paneth cells were also intact in most fields in both third and fourth group. A relative increase in the goblet cells & paneth cells if compared to the second group were also detected. Similar results were reported by[25] who said that there was a selective sparing of goblet cells and paneth cells in the intestine after MTX induced mucsoitis. They added that this sparing is likely to contribute to epithelial defense during increased vulnerability of the intestinal epithelium. On the other hand,[26] have reported that MTX induced a mild goblet cell depletion in the small intestine during a 4-day period of epithelial damage.

It is postulated that pretreatment with ascorbic acid significantly suppressed induced DNA damage in the crypt cells and prevented denudation of intestinal mucosa after injury by whole body irradiation[7]. Moreover,[7&24&23&27] reported that curcumin has also antiapoptotic action which might explain the increased proliferation in the fourth group. This might explain the increased proliferation seen with pretreatment with either ascorbic acid or curcumin.

Appearance of frequent mitotic figures in some fields were observed in the third group if compared to the second group. This might show the advantage of ascorbic acid over curcumin in relieve of the vascular injuries occurred by different injurious agents has described that ascorbic acid pretreatment has a protective effect against the intestinal morphological lesions induced by ischemia-reperfusion injury in rats[7].

The jejunal mucosa of methotrexate & ascorbic acid and curcu-

min treated albino rats (fifith group) showed very little affection of jejunal villi and crypts. Crypts were covered by intact epithelium formed of columnar absorping cells with intact brush border in addition to nearly normal percentage of goblet cells. This might indicate a synergistic cytoprotection acting through different enhancing mechanisms; namely : ascorbic acid (acting mainly as antioxidant) & and curcumin (acting mainly as anti-inflammatory & anti-apoptotic and to lesser extent antioxidant).

Intraepithelial leucocytes were frequentely seen between the columnar absorping ones in fifith group.[28] postulated that mucosal damage and barrier function alterations have been described as consequences of different processes: apoptosis, hypoproliferation, inflammatory response, altered absorptive capacity. Therefore, a pretreatment with both an antioxidant drug in addition to an anti-inflammatory antiapoptic drug might explain the mild affection of the jejunal mucosa after pretreatment with ascorbic acid and or curcumin and also might explain the increased mitotic figures & hyperproliferation & increasd villi length in the pretreated groups.

It was postulated that a defense mechanisms took place in MTX, as shown by an increased gene expression of both secreted (MUC2) and membrane-bound (MUC3 and 4) mucins maximally one week after MTX intake.This might explain the increased goblet cells enhanced by pretreatment of acrobic acid and or curcumin which might enhance the goblet cell by their cytoprotective action [1].

The jejunal mucosa of methotrexate treated albino rats showed weak to negative alkaline phosphatase reaction in most of the brush border of the columnar abrsorping cells of the disrupted villi. Similar results were reported by[29] who said that some components of enterocyte function may be affected more than others. They postulated that there are decreased disaccharidase and alkaline phosphatase, activities after a single intravenous injection of MTX 30 mg/kg.[30] added that alkaline phosphatase activity re-

mained detectable in the brush border of jejunal enterocytes during villus atrophy after MTX treatment despite severe cell loss after MTX treatment.

Most of the columnar abrsorping cells covering the villi of methotrexate and ascorbic acid and of methotrexate and curcumin treated animals showed an alkaline phosphatase reaction that was nearly similar to the control group while those lining the crypts of Lieberkuhn showed moderate reaction. The reaction became strong positive in methotrexate & ascorbic acid and curcumin treated animals. These results again might support the hypothesis of synergistic action between ascorbic acid & curcumin.

The jejunal mucosa of methotrexate treated albino rats revealed severe form of cytopathy in the columnar absroping cells. This was in the form of loss of most of the microvilli, abnormally short or long microvilli with some few remnants of them. The sER was widely dilated and the cytosol showed heterogenous group of abnormal shaped mitochondria. In addition , the cytosol revealed some profiles of abnormal shaped rER with dilated empty vacuoles in between. The lateral borders were disrupted. These findings were in agreement with[1].

The cytopathy of the jejunal mucosa of both methotrexate and ascorbic acid or methotrexate and curcumin treated animals was of mild degree. The microvilli of columnar absroping cells were slightly distorted in shape. The sER was mildly dilated. Fewer mitochondria with abnormal shape were observed while many others were normal in most columnar absroping cells. Also mild distortion of the mucous globule arrangement was observed. This was in agreement with[1], who again attributed the protective effect of curcumin to removal of free radicals by its known antioxidant effect.

Many authors claimed that oxidative stress and mitochondrial dysfunction are evident in MTX treated rats. They added that significant changes were seen in some of lipids in mitochondria, brush border membranes and

surfactant-like particles in response to drug-induced enteropathy. It is mentioned that curcumin attenuated oxidative stress and mitochondrial dysfunction. This might explain the potential mild affection of the jujenal mucosa after pretreatment with curcumin (either alone or with acorbic acid) by its antioxidant effect[31&32]. On the other hand, ascrobic acid is said to be necessary for synthesis of carnitine which is essential for the transport of fatty acids into mitochondria for ATP generation[33]. This might explain the mild cytopathy in the third group although it was reported that acorbic acid resulted in lowered mitochondria production in a study in rats, although the decrease was not statistically significant in humans[35].

The columnar absroping cells in the jejunal mucosa of methotrexate & ascorbic acid and curcumin treated albino rats revealed slight degree of the cytopathy. The columnar absroping cells had numerous intact well fromed microvilli at the top surface. The nuclei of these cells were vesicular with copious amount of euchromatin and nuclear sap with intact nuclear membrane without any invaginations. Frequent nondilated sER and rER were detected. Moreover, in the lateral borders of the cells the contact between cells were intact and not disintegrated. In addition, various forms of goblet cells were seen; one of them is discharging its mucus secertion; other was expanded by a more electron dense mucus globules in the expanded apical part. The protective effect of curcumin is attributed by [34&35] to the removal of free radicals by curcumin plus being a known antioxidant acting synersytically with ascorbic acid.

It was postulated that a number of paneth cells showed striking structural alterations with vacuolar dilatation of the cytoplasm in MTX induced enteritis [36]. Again,[30] has reported that there were selective sparing of paneth cells in the intestine of methotrexate-treated rats. He added that the expression of lysozyme protein and mRNA in them was increased which might act as defense mechanism in MTX treated animals.

The previous postulations and suggestions might shed light on the different mechanisms involved in response of animals to face inetsinal MTX mucositis and the protective effect of the use of ascorbic acid and/or curcumin.

Conclusion and recommendation

1. The structure of jejunal mucosa is affected by treatment with MTX. These structural changes became less obvious and mild when animals were pretreated with either ascorbic acid or curcumin for 2 weeks before methotrexate treatment and became milder when animals were pretreated by both substances simultanously.

2. Pretreatment with both ascorbic acid and curcumin simultanously could be recommended before MTX use, as both could relieve MTX enteritis more effectively than when one of them is being used alone.

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HISTOLOGICAL AND ULTRASTUCTURAL STUDY OF THE PROTECTIVE EFFECT OF ASCORBIC ACID AND CURCUMIN ON METHOTREXATE INDUCED SMALL INTESTINAL MUCOSAL INJURY

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VARUS DEROTATION FEMORAL OSTEOTOMY AND INNOMINATE SALTER OSTEOTOMY IN THE TREATMENT OF LEGG-CALVE-PERTHES' DISEASE. A COMPARATIVE STUDY

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Abstract

Introduction: *The principle of treatment of patients who have Legg-Calve-Perthes disease is functional containment of the femoral head in the acetabulum to minimize deformity of the hip joint and thereby delay the onset of degenerative joint disease in adult life.Many treatment methods have been proposed to achieve this goal,containment may be improved operatively either by redirecting the femoral head into the acetabulum by femoral derotation osteotomy or by redirecting the acetabulum over the femoral head by Salter osteotomy.*

Aim of the work: *The purpose of this study is to compare between the results of femoral derotation osteotomy and innominate Salter osteotomy in management of Legg-Calve-Perthes disease, as well as to assess the factors affecting the results and to record the complications of either procedure.*

Patients & Methods: *30 patients were enrolled for the study. In this study, all children with Legg-calve-Perthes disease who presented to Mansoura university hospital will be treated by femoral or innominate Salter ostotomy. The children will be divided into two groups according to the method of treatment, group A children will be treated by femoral derotation varus osteotomy and group B children will be treated by innominate Salter osteotomy. The children will be selected by systematic random sampling every other case so that patients with odd numbers will treated by femoral osteotomy and those with even numbers by innominate Salter osteotomy.*

Results: *There was no significant difference between Varus group and Salter group as regard the clinical results. Radiographic results showed that acetabular coverage was better in salter group than in Varus group, The residual coxa vara are smaller in Salter's group than in varus group,The trochanteric prominence was closer to normal in Salter's group than in varus group. There was no significant difference between the two groups as regard to the head size (coxa magna).*

Conclusion: *The results of this study show that the femoral varus osteotomy and the Salter innominate osteotomy provide similar clinical results, and the same radiographic results for the size of the femoral head. However, femoral neckshaft angle, articulotrochanteric distance, and acetabular coverage are closer to normal after the Salter innominate osteotomy than after the femoral varus osteotomy. We think that the Salter innominate osteotomy is the better treatment for patients with Perthes' disease to decrease residual problems such as coxa vara, trochanteric prominence, poor acetabular coverage.*

Introduction

The principle of treatment of patients who have Legg-Calve-Perthes disease is functional containment of the femoral head in the acetabulum to minimize deformity of the hip joint and thereby delay the onset of degenerative joint disease in adult life. Many treatment methods have been proposed to achieve this goal. In the past, hip motion exercises and nonweight bearing were popular; however, containment of the femoral head within the acetabulum and weight bearing now is preferred.[1]

The treatment of legg-Calve-Perthes disease is based on two philosophies : noncontainment and containment, Noncontainment treatment includes prolonged bed rest, bed rest with traction, bed rest with immobilization, and nonweight bearing without containment.[2] Containment may be improved operatively either by redirecting the femoral head into the acetabulum by femoral derotation osteotomy[3] or by redirecting the acetabulum over the femoral head by Salter osteotomy.[4]

The advantages of femoral osteotomy are that the operation is done on the affected bone, it provides better lateral coverage than does innomonate Salter osteotomy, and it decreases the force across the joint.[5] The advantages of innomonate Salter osteotomy are that it compensates for any expected femoral shortening and it provides better anterolateral coverage.[6] The disadvantages of the femoral procedure are that it may shorten the limb and may create excessive varus angulation,leading to weakness of the abductors of the hip.[7] The disadvantages of innomonate Salter osteotomy are that it may increase the transarticular pressure and arthritis of the hip later on and is technically more difficult.[8]

Patients and Methods

In the period between January 2009 and October 2012, prospective study was carried out on 30 children with Perthes' disease with deformed noncontainable femoral heads. All the cases were selected from the outpatient clinic of the Orthopaedic Department in the Children's Hospital - Mansoura University. We have used two operative techniques for the selected cases; Varus osteotomy and Salter osteotomy.

The method of treatment has been selected by systematic random sampling every other case so that patients with odd numbers were treated by varus osteotomy and those with even numbers by Salter osteotomy. They were 26 boys and 4 girls. The right side was affected in 10 hips and the left in 20. The mean age at surgery was 7±1.4 years in varus group and 7.3±1.6 years in salter group.

All cases were subjected to clinical and radiological examination:

I) Clinical Examination:

1) General:

In every case general examination was performed to exclude generalized epiphyseal disorders and to detect the presence of other associated anomalies.

2) Local:

All the cases were examined for:

1- Limping. 2- Shortening.
3- Range of motion.
4- Trendlenburg's test.

II) Radiological Examination:

Plain antero-posterior and frog lateral radiographs were assessed for:

1) The radiographic stage of the disease according to Waldenström [9]:
   i) Initial Stage.
   ii) Fragmentation Stage.
   iii) Reossification (Healing) Stage.
   iv) Residual Stage.
2) Extent of radiological involvement according to Catterall Classification:
3) Head at risk signs :
   i) Gage's sign.
   ii) Calcification lateral to the epiphysis.
   iii) Diffuse metaphyseal reaction.
   iv) Lateral subluxation.
   v) Horizontal growth plate.
4) Radiological measurements:
   i) C-E Angle of Wiberg.
   ii) Neck shaft angle:
   iii) Femoral head size ratio :
   v) Difference in Articulotrochanteric distance.

III- Criteria of inclusion in the study:

According to the previous clinical and radiological findings, patients were selected to be included in this study according to the following criteria:

1) Patients above the age of six years and below the age of twelve years will included in this study.
2) Catterall groups III or IV.
3) Patients with radiological evidence of head at risk signs.

IV- Operative Techniques:

1) Varus Osteotomy:

According to the technique described by Müller et al.[10]

2) Salter Osteotomy:

According to the technique described by Salter.[4]

V- After treatment:

Immobilization for 6 weeks then the patients will start active mobilization and gradual weight bearing.

VI- Follow up:

All cases will be followed up clinically and radiologically for average period of 30 month (between 24 and 36 months). Each patient was reviewed every month for the 1st 6 months, then every 3 months for the rest of the follow up. The results were assessed

both clinically and radiologically.In the Salter Osteotomy group, 2 K-wires were used to fix the osteotomy. All the patients were admitted again for K-wire removal under anaesthesia after radiological union of the osteotomy site.

VII-Assessment of the results:

1) Clinically:

We have used the scoring system of Robinson et al [11]:

A) Good clinical result was one in which the involved hip caused no symptoms and had a full, or almost full, range of motion.

B) Fair result was one in which the hips caused no symptoms, but movement of the hips was restricted slightly, especially in internal rotation.

C) Poor clinical result was defined as one in which the findings indicated the presence of pain at rest or pain on motion, with limitation of hip movement.

2) Radiologically:

The preoperative radiological measurements were compared with the same postoperative measurements

Results

This study was conducted on 30 patients 15 had Varus Osteotomy and 15 had Salter's osteotomy. There was no significant difference regarding the age (Table 1).

Tables (2) showed Shortening ranged from 0 to 2 cm in varus group and from 0 to 1.5 cm in salter group it was found significant between both groups.

Table (3) showed that Catterall III cases were more than Catterall IV cases in both groups, 8 hips in Varus group and 11 hips in the salter group were Catterall group III. There was no statistically significant difference concerning Catterall classification between both groups

Table (4) showed the mean preoperative center-edge angle was 18.87° ± 8.13° in Varus group and 20.20° ± 8.76° in Salter group. The mean neck shaft angle was 135° ± 9° in Varus group and 135° ± 8.9° in Salter group. The mean femoral head size ratio was 1.11 ± 0.36 in Varus group and 1.10 ± 0.027 in the Salter group. Statisti-

cal analysis of these measurements showed that there was no significant difference between both groups in each of the 4 measurements.

Table (5) showed the mean post-operative center-edge angle was 24.4° ± 5.79° in Varus group and 30.20° ± 4.64° in Salter group. The mean neck shaft angle was 118.26° ± 6.96° in Varus group and 130.73° ± 7.49° in Salter group. The mean femoral head size ratio was 1.13 ± 0.022 in Varus group and 1.15 ± 0.041 in the Salter group. The diference in articuo-trochanteric distance was 8.60 ± 2.77 in varus group and 4.33 ± 2.66 in Salter group. Statistical analysis of these measurements showed that there was no significant difference between both groups as regard femoral head size ratio but there were significant difference between boths groups as regard other variants.

Table (1): Age at Surgery.

	varus group	Salter group
Mean	**7 ± 1.4**	**7.3 ± 1.6**
Median	**6.5**	**7**
Range	**6-11**	**6-11**

P value = 0.589 (insignificant)

Table (2): Pre-operative Shortening.

	Varus group	Salter group
Mean	1.2	6.40
Range	0 - 2cm	0 -1.5cm

P value = 0.024 (significant)

Table (3): Catterall classification.

Catterall Classification	Varus group		Salter group		Total	
	No.	%	No.	%	No.	%
Catterall III	8	53.3	11	73.3	19	63.3
Catterall IV	7	46.7	4	26.7	11	36.7
Total	15	100	15	100	25	100

P value = 0.256 (insignificant)

Table (4): Pre-operative radiological measurements.

Radiological measurements	Varus group		Salter group		P Value
	Mean	Range	Mean	Range	
C-E angle	18.87° ± 8.13°	6-32	20.20° ± 8.76°	5-35	0.669 insignificant
NS angle	135° ± 9°	119-153	135° ± 8.9°	121-148	0.968 insignificant
Femoral head size ratio	1.11 ± 0.36	1.05-1.17	1.10 ± 0.027	1.06-1.16	0.185 insignificant

Table (5): Radiological Measurements at the Last Follow Up.

Radiological measurements	Varus group		Salter group		P Value
	Mean	Range	Mean	Range	
C-E angle	24.4° ± 5.79°	13-33	30.20° ± 4.64°	23-41	0.006 significant
NS angle	118.26° ± 6.96°	108-129	130.73° ± 7.49°	120-141	0.000 significant
Femoral head size ratio	1.13 ± 0.022	1.09-1.17	1.15 ± 0.041	1.06-1.22	0.115 Insignificant
Diference in ATD	8.60 ± 2.77	4 – 12	4.33 ± 2.66	1 – 9	0.000 significant

Discussion

The most widely used surgical methods for containment treatment of Perthes' disease are the femoral varus osteotomy and the Salter innominate osteotomy. Since Axer[3] introduced subtrochanteric varus femoral osteotomy as a treatment for patients with Perthes' disease in 1965, his technique or modifications have been used consistently.

Salter[4] introduced the Salter innominate osteotomy for treatment of patients with Perthes' disease. The optimum surgical treatment of patients with Perthes' disease is debatable. Numerous authors have described the results of these two procedures for Perthes' disease.[3,11] Clinical residual problems associated with surgical procedures for Perthes' disease are hip pain, restricted hip motion leg length discrepancy, abductor insufficiency. [3,11]

The clinical results of our patients treated by Salter osteotomy showed significant improvement in hip pain as it was relieved completely in 13 out of 15 hips. There was also significant improvement in limping as there is no limbing in 13 cases out of 15 cases. Trendelenburg's test was negative in 13 patients post-operatively. Range of movements which were limited at the time of presentation, improved in Salter's group. Using Robinson et al [11] clinical classification, we found significant improvement as 14 cases (93.3%) were good at the last follow up.

Our clinical results were in agreement with other reports as Sponseller et al[12] compared the clinical and radiographic outcomes of 42 femoral varus osteotomies and 49 Salter innominate osteotomies for treatment of patients with Perthes' disease. Similarly, Moberg et al.[13] investigated the results of both surgeries (16 femoral varus osteotomies and 18 Salter innominate osteotomies). They reported that the clinical results, were the same in the two Groups.

There was no significant difference in pain between the femoral varus osteotomy and the Salter innominate osteotomy groups; There was no significant difference in range of movements between the

femoral varus osteotomy and the Salter innominate osteotomy groups however, the range of internal rotation was limited in both Salter innominate osteotomy group and the femoral varus osteotomy group, There was no significant difference in leg-length discrepancy between the femoral varus osteotomy and the Salter innominate osteotomy groups and the leg-length discrepancy was greater for the femoral varus osteotomy group (0.9cm) than for the Salter innominate osteotomy group (0.8cm). There was no significant difference in trendelenburg sign between the femoral varus osteotomy and the Salter innominate osteotomy groups.

Similarly, Takahiko et al.[14] study investigated the results of both surgeries (46 femoral varus osteotomies and 30 Salter innominate osteotomies). They reported that the clinical results, were the same in the two Groups. There was no significant difference in pain between the femoral varus osteotomy and the Salter innominate osteotomy groups; There was no significant difference in range of movements between the femoral varus osteotomy and the Salter innominate osteotomy groups however.

the range of flexion and abduction was limited in both Salter innominate osteotomy group and the femoral varus osteotomy group, There was no significant difference in leg-length discrepancy between the femoral varus osteotomy and the Salter innominate osteotomy groups and the leg-length discrepancy was greater for the femoral varus osteotomy group (0.6cm) than for the Salter innominate osteotomy group (0.3cm).

Our radiologic results were in agreement with Sponseller et al.[12] compared the clinical and radiographic outcomes of 42 femoral varus osteotomies and 49 Salter innominate osteotomies for treatment of patients with Perthes' disease. Similarly, Moberg et al.[13] investigated the results of both surgeries (16 femoral varus osteotomies and 18 Salter innominate osteotomies). Moberg et al[13] showed that acetabular coverage was better in salter group than in Varus group. The

residual coxa vara are smaller in Salter's group.The trochanteric prominence and the head size (coxa magna) as the head size ratio did not compared in this study.

Our radiologic results were in agreement with Takahiko et al[14] study investigated the results of both surgeries (46 femoral varus osteotomies and 30 Salter innominate osteotomies). The results of our study and the previous three reports[12-14] show that the femoral varus osteotomy and the Salter innominate osteotomy provide similar clinical results, and the same radiographic results for the size of the femoral head. However, femoral neckshaft angle, articulotrochanteric distance, and acetabular coverage are closer to normal after the Salter innominate osteotomy than after the femoral varus osteotomy.

We think that the Salter innominate osteotomy is the better treatment for patients with Perthes' disease to decrease residual problems such as coxa vara, trochanteric prominence, poor acetabular coverage. One of the advantages of surgical treatments for Perthes' disease is to shorten the duration of inconvenience. We choose to do the Salter innominate osteotomy when surgery is indicated for patients with Perthes' disease, based on the results of this study.

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VARUS DEROTATION FEMORAL OSTEOTOMY AND INNOMINATE SALTER OSTEOTOMY IN THE TREATMENT OF LEGG-CALVE-PERTHES' DISEASE. A COMPARATIVE STUDY

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CD142, VEGF AND MICROVASCULAR DENSITY MVD-CD34 EXPRESSION IN HEPATOCELLULAR CARCINOMA OF PATIENTS WITH CIRRHOSIS AND CORRELATION WITH TUMOR GROWTH AND PROGRESSION

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Abstract

Backgroundand Aim: *Angiogenesis is one of the mechanisms most critical to the postoperative recurrence and metastasis of HCC. So,finding the molecular markers associated with angiogenesis may help identify patients at increased risk for recurrence and metastasis of HCC.The aim of this study is to investigate the level of CD142, VEGF, and MVD-CD34 expression in HCC and surrounding cirrhotic liver tissue and their relationship to tumor growth and prognosis.*

Materials and Methods: *This study included forty six patients with clinical, radiological and serological diagnosis of HCC arising on top of cirrhosis. These patients underwent liver transplantation or partial hepatectomy at Mansoura Gastroenterology Surgical Center during the period 2010-2011.Tissue samples were obtained from specimens of resected HCC and the surrounding cirrhotic tissue. Immunohistochemical staining for CD142, VEGF and MVD-CD34 antibodies was performed in HCC tissue specimens and paracarcinamatous cirrhotic tissue. The quantitation of the microvessels identified by anti CD34, VEGF expression identified by anti VEGF monoclonal antibody, and CD142 expression identified by anti TF monoclonal antibody. Each monoclone expression was identified in both HCC tissue, and the surrounding cirrhotic tissue.*

Results: *CD142 and VEGF showed significantly increased expression in HCC compared to LC, and showed increased expression from grade I to grade II to grade III, but no significant difference in their*

expression between grade III and grade IV. There is highly significant association between CD142 and VEGF expression positivity and tumor size, vascular emboli, intrahepatic metastasis and tumor grade (P<0.001). There is highly significant association between CD142 and VEGF expression in both LC and different grades of HCC (P<0.001). MVD-CD34 was increased significantly from LC to HCC and increased significantly from grade I to II to III to grade IV HCC (P<0.001). The MVD-CD34 was significantly higher in tumors with high immunoreactivity for CD142 than in tumors with low immunoreactivity for CD142 (median, 53.26 vs 37.01/HPF, P<0.02).

Conclusion: *Expression of the angiogenic factors CD142, VEGF and MVD-CD34 is increased in HCC relative to LC and correlated with tumor aggressiveness. CD142 has been recognized to be capable of inducing angiogenesis through up-regulation of VEGF. Expression of these factors may be useful as prognostic indicators in patients with HCC.*

Key words: *liver cirrhosis (LC), hepatocellular carcinoma (HCC), hepatocellular carcinoma on top of cirrhosis (HCC-C), vascular endothelial growth factor (VEGF), CD142 (tissue factor), microvascular density (MVD).*

Introduction

Hepatocellular carcinoma(HCC) is the sixth most common cancer worldwide in terms of cases but because of its very poor prognosis it is the third most common cause of death from cancer.[1] The major risk factor is liver cirrhosis associated with chronic hepatitis B and C infection, alcohol and various metabolic disorders.[2] However, in approximately 10-40% of patients the tumor arises from a non-cirrhotic liver.[3] The tumor from patients with cirrhosis tends to be less well differentiated (high grade), and exhibits local portal invasion and metastasis more often than tumors from patients without cirrhosis.[3] Tumor angiogenesis is critical for providing nutrient supply to the tumor and providingthe route for tumor survival, growth, invasion and metastasis of various human solid tumors. In fact, solid tumors can't grow 1 to 2mm in diameter in the absence of angiogenesis.[4]

In HCC, the sinusoid endothelial cells lost their structural and phenotypic characteristics and

adopted the structure and phenotype of normal capillary endothelial cells, a phenomenon known as capillarization.[5] The association of increased expression of vascular endothelial growth factor (VEGF) with the capillarization of sinusoidal endothelial cells demonstrated in recent studies indicates that the capillarization phenomenon is not merely a change of endothelial cell differentiation but rather represents a process of tumor angiogenesis.[6-8]

MVD, a quantitative measurement of tumor angiogenesis, has been shown to be of prognostic value in many types of malignancy including HCC,[9] which became more consistent in dysplastic nodules in cirrhotic liver. Therefore, capillarization of sinusoidal endothelial cells was considered part of the carcinogenesis process in HCC-C.[4] MVD-CD34 has been widely used for the assessment of sinusoidal like neoangiogenesis in HCC.[10]

VEGF is a known marker of angiogenesis.[11] It is thought to be a selective mitogen for endothelial cells. It acts as a link between angiogenesis, immune system and tissue re-modeling.[12] It was found that VEGF secreted by replicating hepatocytes induces sinusoidal endothelial cell proliferation during regeneration after partial hepatectomy in rats.[13] Greene et al[14] in their studies on hepatic regenerative process suggested that the regulation of angiogenesis controls the regenerative process.

CD142 (tissue thromboplastin or tissue factor) is a transmembrane glycoprotein that localize the coagulation serine protease factor VII/VIIa (FVII/VIIa) to the cell surface. The primary function of CD142 is to activate the clotting cascade. Recent studies have shown that CD142 is expressed by tumor cells and contributes to a variety of pathologic processes, such as thrombosis, metastasis, tumor growth and tumor angiogenesis.[15]

The hypervascularity described in HCC varies according to the progression and differentiation of the tumor, suggesting an angiogenic switch during tumor development. Thus, finding the molecu-

lar markers associated with angiogenesis may help identify patients at increased risk for recurrence and metastasis of HCC and thus those who require more aggressive therapy and closer surveillance.[11] Interfering with angiogenesis may be a potential target to avoid progression of liver disease.[16]

This study was designed to investigate whether CD142, VEGF and MVD-CD34 expression could serve as a valid prognostic markers in patients with HCC-C and correlation with angiogenesis, progression and differentiation of HCC-C and so the postoperative recurrence and metastasis.

Material and Methods

This study was conducted on 46 cases with HCC recruited from the Hepatic Oncology Unit at the Specialized Mansoura Medical Hospital, from 2010 to 2011. All patients were subjected to thorough clinical examination, routine laboratory tests, and abdominal ultrasonography and computed tomography (CT) scan. Patients who are candidate for surgical treatment were referred to the Mansoura Gastroenterology Center for either liver transplantation or partial hepatectomy. All the cases were HCC on background of cirrhosis and were HCV positive confirmed by real-time polymerase chain reaction. Cases arising in a normal liver, associated with bilharzial fibrosis only with no evident cirrhosis, or received previous adjuvant therapy or chemoembolization were excluded. Resection specimens, as well as total hepatectomy specimens from the patients who underwent liver transplant surgery were used in the study. No tru-cut biopsy material was used. Liver biopsies were fixed in 10% buffered formalin for 24 hours, and then processed in ascending grades of ethyl alcohol; xylene, wax and paraffin blocks were prepared. Sections (4μm) were cut on albuminized glass slides and stained with Hematoxylin & Eosin stain. All sections were subjected to light microscopic examination for evaluating the histopathological and basic classification of cases. The pathologic diagnosis and classification of variables were based on the criteria recommended in the general rules for clinical and pathological study

of primary liver cancer (Liver Cancer Study Group Of Japan)[17] and included age, gender, surrounding nontumerous liver pathology (cirrhosis), tumor size (<5cm vs ≥5cm), tumor grade, vascular invasion and multiplicity.

Immunohistochemical staining of VEGF:

Formalin-fixed, paraffin embedded sections of tumor tissue and surrounding cirrhotic tissue obtained from the resected liver specimens were cut into 4 microns thick sections and deparafinized in xylene and rehydrated in a graded series of ethanol. Antigen retrieval was performed by using EDTA in case of VEGF for 10m. The slides were then incubated for 1 hour for mouse polyclonal anti VEGF antibody (1:100 dilution, invitrogen code No. 726127A). This is followed by biotin-conjugated goat anti-mouse immunoglobulin and horseradish peroxidase-conjugated streptavidine (UltraVision Detection System) DAB was used as chromogenic substrate. Counterstain the section with Mayer's hematoxylin for 10 minutes then rinsed extensively with distilled water. The brown precipitate was identified as positive staining.

Immunohistochemical Staining of CD142 (tissue factor):

Immunohistochemical staining was performed using the streptavidin-biotin peroxidase complex method. Formalin-fixed, paraffin-embedded sections of 4 μm were deparafinized in xylene and rehydrated in a graded series of ethanol. Endogenase peroxidase was blocked by treating the sections with 3% hydrogen peroxide in methanol for 10 min. The sections were then subjected to antigen retrieval by microwave treatment for 10 min. Afterward, the sections were incubated with normal goat serum for 30 min at 37°C and then with 1:50 diluted TF mouse antihuman monoclonal antibody (American Diagnostica, Inc., product Nos.4509) for 1 h at 37°C. Sections were then incubated with 1:100 diluted biotin-conjugated goat antimouse immunoglobulin-secondary antibody (Zymed Laboratory Inc., SanFrancisco, CA) for 1 h at 37°C and developed in 3,3-diamino-benzidine tetrachloride (Dako, Carpinteria, CA). Counterstain the section with Mayer's

hematoxylin for 10 minutes then rinsed extensively with distilled water.

Immunohistochemical staining of CD34:

Formalin-fixed, paraffin embedded sections of tumor tissue and surrounding cirrhotic tissue obtained from the resected liver specimens were cut into 4 microns thick sections and deparafinized in xylene and rehydrated in a graded series of ethanol. Sections were immersed in citrate buffer (PH 7.0) for antigen retrieval and incubated in a water bath for 40 min 98˚c. After endogenous peroxidase was blocked, the sections were incubated with monoclonal mouse antihuman CD34 antibody (Clone QBEnd/10, Thermo Fisher Scientific, Fremont, CA, USA) for 60 min at room temperature.Tissues were treated with the (EnVision + system, Dako, Glostrup, Denmark) for 30min at room temperature, and the reaction was visualized by DAB until color developed.The cell nuclei were counterstained with Mayer's hematoxylin.

The expression of the three markers was correlated with positive controls (cancer colon for VEGF, cancer breast for TF, and internal control, vessels in the portal tracts and fibrous septa for CD34) and negative controls. The expression of three markers was correlated with pathological data collected. For this purpose patients were divided into two age groups (<60years & ≥60 years), size (<5cm & ≥5cm), and tumors were graded according to Edmonson and Steiner four tiered grading system.[18,19]

Immunohistochemical scoring of VEGF and tissue factor:

For VEGF, cytoplasmic staining of more than 10% of the tumor cells was defined as positive. The intensity of immunoreactivity was graded as weak (+), moderate (++) and marked (+++).[20] The immunoreactivity of TF was classified as high if >50% of the tumor cells were stained positively and low if <50% of the tumor cells were stained positively.[9]

Determination of microvascular density (MVD):

MVD was evaluated according to the method described by

Weinder et al[21] Brown-stained endothelial cell or endothelial cell cluster, which was clearly separate from adjacent microvessels and other connective tissue elements, was considered a single, countable blood vessel. Screening of the cores was first performed at a low power (40 X) to identify areas of the highest MVD. Counting was performed in the three highest MVD areas at high power (400 X). The mean value of the counted three fields was considered as the MVD of an individual case.

Statistical analysis:

The Statistical Package for Social Sciences (SPSS) for Windows (version 10) computer program was used for statistical analysis. For comparison of more than 3 group's means, one-way ANOVA test was used with Post Hoc test for internal comparisons. Comparison between positive cases was calculated by Chi-square test. P value <0.05 was considered statistically significant.

Results

Patients were 40 males (87%) and 6 females (13.0%), with age range (41-69 years) with a mean of 52.91±6.98 years. Frequency of vascular invasion was insignificantly higher (P>0.05) in patients with tumor size >5cm (87.5%) compared to tumor size <5cm (76.7%). On the other hand, there was significant association between vascular emboli and high tumor grade (P=0.03).

Immunohistochemical expression of VEGF :

Immunohistochemical staining of 46 cases for VEGF shows expression of the biomarker in both tumor tissue and the surrounding cirrhotic tissue, and the endothelial cells lining the blood vessels in the tumor area mainly and in the nearby periphery of the tumor. Two cases (4.3%) were negative for VEGF and 44 (95.7%) cases were positive for VEGF.

In the cirrhotic liver, 12(26.1%) cases showed weak staining (+), 18(39.1%) cases were of moderate staining (++) and 14(30.4%) cases showed marked staining (+++). In patients with HCC, 4 cases (8.7%) showed weak staining, 13 cases (28.3%) showed moderate staining, and 27 cases (58.7%) showed marked staining

(Fig. 1). In 15(39.1%) cases, the stain was more intense at the periphery of the tumor than in the center (Fig. 2).

In our study, there was no significant association between VEGF expression positivity both in cirrhosis and tumor tissue and age or sex (P>0.05). However, there is highly significant association between VEGF expression positivity and tumor size, vascular emboli, intrahepatic metastasis and tumor grade (P<0.001).

There is significant increase in VEGF expression in HCC cases relative to LC (P<0.05). VEGF expression increased significantly from grade I to grade II to grade III (p<0.05). But there was no significant difference in VEGF expression between grade III and grade IV (Table 1).

Immunohistochemical expression of CD142 :

Immunohistochemical staining of 46 cases for CD142 showed expression of the biomarker in both tumor tissue and the surrounding cirrhotic tissue. There were 2 cases (4.3%) negative for CD142 and 44 (95.7%) of cases were positive for CD142.

As regard CD142 expression in cirrhotic tissue, there were 30 cases (65.2%) with low expression (Fig. 3), and 14 cases (30.4%) showed high expression (Fig. 4). However, there were 17(37.0%) cases with low CD142 expression and 27(58.7%) cases showed high CD142 expression in HCC (Fig. 5). There was significantly higher CD142 expression in HCC cases relative to LC (P<0.05).

In our study, there was no significant association between CD142 expression positivity both in cirrhosis and tumor tissue and age or sex (P>0.05). However, there is highly significant association between CD142 expression positivity and tumor size, vascular emboli and tumor grade (P<0.001).

CD142 expression increased significantly from grade I to grade II to grade III. But there was no significant difference in CD142 expression between grade III and grade IV (Table 2).

Immunohistochemical staining of MVD-CD34

Immunohistochemical staining of 46 cases for CD34 showed expression of the biomarker in both tumor tissue and the surrounding cirrhotic tissue. In liver cirrhosis, there was one case (2.17%) negative for CD34 and 45 (97.83%) cases were positive for CD34, while all cases of HCC were positive for CD34.

There was significant increase ($P<0.05$) in CD34 expression fromcirrhotic nodules to dysplastic nodules to HCC (Fig. 6).

In liver cirrhosis, CD34 staining was confined to large vessels in the portal tracts. In cases of HCC-C the density of microvessels was higher in the peripheral tumor tissue close to the margin than in the central areas.

The mean MVD-CD34 in liver cirrhosis was significantly lower than the MVD-CD34 in HCC (8.28±6.62 vs 46.24±24.04, $P<0.001$) (Table 3). Specific staining of capillary-like vessels by anti-CD34 antibody was observed in tumor specimens and between cancer cells. CD34 expression was found to be significantly increased with the tumor grade ($P<0.05$) (Fig. 7, 8).

Correlation between CD142 immunoreactivity and MVD-CD34:

Immunohistochemical staining of the tumor sections showed that CD142 was expressed to a variable extent by HCC tumor cells in 95.7% of the tumors studied, with high immunoreactivity in 27 patients and low immunoreactivity in 17 patients. Specific staining of capillary-like vessels by anti-CD34 was observed in 97.82% of tumor specimens. The mean tumor MVD was 52.1/HPF (range, 0-78.9). The MVD was significantly higher in tumors with high immunoreactivity for CD142 than intumors with low immunoreactivity for CD142 (median, 53.26vs 37.01/HPF, $P<0.02$).

Correlation between CD142 immunoreactivity and VEGF:

Highly statistically significant ($P<0.001$) association was found between VEGF and CD142 expression in both LC and different

grades of HCC. All cases of LC and HCC with high CD142 immunoreactivity also showed marked VEGF expression. On the other hand, all cases of LC and HCC with low CD142 immunoreactivity also showed mild to moderate VEGF expression. Furthermore, cases with negative CD142 immuno-reactivity showed negative expression for VEGF in both LC and HCC.

Table 1: VEGF expression levels in the paracarcinomatous cirrhotic liver and in different grades of HCC-C.

Groups	VEGF			
	No. of positive cases for VEGF	Percentage of positive area /3 Microscopic fields		
		Mild	Moderate	Marked
LC (n=46)	44/46	12 (26.1)	18(39.1)	14(30.4)[a]
HCC(n=46)	44/46	4(8.7)	13(28.3)	27(58.7)
GI (n=3)	2/3	0(0.0)	1(33.33)	1(33.33)[b]
GII (n=23)	22/23	2(8.7)	11(47.8)	9(39.1)[c]
GIII (n=14)	14/14	2(14.3)	0(0.0)	12(85.7)[c,d]
GIV(n=6)	6/6	0(0.0)	1(16.7)	5(83.3)[c,d]

[a]P value <0.05 LC relative to HCC --[b]P<0.05 between different HCC grades.
[c]P<0.05 GI relative to GII, GIII, GI -- [d]P<0.05 GII relative to GIII, GIV.

Table 2 : CD142 expression levels in paracarcinomatous cirrhotic liver and in different grades of HCC-C.

Groups	CD142		
	No. of positive cases for CD142	Percentage of positive cases	
		Low	High
LC (n=46)	44/46	30(65.2)	14(30.4)[a]
HCC(n=46)	44/46	17(37.0)	27(58.7)
GI (n=3)	2/3	1(33.33)	1(33.33)[b]
GII (n=23)	22/23	13(56.5)	9(39.1)[c]
GIII (n=14)	14/14	2(14.3)	12(85.7)[c,d]
GIV(n=6)	6/6	1(16.7)	5(83.3)[c,d]

[a]P value <0.05 LC relative to HCC - [b]P<0.05 between different HCC grades.
[c]P<0.05 GI relative to GII, GIII, GIV - [d]P<0.05 GII relative to GIII, GIV.

Table 3: MVD-CD34 expression levels in paracarcinomatous cirrhotic liver and in different grades of HCC-C.

Groups	MVD-CD34	
	No. of positive cases for CD34	MVD μ^2/ 3 microscopic field Mean ± SD
LC (n=46)	45/46	8.28±6.62[a]
HCC(n=46)	46/46	46.24± 24.04
GI (n=3)	3/3	13.53±18.80[b]
GII (n=23)	23/23	44.77±22.94[c]
GIII (n=14)	14/14	50.31±19.99[c]
GIV(n=6)	6/6	57.32±18.64[c]

[a]P<0.001 LC relative to HCC–[b]P<0.05 between different HCC grades
[c]P<0.05 GI relative to GII, GIII, GIV.

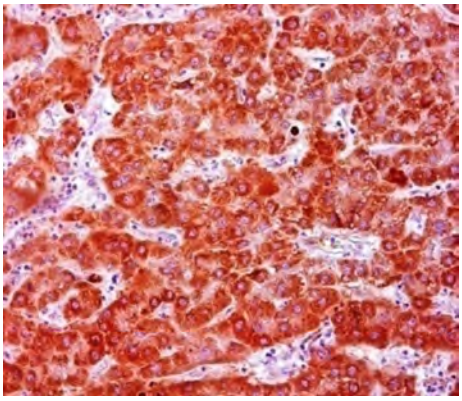

Fig. 1 : Marked intensity of VEGF immunostaining in case of HCC (original magnification X 200).

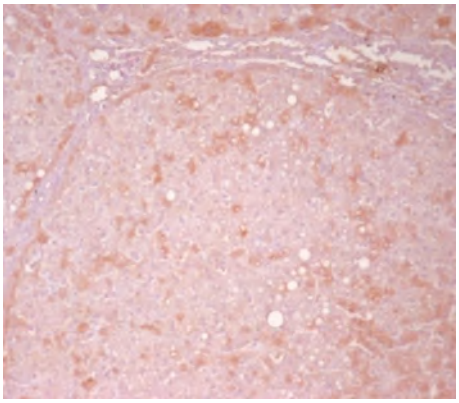

Fig. 3 : CD142 immunostaining (low expression) in liver cirrhosis. (original magnification X 100).

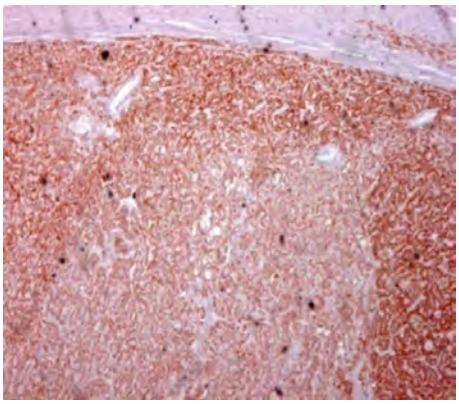

Fig. 2 : Increased intensity of the VEGF staining at the periphery of the tumor. (Original magnification X 100).

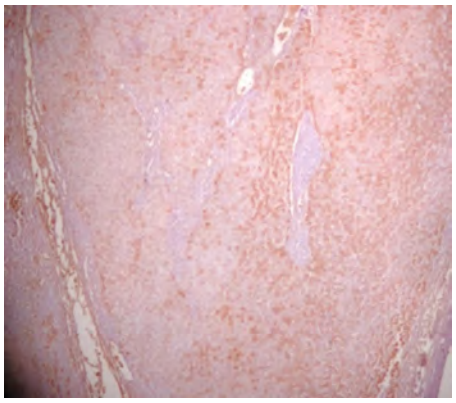

Fig. 4 : CD142 immunostaining (high expression) in liver cirrhosis. (original magnification X 100).

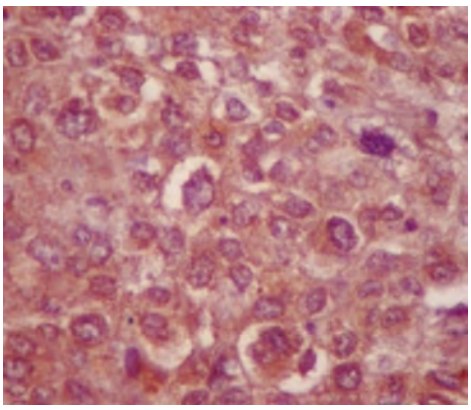

Fig. 5 : CD142 immunostaining (high expression) in grade III HCC. (original magnification X 400).

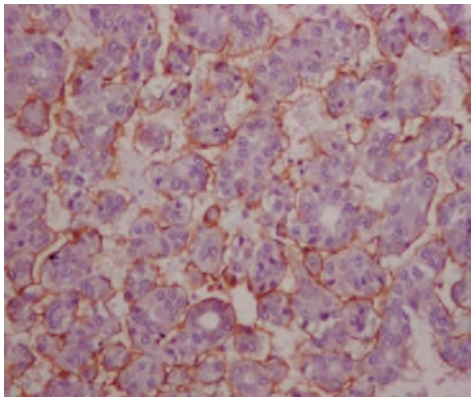

Fig. 7 : A case of grade I HCC stained by anti CD34 immunostaining showing high MVD. (original magnification X 200).

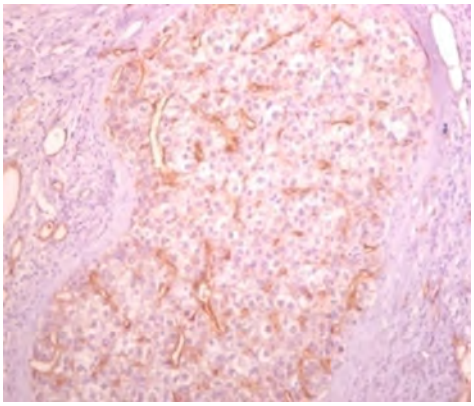

Fig. 6 : A case of dysplastic cirrhotic nodule showing high MVD as assessed by anti-CD34 immunostaining. (original magnification X 200).

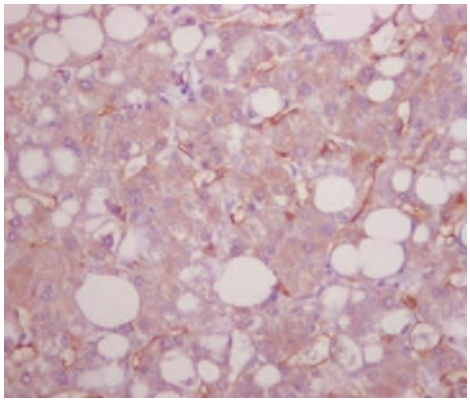

Fig. 8 : A case of grade III HCC stained by anti CD34, showing high MVD. (original magnification X 200).

Discussion

HCC is a highly malignant tumor with a propensity for vascular invasion and metastasis. Hepatic resection is the treatment of choice for HCC, but the prognosis after resection remains unsatisfactory because of a high incidence of recurrence related to tumor metastasis.[22] Tumor angiogenesis is an important determinant of invasiveness and progression of HCC.[23-25] However, little is known about the regulatory mediators of angiogenesis in HCC.

This study showed a positive correlation between VEGF degree of staining and vascular emboli (P<0.001). There was 27 cases (58.7%) associated with vascular invasion and showed marked degree of VEGF staining. Deli et al [26] found that VEGF expression in HCC has a significant correlation with vascular invasion, intrahepatic metastasis and shortened survival rates. Also, this study has demonstrated a positive correlation between tumor size and intrahepatic metastasis and expression of VEGF and this is consistent with that reported by the Moon et al study.[20]

According to Saaristo et al,[27] VEGF is a cell specific mitogen and is a major inducer of angiogenesis in human cancers. There is some evidence that VEGF is an important angiogenic mediator of HCC.[21,28-30] Yoshiji et al[31] have also shown that VEGF expression increases significantly during fibrogenesis and carcinogenesis and that the combined effect of VEGF & its receptor reflect the combined effect of both on hepatic stellate cells and endothelial cells. Ivarone et al[42] concluded that VEGF appears to be involved in the development of HCC and could be a predictor of HCC development in patients with cirrhosis.

In our study, there was positive correlation between grades of the tumor and degree of VEGF expression (P<0.05). In agreement with our results, Yoo et al[33] reported increased expression of VEGF in poorly differentiated tumors and decreased in well differentiated ones. However, Hammam et al[34] found that VEGF expression showed insignificant increase in HCC compared to LC. Moreover, they found that with differentia-

tion of HCC, VEGF showed high expression in grade I and decreased in grade II and grade III.

Increased expression of VEGF receptors in HCC was demonstrated on different levels, including mRNA and protein.[35,36] The patterns of VEGF expression (mRNA or protein expression) in HCC and surrounding liver tissue are still-controversial. Most of the studies report that the mRNA VEGF expression level is higher in HCC than in the surrounding hepatic tissue.[37,38] The expression of VEGF protein is inconsistent, demonstrating a higher level either in HCC or in the surrounding liver tissue.[39,40]

In our study, VEGF expression is increased significantly in HCC relative to LC. Shimamura et al[35] and Hassan et al[41] studies concluded that the increased level of VEGF in HCV-related HCC is the result of VEGF gene amplification. In the study of Tseng [42] the presence of viremia (either HBV or HCV) was associated with VEGF overexpression in patients with HCC, being a poor prognosis factor in these patients. As all our cases were HCV positive, this may explain the high expression levels of VEGF (95.7% of cases) in this study.

In our study, 39.1% of cases showed that VEGF expression was more intense at the marginal area of the tumor than in the center. This can be explained by the fact that rapid cell proliferation in the center of a tumor can lead to increased interstitial fluid pressure, which may result in compression closure of capillaries and consecutive tissue necrosis. Central necrosis areas cause a suppression of VEGF protein synthesis.[26]

This study showed higher expression of VEGF in the surrounding cirrhotic liver tissue compared to HCC in some cases. This is explained by Deli et al,[26] who suggest that the sustained mechanically-reduced blood flow affects the hepatocytes at the cirrhotic area. This leads to decreased oxygen pressure and strongly upregulates VEGF transcription and protein synthesis in the cirrhotic area.

By immunohistochemical stain-

ing, we showed that CD142 wasexpressed by HCC tumor cells in 44(95.7%) of the 46 specimens. However, there was a wide variation in CD142 expression among different grades of tumor. In our analysis, we observed significantly high (P<0.05%) expression of CD142 in HCC compared to LC. There are no previous studies for CD142 expression in cirrhotic liver tissue adjacent to HCC. Also, there was positive correlation between grades of the HCC and degree of CD142 expression (P<0.05). This result is consistent with the Poon et al study[43] who concluded that TF expression was up-regulated in moderately or poorly differentiated HCC compared with well differentiated HCC.

Our study showed a positive correlation between CD142 and VEGF expression in both cirrhosis and the tumor. This may explain the possible pathway through which CD142 is involved in the angiogenesis of HCC. Rickles et al.[44] has reported that CD142 mayregulate tumor angiogenesis in HCC via up-regulation of VEGF.

According to our results, CD142 expression has a significant correlation with vascular invasion, intrahepatic metastasis and tumor size. On the other hand, Poon et al[43] found no significant correlation between tumor CD142 expression and tumor size and reported that tumor CD142 expression may influence tumor invasiveness independent of tumor size.

In our study, there was significant increase (P<0.05) in MVD-CD34 expression from cirrhotic nodules to dysplastic nodules to HCC. This result is similar to that obtained by Park et al[45], Kim & Hu,[46] and Ma Jee[47] study. CD34 was closely associated with neo-vascular process in cirrhosis and hepatocellular carcinoma. Di Carlo,[48] studied by immunohistochemistry, the expression and distribution of CD34 in liver cirrhosis and HCC. They found that the sinusoids of the liver showed no or focal immunoreactivity for CD34, an increased immunoreactivity was observed in the periportal sinusoids of cirrhotic nodules, whereas diffuse and strong staining was in overall

HCC. They concluded that immunoreactivity for CD34 represents an effective method to evaluate angiogenesis. Ma Jee et al[47] concluded that CD34 is a useful marker for distinguishing HCC from non-cancerous liver tissue.

The study by Namashima et al[49] using CD34 staining for MVD-CD34, concluded that MVD representing tumor angiogenesis offers a new candidate prognostic factor in HCC to predict tumor recurrence and patient survival. Also, Poon et al[43] found that high MVD-CD34 is predictive of early post-resection recurrence in patients with HCCs ≤ 5 cm.

According to the results of our study, MVD-CD34 expression was significantly increased ($P<0.05$) from grade I to grade II to grade III to grade IV. Park et al[45] studied CD34 in HCC and found that 14 out of 21 cases of moderate to poorly differentiated HCC showed strong positivity, while it was seen in three out of 11 cases of well differentiated HCC. The importance of neovascularization in the progress of HCC has been high lightened suggesting that microvessels increase gradually from cirrhotic nodules through low grade and high grade dysplastic nodules with the greatest number recorded in HCC. Ma Jee et al[47] found significant difference ($P<0.05$) in the expression of CD34 between well differentiated and moderately differentiated HCC. Amarapukar et al[10] suggested that angiogenesis as assessed by CD34 expression play an important role in carcinogenesis.

According to our analysis, MVD-CD34 expression has a significant correlation with vascular invasion and tumor size. This result is similar to that obtained by Messerini et al.[50]

This study has demonstrated that MVD-CD34 was significantly higher in tumors with high immunoreactivity for CD142 than in tumors with low immunoreactivity for CD142. Also, we found highly significant association between VEGF and CD142 expression in both LC and different grades of HCC. All cases of LC and HCC with high CD142 immunoreactivity also showed marked VEGF expression. On the other hand, all

cases of LC and HCC with low CD142 immunoreactivity also showed mild to moderate VEGF expression. Furthermore, cases with negative CD142 immunoreactivity showed negative expression for VEGF in both LC and HCC. To our knowledge, this is the first study that evaluated the CD142 expression in LC and its relationship with angiogenic factors VEGF and MVD-CD34 expression in LC and different grades of HCC.

Conclusion

Overexpression of CD142, VEGF and CD34 is correlated with the factors of poor prognosis, like tumor size, vascular invasion and intrahepatic metastases. Expressionof CD142 in HCC is related to tumor angiogenesis and invasiveness through up-regulation of VEGF and MVD-CD34. This novel finding may provide insight into a new therapeutic strategy for HCC by inhibiting CD142 expression as it plays an important role in the development and progression of HCC. Also, there is a link between HCV infection, angiogenesis and hepatocarcinogenesis.

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CD142, VEGF AND MICROVASCULAR DENSITY MVD-CD34 EXPRESSION IN HEPATOCELLULAR CARCINOMA OF PATIENTS WITH CIRRHOSIS AND CORRELATION WITH TUMOR GROWTH AND PROGRESSION

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A RANDOMIZED TRIAL OF CARBOPLATIN AND WEEKLY PACLITAXEL VERSUS CISPLATIN-CYCLOPHOSPHAMIDE IN PATIENTS WITH ADVANCED OVARIAN CARCINOMA : LONG-TERM EFFICACY RESULTS

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Abstract

Background: *Chemotherapy combinations that include an alkylating agent and a platinum coordination complex have high response rates in women with advanced ovarian cancer. Such combinations provide long-term control of disease in only few patients. Therefore, we compared the long- term efficacy and toxicity of the two combinations, cisplatin and cyclophosphamide versus carboplatin and paclitaxel, in women with advanced ovarian cancer.*

Methods: *We randomly assigned 75 women with advanced ovarian cancer and residual masses after initial surgery to receive either cisplatin (75 mg/m2) with cyclophosphamide (750 mg/m^2), repeated every 3 weeks or paclitaxel (80 mg/m^2 over a period of one hour weekly for three weeks with one week rest) with carboplatin at a dose of AUC 6 day 1, repeated every 4 weeks. Progression-free survival was the primary end point.*

Results: *The overall response rate was 80.5% in the paclitaxel group and 59% in the cyclophosphamide group; the complete remission rates were 44.4% and 20.5%, respectively; both differences were statistically significant (P=0.030, P=0.028, respectively). At a median follow-up of 38.5 months, a longer progression-free survival (long-rank P=0.027; median of 17 months versus 11 months) and a longer overall survival (long-rank P=0.001; median of 36 months versus 23 months) were*

seen in the paclitaxel regimen compared with the cyclophosphamide regimen. There were also statistically significant differences in 3-years and 5-years survival rate (50% and 27.8% vs 20.5% and 5.1%; P=0.001 and P=0.002) and 3-years and 5-years progression-free survival rate (27.7% and 13.9% vs 15.4% and 2.6%; P=0.028 and P=0.036) between paclitaxel group versus cyclophosphamide group, respectively.

Conclusions: *Incorporating paclitaxel into first-line therapy improves the duration of progression-free survival and of overall survival in women with incompletely resected stage IIB, IIC, III and IV ovarian cancer. Also, weekly paclitaxel associated with reduced toxicity. Our study support paclitaxel-carboplatin as a standard regimen for treatment of patients with advanced ovarian cancer.*

Key words: *Carboplatin; Paclitaxel; Cisplatin-Cyclophosphamide; advanced ovarian carcinoma.*

Introduction

Epithelial ovarian cancer (EOC) is the second most common gynecologic malignancy in the United States and it is still the most common cause of death from gynecological cancer[1,2]. Despite ongoing research in screening, the majority of patients continue to be diagnosed with advanced-stage disease. For these patients, treatment consists of a combination of surgical debulking and chemotherapy[3]. Platinum-based chemotherapy remains the cornerstone of treatment for ovarian carcinoma, and over the last 20 years surgical cytoreduction plus chemotherapy has improved the 5-year survival in the United States [4].

In patients with advanced ovarian cancer, a combination of cisplatin and cyclophosphamide was standard treatment. Unfortunately, long-term disease control with this regimen occurs in less than 10 percent of women with incompletely resected stage III disease and less than 5 percent of women with stage IV disease [5].

The Gynecologic Oncology Group (GOG) 111 study established cisplatin and paclitaxel as the standard front-line chemotherapy [6]. Because this combination requires a 24-hour hospitalization and given the significant oto-, nephro-, and neurotoxicity associated with cisplatin, carbop-

latin was substituted for cisplatin in GOG 158, with an equivalent response rate and a better toxicity profile[7]. A cumulative analysis of seven phase I/II trials, evaluating the combination of carboplatin and paclitaxel, showed an overall response rate of 81.4% with a complete response rate of 47.2%, further suggesting its equivalency with cisplatin and paclitaxel in the front-line therapy of ovarian cancer[8].

The combination of carboplatin and 3-hour paclitaxel was found to be a safe and highly active regimen in a phase 1 trial conducted by the GOG[9]. On the basis of this small study and the ease and safety of outpatient administration, the combination had been adopted by most physicians even before any study was performed to demonstrate its equivalence with the longer infusion of paclitaxel with cisplatin. Although carboplatin was equivalent to cisplatin in combination with cyclophosphamide, assuming that the same will be true in combination with paclitaxel is somewhat dangerous. There is a well-known interaction between carboplatin and paclitaxel on the megakaryocyte such that carboplatin causes less thrombocytopenia in combination with paclitaxel than when carboplatin is used alone. A similar effect could also be operational at the level of the tumor cell. Thus, a GOG study was critically important; it compared these two regimens in patients with optimally debulked disease; that is, short-infusion (3-hour) paclitaxel 175 mg/m^2 and carboplatin (area under the curve [AUC] 7.5) with long-infusion (24-hour) paclitaxel 135 mg/m^2 and cisplatin 75 mg/m^2. Recent data from that study strongly indicate that the carboplatin and paclitaxel doublet will not be less effective than the cisplatin and paclitaxel doublet and will have a better toxicity profile [8,9].

A second study performed by a German consortium (Arbeitsgemeinschaft Gynakologische Onkologie) asked only the question of which platinum analog was preferred (stages IIB to IV), with all patients receiving paclitaxel 185 mg/m^2 for 3 hours and either cisplatin 75 mg/m^2 or carboplatin (AUC 6)[10]. Again, no significant

outcome differences were noted, although the hazard ratio of 0.86 favored the cisplatin arm compared with the carboplatin arm, which is the reverse of the results seen in GOG-158. Some investigators have suggested that differences in dose-intensity of carboplatin in the two trials may explain these opposite trends. However, grade 1 or greater neurotoxicity was significantly greater in the cisplatin arm (56% v 35%) and was slower to resolve, demonstrating once again that the doublet of short-infusion paclitaxel with cisplatin is excessively neurotoxic and cannot be recommended [7, 11].

In order to minimize toxicity, paclitaxel can be given weekly instead of 3-weekly[12,13,14]; this results in a higher dose intensity of the drug[15]. Weekly paclitaxel is a highly active and well tolerated regimen that is increasingly being adopted for the treatment of relapsed ovarian cancer. This regimen is usually administered at 80-90 mg/m^2/week, and the use of a 1h infusion helps minimize myelosuppression. When compared with the 3-weekly schedule, weekly paclitaxel is better tolerated, with a reduced frequency of grade 3-4 toxic effects (12). Two non-randomized trials 16, 17 have suggested that the activity of paclitaxel in EOC is dose-dependent, and a randomized trial 14 has shown reduced toxicity with weekly scheduling without any detriment to efficacy.

Because of the poor long-term results of standard therapy, we conducted this prospective, randomized trial in order to assess the antitumor efficacy, tolerability and safety of combined carboplatin and weekly paclitaxel as first-line treatment in women with incompletely resected stage IIB, IIC, III or IV ovarian cancer and compare its efficacy with the standard therapy of cisplatin - cyclophosphamide.

Patients and methods

Between January 2005 and August 2007, eighty patients with advanced ovarian carcinoma who attended at department of Clinical Oncology and Nuclear Medicine at Mansoura University Hospital and Health Insurance Hospital were enrolled in our study.

Eligibility Criteria:

All patients included in the study had histologically proven epithelial ovarian cancer and FIGO stage IIB, IIC, IIIA, IIIB, or IV disease. Women had to have their initial surgical procedure within less than 8 weeks of recruitment, and their initial surgical procedure could have consisted of an optimal (≤ 1-cm residual mass) or a suboptimal (> 1-cm residual mass) tumor cytoreduction. Informed consent was obtained from all patients according to the requirements local human biomedical ethics committees.

Other criteria for inclusion were as follows: chemonaivety, a performance status (WHO) of 0-2, age of between 18 and 75 years, leukocytes >4.0 x 10^9/l and <12.0 x 10^9/l, platelet count >100 x 10^9/l, Hgb >10 g/dl, total bilirubin <1.5 mg/dl, serum creatinine <1.5 mg/dl, serum GOT/GPT <2x upper limit of normal, 24 h creatinine clearance >60 ml/min, absence of cardiovascular disease, life expectancy of at least 3 months and absence of any past or current history of other neoplasma.

Exclusion criteria were previous pelvic irradiation (limited prior radiotherapy, for example a single fraction for bone pain, was allowed); borderline histology; central or peripheral neuropathy, common toxicity criteria (CTC) grade 2; bowel obstruction; significant comorbidities; history of a second malignancy, cerebral metastases and pregnancy or lactation.

Pretreatment Evaluation:

Baseline examinations included a complete history and physical examination with documentation of all measurable disease. Further analyses included : a complete blood count, blood chemistry analyses including liver and renal function test, ovarian tumor marker CA-125, electrolytes, urine analysis, chest X-ray, electrocardiogram and a CT scan to document measurable disease.

Treatment:

Patients were randomized to standard-therapy (arm A) received cyclophosphamide (750 mg/m^2 intravenously) and cisplatin (75 mg/m^2 intravenously at the rate of 1 mg per minute) repeated every

three weeks or paclitaxel was administered weekly in a planned dose of 80 mg/m^2/week for three weeks with one week rest and carboplatin was given at a dose of AUC 6 on day 1 (arm B). Treatment was administered every 4 weeks for a minimum of six cycles unless there was progression, unacceptable toxicity or patient refusal. In both treatment groups, after six courses the patients could continue therapy for three further cycles if, in the opinion of the attending physician, further clinical benefit could be expected.

Stratification factors included the FIGO stage (IIB-C, III, or IV), the amount of residual disease (1 cm, or >1 cm), the WHO performance status (0, 1 or 2), and the tumor grade (well differentiated, moderately differentiated, poorly differentiated).

Treatment was repeated if blood counts recovered (neutrophils 1500/mm^3 and platelets 100 000/mm^3) and non-hematological toxicity recovered to grade 1. A delay of more than 42 days in any one course of treatment administration was sufficient to have the patient transferred out of the study.

Paclitaxel was given over 1-hour prior to platinum compounds. All patients were premedicated 30 to 60 minutes before paclitaxel with dexamethasone 20mg intravenously (iv), diphenhydramine 50 mg iv, and a histamine 2 blocker (such as cimetidine 300 mg or ranitidine 50 mg iv). The recommended antiemetics schedule comprised 5-HT3 inhibitors (administered IV at the begining of infusion, 4 hours and 8 hours afterwards). The AUC (area under the plasma concentration versus time curve) was calculated according to the Calvert formula[18]. The carboplatin dose was determined by the AUC method of Calvert [dose in mg = AUC x (GFR + 25)]. The AUC chosen was 6 in arm B. Carboplatin was diluted in 250 ml of 5% dextrose and infused over a period of 30-60 min. Premedications with corticosteroids, antiemetics and hydration were administered before and after cisplatin administration in arm A. Antiemetics were mandatory. The recommended antiemetics schedule comprised 5-HT3 inhibi-

tors (administered iv at the begining of infusion, 4 hours and 8 hours afterwards).

Evaluation during the study included: (i) before each course of therapy : a history and physical examination, a complete blood count, differential count, platelet count, blood chemistry survey with renal and liver function tests, and evaluation of measurable disease by physical examination; (ii) CA-125 was measured following every two courses; and (iii) appropriate imaging studies were performed to assess measurable disease every three cycles.

WHO response criteria were employed for evaluation of measurable disease[19]. All adverse effects were graded according to the toxicity criteria of the Gynecologic Oncology Group[9].

Criteria for withdrawal from the study:

Patients were removed from the study for any one of the following reasons: (i) evidence of disease progression after a minimum of one cycle of therapy; (ii) development of unacceptable toxicity; or (iii) patient refusal or inability to comply with protocol requirements.

After three cycles of therapy, a formal assessment had to be made, and patients had to be categorized with regard to their current disease status. By use of clinical, CA(125) and radiological assessment, the patients were assigned to one of four subgroup categories: those who progressed clinically or who were unchanged clinically or those who showed partial clinical response or complete clinical response. Patients categorized as progressed clinically finished the protocol treatment and were allowed to receive any secondary treatment. All of the other patients were scheduled to receive three further cycles of protocol treatment.

After six cycles of protocol treatment, the patients had to be categorized with regard to their final response status with the use of clinical, CA(125) and radiologic assessments and/or second-look surgery assessment and the same subcategories as defined above. Patients not showing dis-

ease progression at this point could cease all cytotoxic therapy or could receive three additional cycles of protocol treatment.

Radiologic investigations to document the status of all measurable lesions noted at baseline had to be repeated after three, six, and nine cycles of chemotherapy. Once patients were off the protocol therapy, they were monitored for assessment of disease status every 3 months for 2 years and every 6 months thereafter. Monitoring comprised clinical examination and CA(125) estimation; routine computed tomography scans were required and were requested also if the CA(125) level rose and/or symptoms developed.

The changes in CA(125) levels during chemotherapy post-surgery in patients were analyzed. CA(125) level reduction or increased used as an early sign of response or progression, respectively. However, this finding must be confirmed by radiological image.

End points

Progression-free survival (PFS), the primary end point, was defined as the interval between the date of randomization and the date of progression of the disease. Other study end points included clinical response rate, overall survival (OAS) and the potential use of CA125 as a surrogate for patient outcome. Overall survival was defined as the interval between the date of randomization and the date of death or last follow-up. A complete response (CR) was defined as the disappearance of all clinical evidence of tumor, including normalization of CA125 level. A partial response (PR) was defined as a 50% or greater decrease in the sum of the products of the perpendicular diameters of the measured lesions. Stable disease was defined as a steady state of response less than a PR or progression less than 25% lasting at least 4 weeks. Progression disease (PD) was defined as the unequivocal increase of at least 25% in the sum of the products of the perpendicular diameters of the measured lesions.

Statistical analysis:

Pre-treatment patients' characteristics of both treatment groups were compared using the Chi-

square test. Responses were compared using the Pearson's Chi-square test. Mann-Whitney U test was used to compare the median response, survival and progression-free survival in both treatment groups. Survival curves were generated by the Kaplan-Meier life table method and were compared using the Log-rank test. The relative risk and corresponding confidence intervals (CIs) were calculated using Chi-square test. Prognostic factors related to response, survival and progression-free survival were studied by univariate and multivariate analysis using Cox proportional hazards regression model. Multivariate analysis of variables predictive of survival was based on a logistic regression model. The response rate and survival outcomes of patients with various levels of CA (125) were studied using Kaplan-Meier method. Multivariate Cox regression model was used to assess the correlations between survival and the change in CA(125) level during chemotherapy and other prognostic factors. SPSS Version. 9 was used for calculations. P value is considered significant if it is <0.05.

Results

Characteristics of the Patients

Between January 2005 and August 2007, 80 patients with epithelial ovarian cancer entered this trial, 40 patients in each treatment group. Five patients were ineligible: two patients of which had the wrong primary tumor, one patient had a history of cancer, and two patients had inappropriate stage. The remaining 75 eligible women were randomly assigned to either the cisplatin-cyclophosphamide group (no. = 39 patients, group A) or the carboplatin-paclitaxel group (no. = 36 patients, group B). The two groups were balanced for several prognostic factors (Table1).

Chemotherapy Administration

A total of 458 cycles were administered; 225 to the 39 patients treated with cisplatin- cyclophosphamide and 233 to the 36 patients treated with paclitaxel-carboplatin. A median number of six courses (cycles) in both groups, with a range from 3 to 9, was given to each treatment group. The proportions of patients continued treatment to cycle 6 was 51.3% in the cisplatin- cyclo-

phosphamide group versus 61.1% in the paclitaxel-carboplatin group; 25.6% in the cisplatin- cyclophosphamide group and 30.6% in the paclitaxel-carboplatin group received treatment from 7 to 9 cycles. Nine patients (23.1%) in cisplatin- cyclophosphamide group and three patients (8.3%) in paclitaxel–carboplatin group did not complete all six cycles of therapy because of disease progression, toxicity or death. Dose reduction was uncommon, occurring in 11.1% of cycles in arm A and 8.6% of cycles in arm B. For patients who did experience the previously defined adverse hematologic toxic reaction, a 20% reduction in the paclitaxel or cyclophosphamide dosages was planned, with no reduction in the cisplatin or carboplatin dose. The use of granulocyte colony-stimulating factor was accepted only if adverse hematologic toxic effects recurred despite an initial dose reduction. However, dose delay was significantly more frequent in the cisplatin- cyclophosphamide arm (38.2% versus 19.7% of the cycles in arm A versus arm B, respectively; P=0.006). The main reason for delay was absence of hematological recovery (granulocytes or platelets) by day 21 in arm A and by day 28 in arm B.

Toxicity

Analysis of toxicity has been carried out in 75 patients who started their treatment and had at least one course documented for the occurrence of treatment-related side effects. The percentage of patients with grade 1-4 adverse effects is displayed in Table 2 according to treatment group; to facilitate comparisons with the GOG study. Significant (grade 1-4) non-hematological toxicity was more commonly associated with the paclitaxel-carboplatin combination. The more relevant toxicities observed in arm B were: grade 1-2 alopecia in 38.8%, grade 1-2 myalgia/arthralgia in 33.3% and 24.9% of patients and peripheral neurosensory toxicity in 36.1% of patients. All these toxicities were significantly more frequent in arm B than in arm A. All patients with peripheral neuropathy had grade 1-2 toxicity, and no grade 3-4 was observed.

In contrast, grade 3 vomiting

was considerably more frequent in the arm A. Other toxicities of nausea and vomiting, asthenia and anorexia were of mild to moderate intensity, and more commonly associated with cisplatin-cyclophosphamide combination. Of note, ototoxicity was encountered in 5.1% of the patients in the cisplatin-cyclophosphamide group. A rise in the creatinine level above the upper normal limit (grade 1 or grade 2) was also a relatively infrequent event : 10.2% in the arm A and 2.7% in arm B. The general acceptance of the two regimens was reflected by the low proportion of patients' prematurely discontinuing treatment for toxicity.

It is interesting that, in this trial in which a weekly 1-hour paclitaxel infusion time was used, febrile neutropenia (defined as fever 38°C with a neutrophil count of <0.5 x 10^9/L) was rare and occurred only in one patient in arm A and arm B. No toxic deaths were observed. Grade 3 or 4 toxicity rates for white blood cell counts, granulocyte counts, platelet counts, and hemoglobin levels were lower in the arm B than in the arm A, but the difference was statistically insignificant.

Clinical Response

Clinical response could be assessed in 75 patients who entered the study with clinically or radiologically measurable disease. The overall response rate was 80.5% in the arm B and 59% in arm A (P=0.030). Complete clinical responses were more frequent among women treated with carboplatin and paclitaxel (44.4%) than among those treated with cisplatin and cyclophosphamide (20.5%) (P =0.028), the differences were statistically significant. Five Patients (13.9%) in arm B and arm A (12.8%) had stable disease. Two Patients (5.6%) in the arm B versus eleven patients (28.2%) in arm A had progression of disease (table 3).

Progression-free Survival and Overall Survival

After a median follow-up of 38.5 months, both progression-free survival and overall survival were statistically significantly longer for the patients in the paclitaxel-carboplatin group. Fig. 1 and Fig. 2 show the progression-

free and overall survival curves, respectively, for all patients entered in the trial. The median PFS was 17 months (95% CI 8.194 - 25.806) for patients in the arm B and 11 months (95% CI 8.023 - 13.977) for patients in the arm A (P=0.027). The estimated 2-years, 3-years and 5-year PFS rates were 33.3%, 27.7% and 13.9% versus 20.5%, 15.4% and 2.6%, respectively. An approximately 13-months difference in median overall survival was particularly substantial in favor of the paclitaxel-carboplatin arm (log-rank P=0.001; median of 36 months (95% CI 26.180 - 43.820) for the arm B versus 23 months (95% CI 18.054 - 27.946) for the arm A). The estimated 2-years, 3-years and 5-year OS rates in arm B and arm A were 61.1, 50% and 27.8% versus 33.3%, 20.5% and 5.1%, respectively.

Normalization of CA(125) level after 3 cycles of chemotherapy had highly statistically significant difference as regard prediction of response, survival and progression free survival in arm A (P=0.0001, P=0.0001, P=0.0001, respectively) and arm B (P=0.0001, P=0.0001, P=0.0001, respectively) than those with persistence or elevated CA(125) after 3 cycles of chemotherapy. CA(125) level after three cycles of chemotherapy post-surgery is an independent predictor of survival for advanced ovarian carcinoma in both groups. Whatever the patients undergo, optimal or suboptimal cytoreduction, if the CA(125) becomes normalized after three cycles of chemotherapy, they would have more favorable prognosis than those with elevated CA (125) after three cycles of chemotherapy in both treatment groups.

On univariate analysis, the most important predictor of response was stage (P=0.001), amount of residual disease (P=0.003), number of chemotherapy cycles (P<0.0001) and level of CA125 changes after 3 cycles of chemotherapy (P<0.0001) in arm A and was stage (P<0.0001), amount of residual disease (P=0.001), number of chemotherapy cycles (P<0.0001) and level of CA125 changes after 3 cycles of chemotherapy (P<0.0001) in arm B. However, the most important predictor of progression-

free survival and survival were stage (P=0.029), (P=0.003), amount of residual disease (P=0.001), (P=0.001), response rate (P=0.002), (P=0.007), grade (P=0.002), (P=0.020) and level of CA125 changes after 3 cycles of chemotherapy (P=0.002), (P=0.001) in arm A and was stage (P<0.0001), (P<0.0001), amount of residual disease (P<0.0001), (P<0.0001), response rate (P<0.0001), (P<0.0001), grade (P<0.0001), (P<0.0001), pathology (P=0.001), (P=0.006) and level of CA125 changes after 3 cycles of chemotherapy (P<0.0001), (P<0.0001) in arm B.

Results of the Cox regression multivariate analysis for OAS revealed that several prognostic factors including worse performance status (1 versus 0: HR = 1.3, 95% CI 0.85-1.85, P=0.24; 2 versus 0: HR = 2.5, 95% CI 1.5-4.0, P <0.001), worse stage (III versus IIC: HR=3.1, 95% CI 1.24-7.72, P=0.02; IV versus IIC: HR = 5.2, 95% CI 1.94-13.79, P=0.001) and high level of CA125 after 3 cycles of chemotherapy (high level versus decreased (normalized) level: HR = 1.6,95% CI 1.1-2.3, P=0.01), were related to significantly poorer survival.

Regarding PFS, the hazard of disease progression at any time was significantly higher with worse performance status (1 versus 0: HR=1.1, 95% CI 0.75-1.53, P=0.7; 2 versus 0: HR=2.1, 95% CI 1.34-3.33, P=0.001), worse stage (III versus IIc: HR=2.6, 95% CI 1.25-5.52, P=0.01; IV versus IIc: HR=4.0, 95% CI 1.74-9.1, P=0.001), presence of residual disease (>1 cm versus 1 cm: HR=2.4, 95% CI 1.61-4.21, P=0.001) and high level of CA125 after 3 cycles of chemotherapy (high level versus decreased (normalized) level: HR = 2.5, 95% CI 1.20-5.48, P=0.01).

Table 1 : Patients' characteristics according to treatment groups.

Characteristic	Arm A (N=39) No.	%	Arm B (N=36) No.	%	P value
Age:					
≤ 60 years	29	74.4	26	72.2	0.834
> 60 years	10	25.6	10	27.8	
Median age (range)	50 (18-75)		51 (23-74)		
WHO performance status:					
0	11	28.2	10	27.8	
1	19	48.7	18	50	0.895
2	9	23.1	8	22.2	
FIGO stage:					
IIB or IIC	9	23.1	14	38.9	
III	19	48.7	19	52.8	0.130
IV	11	28.2	3	8.3	
Amount of residual disease:					
≤ 1 cm	20	30.8	18	50	0.218
> 1 cm	27	69.2	18	50	
Cell type:					
Serous adenocarcinoma	14	35.9	16	44.4	
Endometroid adenocarcinoma	9	23.1	6	16.7	0.849
Mucinous adenocarcinoma	8	20.5	9	25	
Clear-cell adenocarcinoma	3	7.7	2	5.6	
Other	5	12.8	3	8.3	
Tumor grade:					
1: well differentiated	12	0	1	2.8	0.200
2: moderately differentiated	0	43.6	13	36.1	
3: poorly differentiated	22	56.4	22	61.1	
Number of cycles of chemotherapy:					
≤ 4 cyles	•	23.1	3	8.3	
6 cycles	9	51.3	22	61.1	0.238
> 6 cycles	10	25.6	11	30.6	

Arm A : Cisplatin + Cyclophosphamide. **Arm B : Paclitaxel + Carboplatin.**

Table 2 : Toxicity effects according to severity and treatment groups.

Toxic Effects	Arm A Grade of severity (%)				Arm B Grade of severity (%)			
	1	2	3	4	1	2	3	4
Neutropenia	15.4	25.6	35.9	25.6	19.4	25	30.5	19.4
Thrombocytopenia	41	10.2	5.1	2.5	30.5	5.5	2.7	0
Anemia	15.4	35.9	5.1	0	25	11.1	5.5	0
Gastrointestinal symptoms								
Nausea	25.6	15.4	5.1	0	19.4	0	0	0
Vomiting	41	30.7	5.1	0	25	11.1	0	0
Alopecia	20.5	5.1	-	-	33.3	5.5	-	-
Arthralgia	10.2	2.5	0	0	19.4	5.5	0	0
Myalgia	15.4	2.5	0	0	25	8.3	0	0
Neurologic symptoms	5.1	0	0	0	25	11.1	0	0
Allergic reaction	2.5	2.5	0	0	2.7	2.7	0	0
Renal symptoms	5.1	5.1	0	0	2.7	0	0	0

Arm A : Cisplatin + Cyclophosphamide. **Arm B : Paclitaxel + Carboplatin.**

Table 3 : Response rate according to treatment groups.

Response	Arm A (N=39) No.	%	Arm B (N=36) No.	%	P value
Overall response rate(OAR)	23	59	29	80.5	0.030
Complete response(CR)	8	20.5	16	44.4	0.028
Partial response (PR)	15	38.5	13	36.1	
Stable disease (SD)	5	12.8	5	13.9	
Progressive disease (PD)	11	28.2	2	5.6	

Arm A : Cisplatin + Cyclophosphamide. Arm B : Paclitaxel + Carboplatin.

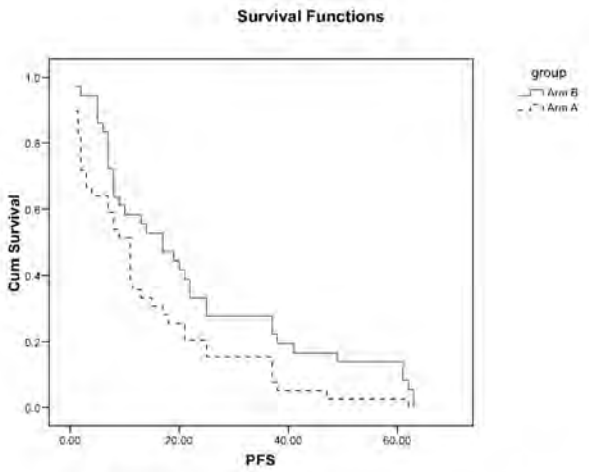

Fig. 1 : Progression free survival of both treatment groups.

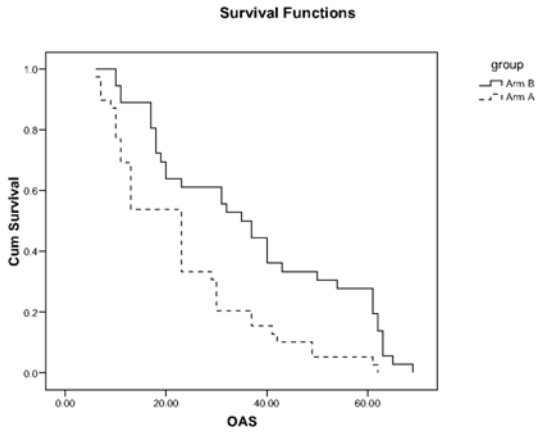

Fig. 2 : Overall survival of both treatment groups.

Discussion

The standard chemotherapy regimen for ovarian cancer in the last two decades has been cyclophosphamide-cisplatin ± adriamycin combination chemotherapy[20,21]. However, as a result of study 111 of the Gynecologic Oncology Group (GOG), it was found that a combination therapy of paclitaxel (135 mg/m^2, 24 h DIV) and cisplatin has a significantly better response rate and PFS than that of cyclophosphamide-cisplatin[5,6]. In 1994, a European and Canadian multicenter joint research project found these results to be impressive but not conclusive enough. They believed that (a) further data were required before the paclitaxel/cisplatin combination could be adopted as the new standard first-line chemotherapy regimen for this disease, (b) the paclitaxel/cisplatin regimen could be improved by increasing the dose of paclitaxel and shortening its infusion time. Using a 3 h intravenous paclitaxel infusion, side effects such as bone marrow suppression were significantly reduced. (c) more knowledge was needed regarding the comparative quality of life and economic impacts of these competing regimens. An allergic reaction to paclitaxel was also controlled by premedication. This joint research group reported that in the comparative test between the carboplatin-paclitaxel 175 mg/m^2 (3 h DIV) chemotherapy and the cyclophosphamide-cisplatin chemotherapy, the PFS was extended significantly in the former, verifying the GOG 111 study. The subjects were 680 ovarian cancer patients, including patients who had previously been treated with other regimens. They concluded that carboplatin-paclitaxel therapy was highly effective for the treatment of ovarian cancer [11].

A number of studies on combination chemotherapies of platinum agents and paclitaxel have been carried out in Western countries (5-7, 11, 20-25). The rationale behind the current use of carboplatin in this combination chemotherapy is as follows. (i) Carboplatin alone is as effective as cisplatin alone for the treatment of ovarian cancer [26,27]. (ii) Carboplatin is significantly less nephrotoxic than cisplatin. Hydration is not necessary when

administering carboplatin, unlike with cisplatin[18]. (iii) The non-hematological toxicity of carboplatin is less than that of cisplatin; this is important because there is concern about the anxiety produced by the peripheral nervous toxicity in cisplatin-paclitaxel combination chemotherapy[7,10]. (iv) Using the carboplatin dose obtained from AUC and GFR by Calvert's equation, it is possible to control platelet reduction [18,28].

In randomised trials[14,16], chemotherapy-induced myelo- and neurotoxicity in patients with EOC was significantly reduced without any detriment to efficacy when paclitaxel was administered weekly instead of 3-weekly. Furthermore, in vitro cancer models have demonstrated that paclitaxel can have antiangiogenic effects and these are thought to be more pronounced with weekly scheduling. Remarkably, a recently published phase II trial showed responses to weekly paclitaxel in EOC patients' refractory to 3-weekly paclitaxel-platinum[12]. In our study, the overall response rate was significantly higher in carboplatin/weekly paclitaxel arm (80.5%) than cisplatin/cyclophosphamide arm (59%). In addition, the complete response rate was significantly higher in weekly paclitaxel plus carboplatin arm (44.4%) than cisplatin plus cyclophosphamide group (20.5%). These findings go ahead with many studies which reported significantly higher overall response rate and complete response rate in weekly paclitaxel plus carboplatin arm than cisplatin plus cyclophosphamide arm[5,6,7]. Moreover, concerning high overall response rate and complete response rate in weekly paclitaxel plus carboplatin in our trial go ahead with most previously published trials[12-17,29,30]. Furthermore, weekly paclitaxel plus carboplatin group offers higher overall response rate than reported in other studies using 3-weeks paclitaxel [11,17,31-35].

In our study, paclitaxel was administered weekly in a dose of 80 mg/m^2 in order to reduce toxicity whilst maintaining dose intensity. Dose limiting toxicities (DLTs), occurring at paclitaxel 90 mg/m^2/week, consisted of prolonged febrile neutropenia and grade 3

peripheral neuropathy [33]. Myelotoxicity was the DLT in most previously published trials[29,32,33] for the combination of platinum-taxane.

Non-haematological DLT in our current study was peripheral neuropathy. However, all our patients with peripheral neuropathy had grade 1-2 toxicity and were observed in nine and four patients, respectively and no grade 3-4 was observed. Peripheral neurosensory toxicity was observed in 36.1% of patients and grade 1-2 arthralgia/myalgia toxicity in 24.9% and 33.3% of patients, respectively. All peripheral neurosensory toxicity and arthralgia/myalgia toxicities were statistically significantly more frequent in weekly paclitaxel plus carboplatin arm than in cisplatin plus cyclophosphamide arm. These findings are consistent with other studies using weekly paclitaxel[29-31] regimen and much lower than studies using 3-weekly paclitaxel[5,7,34,35].

Grade 3 or 4 toxicity rates for white blood cell counts, granulocyte counts, platelet counts, and hemoglobin levels were lower in the weekly paclitaxel plus carboplatin arm than in the cisplatin plus cyclophosphamide arm, but no difference between the two treatment groups. Febrile neutropenia was rare and occurred only in one patient in cisplatin plus cyclophosphamide arm and weekly paclitaxel plus carboplatin arm. These findings are consistent with other studies using weekly paclitaxel[15,30-32] and much lower than other studies using three-weekly paclitaxel plus carboplatin compared with cisplatin plus cyclophosphamide[5-7].

A reduction in toxicity, one of the rationales for using weekly paclitaxel in this schedule, was achieved. Weekly paclitaxel is more effective in EOC than three-weekly paclitaxel[34,35] is established in our study and is confirmed in many studies[14,15,30,31,34,35], our schedule merits further investigation.

The present trial provides clear evidence that weekly paclitaxel plus carboplatin offers a progression free survival advantage compared with cisplatin plus cyclophosphamide. The median

progression free survival was higher in weekly paclitaxel plus carboplatin arm (17 months) than cisplatin plus cyclophosphamide (11 months). The percentage of patients who remained free for 2-years, 3-years and 5-years were 33.3%, 27.7% and 13.9% in weekly paclitaxel plus carboplatin arm versus 20.5%, 15.4% and 2.6% in cisplatin plus cyclophosphamide arm, respectively. These findings were in agreement with many studies that reported relatively similar results [5-7]. Furthermore, our study reported progression free survival in weekly paclitaxel plus carboplatin arm higher than reported in other studies using 3-weeks paclitaxel [34,35].

Our trial provides an approximately 14 months difference in median overall survival in favor weekly paclitaxel plus carboplatin. The median overall survival was higher in weekly paclitaxel plus carboplatin arm (36 months) than cisplatin plus cyclophosphamide (23 months). This relatively high median overall survival in weekly paclitaxel plus carboplatin arm versus cisplatin plus cyclophosphamide arm go ahead with many studies[5-7]. Futhermore, the 2-years, 3-years and 5-years survival rates in our study were 61.1%, 50% and 27.8% in weekly paclitaxel plus carboplatin arm versus 33.3%, 20.5% and 5.1% in cisplatin plus cyclophosphamide arm which are in accordance with other studies that reported similar results [5-7].

CA(125) level after three cycles of chemotherapy post-surgery is an independent predictor of response, survival and progression-free survival for advanced ovarian carcinoma. Our findings are coinciding with many other studies that evaluate the prognostic value of serum CA(125) level change during chemotherapy in patients with ovarian carcinoma [36-41].

In conclusion, the combination of carboplatin and weekly paclitaxel in patients with EOC is active and its main toxicity was peripheral neuropathy and arthralgia; grade 1 or 2. However, neurotoxicity was more lowered in weekly paclitaxel than 3-weeks paclitaxel. We therefore conclude that this combination chemotherapy using weekly paclitaxel 80

mg/m^2 and carboplatin AUC 6 is well tolerated and highly effective in the treatment of advanced ovarian cancer.

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BENHA MEDICAL JOURNAL

A RANDOMIZED TRIAL OF CARBOPLATIN AND WEEKLY PACLITAXEL VERSUS CISPLATIN-CYCLOPHOSPHAMIDE IN PATIENTS WITH ADVANCED OVARIAN CARCINOMA : LONG-TERM EFFICACY RESULTS

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OXIDATIVE STRESS AND CYTOPROTECTIVE ENZYMES AS NONINVASIVE BIOMARKERS IN PREDICTION OF NONALCOHOLIC STEATOHEPATITIS (NASH) IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Abstract

In light of the dramatic increase in the prevalence of nonalcoholic fatty liver disease (NAFLD), noninvasive, simple, reproducible, and reliable biomarkers that can allow identifying patients with nonalcoholic steatohepatitis (NASH) among NAFLD patients are greatly needed. The aim of our study was to highlight the role of oxidative stress and cytoprotective enzymes as noninvasive predictors for NASH, aiming to reduce the use of liver biopsy.

Our study included 4 groups: group I (18 patients) with simple liver steatosis, group II (26 patients) with mild NASH, group III (16 patients) with Severe NASH, and group IV (30 healthy subjects).

We investigated oxidative stress by measuring serum malondialdehyde (MDA) and nitric oxide (NO) and we investigated cytoprotective enzymes by measuring serum glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD). Serum levels of MDA, NO, GSH-Px, and SOD were determined by spectrophotometric methods.

We found that serum MDA, and NO levels were significantly increased in patients with NASH compared to simple liver steatosis group and healthy control group indicating increased oxidative stress, while serum GSH-Px, and SOD levels were significantly decreased in patients with NASH compared to simple liver steatosis group and healthy control group indicating impaired hepatic antioxidant enzymatic defense

system in NASH.

The balance between oxidative stress and cytoprotective enzymes may be impaired by depletion of cytoprotective enzymes and increased serum levels of MDA and NO in patients with NASH. So, oxidative stress and cytoprotective enzymes can be used in identification of patients with nonalcoholic steatohepatitis (NASH). Failure of cytoprotective enzymes against oxidative stress may be an important factor in the pathogenesis of NASH.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is currently the most common form of chronic liver disease affecting both adults and children, and is strongly associated with obesity and insulin resistance[1,2]. The term NAFLD represents a group of conditions ranging from simple liver steatosis, usually asymptomatic, to nonalcoholic steatohepatitis(NASH), which is characterized by the presence of apoptosis / inflammation and fibrosis, and also by a progressive course, evolving to cryptogenic cirrhosis [3-5].

The pathogenesis of NASH involves two major stages; accumulation of triglyceride within hepatocytes as the first step, and oxidative stress that leads to inflammation, cellular injury, and progressive fibrosis as the second step. Cellular damage induced by mitochondrial oxidative stress is a widely accepted explanation for steatosis to the progression of steatosis to necroinflammation and fibrosis[6,7].

The body protects itself from oxygen free radical toxicity by cytoprotective enzymes (eg, glutathione peroxidase, GSH-Px; glutathione reductase, GR; superoxide dismutase, SOD; and catalase) and by non-enzymatic antioxidants (eg, vitamins, uric acid, albumin, bilirubin, and many others) [8].

The goal of diagnostic procedures is to identify the patients with NASH before the stage of advanced fibrosis. Currently, the diagnosis of NASH requires an invasive liver biopsy with drawbacks of sampling and interpretation errors[9]. Hence the development of noninvasive simple, reproducible,

and reliable biomarkers that can allow identifying patients with nonalcoholic steatohepatitis (NASH) among NAFLD patients are greatly needed. Therefore, the aim of our study was to highlight the role of oxidative stress and cytoprotective enzymes as noninvasive predictors for NASH, aiming to reduce the use of liver biopsy.

Subjects and Methods

Subjects:

The present study included 90 subjects selected from attendants of out and/or inpatients, medical Clinics, Mansoura University Hospitals (MUH) during the period between March 2011 to September 2012.

They were 52 males and 38 females with age ranged from 32 to 64 years with a mean value 49.26 ± 7.87 years.

Exclusion criteria:

- Patients with known history of alcohol consumption(alcohol abuse >20g/day).
- Patients with history of drugs causing rise in serum transaminases or hepatomegally to exclude drug induced liver disease.
- Viral hepatitis seropositivity (HBS Ag & HCV Ab).
- Positive immunological markers for autoimmune hepatitis.
- Pregnancy and lactation.
- Patients under weight reduction programs or using appetite suppressing drugs.

The subjects were divided into four groups:

Group I : 18 patients with simple liver steatosis.

Group II : 26 patients with mild NASH.

Group III : 16 patients with severe NASH.

Group IV : control group of 30 healthy volunteers (They were workers who gave no history of liver disease or alcohol comsumption, liver function tests and sonographic liver examinations were normal).

Methods:

All patients were already diagnosed as (Simple Steatosis / NASH) by liver biopsy & imaging techniques. All subjects were subjected to:

A) Proper thorough history

taking with special stress on:

- Alcohol consumption or drug induced liver diseases (DILD).
- Significant medications in the last 2 years.
- Past history suggestive of hepatitis.
- Presence or absence of symptoms suggestive of liver disease.

B) Clinical evaluation:

- Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Patients were considered overweight or obese when their BMI > 30 kg/m^2 [10].

C) Biochemical evaluation:

- Routine liver function tests (S.ALT, S.AST, S. bilirubin, S. albumin, INR).
- Fasting blood glucose and complete lipid profile:
  - S. cholesterol (total, HDL & LDL).
  - S. triglycerides (TG).
- Serological markers for viral hepatitis (HBS Ag & HCV Ab).
- Immunological markers for autoimmune hepatitis (ANA & Anti ds-DNA).
- Serum malondialdehyde levels (MDA).
- Serum Nitric oxide levels (NO).
- Serum glutathione peroxidase levels (GSH-Px).
- Serum superoxide dismutase levels (SOD).

Assay procedure:

MDA was determined by the thiobarbituric acid method [11]. Aliquots of 0.2 ml of serum were mixed thoroughly with 0.8 ml of phosphate - buffered saline (pH 7.4) and 25 µl of butylated hydroxytoluene solution. After addition of 0.5 ml of 30% trichloroacetic acid, the samples were placed on ice for 2 hr and then centrifuged at 2000 x g at 25°C for 15 min. One ml of supernatant was mixed with 0.075 ml of 0.1 mol/L EDTA and 0.25 ml of 1% thiobarbituric acid in 0.05 N sodium hydroxide. The samples were kept in boiling water for 15 min, cooled to room temperature, and the absorbance at 532 nm was measured. Total thiobarbituric acid-reactive substances (TBARS) were expressed as MDA, using a molar extinction coefficient for MDA of 1.56 x 105 cm^{-1} M^{-1};

results were expressed as nmol/ml.

Serum NO levels were measured according to green et al[12]. In acid medium and in presence of nitrite, the formed nitrous acid diazotise sulphanilamide and the product is coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright- purple colur,which can be measured Spectrophotometrically at 540 nm. Sodium nitrate was used as the standard and results were expressed as mmol/L.

Serum SOD activity was assayed according to Sun et al [13]. The SOD activity was measured at 560 nm by detecting the inhibition of the nitroblue tetrazolium (NBT) reduction rate. One SOD unit was defined as the enzyme activity that caused 50% inhibition of the NBT reduction rate. SOD activity was expressed as U/ml.

GSH-Px activity was measured according to Paglia and Valentine[14]. When oxidized glutathione was converted to the reduced form in the presence of glutathione reductase (GR), NADPH was oxidized to NADP. The diminished absorbance of NADPH was measured at 340 nm. By measuring the ΔA/min and using the molar extinction coefficient of NADPH, GSH-Px activity was calculated and expressed as IU/L.

Statistical Analysis:

Data was analyzed using SPSS (Statistical Package for Social Sciences) version 10. Quantitative data were presented as mean ± SD and the Student t test was used to compare means of two groups and the One-Way ANOVA procedure was used to compare means of more than two groups with Post Hoc test for internal comparisons. Qualitative data were presented as number and percentage and chi-square (χ^2) was used to test the association between raw and column variables. Pearson's correlation coefficient was used to test correlation between variables. A statistical index of the degree of linear dependence between the pair of values taken by observation of two variables. By definition, this must lie between +1 and -1, being positive if the two variable increase or decrease together. A zero correlation coeffi-

cient (r) implies a complete absence of correlation. $P < 0.05$ was considered to be statistically significant and $P < 0.001$ was considered to be statistically highly significant.

Results

Results are shown in the following tables and figures:

Table 1 showed that; sex distribution shows statistically significant difference between the four studied groups (P=0.047), while age and BMI show highly significant difference between the four studied groups.

Table 2 and 3 showed that; all the parameters of oxidative stress & cytoprotective enzymes show statistically significant difference in all studied patient groups and control group.

Table 4 and 5 showed that; all laboratory investigations data used show statistically significant difference in all studied patient groups and control group except INR which show statistically non-significant difference in all studied patient groups (P=0.059).

Table 6 showed that; most of NASH patients had minimal or mild hepatic inflammation and minimal evidence of liver fibrosis; none had severe of hepatic inflammation and fibrosis.

Figure 1 showed that; serum MDA, and NO levels were significantly increased in patients with NASH compared to simple liver steatosis group and healthy control group, while Serum GSH-Px, and SOD levels were significantly decreased in patients with NASH compared to simple liver steatosis group and healthy control group.

Table (1) : Demographic data of all studied groups.

	G I N = 18		G II N = 26		G III N = 16		G IV N = 30		χ^2	P. value
	No	(%)	No	(%)	No	(%)	No	(%)		
▪Sex:										
•Male	8	44.4	20	76.9	6	37.5	18	60	7.98	0.047
•Female	10	55.6	6	23.1	10	62.5	12	40		
▪Age (year)										
• 30 – 40	6	33.3	2	7.7	-	-	6	20	32.31	<0.001
• 40 – 50	12	66.7	6	23.1	4	25	16	53.3		
• 50 – 60	-	-	13	50	9	56.3	6	20		
• > = 60	-	-	5	19.2	3	18.8	2	6.7		

	GI N = 18		GII N = 26		GIII N = 16		GIV N = 30		Test of sig.	P. value
	Mean ± S D		Mean ± S D		Mean ± S D		Mean ± S D			
▪Weight (Kg)	108.83	13.36	98.23	6.63	91.00	6.76	82.87	6.67	16.36	0.009
▪Height (m)	1.68	0.09	1.63	0.05	1.62	0.06	1.73	0.06	4.79	0.012
▪BMI (Kg/m^2)	38.48	4.53	36.97	2.33	34.81	2.03	27.82	1.28	5.99	0.006

G I : Simple steatosis group. G II : Mild NASH group.
G III : Severe NASH group. G IV: Control group.

Table (2): Oxidative stress & Cytoprotective enzymes data of all patients and control group.

	Patients N = 60		Control gp N = 30		Test of sig.	P. value
	Mean ± SD		Mean ± SD			
NO (mmol/L)	130.95	15.74	92.74	10.72	11.97	<0.001
MDA (nmol/ml)	10.97	3.08	4.25	0.95	15.48	<0.001
GSH- Px (IU/L)	1030.63	29.99	1049.83	34.42	2.72	0.012
SOD (U/ml)	1.10	0.15	1.48	0.22	8.57	0.004

Table (3): Oxidative stress & Cytoprotective enzymes data in patients groups.

	GI N = 18		GII N = 26		GIII N = 16		Test of sig.	P. value
NO (mmol/L)	125.11	12.39	129.19	13.11	140.38	19.30	4.82	0.012
MDA (nmol/mL)	9.06	2.27	10.77	2.70	13.44	2.90	11.84	0.001
GSH-Px (IU/L)	1039.90	28.63	1033.73	31.85	1015.19	23.32	3.37	0.041
SOD (U/mL)	1.20	0.11	1.09	0.12	0.99	0.15	13.00	<0.001

Table (4): Laboratory investigations data of all patients and control group.

	Patients N = 60		Control gp N = 30		Test of sig.	P. value
	Mean ± SD		Mean ± SD			
FBS (mg/dl)	119.42	25.57	84.87	6.48	7.27	<0.001
ALT (U/L)	80.07	16.70	25.83	6.15	22.31	<0.001
AST (U/L)	70.68	16.18	24.87	3.83	15.25	<0.001
GGT (U/L)	58.82	9.25	21.43	3.61	27.40	<0.001
Bilirubin (µmol/L)	17.96	3.63	12.15	1.59	8.38	<0.001
Albumin (gm/dl)	3.85	0.26	4.35	0.20	9.95	<0.001
INR	1.17	0.10	1.01	0.03	8.36	0.012
Cholesterol (mg/dl)	231.55	30.80	135.10	11.76	16.52	<0.001
TG (mg/dl)	215.47	28.86	100.43	12.01	26.61	<0.001
HDL-Cho (mg/dl)	48.85	5.14	54.20	3.89	5.02	0.002
LDL-Cho (mg/dl)	139.61	26.64	60.81	7.50	21.29	<0.001

Table (5): Laboratory investigations data in patients groups.

	GI N = 18		GII N = 26		GIII N = 16		Test of sig.	P. value
	Mean ± SD		Mean ± SD		Mean ± SD			
FBS (mg/dl)	109.06	13.52	112.85	20.94	141.75	29.97	11.42	0.001
ALT (U/L)	63.44	5.59	79.12	8.59	100.31	12.96	67.31	<0.001
AST (U/L)	55.56	5.90	67.50	6.49	92.88	10.15	109.53	<0.001
GGT (U/L)	51.06	4.29	57.81	6.47	69.19	7.57	36.33	0.001
Bilirubin (µmol/L)	15.55	1.44	17.12	2.36	22.06	3.74	29.05	0.001
Albumin (gm/dl)	4.04	0.23	3.81	0.23	3.72	0.23	8.95	0.012
INR	1.12	0.10	1.18	0.09	1.20	0.09	2.98	0.059
Cholesterol (mg/dl)	212.00	24.80	239.19	31.54	241.13	26.80	6.10	0.004
TG (mg/dl)	199.50	28.07	217.65	27.73	229.88	23.76	5.57	0.006
HDL-Cho (mg/dl)	50.22	3.35	49.96	5.16	45.50	5.51	5.33	0.008
LDL-Cho (mg/dl)	121.88	23.24	145.70	26.45	149.65	21.54	6.98	0.002

Table (6): Histologic findings in liver biopsy in patients group.

	GI N = 18		GII N = 26		GIII N = 16		χ^2	P. value
	No	(%)	No	(%)	No	(%)		
•Steatosis								
- None / minimal	-	-	-	-	-	-	10.23	0.006
- Mild	12	66.7	11	42.3	2	12.5		
- Moderate	6	33.3	15	57.7	14	87.5		
- Severe	-	-	-	-	-	-		
•Inflammation								
- None / minimal	12	66.7	4	15.4	-	-	38.17	<0.001
- Mild	6	33.3	18	69.2	5	31.3		
- Moderate	-	-	4	15.4	11	68.7		
- Severe	-	-	-	-	-	-		
•Ballooning								
- None	17	94.4	17	65.4	1	6.2	30.01	<0.001
- Few	1	5.6	9	34.6	13	81.3		
- Many	-	-	-	-	2	12.5		
•Fibrosis								
- F0	18	100	7	26.9	-	-	91.57	<0.001
- F1	-	-	19	73.1	-	-		
- F2	-	-	-	-	9	56.3		
- F3	-	-	-	-	7	43.7		
- F4	-	-	-	--	-	-		

Table (7): Correlations between histologic findings in liver biopsy and different studied biochemical parameters.

	Steatosis		Inflammation		Ballooning		Fibrosis	
	r	P	r	P	r	P	r	P
▪BMI	0.082	0.534	0.042	0.751	-0.221	0.090	-0.336	0.009
• NO	0.415	0.001	0.621	<0.001	0.361	0.005	0.394	0.002
•MDA	0.532	<0.001	0.731	<0.001	0.445	<0.001	0.519	<0.001
•GSH-Px	-0.506	<0.001	-0.649	<0.001	-0.280	0.030	-0.322	0.012
•SOD	-0.549	<0.001	-0.772	<0.001	-0.478	<0.001	-0.515	<0.001
•FBS	0.481	<0.001	0.672	<0.001	0.488	<0.001	0.520	<0.001
•ALT	0.463	<0.001	0.729	<0.001	0.604	<0.001	0.790	<0.001
•AST	0.358	0.005	0.627	<0.001	0.657	<0.001	0.875	<0.001
•GGT	0.381	0.003	0.644	<0.001	0.560	<0.001	0.716	<0.001
•Bilirubin	0.212	0.103	0.404	0.001	0.467	<0.001	0.662	<0.001
•Albumin	-0.264	0.042	-0.358	0.005	-0.388	0.002	-0.442	<0.001
•INR	0.198	0.129	0.226	0.083	0.307	0.017	0.294	0.022
•Cholesterol	0.449	<0.001	0.672	<0.001	0.350	0.006	0.311	0.016
•TG	0.672	<0.001	0.535	<0.001	0.347	0.007	0.321	0.012
•HDL-Cho	-0.038	0.772	-0.005	0.968	-0.122	0.352	-0.284	0.028
•LDL-Cho	0.381	0.003	0.663	<0.001	0.353	0.006	0.345	0.007

- $P < 0.05$ was considered to be statistically significant.
- $P < 0.001$ was considered to be statistically highly significant.
- The correlation is weak at r =0.5, moderate at r =0.55 - 0.75, and strong at r =0.8 - 1.0.

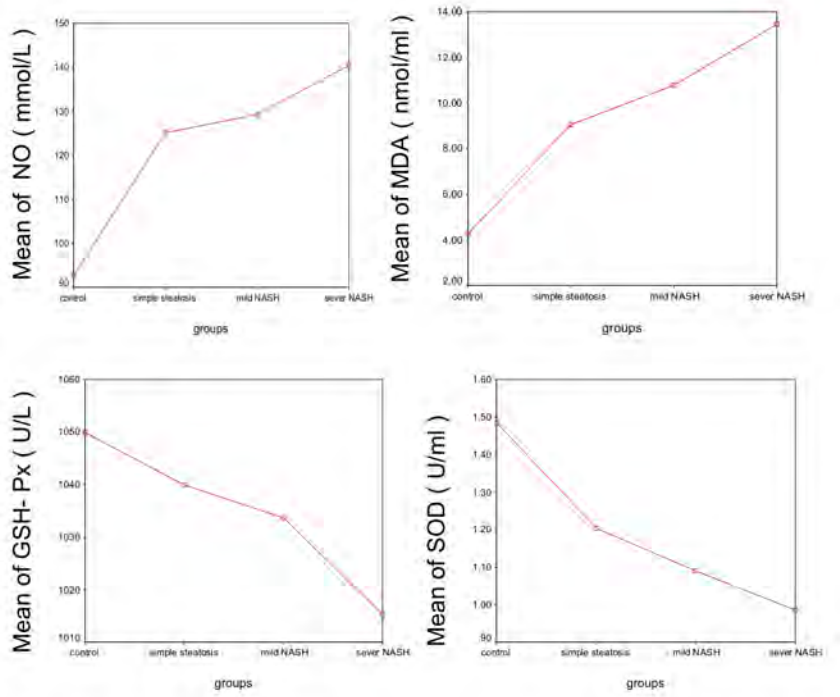

Figure (1): The mean of (NO - MDA - GSH-Px - SOD) in all studied groups.

Discussion

Nonalcoholic steatohepatitis represents the progressive form of NAFLD, with greater potential for progressing to end-stage liver disease. In this context, early diagnosis of patients with NASH by simple noninvasive method prior to the onset of advanced fibrosis would be helpful in guiding aggressive intervention.

The aim of this study is to high light the role of oxidative Stress and cytoprotective enzymes as noninvasive predictors for NASH, aiming to reduce the use of liver biopsy.

We found that NAFLD is more common in obese males during 5^{th} and 6^{th} decades of life, this is in agreement with Patel (2002)[15], who found that NAFLD and NASH are common findings in obese male subjects especially

with type 2 DM. On the contrary, Luyckx et al. (1998) [16] found that the incidence of NAFLD and NASH was more common in females than males regarding the sex. Also, they proved that the female sex is a possible risk factor for NASH due to the nature of the disease itself and/or hormonal effect.

Although BMI was significantly different among all studied groups (P = 0.006), we did not find correlations between the histologic severity of the disease and BMI. Park et al used the clinical BMI value to diferentiate the simple steatosis from NASH, establishing a threshold value for BMI as indicator for NASH[17].

Insulin resistance, oxidative stress, and an inflammatory cascade are believed to play integral roles in the pathogenesis and progression of NAFLD[18,19].

The origin of oxidative stress in patients with NASH is represented by mitochondrial dysfunction, followed by increased reactive oxygen species (ROS) production, which, in turn, initiates a positive feedback with organelle damage. ROS damage mitochondrial DNA (mtDNA) and activate cellular lipid peroxidation, generating malondialdehyde and 4-hydroxynonenal [20-22].

Evidence of lipid peroxidation in the form of increased MDA production, a surrogate marker of oxidative stress, has been noted in previous studies, and serum levels of MDA have been correlated with the severity of chronic hepatitis [23,24].

In the present study, serum MDA levels were significantly increased in patients with NASH, indicating increased oxidative stress, but weak to moderate correlations were found between serum MDA levels and the histopathologic findings in NASH patients. This may reflect the fact that most of NASH patients had minimal or mild hepatic inflammation and absent or minimal evidence of liver fibrosis; none had severe of hepatic inflammation and fibrosis.

Reports concerning the role of NO in liver damage during inflammatory conditions are contradicto-

ry. Zhu and Fung [25] found that NO protects against liver injury by scavenging lipid radicals and inhibiting the lipid peroxidation chain reaction. On the other hand, Sass et al[26] reported that iNOS-derived NO regulates proinflammatory genes in vivo, contributing to inflammatory liver injury. Other investigators have reported that, in the pathogenesis of NASH, NO may potentiate cytotoxicity by reaction with superoxide anion to form peroxynitrite, a strong oxidant that promotes nitration of tyrosine to form nitrotyrosine [27,28].

The finding that intrahepatic accumulation of nitrotyrosine is associated with the histological severity of NASH strongly suggests that oxide-related oxidative injury may play a significant role in the pathogenesis of NASH[29]. Although our NASH patients had increased serum levels of NO, we found weak correlations between the histologic severity of the disease and serum NO concentration. This may reflect the early stage of the disease in most of our NASH patients. In addition, NO may have a protective role during an early stage of NASH. At the begining of hepatic injury, when only a small amount of NO is being produced, NO may protect the liver through vasodilatatory, antioxidative, and antiapoptotic effects. However, in the presence of massive injury (eg, high level of inducers and elevated oxidative stress), greatly increased NO production might induce the hepatocytes to progress to irreversible channel necrosis and cell death [29].

The defenses against free radical mediated injury include enzymatic deactivation and direct reaction with free radicals [30]. Cells have various antioxidant systems (eg, SOD, GSH-Px, GR, and catalase) and non-enzymatic scavengers (eg, vitamins E, A, and C; carotenoids, flavonoids, and thiols)[8].

SOD, the first line of defense against oxygen-derived free radicals, converts superoxide anion into H2O2, forming as neutral products O2 and H2O.

GSH-Px catalyses reductive destruction of hydrogen and lipid

hydroperoxides, using glutathione as an electron donor [31].

It is now generally accepted that oxidative stress due to increased ROS production has a role in the pathogenesis of NASH. Hepatocytes are continuously exposed to ROS and are protected from oxidative injury by a range of antioxidant pathways[32]. The state of oxidative stress exists when there is imbalance between pro-oxidant and antioxidant chemical species.

In our study, we observed decreased serum SOD and GSHPx activitiy. This findings may indicate that the hepatic antioxidant enzymatic defense system in NASH is impaired. Previous studies suggested that mitochondrial ROS can deplete hepatic antioxidants, allowing the accumulation of more ROS[33,34].

Robertson et al [35] reported that when antioxidant reserves are depleted, hepatic CYP 2E1 and 4A, which are microsomal oxidases involved in fatty acid oxidation, could induce the cellular injury from oxidative stress in NASH. Moreover, down-regulation of the cytosolic isoform of SOD sensitizes tissues and organs, including the kidney and liver, to superoxide mediated tissue injury [36].

The balance between oxidative stress and cytoprotective enzymes may be impaired by depletion of cytoprotective enzymes and increased serum levels of MDA and NO in patients with NASH. So, oxidative stress and cytoprotective enzymes can be used in identification of patients with nonalcoholic steatohepatitis (NASH) . Failure of cytoprotective enzymes against oxidative stress may be an important factor in the pathogenesis of NASH.

Treatment approaches that address the antioxidant enzymes and the antioxidant vitamins may be helpful in the therapy of patients with NASH.

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BENHA MEDICAL JOURNAL

OXIDATIVE STRESS AND CYTOPROTECTIVE ENZYMES AS NONINVASIVE BIOMARKERS IN PREDICTION OF NONALCOHOLIC STEATOHEPATITIS (NASH) IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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FUNCTIONAL MIDDLE EAR AND MASTOID SURGERY (FMMS)

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Abstract

This study presents a trial for application of TMC staging and classification for cholesteatoma that allow standardization and management of cases. All previous attempts failed to gain acceptance because of the lack of clinical relevance.

Objective: *Trial to standardize a preoperative management strategy for middle ear cholesteatoma.*

Study design: *A prospective study, from October 2009 to September 2012. All patients with cholesteatoma (61 ears) were graded according to modified TMC system preoperatively using CT. The choice of type of surgery was chosen according to the staging. Comparison of the preoperative with the intra-operative grading was done. Otoendoscopy was used in all cases.*

Results: *The preoperative grading was correct compared to the intra-operative grading in 88% of cases. This demonstrates the potential value of this grading system.*

Conclusion: *FMMS is an effective preoperative management strategy for middle ear cholesteatoma depending on preoperative CT that detect cholesteatoma extension and presence of complications. It can be applied as standard preoperative management strategy for middle ear cholesteatoma.*

Keywords: *Cholesteatoma,atticotomy,mastoidectomy,sinus tympani.*

Introduction

The primary goal of management of middle ear cholestestoma is to eradicate the disease and make the ear safe and dry. There are two main techniques in man-

agement of cholesteatoma; the canal wall down (CWD) and the intact canal wall (ICW) techniques. The second objective is to restore hearing by means of tympanoplasty.[1] Besides that, it also aims single or minimum number of operations, minimal recurrence rate and, in general, a good quality of life. In general, ICW surgery is designed to maintain the normal anatomy and is proposed by a number of surgeons as the most appropriate surgical technique in most of their cases.[2] It usually needs shorter time period to heal in comparison to CWD methods.[3] Although there is a general belief that ICW is associated with high recurrent and residual cholesteatoma, some surgeons who use both techniques find little difference in the incidence of residual disease.[4] Another benefit of ICW technique is the maintenance of the self-cleaning ability of the ear. When a cavity is created it is usually necessary to clean every 3 or 6 months for the rest of patients' life. Furthermore, precautions to getting water in the ear are necessary in CWD cases. Fitting a hearing-aid is easier in ICW procedures.[5]

Canal wall-down mastoidectomy is the most widely used surgical method worldwide. It is supposed to be easier, of shorter duration, necessitates less surgical experience than the ICW procedures, and has low recurrence and residual rate and the rate of complications is acceptably low.[6] Certain factors are strong indications for CWD surgery. These include extensive damage of the posterior canal wall, a severely contracted mastoid, only hearing ear and labyrinthine fistula. Another relative indication is recurrent cholesteatoma. The anatomy of a CWD mastoidectomy makes retraction pocket recurrences of cholesteatoma unlikely. Follow-up in CWD procedures is usually easy through good meatoplasty as recurrent or residual disease may be easily visualized in a mastoid cavity.[5] In general, there has been a long controversy between these two techniques. Various modifications have been made in the classic ICW technique for improvement in results and prevention of second stage as posterior transmastoid facial recess approach and anterior atticotympanotomy.[7,8] Nevertheless, the

debate is still on due to new evidence, better imaging, high-tech endoscopes and intraoperative use of facial nerve monitoring.(5)

Regardless of the technique, the secret to surgical success is complete eradication of the disease. The selection of the procedures is based on type, grade and extension of cholesteatoma.(9) No staging system for cholesteatoma has been universally adopted because of the lack of clinical relevance. Traditionally, cholesteatomas are classified as congenital and acquired and they are subdivided into primary and secondary. Tos and Lau proposed another classification based on site of origin of cholesteatoma (attic, tympanic sinus and pars tensa).(10) Saleh and Milles Proposed SOC staging, according to the site affected by the cholesteatoma, ossicler chain condition and preoperative complications. They proposed the type of surgery required according to the staging system.(11)

While the incidence of extensive or complicated cholesteatoma has dramatically decreased over the last decades, the early staged or less extensive cholesteatoma is more frequently found. Beside the controversy about the appropriate technique, whether or not to do mastoidectomy became another controversy. This concept was supported by Chun et al. who suggested the term "Functional middle ear surgery". It was based on safe marsupialization of cholesteatoma, mucosal preservation and adequate ventilation.(12)

Aim of the work

This study aims at outlining a strategy for management of middle ear cholesteatoma, by performing one-stage functional middle ear surgery with or without mastoidectomy. This is done using different surgical techniques according to a proposed staging system for cholesteatoma. The surgical findings will be correlated with the staging and outcome of surgery will be evaluated regarding complete disease eradication, restoration of normal ear function and minimizing recurrence or residual disease.

Patients and Methods

This is a prospective study carried out from October 2009 to

September 2012 at Mansoura university otolaryngology department. 61 cases of chronic otitis media with cholesteatoma irrespective of the presence or absence of complications were selected for the study. Cases of congenital cholesteatoma and residual or recurrent cholesteatomas were excluded from this study.

We have modified the TMC classification and staging system so as to allow us to categorize the cholesteatoma (which is mainly diagnosed clinically) according to its extent and presence of complications based on preoperative radiological examination using HRCT. As staging by Belal et al.[13], It has been referred to involvement of the tympanic cavity as (T), mastoid (M) and presence of complications (C) as follows:

1) Tympanic cavity involvement: (T)

T1: Localized cholesteatoma in attic region.

T2: Localized cholesteatoma in tympanic region.

T3: Attico-tympanic cholesteatoma extending from one region to another.

T4: Holotympanic cholesteatoma filling the whole middle ear.

2) Mastoid cavity involvement: (M)

M0: No mastoid cavity involvement.

M1: Cholesteatoma extending to the mastoid antrum only.

M2: Cholesteatoma extending to mastoid cavity (beyond antrum).

3) Presence of complications: (C)

C0: Uncomplicated Cholesteatoma.

C1: Mastoidits, mastoid abscess and fistula.

C2: Other cranial and intracranial complications.

Staging:

Stage 1: T1 or T2 (one region of middle ear cavity) without mastoid involvement or complications.

Stage 2: T3 or T4 (Attico-tympanic or Holotympanic cholesteatoma) and or M1 or M2 (mastoid involvement) and or

C1 (mastoidits, mastoid abscess and fistula).

Stage 3: C2 (other cranial and intracranial complications).

An example of our classification would be (T3 M1 C0) when a cholesteatoma has extended through the attic and the mesotympanum regions and mastoid antrum with no complications. See figure (1). The selected cases were appropriately investigated. HRCT temporal bone with zooming axial and coronal thin cuts was done for all cases. Informed consent was obtained from each patient after counseling them and their relatives regarding the nature of the disease and surgery. All cases were operated with one surgeon. All of the cases underwent tympanomastoidectomy by a postauricular approach. Temporalis fascia was the graft material taken in all cases. Reconstruction of hearing mechanism was undertaken in all possible cases. The patients were divided into 3 groups according to the staging system used. Group 1 included stage 1, Group 2 included stage 2 and Group 3 included stage 3. We added to the third group cases with other indications of CWD mastoidectomy (as contracted mastoid or low set dura preventing adequate surgical access, destructed posterior bony canal wall and dead ears).[14] Group I was managed by postauricular permeatal atticotomy, group II was managed by Tos modified intact canal wall tympanomastoidectomy and group III was managed by canal wall down (modified radical) mastoidectomy.

Surgical technique:

Atticotomy:

First, a postauricular incision is made, then a posterior permeatal incision to enter the external auditory canal. Then a tympanomeatal flap along with the fibrous annulus is elevated and the posterior-superior bony annulus was drilled ('otosclerosis drilling'). Transmeatal atticotomy with preservation of a thin bridge. Cholesteatoma in the middle ear is targeted first and followed to posterior attic.

Tos modified intact canal wall tympanomastoidectomy:

It starts as atticotomy, then

cortical mastoidectomy and posterior atticotomy with intact and thin canal wall were performed Cholesteatomas extending into the attic, antrum, mastoid process, were removed. 30 degree otoendoscope is used to assess sinus tympani for residual cholesteatoma. The eardrum was grafted and atticotomy was then reconstructed with conchal cartilage or cortical bone.

Canal wall down (modified radical) mastoidectomy:

Complete mastoidectomy is performed followed by removal all the posterior bony canal wall with lowering of the facial ridge. Cholesteatoma is completely removed. 30 degree otoendoscope is used to assess sinus tympani for residual cholesteatoma. A wide meatoplasty is done in all cases.

Results

This study included 61 patients (61 ears) of middle ear cholesteatoma. The mean patient follow-up period after surgery was 12 months (ranging from 6–23 months). The study included 61 patients, 32 were males (52.5%) and 29 were females (47.5%) with ratio of 1.11:1. The mean age was 26.82 years (± 14.49) years with range of (5-57) years. There were 24 right (39.3%) and 37 left (60.7%) middle ear cholesteatoma. There were 34 attic (55.7%) and 27 tympanic (44.3%) middle ear Cholesteatoma. 13 cases (21.3%) were presented with aural polyp. There were 6 cases presented with mastoidits and mastoid abscess, 4 of them with mastoid postauricular fistula (C1). There were 2 cases complicated with brain abscess, 2 cases with extradural abscess and one case with presinus abscess (C2).

TMC classification

Tympanic involvement (T)

By preoperative HRCT, we found that T4 cases was the most frequent (20 cases), followed by T3 (17 cases), then T2 (13 cases) and T1 (11 cases). This arrangement had changed by intraoperative exploration. It became: T3 the most frequent (21 cases), followed by T4 (17 cases), then T2 (12 cases) and T1 (11 cases). There were 6 cases with under estimation (10%); 2 cases in T1, 3 cases in T2 and one case in T3, while there was overestimation in 4 cases (5%) in T4.

The sensitivity of HRCT in detection of extension of cholesteatoma was 85%. There was a good to excellent correlation between HRCT and intra operative findings regarding T classification (r= 0.777, p= 0.000).

Mastoid involvement (M)

In our study preoperative CT showed no mastoid involvement (M0) was in 6 cases that were reduced to 4 cases by intraoperative exploration where the other 2 cases found to be M1. There were 35 cases of M1 detected by preoperative CT that were reduced to 30 with 5 cases found to be M2 by intraoperative exploration. There were 20 cases of M2 detected by preoperative CT that were reduced to 17 with 3 cases found to be M1 by intraoperative exploration. There were 7 cases with under estimation (16%); 2 cases in M0 and 5 cases in M1, while there was overestimation in 3 cases (5%) in M2. The sensitivity of HRCT in detection of mastoid extension of cholesteatoma was 79%. There was a good correlation between HRCT and intra operative findings regarding M classification (r= 0.734, p= 0.000).

Presence of complications (C)

In our study we did a slight modification in Belal et al. TMC staging.[13] We referred for absence of complication (C0), for mastoidits, mastoid abscess and mastoid fistula (C1) and for other cranial and intracranial complications (C2). This modification aimed at standardizing the preoperative surgical plan for middle ear cholesteatoma cases. For C2 cases canal wall down mastoidectomy were done[13]. In this study there were 50 cases with C0, 6 cases with C1 and 5 cases with C2. The sensitivity of HRCT in detection of complications was 100%. There was an excellent correlation between HRCT and intra operative findings regarding C classification (r= 1.0, p= 0.000).

TMC staging :

According to TMC staging there were 4 cases in stage (1), 52 cases in stage (2) and 5 cases in stage (3) as shown in table(1).

Preoperative-operative TMC correlation :

The overall sensitivity of HRCT in TMC staging was 88% with 9% underestimation and 3% overesti-

mation. There was an excellent correlation between HRCT and intra operative findings regarding TMC classification and staging (r=0.863, p=0.000). Table (2) shows correlation details. No under or overestimation had affected the surgery decision except one case T1M0C0 that was under estimated, intraoperative finding showed T1M1C0 and decision was changed and ICW tympanomastoidectomy was done.

Study groups :

Group I: It includes cases of stage 1 (4 cases).

Group II: It includes cases of stage 2 (52 cases) 14 cases of this group were reclassified to group III due to presence of an indication for CWD tympanomastoidectomy, thus group II became 38 cases.

Group III: It included cases of stage 3 (5 cases) in addition to cases of other stages with indications of CWD tympanomastoidectomy (14 cases), thus group III became 19 cases. CWD tympanomastoidectomy indications were in 14 cases as following: 5cases with dead ear, 6 cases with contracted mastoid (inadequate access) and 3 cases with destructed posterior bony canal wall.

Attic reconstruction :

It was done in all 42 cases of groups I and II. Conchal cartilage was used in 28 cases, cortical bone was used in 10 cases and bone cement with bone pate was used in 4 cases.

Cholesteatoma recidivism :

Follow up ranged from 6 to 23 months with mean of 12 months (+4). There were 3 cases of Cholesteatoma recidivism in group II (7.7%), and one case in group III (5.3%) and no recidivism in group I.

Table (1) : Preoperative staging.

Stage	Cases number	Percentage
Stage 1	4	6.5%
Stage 2	52	85%
Stage 3	5	8.1%

Table (2): Correlation between preoperative HRCT and intraoperative findings.

Stage	Cases	Good Correlation	Underestimated cases	Overestimated cases	Correlation (%)	Average correlation
T1	11	9	2	0	89%	85%
T2	13	10	3	0	78%	
T3	17	16	1	0	94%	
T4	20	16	0	4	80%	
M0	6	4	2	0	66%	79%
M1	35	30	5	0	86%	
M2	20	17	0	3	85%	
C0	50	50	0	0	100%	100%
C1	6	6	0	0	100%	
C2	5	5	0	0	100%	
Total cases	61	88%	9%	3%		

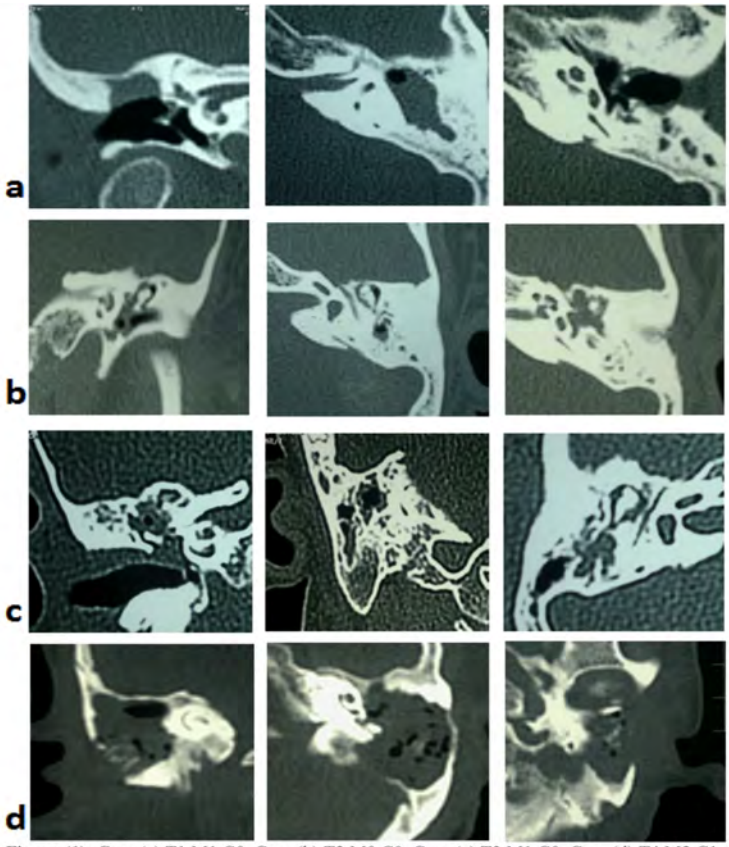

Figure (1): Case (a) T1 M1 C0, Case (b) T2 M0 C0, Case (c) T3 M1 C0, Case (d) T4 M2 C1.

Discussion

Documents reporting results of cholesteatoma surgery would be more meaningful if standardized nomenclature were used in describing the type of surgery performed. Standardized nomenclature for surgery type and classifications for chronic otitis media does not exist.[15] In 1999, Saleh and Milles proposed "SOC staging" depending on the extent of the lesion, ossicular condition and preoperative complications. It depended mainly on intraoperative findings and it has taken into consideration other factors that are difficult to be evaluated preoperatively as ossicular chain damage which may not affect the decision of the type of surgery. They recommended the possibility of using the stage (S) alone for simplicity. But this staging can't be applied preoperatively and did not propose a management strategy for cholesteatoma.[11]

Another staging system for cholesteatoma (TMC staging) was proposed by Belal et al. that depended on clinico-radiological correlation between Tos otoscopic classification and preoperative HRCT to categorize the extent of the disease and presence of complications.[13] They did not consider pathological changes that can't be evaluated preoperatively as damage of the ossicular chain. And this staging has considered presence of preoperative complications that may change the decision of surgery. They have graded tympanic extension of cholesteatoma into a very anatomically descriptive classification rather than clinically applied. Also they did not consider in the C1 stage the management for different cranial complications. Also they did not propose a management strategy for cholesteatoma according to the staging.

In this study, TMC system has been modified so as to allow categorizing the middle ear cholesteatoma (which is first diagnosed clinically) according to its extent and presence of complications based on preoperative radiological examination using HRCT alone. We have changed T classification. This modification made the staging more clinically applied in description of the tympanic extension of cholesteatoma in addition

to plan the type of surgery. By preoperative HRCT, we found that T4 was the most frequent, followed by T3, then T2 and T1. This arrangement had changed by intraoperative exploration. T3 became the most frequent. We think that the cause of overestimation in T4 cases was presence of aural polys. There was a good to excellent correlation between HRCT and intra operative findings regarding T classification. Our results were close to Belal et al. study, as the correlation was 87.3%.[13]

In this study, and according to Belal et al. TMC staging, we have staged the mastoid extension of cholesteatoma using the preoperative HRCT into 3 levels. The aim of this staging is to detect the extension of cholesteatoma and to determine the extent of mastoid air cells to be exenterated. The aim of mastoidectomy, besides eradicating the disease, it is an approach to follow cholesteatoma to the posterior attic. The correlation between HRCT and operative findings for M staging was 79%. We think the cause of overestimation in HRCT may be due to presence of granulation tissue or effusion in mastoid air cells, while the cause of underestimation was the difficult sharp anatomical demarcation between the mastoid antrum. Our results were close to the study by Belal et al. as the correlation was 77.7%.[13]

In our study we did a slight modification in C of Belal et al. TMC staging. This modification aimed at standardizing the preoperative surgical plan for middle ear cholesteatoma cases. For C2 cases CWD mastoidectomy were done. The correlation between HRCT and operative findings for C staging was 100%. That was close to the study by Belal et al., where the correlation was 96.6%. The overall correlation between HRCT and operative findings for modified TMC classification and staging was 88%. That was very close to Belal et al. study in which the overall correlation was 87%. No under or overestimation had affected the surgery decision except one case T1M0C0 that was under estimated, intraoperative finding showed T1M1C0 and decision was changed and ICW tympanomastoidectomy was done. [13]

The goals of surgery for middle ear cholesteatoma are to completely remove the disease without leaving residual cholesteatoma, to restore the ear anatomy properly in order to prevent recurrent cholesteatoma and to improve the quality of life of the patient (good hearing and no cavity problems).[16] The CWD approach can give improved exposure but produces significant clinical problems after surgery, such as late healing, postoperative hearing loss. It also produces a large mastoid cavity (bowel) needing frequent cleaning. Also it may lead to postoperative dizziness. Because of these problems, patients usually require long-term postoperative management, must limit their exposure to water, and experience problems with wearing hearing aids, thus it can have a negative impact on a patient's quality of life.[17,18] The advantages of the ICW technique are rapid wound healing, being more cosmetic (no meatoplasty), no need to clean the ear periodically and preservation of the external ear canal for hearing aid if needed. However, it has been found that residual and recurrent cholesteatomas are common after this technique. This is because it is difficult to access some areas as the epitympanum and sinus tympani.[19] Therefore, a technique combining the advantages of both ICW and CWD techniques, which provides good visualization and removal of cholesteatoma from hidden areas as sinus tympani and anterior attic, with a reconstruction technique that prevents postoperative recurrence, seems to be ideal.

According the extension of the disease we have chosen the surgical technique appropriate. In stage I the cholesteatoma was limited to one region of the middle ear, so the surgery was limited to postauricular permeatal atticotympanotomy, and no mastoidectomy needed. In group III, we have used CWD procedure. This group includes cases of stage III where there are cranial and or intracranial complications beyond mastoidits, in addition to cases with absolute indications of CWD. The rational of choosing this technique in cases with complications is that in these cases cholesteatoma has broken the anatomical boundaries of middle ear cleft. And these

patients became at high risk and life threatened. So the choice of the surgery had to be radical surgery creating wide cavity allowing growth of possible residual cholesteatoma into it thus preventing further complications and a good meatoplasty can allow follow up and its early detection. In these cases we also used 30° endoscope to be sure of complete removal of cholesteatoma especially from sinus tympani.

The debate is usually on the atticotympanic cholesteatoma reaching the mastoid air cells (representing cases of stage II). This is usually the common extension of middle ear cholesteatoma presented clinically (62% of our cases). This is focused mainly on the degree of visualization of the cholesteatoma and thus its complete removal, and how much better the visualization offered by CWD technique with lower incidence of recidivism compared to ICW technique. For these cases, we have used Tos modified ICW tympanomastoidectomy assisted with 30° otoendoscope. Tos modification consists of "otosclerosis drilling" of the postero-superior bony annulus, transcanal atticotomy with preservation of thin bridge and cortical mastoidectomy. It has been claimed to provide good exposure to sinus tympani and anterior attic when compared with the classic ICW mastoidectomy with safer and easier removal of cholesteatoma.[20] This was supported in the study by Uzan and Kutoglo, where visualization of sinus tympani and anterior attic were objectively assessed using classic ICW, CWD and Tos techniques in cadavers. They found that Tos technique provided similar visualization of sinus tympani and anterior attic but significantly better than the classic ICW tympanomastoidectomy. [21]

In our study we have used 30° endoscope to visualize ST in all cases even CWD cases. We agree with Badr-El-Dine that removal of the canal wall does not affect the frequency of residual disease and its incidence in the attic and mesotympanum will be the same in ears of both ICW and CWD.[22] Wide-angle endoscopes particularly 30° give a panoramic view of sinus tympani even with preservation of posterior canal wall and

with no need for posterior tympanotomy. If the atticotomy can be performed widely enough to expose the whole extent of the cholesteatoma, the mass can be removed without leaving any residual matrix. However, the opened epitympanic area will act as a space for postoperative retraction and might allow the development of a recurrent cholesteatoma.[19,23] In a study by Sanna et al. they suggested that reconstruction of the attic bony defect was more important than procedures that target the tubal insufficiency as a means of preventing retraction pockets.[24] Hinohira et al. have come to the conclusion that reconstructing the scutum bony defect is essential for preventing postoperative retraction pocket in ICW tympanoplasty. In our study we have used conchal cartilage and cortical bone in attic reconstruction.[23]

In this study, it was tried to fulfill all factors so as to prevent cholesteatoma recidivism. We have used otoendoscopy in all cases; also we did scutoplasty for atticotomy and ICW cases. In this study there were 3 cases of cholesteatoma recidivism in group II (7.7%), and one case in group III (5.3%) and no recidivism in group I.

Conclusion

FMMS is an effective preoperative management strategy for middle ear cholesteatoma depending on HRCT that detect cholesteatoma extension and presence of compilcations.

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THE APPLICATION OF LIGHTWEIGHT MESH SUPPORT IN INFERIOR PEDICLE BREAST REDUCTION; CLINICAL, PATHOLOGICAL AND RADIOLOGICAL EVALUATION

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Abstract

Background: *Numerous techniques have been described for the treatment of breast hypertrophy and ptosis. Unfortunately, recurrent ptosis after mammaplasty can occur regardless of the technique used. To avoid this problem, different kinds of supporting devices have been described with variable rates of success. However, the true implications of incorporating prosthetic materials into breast surgery have never been clarified. Therefore, surgeons have traditionally been reluctant to apply any kind of prosthetic material to the breast, fearing inflammation, an unfavorable aesthetic outcome, palpable or visible deformities, and interference with the mammographic evaluation of breast cancer. This study analyzed the clinical, pathological and mammographic evaluations of using lightweight mesh as a supportive device in inferior pedicle breast reduction.*

Methods: *For this study, 30 healthy adult female patients with age range from (23-40 years) with breast hypertrophy, ptosis, or both were divided into two groups, each group formed from 15 patients. Group one were managed by bilateral inferior pedicle breast reduction with lightweight mixed (60% Polyglactine and 40% polypropylene) mesh support while group two were managed by bilateral inferior pedicle breast reduction without mesh support. Clinical and aesthetic outcome were done immediate and one year postoperative for recurrent ptosis, con-*

tour irregularities and postoperative complications by digital photography, measurement of the length of the vertical limb, distance from suprasternal notch to nipples. Thickening or widening of the scar with extrusion of the mesh, and any palpable or hardened areas. Bilateral soft tissue mammography was done one year postoperatively for all patients analyzed by both the surgeon and an expert radiologist. The evaluated factors were calcifications, parenchymal redistribution, mesh detection and elevation of NAC. Incision biopsies one year postoperative were done by open excision of a piece of breast tissue including part of the mesh for evaluation of the degree of tissue reaction to the mesh.

Results: *According to the clinical observations immediate and one year postoperative; there were no mesh related abnormalities and complications in the breast, the mesh was not palpable, the breast was soft and pliable and there was no recurrent ptosis in those patients with mesh support. In terms of mammographic imaging, there were no difference between both groups, the mesh was not detected, the mesh did not interfere with the visualization and analysis of the breast's parenchyma, parenchymal redistribution were noted in (93.3%) of all patients, localized microcalcifications were detected in (40%) of patients. Histopathologic analysis was done one year post operative and it shows the non absorbable polypropylene fiber of the mesh imbedded in adipose tissue surrounded by thin layer of reactive collagenous tissues and mild inflammatory reaction which is expected finding.*

Conclusions: *The use of mesh support in breast surgery can enhance the aesthetic results without inducing visible or palpable deformities or mammographic abnormalities that interfere with diagnostic imaging of the breast.*

Key words: *Inferior pedicle, breast reduction, mesh support.*

Introduction

Numerous different techniques have been described for the treatment of breast hypertrophy and ptosis [1]. The resection of excess skin and breast tissue usually has been performed through different combinations of periareolar and vertical skin incisions, with an associated horizontal incision of variable length[2].

Inferior pedicle technique was first introduced by Riberio in 1975 where a dermoglandular flap based on the inferior aspect of the breast mound, and was used to build up the new breast after superior, medial lateral resections[3]. Unfortunately, pseudoptosis is a common problem after reduction mammaplasty. This is especially true after the inferior pedicle reduction technique. Many surgeons continue to use the inferior pedicle technique because it is easy to perform, familiar, and useful for very large breasts. Most surgeons would agree that when the nipple must be transposed a long distance, the inferior pedicle technique is the most reliable[4].

Given the utility of and need for the inferior pedicle technique, surgeons have tried numerous ways to prevent the phenomena of postoperative "bottoming out". These include suture suspension of the pedicle to the chest wall, dermal bra, dermal strips, superficial fascial suspension, fascia lata, and muscular slings [5].

The use of mesh support technique for preventing bottoming out and recurrent ptosis was championed by Goes, who first described the use of mesh for breast surgery in 1992. His initial report described the use of an absorbable polyglactine mesh, but since then he has described the use of a polyglactine and polyester mesh to add a component of permanent mesh because of recurrence of ptosis seen with the former technique[5]. The mesh helps to maintain long-lasting anterior projection and to avoid the recurrence of ptosis also it works by inducing a scarring reaction, which functions as an internal brassiere[5]. However, the true implications of incorporating mesh into breast surgery have never been clarified. Therefore, most surgeons have been reluctant to apply any kind of prosthetic material to the breast, fearing inflammation, an unfavorable aesthetic out-come, palpable or visible deformities, and interference with the mammographic evaluation of breast cancer[6].

This study analyzed the aesthetic, clinical, pathological and mammographic evaluation of using lightweight mixed mesh as a

supportive device in inferior pedicle breast reduction.

Patients and Methods

A total number of thirty female patients were admitted to plastic surgery department, Mansoura University Hospital for bilateral breast reduction. The mean age was 31.4 with a range of 23 - 40 years, with bilateral breast hypertrophy, ptosis or both. They were divided into two groups according to the procedure done: Group one (15 patients) bilateral inferior pedicle breast reduction with application of light weight mesh support and Group two (15 patients) bilateral inferior pedicle breast reduction without application of light weight mesh support.

Surgical technique; after marking and preparation of the inferior pedicle, this followed by application of two straps of light weight mesh 2 cm in width and 30 cm in length. The proximal strap was fixed at the junction of the proximal and middle third of the pedicle and the distal strap was fixed at the junction of the middle and distal third of the pedicle (Fig 1). Fixation of the mesh in the pedicle by creating a subdermal tunnel of the pedicle to bypass the mesh, then to be fixed by single non-absorbable polypropylene 2/0 stitch to the lateral edges of the pedicle. Creation of an opening 2 cm in the pectoralis fascia midway between the parasternal line and midclavicular line at the 2nd and 4th space then suturing the mesh strap by two stitches of polypropylene 0/0 suture to the periostium of the rib and suturing the opening in the fascia to the mesh strap by the same suture. The same were done on the same levels but midway between the midclavecular line and anterior axillary line to fix the other end of the mesh straps. Closure of the pillars in layers with fixation of no. 16 suction drain in each breast.

Clinical and aesthetic outcome was performed immediate and one year postoperative for recurrent ptosis, contour irregularities and postoperative complications by digital photography, measurement of the length of the vertical limb, distance from suprasternal notch to nipples. Thickening or widening of the scar with extrusion of

the mesh, and any palpable or hardened areas.

Bilateral soft tissue mammography was performed one year postoperatively for all patients analyzed by both the surgeons and an expert radiologist. The evaluated factors were calcifications, parenchymal redistribution, elevation of NAC, mesh detection, skin retractions, as well as any abnormality that precluded an adequate mammographic interpretation were reported.

Incision biopsies one year postoperative were done by open excision of a piece of breast tissue including part of the mesh for evaluation of the degree of tissue reaction to the mesh.

Results

All the 30 patients had age range from 23 to 40 years with a mean of 31.4, there BMI ranged from 25 to 34 with a mean of 29.7, and had complaint of cosmoses in 26 patients (86.7%), followed by shoulder pain, grooving and bra-strap discomfort in 17 patients (56.7%), the husband request came next in order in 14 patients (46.7%), and finally came the inframammary intertrigo in 13 patients (43.3%). The amount of excised tissue per side ranged from 500 to 4000 gm with a mean of 1208 gm.

In this work the preoperative measurement of the nipple to SSN ranged from 29 to 53 cm with a mean of 34.6, with a significant change of the immediate and one year postoperative mean from 22.8 to 21.1, this means shortening of the distance from nipple to SSN around 1.7 cm. The length of the inferior pedicle ranged from 14 to 38 cm with a mean of 23.1 (Fig 2-1, 3-1).

The length of the vertical limb immediately post operatively was 7 cm in both groups as planned in the marking preoperatively, while one year post operatively there was a great significant difference between both groups as it ranged from 7.5 to 8 cm with a mean of 7.7 cm in group one while in group two it ranged from 8.5 to 10 cm with a mean of 9.1 cm, this is because of the application of mesh in the group one (Table 1, Fig 4). Also the inferior pole were

rounded in group one than in group two (90% versus 43.3%) due to fixation of the inferior pedicle in the central part of the breast by mesh. The longest follow-up period is 30 months. There were no bottoming out could be noted in the group one with mesh while there was a different grades of bottoming out in group two.

The postoperative mammographic study reveals (Fig 2-2, 3-2) no mesh detection in all patients. Parenchymal redistribution in 14 cases (93.3%), detection of oil cyst, dermoid cyst, retroareolar fibrosis and microcalcifications with no significant difference between both groups. Our results also demonstrate that the presence of mesh did not lead to surgical complications, palpability after the operation, or the formation of densities, specula-tions, or microcalcifications.

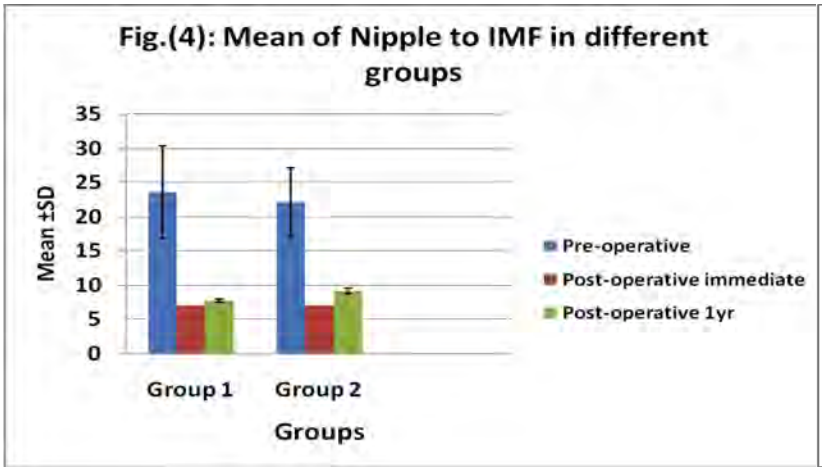
Histopathological examination shows (Fig 2-3, 3-3) the non absorbable polypropylene fiber of the mesh imbedded in adipose tissue surrounded by thin layer of reactive collagenous tissues and mild inflammatory reaction which is expected finding.

Postoperative complications includes the following: Single patient with unilateral complete loss of NAC, (3.3%), another patient with bilateral loss of sensation of the NAC but this one because she already did amputation mammaplasty with NAC graft, another single patient develop unilateral subareolar breast abscess in group two (3.3%) and three patients in each group develops a unilateral partial wound dehiscence (10%).

Table (1): Mean of nipple to IMF (pre-operative, immediate post-operative and 1-year post-operative) in group 1 and group 2.

	Nipple to IMF		
	Pre-operative	**Post-operative immediate**	**Post-operative1y**
Group 1	23.6±6.8	7	7.7±0.25
P1		<0.05*	<0.05*
P2			<0.05*
Group 2	22.06±5.1	7	9.1±0.4
P1		<0.05*	<0.05*
P2			<0.05*
P3	>0.05	>0.05	<0.05*

All results are expressed as mean ± standard deviation (SD).
Significant (*): at $P < 0.05$.
P1 significance between Pre-operative & Post-operative immediate and post-operative 1yr in group 1 and group 2.
P2 significance between Post-operative immediate and post-operative 1yr in group 1 and group 2
P3 significance between Group 1 and Group 2 in Pre-operative , post-operative immediate and post-operative 1yr groups.



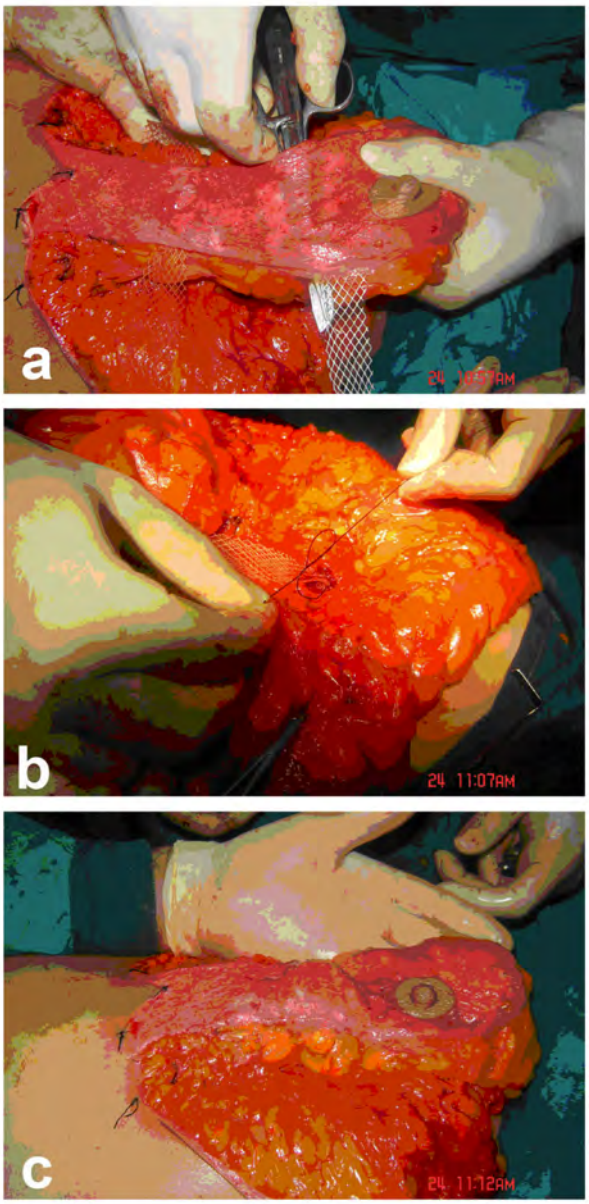


Fig 1. (a) Subdermal tunnel for mesh fixation to the pedicle. (b) Fixation of mesh to the rib periostium, (c) Inferior pedicle after fixation of two mesh straps.

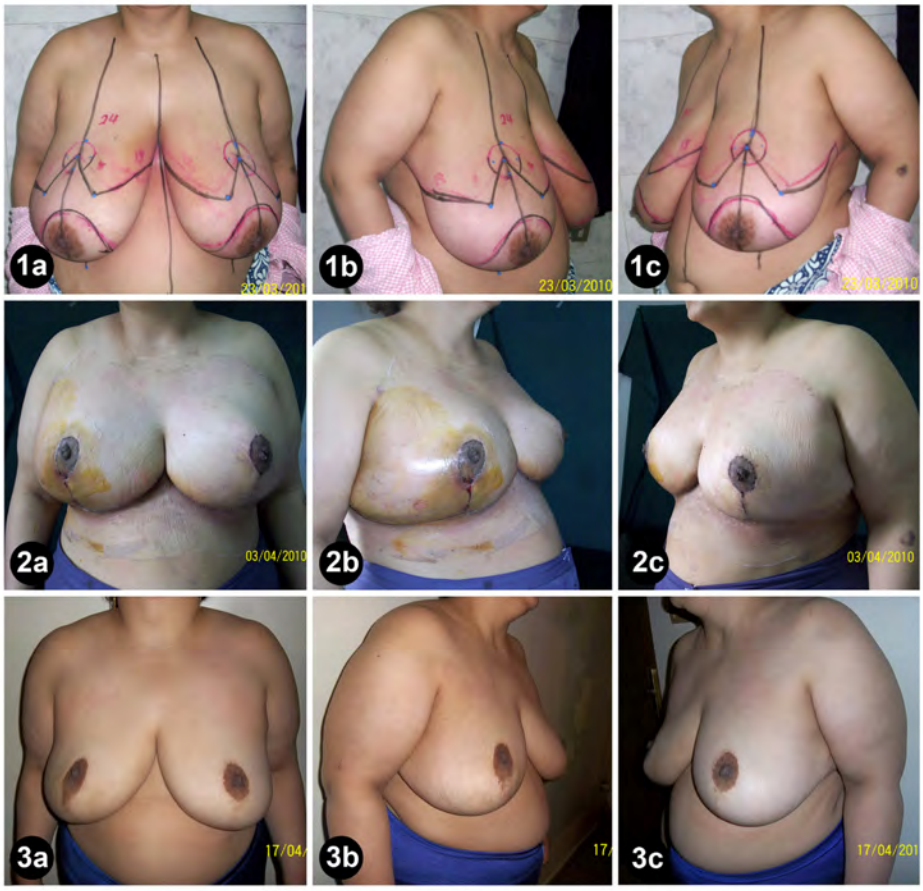


Fig 2-1. A 40 years-old female with breast hypertrophy, nipple at 35 cm before (1a, b, c), immediate postoperative (2a, b, c), and 1-year after inferior pedicle reduction mammaplasty with mesh support (3a, b, c). Total amount of tissue removed: 1200 g for each breast.

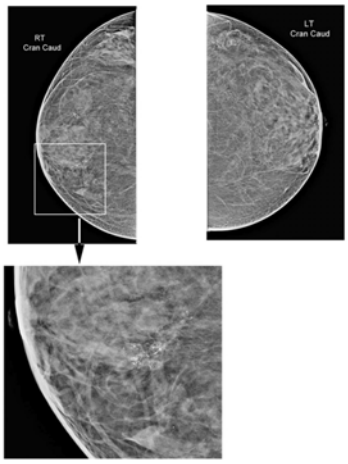


Fig 2-2. Bilateral soft tissue mammography in Cranio-Caudal view one year post-operative of a patient treated with inferior pedicle breast reduction with light weight mesh support. The mesh was fixated to the pectoralis fascia using nylon sutures and is not visible. Punctat benign appearing calcification, Parenchymal redistribution, nipple elevation, sub-areolar thickening, maintaining the continuity of the sub-areolar ducts to the nipple. The gross calci-fications present in the minority of cases were seen in the glandular breast tissue far from the mesh.The mesh is not visible and did not interfere with the detection of minute and diffuse benign microcalcifications present in this case within an area of scarring (i.e., unrelated to the presence of the mesh). This demonstrates that accurate mammographic interpretation is possible after this operation.

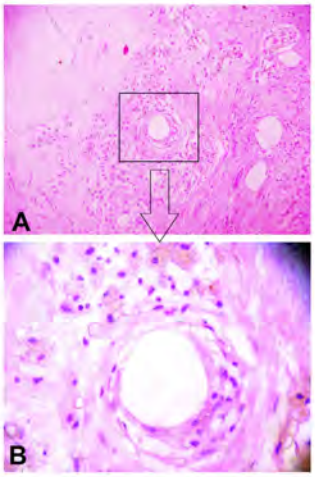


Fig 2-3.

A pice of breast tissue was taken one year postoperative in patient with inferior pedicle breast reduction with lightweight mesh (UltraPro) support. (A) Shows the non absorbable polypropylene fiber of the mesh imbedded in adipose tissue surrounded by thin layer of reactive collagenous tissues and mild inflammatory reaction with few infiltration of chronic inflamatory cells at the interface between the mesh fiber and host tissue which is expected finding (H&E, 100x) . (B) magnifications at 400x that revels fibroplastic infiltration and thin collagen fibers around the polypropyline fiber.

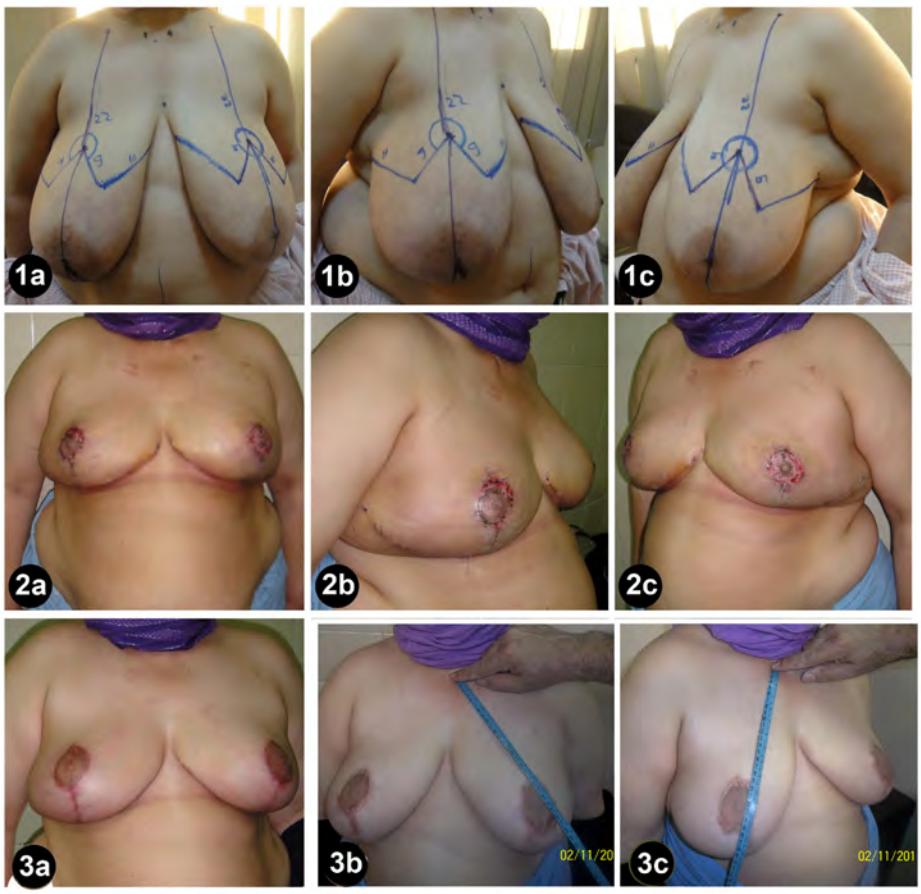


Fig 3-1. A 36 years-old female with breast hypertrophy, nipple at 40 cm before (1a, b, c), immediate post-operative (2a, b ,c), 3 months (3a), and one-year (3b, c) after inferior pedicle reducion mammoplasy with mesh support. Total amount of tissue exised 1300 g per breast.

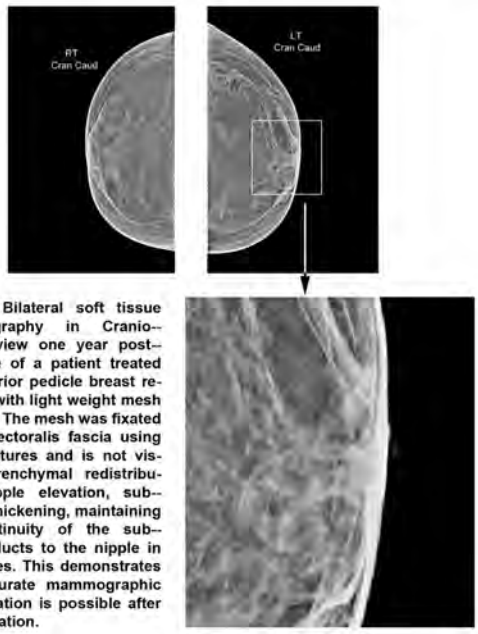


Fig 3-2. Bilateral soft tissue mammography in Cranio--Caudal view one year post--operative of a patient treated with inferior pedicle breast reduction with light weight mesh support. The mesh was fixated to the pectoralis fascia using nylon sutures and is not visible. Parenchymal redistribution, nipple elevation, sub--areolar thickening, maintaining the continuity of the sub--areolar ducts to the nipple in both sides. This demonstrates that accurate mammographic interpretation is possible after this operation.

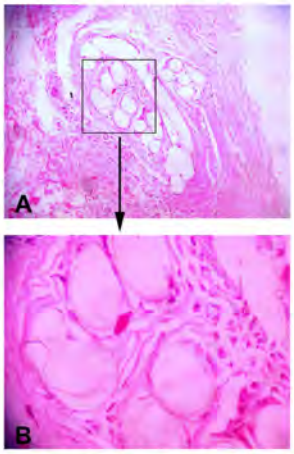


Fig3-3. A pice of breast tissue was taken one year postoperative in patient with inferior pedicle breast reduction with lightweight mesh (UltraPro) support. (A) Shows the non absorbable polypropylene fiber of the mesh imbedded in adipose tissue surrounded by thin layer of reactive collagenous tissues and mild inflammatory reaction with few infiltration of chronic inflamatory cells at the interface between the mesh fiber and host tissue which is expected finding (H&E, 100x) . (B) magnifications at 400x that revels minor foreign body reaction around the mesh fibers as fibroplastic infiltration and thin collagen fibers around the polypropyline fiber, apperance of multinucleated giant cels and thin collagen fibers around the polypropyline fiber.

Discussion

The inferior pedicles are the most popular technique used among plastic surgeon as Okoro at 2008 concludes that (69 %) of plastic surgeon prefer the inferior pedicle technique for breast reduction especially in North America [7].

Pseudoptosis is a common problem after reduction mammaplasty. This is especially true after the inferior pedicle reduction technique. Many surgeons continue to use the inferior pedicle technique because it is easy to perform, familiar, and useful for very large breasts [4].

Given the utility of and need for the inferior pedicle technique, surgeons have tried numerous ways to prevent the phenomena of postoperative "bottoming out". These include suture suspension of the pedicle to the chest wall, dermal bra, dermal strips, superficial fascial suspension, fascia lata, and muscular slings [5].

The use of mesh to support the breast parenchyma was first described by Goes in 1992. His initial report described the use of an absorbable polyglactine mesh, but since then he has described the use of a polyglactine and polyester mesh to add a component of permanent mesh because of recurrence of ptosis seen with the former technique [5].

In our study we are using synthetic, partially absorbable, mixed and lightweight mesh to overcome the loss of autologous grafts, maintain the results, and reduce the tissue reaction. This is proved by follow up of our patients and they are fully satisfying with the result as they have soft pliable breasts, no mish extrusions, no added infections because of the mesh, no interference with the mammographic study of the breast, long standing result with no bottoming out as the longest follow up periods was 30 month.

In the current study we use two straps of mixed lightweight mesh 2 cm width and 30 cm length to support the inferior dermoglandular pedicle and fix it to periostum of the ribs, pectoralis muscle and fascia. Outcomes improved by using a mixed mesh composed of

60% Polyglactine and 40% nonabsorbable polyester. This mesh combined the initial shaping properties of the breast with the durability. As a result, the ideal shape and anterior projection of the newly shaped breast were maintained for longer periods of time. There were no bottoming out could be noted in the group one with mesh while there was a different grades of bottoming out in group two.

Mammography remains the ideal screening method for detecting no palpable breast cancer, and any factor that can possibly interfere with this examination should be avoided. Although the application of nonabsorbable elements has never been shown to be such a factor, some authors believe that avoiding them is the only guarantee for a reliable clinical and radiologic surveillance of breast cancer [6].

The postoperative mammographic study reveals no mesh detection in all patients. Parenchymal redistribution in 14 cases (93.3%), detection of oil cyst, dermoid cyst, retro areolar fibrosis and microcalcifications with no significant difference between both groups. This is in agreement with Góes JC [6].

Our results demonstrate that the presence of mesh did not lead to surgical complications, palpability after the operation, or the formation of densities, speculations, or microcalcifications. The gross calcifications present in the minority of cases were seen in glandular breast tissue far from the mesh. Additionally, mammographic interpretation was performed uneventfully, and there was no interference with the diagnosis and treatment of even minute lesions such as benign localized microcalcifications.

Histopathological examination was done one year postoperative that results in significantly decreased typical chronic inflammatory reaction, the fibrotic reaction around the mesh in total as well as around single mesh fiber is greatly reduced, and it shows the non absorbable polypropylene fiber of the mesh imbedded in adipose tissue surrounded by thin layer of reactive collagenous tissues and mild inflammatory re-

action which is expected finding, this is agreeing with the study of Bernd Klosterhalfen 2005[8].

Our results demonstrate that the presence of mesh did not lead to surgical complications, palpability after the operation, or the formation of densities, spiculations, or microcalcifications. The gross calcifications present in the minority of cases were seen in 6 cases (40%). The Application of Mesh Support the glandular breast tissue was far from the calcifications. Additionally, mammographic interpretation was performed uneventfully, and there was no interference with the diagnosis and treatment of any breast lesions.

Finally, we hope that the evidence presented in this report demonstrates the safety of using lightweight mesh in inferior pedicle breast reduction surgery. In this way, some of the most unfavorable long-term outcomes such as loss of projection and recurrence of ptosis may be attenuated. Additionally, the availability of a stronger supporting system may lead to the development of even more satisfactory breast surgery techniques in future.

Conclusion

The use of lightweight mesh as a supporting system in the inferior pedicle technique has significant reduction in the bottoming out that follow inferior pedicle technique and did not result in visible or palpable deformities, surgical complications, or interference with mammographic interpretation.

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THE APPLICATION OF LIGHTWEIGHT MESH SUPPORT IN INFERIOR PEDICLE BREAST REDUCTION; CLINICAL, PATHOLOGICAL AND RADIOLOGICAL EVALUATION

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METRONOMIC CAPECITABINE FOR ELDERLY ADVANCED GASTRIC CARCINOMA PATIENTS PRETREATED WITH ONE OR TWO SYSTEMIC CHEMOTHERAPY LINES

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Abstract

Purpose The aim of this study was to investigate the efficacy and safety of metronomic capecitabine chemotherapy in pretreated elderly patients with advanced gastric cancer (AGC).

Patients and Methods Eligible patients were treated with capecitabine at a fixed dose of 1000 mg daily until disease progression or toxicity. Endpoints were overall response rate (ORR), safety ,progression-free survival (PFS) and overall survival (OS).

Results Thirty -eight patients were enrolled from June 2004 to Oct. 2011. Median age was 69 years (range : 67-75). The majority were males (74%). Two patients received just 2 weeks of treatment and then refused continuation due to deterioration of general condition. Consequently, 36 out of 38 patients were assessed for response. No complete response was observed, 6 patients (17%) achieved partial response, 13 patients (36%) showed stable disease and 17 patients (47%) showed progression of disease during treatment. The disease control rate (response rate +stability rate) was 53%.

Treatment was well tolerated. The most common hematologic grade 3 or 4 adverse effects was neutropenia [3/38 patients (8%)], followed by mucositis [2/38 patients (5%)]. There was one reported case of neutropenic fever. The reported hand- foot syndrome cases were all <grade 3. No treatment -related death existed. Treatment delays were observed in 8 patients.

Symptomatic improvement was evident in 18 of 36 (50%) patients.

Median follow up period was 6.5 months. Median time to progression was 3 months (range:0-7 months) while the median overall survival was 7 months (range: 3-15 months).

Conclusion : *Metronomic capecitabine was effective and tolerable in elderly patients with pretreated AGC.*

Key words: *Metronomic capecitabine, advanced gastric cancer, elderly cancer patients.*

Introduction

A number of randomized clinical trials have established the role of chemotherapy in the treatment of patients with advanced gastric cancer (AGC). In the nineties of the previous century , trials that compared chemotherapy plus best supportive care with best supportive care alone reported statistically significant longer survival in the former[1,2]. Moreover, quality of life, was proved to be statistically superior in the chemotherapy-treated groups over best supportive care alone [3].

Metronomic chemotherapy refers to the frequent administration of chemotherapeutics at doses significantly less than the maximum-tolerated dose, with no prolonged drug-free breaks[4]. An antiangiogenic activity is prominent with the protracted exposure to low doses of chemotherapeutics if compared with their cyclic administration at the maximum-tolerated dose[5]. Capecitabine is an oral fluoropyrimidine which is enzymatically converted to 5-fluorouracil (5-FU), when absorbed from the gastrointestinal tract to be generated preferentially at the tumor site[6]. Metronomic capecitabine has been used as a single agent for several advanced tumors as gastrointestinal tract tumors and breast cancer after prior treatment failure[7,8]. This study aimed at investigating the efficacy and safety of metronomic capecitabine in elderly patients with AGC after failure of one or more chemotherapy regimens.

Patients and Methods

This prospective, phase II trial was accomplished at the Clinical Oncology & Nuclear Medicine Department of Mansoura University and the outpatient clinic of East Delta Insurance Institute of Mansoura. Eligibility criteria were patients above 65 years of age with histologically proven AGC, after failure of first or second lines of treatment, existence of at least one measurable lesion, elapsing of at least 4weeks since prior chemotherapy or radia-

tion therapy, life expectancy greater than 3 months, Eastern Cooperative Oncology Group (ECOG) performance status ≤2, normal marrow and organ function (leukocytes ≥3,000/μL, absolute neutrophil count ≥1,500/μL, platelets ≥100,000/μL, total bilirubin within normal institutional lmits, AST/ALT ≤2 x institutional upper limit of normal, creatinine within normal institutional limits). Cases with only brain metastasis were excluded. The trial was approved by the local ethics committee. Written informed consent was required.

Study Schedule:

Before treatment,each patient gave a complete history. Physical examination, complete blood count, liver and renal function tests, electrolytes assessment, ECG, chest radiograph and computed tomography (CT) scanning of the bdomen and pelvis were done. Metronomic capecitabine was administered orally at a dose of 1000mg daily contiuously until disease progression or toxicity. Biweekly evaluation during treatment included physical examination, assessment of performance status,and complete laboratory profile. Treatment was interrupted in patients with grade III or IV toxicity. Growth factors were given if indicated and not prophylactically.

Response Evaluations

Response to treatment was evaluated every 8 weeks using the Response Evaluation Criteria in Solid Tumors[9]. Toxicities were graded using the National Cancer Institute Common Terminology Criteria of Adverse Events version 3[10].

Statistical Analysis :

The end points of this study included clinical tumor response, treatment toxicity, and survival. Post-treatment toxicities were coded as the worst grade observed after the start of treatment. Symptomatic improvement was defined as subjective improvement of at least one tumor-related symptom (e.g., dysphagia, weight loss, loss of appetite and pain) without worsening of any other symptom or appearance of any new tumour-related symptom.

Patient characteristics and adverse events were presented as percentages or median values with ranges.

The Kaplan-Meier method was used to generate progression -free and overall survival curves. Progression - free survival was measured from the date of entry into the study until the date of progression or death. Overall survival was measured from the date of entry into the study until the date of death. The Log rank test was used to determine the statistical significance of difference between survival curves. Statistical analysis were performed using SPSS version 10.

Results

Patient characteristics

Thirty-eight eligible patients were enrolled during the predetermined period of study(from June 2004 to Oct. 2011). Tables (1&2) lists the baseline characteristics and previous treatment received respectively. Median age was 69 years (range : 67-75). The majority were males (28 patients representing 74%). The most common site of disease was the lymph nodes (37%). The most common regimens used were 5FU - based. First - line regimens were received by all the cases while second - line regimens were received by 4 patients. Median time to tumor progression while on the preceding line received was 9 months.

Response:

Two patients received just 2 weeks of treatment and then refused continuation in the study due to worsening of clinical conditions. Consequently, 36 out of 38 patients were assessed for response. No complete response was observed while 6 patients (17%) achieved partial response. Thirteen patients (36%) showed stable disease and 17 patients (47%) showed progression of disease during treatment (Table 3). The disease control rate (response rate + stability rate) was 53%.

Tolerability:

Treatment was well tolerated. Adverse effects are listed in Table (4). The most common hematologic grade 3 or 4 adverse effects was neutropenia [3/38 patients

(8%)], followed by mucositis [2/38 (5%)]. There was one reported case of neutropenic fever. The reported hand- foot syndrome cases were all of grade 1. No treatment -related death existed. Treatment delays were observed in 8 patients. Progression of disease was the cause of stopping treatment in 36 patients.

Symptomatic improvement and survival:

Symptomatic improvement was evident in 18 of 36 (50%) patients.

The median follow up period was 6.5 months.The median progression -free and overall survivals were 3 months (range:0-7) and 7 months (range 3-15) respectively.

Table 1 : Baseline clinicopathological characteristics for enrolled patients (n = 38)

Variable	*No.*	*%*
Median age:	69(range:67-75)	
Gender:		
Male	28	74
Female	10	26
ECOG performance status:		
0/1	21	55
2	17	45
Histology:		
Adenocarcinoma, well differentiated	1	3
Adenocarcinoma, moderately differentiated	7	18
Adenocarcinoma, poorly differentiated	22	58
Signet ring cell carcinoma	8	21
No. of metastatic sites:		
1	10	26
2	17	45
>or=3	11	29
Organs involved:		
Lymph nodes	14	37
Liver	6	16
Peritoneum	7	18
Lung	5	13
Bone	3	8
Abdominopelvic mass	7	18

NB: ECOG, Eastern Cooperative Oncology Group; PS, performance status; 5-FU, 5 fluorouracil

Table 2 : Prior treatment of enrolled patients (n = 38)

Surgery	20(53)
Preoperative chemoradiotherapy(5-FU/cisplatin)	6 (16)
Postoperative chemoradiotherapy(5-FU/leucovorin)	4 (11)
Perioperative chemotherapy(epirubicin/cisplatin/5-FU)	5 (13)
First-line (epirubicin/cisplatin/5-FU)	9 (24)
First-line (paclitaxel/cisplatin)	9 (24)
First-line (5-FU /oxaliplatin)	6 (16)
First-line (paclitaxel/carboplatin)	7 (18)
First-line single agent paclitaxel	7 (18)
Second-line (irinotecan/5FU)	2 (5.3)
Second –line(irinotecan/cisplatin)	2 (5.3)

Table 3 : Best overall response of 36 patients with measurable disease

Response	*No. patients*	*%*
Complete response	0	0.0
Partial response	6	17
Stable disease	13	36
Progressive disease	17	47

Table (4): Toxicities in 38 patients.

Toxicity type	**Toxicity Grades**			
	Grade 1 n (%)	***Grade 2 n (%)***	***Grade 3 n (%)***	***Grade 4 n (%)***
Neutropenia	10(26)	4 (11)	2 (5)	1(3)
Anemia	6 (16)	2 (5)	1(3)	-
Thrombocytopenia	6 (16)	1 (3)	-	-
Anorexia	10 (26)	5 (13)	1 (3)	-
Neusea	5 (13)	1 (3)	-	-
Vomiting	3(8)	-	-	-
Abdominal pain	2 (5)	2(5)	-	-
Mucositis	6 (16)	4(11)	2(5)	-
Diarrhea	6 (16)	2(5)	1(3)	-
Hand -foot syndrome	6(16)	-	-	-
Liver	2 (5)	-	-	-
Sensory neuropathy	2 (5)	-	-	-
Fatigue	5 (13)	5(13)	1(3)	-

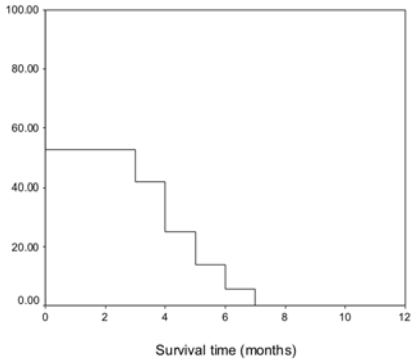

Fig. (1): Progression-free survival of 36 patients.

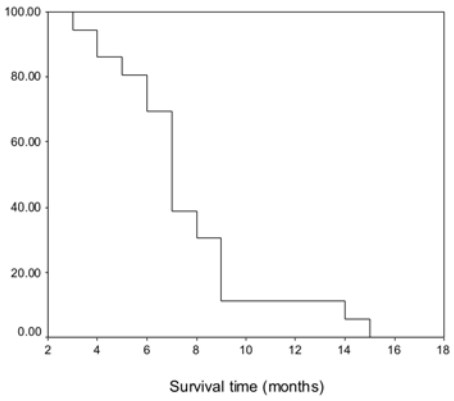

Fig. (2) : Overall survival of 36 patients.

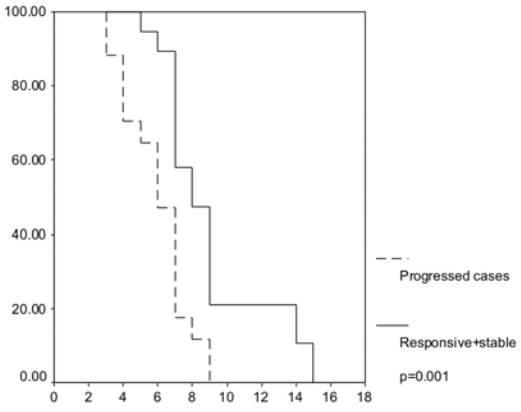

Fig. (3): Overall survival of cases showing progression versus that of others.

Discussion

During the 1980s, the reference treatment for advanced gastric carcinoma was the combination 5-FU, doxorubicin and mitomycin C (FAM). In 1991, a randomised European Organisation for Research and Treatment of Cancer (EORTC) clinical trial found that the 5-FU, doxorubicin and methotrexate combination (FAMTX) was superior to FAM[11]. In 1994, a similar randomised clinical trial found that the same was true of PELF regimen (cisplatin, epirubicin, leucovorin, 5-FU) designed by the Italian Oncology Group for Clinical Research (GOIRC)[12]. A number of relatively new agents (particularly the taxanes, irinotecan and oxaliplatin) have also been found to have significant activity in advanced gastric carcinoma[13]. As a consequence, a number of phase II and few phase III trials have been conducted combining paclitaxel, docetaxel or irinotecan with 5-FU (plus or minus leucovorin), cisplatin or both[14-16]. Other phase II studies have combined oxaliplatin with 5-FU and leucovorin[17].

Frequent administration schedules of cytotoxics, without protracted breaks (metronomic delivery), has been tested to optimize the antiangiogenic effects and minimize toxicity[18,19]. Metronomic low-dose chemotherapy is increasingly recognized as a useful tool for the treatment of several types of cancer, such as hormone-refractory prostate cancer[20] heavily pretreated sarcoma[21] melanoma[22] ovarian cancer[23] and breast cancer[24].

The results of the present study indicated that a clinically relevant fraction of AGC patients (53%) achieved a control of the disease (response +stability). Our results are in line with results of He et al[25]. They evaluated metronomic capecitabine at a dose of 1,000mg daily from day1-28 every 5 weeks in 45 pretreated elderly AGC patients. The disease control rate was 51% and median progression-free and overall survival were 3.6 and 7.6 months respectively.

The limited grade III and IV toxicities in the present study agrees with other trials using metronomic capecitabine alone like that of Ballardini et al[26] who evaluated

metronomic capecitabine at a dose of 1,000mg daily in metastatic HCC associated with Child-Pugh class B cirrhosis and Fedele P et al[27] who evaluated metronomic capecitabine at a dose of 1500mg daily in 60 heavily pretreated metastatic breast carcinoma.

Hand-foot syndrome (HFS) is a relatively common dermatologic toxic reaction to certain anticancer therapies. Although not life-threatening, this complication can reduce patient quality of life. Dose modification of the inciting agent serves as the most effective management[28]. Data of phase II and III trials have shown that the incidence of grade 1 to 3 HFS with capecitabine in general is in the range of 43% to 71%. Grade 3 has been observed in 5% to 24% of these patients[29,30]. In the present study just grade one hand and foot syndrome was reported with good response to topical emollients and oral systemic corticosteroids. No patients withdrew from treatment because of such toxicity.

Respecting the palliative aim in the present study, to what extent would the severity of toxicity be if a combination regimen based on metronomic capecitabine rather than metronomic capecitabine alone was used in the present trial?. In fact this issue was investigated in the literature. As an example, Sperone et al[31] assessed the activity and toxicity of gemcitabine plus metronomic fluoropyrimidines in heavily pretreated advanced adrenocortical carcinoma (ACC)patients. From 1998 to 2008, 28 patients with advanced ACC progressing after mitotane plus one or two systemic chemotherapy lines were enrolled. They received a combination of i.v. gemcitabine (800 mg/m(2), on days 1 and 8, every 21 days) and i.v. 5-fluorouracil protracted infusion (200 mg/m(2)/daily without interruption until progression) in the first six patients, or oral capecitabine (1500 mg/daily) in the subsequent patients. Mitotane administration was maintained in all cases. A relatively high rate of grade III and IV hematologic toxicity in the form of leukopenia (21.4%) was reported which is indeed a considerable toxicity rate if occurs in the elderly.

An attractive extension of the present work could be testing the application of metronomic capecitabine with target therapy, in particular antiangiogenic drugs. Such combinations are particularly appealing because high local concentrations of vascular endothelial growth factor (VEGF) in the tumor environment was found to promote multidrug resistance in tumor endothelium[4,32,33]. Hurwitz et al.[34] reported a large interesting study in which bevacizumab (humanized monoclonal antibody against VEGF) combined with standard chemotherapy regimens significantly improved survival for patients with advanced-stage metastatic colorectal carcinoma. Of 813 patients with previously untreated metastatic colorectal cancer, they randomly assigned 402 to receive irinotecan, bolus fluorouracil, and leucovorin (IFL) plus bevacizumab (5 mg per kilogram of body weight every two weeks) and 411 to receive IFL plus placebo. The median duration of survival was 20.3 months in the group given IFL plus bevacizumab, as compared with 15.6 months in the group given IFL plus placebo. ($P<0.001$). These benefits may extend to the combination of antiangiogenic agents with metronomic regimens of cytotoxic agents aiming at a more tolerable toxicity profile.

Moreover, combined therapy of continuous low dose capecitabine and high dose celecoxib targeting angiogenesis was used in a phase II trial to treat advanced cancer patients with different types of tumors[35]. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was used to monitor antiangiogenic effects. Therapy consisted of 2 x 500 mg oral capecitabine / day and 2 x 400 mg oral celecoxib / day continuously until progression of disease. Thirty -seven patients were treated. Therapy was well tolerated without grade 3 and 4 toxicities. Disease stabilization after 3 cycles was seen in 11 patients. DCE-MRI demonstrated a reduction of tumor vessel permeability and blood flow in patients who reached stable disease or some minor regression.

Another example is that 46 patients with advanced breast cancer were candidates to receive

metronomic oral capecitabine (500mg thrice daily), cyclophosphamide (50 mg daily) and bevacizumab (10 mg/kg every 2 weeks). Overall response rate was 48%, median time to progression was 42 weeks and toxicity was generally mild. Grade 3 or 4 non-hematologic adverse effects included hypertension (n = 8), transaminitis (n = 2), and nausea/vomiting (n = 2)[36].

Such extension of the present study should be in the form of phase III study to be able to evaluate the real benefit of using target therapy plus metronomic capecitabine. However, it did not start due to economic reasons.

The median overall survival in the present study was 7 months a figure larger than that reported in relatively recent respectable phase III trials of AGC in their best supportive care arms[37,38]. Between 10/2002 and 12/2006, 40 pts with metastatic or locally advanced gastro-oesophageal junction or gastric adenocarcinoma were randomised. Treatment arms were Arm A: Irinotecan 250mg/m (2)q3w (first cycle) to be increased to 350mg/m(2), depending on toxicity, while arm B: best supportive care. Rate of Improvement of tumour related symptoms: was 50% in arm A and 7%. In arm B. Median survival of arm A was 4.0months (95% CI 3.6-7.5) and for arm B: 2.4months (95% CI 1.7-4.9)[37]. While in another study,patients with AGC with one or two prior chemotherapy regimens involving both fluoropyrimidines and platinum and with an ECOG 0 or 1 were randomly assigned in a ratio of 2:1 to salvage chemotherapy plus best supportive care or best supportive care alone. Primary end point was overall survival. Median OS was 5.3 months among 133 patients in the salvage chemotherapy arm and 3.8 months among 69 patients in the best supportive care arm (P = .007). The overall survival benefit for salvage chemotherapy arm was consistent in most of the prospectively defined subgroups, including age, PS, number of prior treatments, metastatic sites, hemoglobin levels, and response to prior chemotherapy[38].

Conclusion

Our results indicate that

metronomic capecitabine may be considered a safe and valid treatment option for advanced gastric cancer patients after failure of previous lines of chemotherapy, especially when the aim of medical treatment is to arrest tumour growth on an outpatient base without hospitalization and without affecting the patient's quality of life. Nevertheless, further clinical studies, as well as a greater clinical experience are required in order to better define the role of the metronomic treatment strategy in medical oncology.

Authors' Disclosures Of Potential Conflicts Of Interest

The author(s) indicated no potential conflicts of interest.

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METRONOMIC CAPECITABINE FOR ELDERLY ADVANCED GASTRIC CARCINOMA PATIENTS PRETREATED WITH ONE OR TWO SYSTEMIC CHEMOTHERAPY LINES

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RECTAL ADVANCEMENT FLAP : A SIMPLE AND EFFECTIVE APPROACH FOR SOME CASES OF RECTOURETHRAL FISTULA

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Abstract

Background : *Rectourethral fistula (RUF) is a rare entity that represents a complex management problem for any surgeon. Current study analyzed the outcome of rectal advancement flap (RAF) as a simple and effective approach for some cases of rectourethral fistula.*

Methods : *A total of 7 patients with RUF were included and assigned to RAF.*

Results : *Mean hospital stay was 7(±0.74) days with a range of 2 days. No postoperative mortality was seen. Mean follow up was12.14 (±3.98) months with a range of 10 months. The results of mean anal pressure measurements postoperatively showed no significant differences compared with preoperative measurements. The results of postoperative WIS showed high significant improvement(p value = 0.016). Successful primary closure was achieved in only two (28.57 percent) of seven patients. All five patients with unsuccessful repairs underwent subsequent RAF repair. Three patients subsequently had successful closure, for an overall success of 71.42 percent (5/7). By the time of the last follow-up, two persistent fistulas were noticed, for a total failure rate of 28.57 percent (2/7).*

Conclusion : *Although not applicable to all rectourethral fistulas, we advocate RAF repair for the primary and repeat repair of RUF, when technically feasible. The transanal approach is the simplest procedure and also happens to be less invasive for fistulas within approximately 6 cm from the anal verge. Before fistula repair RAF should be covered by*

the use of proximal fecal diversion with the consideration of a suprapubic catheter in the majority of patients.

Key words : *Urethra; Rectum; Fistula; Rectal advancement flap.*

Introduction

Rectourethral fistula (RUF) is a rare entity that represents a complex management problem for any surgeon[1]. Its aetiology is divided into congenital origin accompanied by anal atresia, and other acquired origins as trauma, prostatic or rectal surgery, pelvic radiation, cryotherapy, radiofrequency hyperthermia for benign prostatic hypertrophy and Crohn's disease[2].

The prostatic urethra being adjacent to the rectal wall is the commonest site for fistulation to occur[3]. Less commonly the bulbous and membranous urethra may be involved.

Rectourethral fistula may result in faecaluria, pneumaturia, recurrent urinary tract infection and/or drainage of urine per anum[4].

As with any rare or complicated surgical problem, many types of repairs have been described, including transperineal, transsphincteric,and transanal flap repairs; gracilis muscle interposition; transabdominal repair; and transanal endoscopic repair(5). These repairs are supported by data from many studies[6-11]. Other studies report modified repairs including a combination of the transsphincteric approach with advancement flap closure, combination rectal advancement flap and gracilis muscle interposition, transsphincteric approach without diversion and perineal approach with gracilis muscle interposition[12-16]. Conservative management alone, with diversion and antibiotic therapy without any attempt at repair, has also been described[17].

The aim of the present study was to review our experience with rectal advancement flap(RAF) for the treatment of some cases of RUF.

Patients and methods

The potential study population comprised 11 male patients with recto-urethral fistulae referred to

our Colorectal Surgery Unit, Mansoura University Hospital in the period from January 2005 to January 2012 with one or more of the following symptoms: urine per rectum, pneumaturia, fecaluria and/or reccurent UTI. Patients with Crohn's disease with active proctitis, malignant or radiation-related fistula, stricture of the anorectum or an external sphincter defect were excluded from the study. A total of 7 patients fulfilled the study criteria. Three of the fistulas had recurred after failed previous repairs at other hospitals, and data were not available as to the exact nature of these repairs. The Ethics Committee of the University of Mansoura approved the study protocol. All patients participating in the study gave a written informed consent.

All patients were evaluated preoperatively by clinical interview including Wexner Incontinence Score(WIS)[18]. Anorectal manometry using perfusion catheter systems (Synectics, Stockholm, Sweden) was done for all patients with evaluation of mean anal resting pressure (MARP), mean anal squeezing pressure (MASP) and recto-anal inhibitory reflex (RAIR). Preoperative ascending urethrocystogram and/or rectal contrast studies were done for all patients to estimate the recto-urethral fistulas. EUA combined with proctoscopy and cystoscopy by a urologist were done for all patients to estimate the fistulous openings' diameters and distances from the anal verge.

Diverting colostomies and urostomies were used in five patients. Preoperative antibiotics and bowel preparation (phosphate enemas) were routinely administered. All patients were catheterized.

The procedure was performed under general anaesthesia with the patient in the prone jackknife position. Rectal retractors were placed to expose the fistula opening. A 1:100,000 epinephrine solution was injected submucosally for hemostasis, and a partial-thickness flap incorporating the mucosa and a part of the internal circular muscle layer (muscular mucosa) was created with a broad proximal base (base to apex = 2 to 1). The fistulous tract was excised, and any granulation

tissue was removed. The urethral defect was carefully closed with vicryl 5/0 (Vicryl; Ethicon, Somerville, NJ, USA). The intervening internal muscle layer was approximated using vicryl 3/0. The rectal opening of the fistula was excised from the raised flap; the flap was pulled down and sutured in place, distal to the muscle repair, using vicryl 3/0. Antibiotics were given postoperatively for a variable amount of time. Postoperatively, colostomy patients were permitted to eat and drink and were discharged with the catheter in situ once they were fully mobile. In non-colostomy patients, oral intake was restricted with intravenous fluids for five days followed by clear oral fluids for other five days with gradual return to food intake.

A cystogram was performed at 6 weeks, and if no fistula was demonstrated and the patient was asymptomatic, the urethral catheter was removed.

Reversal of any preoperative diversion was performed at a variable amount of time postoperatively (typically 6-12 weeks) after voiding cystogram and rectal contrast studies were performed to rule out persistent fistula. Persistent fistulas were defined as failure of the operative procedure to eliminate the fistula before closure of any preoperative diversion.

Statistical analysis: data were processed using SPSS version 10 under Microsoft windows XP. P value < 0.05 was significant.

Results

Between January 2005 and January 2012, 7 male patients with an average age of 19.57 (±13.32) years and a range of 35 years presented to our colorectal surgery unit with RUF. Three of the fistulas had recurred after failed previous repairs at other hospitals, and data were not available as to the exact nature of these repairs. The etiology of RUFs included road traffic accidents with fracture pelvis in two patients, falling from height with fracture pelvis in one patient, bullet injury in one patient and anal atresia in three patients. All patients had symptoms of urine per rectum during micturition,

pneumaturia, fecaluria and/or recurrent UTI (Table 1). Openings of the fistulas were all located above the anal sphincter complex with a mean distance being 4 cms from the anal verge and a mean rectal side opening diameter 3.71 mms. The preoperative MARP and MASP were normal in all patients. Preoperative RAIR was delayed in the three post anal atresia patients.

Symptoms occurring after the surgery included discomfort in the anus in all patients, tenesmus without diarrhea in four patients, and feeling of incomplete evacuation in two patients. Mean hospital stay was 7 (±0.74) days with a range of 2 days. All postoperative symptoms spontaneously disappeared within 3 weeks after surgery. No postoperative mortality was seen.

Mean follow up was 12.14 (±3.98) months with a range of 10 months (table 1). Patients were followed up by clinic or telephone interview with specific questionnaires to collect the information about status of fecal control, symptoms of urine per rectum during micturition, pneumaturia, fecaluria and/or recurrent UTI. The interview was scheduled twice a month for the first 3 months, followed by once a month for 6 months. Functional results were evaluated after 6 months by anal manometry and a questionnaire reflecting the symptomatic results involving the Wexner incontinence score.

Postoperative mean anal pressure measurements showed no significant differences compared with the preoperative measurements (Tables 2). Postoperative WIS showed high significant improvement (p value= 0.016) table 3. Postoperative RAIR was persistently delayed in the three post anal atresia patients. Successful primary closure was achieved in only two (28.57 percent) of seven patients. All five patients with unsuccessful repairs underwent subsequent RAF repair. Three patients subsequently had successful closure, for an overall success of 71.42 percent (5/7). By the time of the last follow-up, two persistent fistulas were noticed, for a total failure rate of 28.57 percent (2/7).

Table 1 : Patient characteristics and follow-up data of the 7 patients.

patient	Age in years	aetiology	presentation	diversion	First repair	Second repair	morbidity	Length of stay (days)	Follow up (months)
1	25	RTA Fracture pelvis	urine per rectum	Colostomy	successful	-	-	7	7
2	40	RTA Fracture pelvis	urine per rectum	Colostomy	failed	succeeded	-	7+6	13
3	18	FFH Fracture pelvis	pneumaturia, fecaluria	Suprapubic cystocath	failed	succeeded	-	6+8	14
4	5	congenital	Reccurent UTI	Colostomy+suprapubic cystocath	failed	succeeded	-	7 +8	14
65	7	congenital	pneumaturia, fecaluria	Suprapubic cystocath	succeeded	-	-	8	6
6	10	congenital	urine per rectum, pneumaturia, fecaluria	-	failed	failed	failure	7+6	16
7	32	Bullet injury	urine per rectum, pneumaturia, fecaluria	-	failed	failed	failure	7+7	15

Table 2: comparison between preoperative and postoperative motility studies changes in our patients:

	Preoperative	**Postoperative**	***p* Value**
MARP	62.29±15.64	61.43±16.36	Non-significant
MASP	106.57±12.47	105.71±12.13	Non-significant

MARP = mean anal resting pressure MASP = mean anal squeezing pressure

Results of the two-tailed t test for paired samples (preoperation vs postoperation). The reference values of anal rest pressure and squeeze pressure in our laboratory were 35-70 and 95-175 mmHg, respectively.

Table 3: Comparison between preoperative and postoperative Wexner Incontinence Score in our patients:

	Preoperative	Postoperative	*p* Value
WIS	8.57±3.21	2.86±4.60	0.016

WIS= Wexner Incontinence Score

Discussion

Recto-urethral fistula (RUF) is a rare entity that represents a complex management problem for any surgeon[1]. Its etiology is divided into congenital origin accompanied by anal atresia, and other acquired origins as trauma, prostatic or rectal surgery, pelvic radiation, cryotherapy, radiofrequency hyperthermia for benign prostatic hypertrophy and Crohn's disease [2].

The prostatic urethra being adjacent to the rectal wall is the commonest site for fistulation to occur. Less commonly the bulbous and membranous urethra may be involved [3].

Various operations from the first description of surgical treatment by Cooper [20]. These include: transperineal [8], transsphincteric [19], transrectal [21] and perianal [22] approaches. Abdominoperineal omental interposition [1, 2], mucosal pull-through [3] and the Kraske's posterior perineal approach [7] have also been used. In the last 30 years, the York–Mason method has been the most popular technique [5, 23]. Although effective, this method involves transection of the anal sphincter mechanism and has usually been combined with a temporary colostomy [23].

Advancement rectal flaps have been used with success for the treatment of fistula-in-ano and rectovaginal fistulas [22–24]. The

potential advantages of RAF are obvious. There is no cutaneous incision, and thus pain is minimal, exposure is adequate, blood loss is minimal, and operative time is short, avoids any division of the sphincteric mechanism and it does not complicate future operative interventions.

In our study, postoperative mean anal pressure measurements showed no significant differences compared with the preoperative measurements. MARP is not reduced, going with Finan PJ 1996[25], due to intact external sphincter and key hole deformity is not created.

Most studies advocate the use of urinary diversion, often with concomitant use of fecal diversion, as the primary stage in the management of RUF [5, 13]. In our study, we noted that only two patients underwent repair without any type of diversion, both patients had persistent fistulas and ultimately required permanent diversion.

Garofalo et al. [5] reported a 20-year experience at the Cleveland Clinic, wherein primary success was reported in 8 of 12 (67%) patients, rising to 83% after repeat procedures. In our study, Successful primary closure was achieved in only two of seven patients (28.57 percent). All five patients with unsuccessful repairs underwent subsequent RAF repair. Three patients subsequently had successful closure, for an overall success of 71.42 percent (5 of 7 patients) and high significant postoperative improvement of WIS.

None of the patients in the present series had had radiotherapy and are thus good candidates for a primary repair, without the use of additional tissue grafting.

Although the transanal approach is the simplest procedure and also happens to be less invasive, this approach may only be adopted for fistulas within approximately 6 cm from the anal verge.

Conclusion

Although not applicable to all rectourethral fistulas, we advocate RAF repair for the primary and repeat repair of RUF, when

technically feasible. The transanal approach is the simplest procedure and also happens to be less invasive for fistulas within approximately 6 cm from the anal verge. Before fistula repair RAF should be covered by the use of proximal fecal diversion with the consideration of a suprapubic catheter in the majority of patients.

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RECTAL ADVANCEMENT FLAP : A SIMPLE AND EFFECTIVE APPROACH FOR SOME CASES OF RECTOURETHRAL FISTULA

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CALCANEOSTOP VERSUS CALCANEAL LENGTHENING IN TREATMENT OF FLEXIBLE FLAT FEET IN CHILDREN

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Abstract

Infants are born with flexible flat feet, and the normal foot arch develops in the first decade of life. Although flexible flat feet rarely cause disability, it is still one of parents' major concerns. Children with flexible flat feet will be brought to consult pediatric orthopedists, and it is generally believed to lead to gait disorders later. This has long been a topic that questions what is normal and what is pathologic, when to treat and when to observe, what is the best conservative treatment, and when to intervene surgically and with what approach (1, 2,3).

Thirty idiopathic flexible flat feet in sixteen patients were included in this study; fourteen were bilateral and two were unilateral. Ten patients were females and six were males. The mean age at surgery was 11.56 years (range 6 -13 years). All patients presented with pain, limitation of daily activity and history of failed conservative treatment. We had 2 groups; fifteen feet in each group, to avoid bias random sampling method was used. Odd number patients treated with calcaneostop as described by Jerosch et al(4) and even number were treated with anterior calcaneal osteotomy as described by Mosca(5). All feet were assessed radiologically by measuring the anetro-posterior and lateral talo-calcaneal angles, and the antero-posterior talo-first metatarsal angle in standing antero-posterior and lateral views of the foot and ankle. The radiographic data were reported at time of surgery and at follow-up after surgery.

The mean follow up period was 27.43 months (range 24-34 months). When comparing the radiological angles in both AP and lateral view we found that the calcaneostop could correct the lateral and lateral talo-calcaneal angle but not the AP talo-first metatarsal angle. On the opposite hand the anterior calcaneal lengthening corrected all three angles.

Introduction

Till now there is no universal agreement about the indications of surgical treatment of idiopathic flexible flat feet. Flexible flat feet are still the most common cause of seeking pediatric orthopaedic consultation. This is due to the concern of the parents about the shape of the feet of their child. Although most of the time it is only the shape of the feet is the main concern of the parents but sometimes it is the abnormal gait or easily fatigability of the child. Most of those flat feet are the flexible ones; where the arch of the foot is reconstructed in the non-weight bearing position and disappear when the child is standing.

One of the first questions most parents want to be answered is the need of treatment of such deformity and shall it lead to a long lasting disability to their child. One of the good points while talking to the family is to inquire about the family history of flat feet. If there is a family history this will help in giving a prospective view of the natural history of the disease. The most classic paper in the natural history of flat feet is that written by Edwin and Harris[6] on the Canadian recruits in 1948. This is the largest and longest study discussing this issue to our knowledge. They concluded that flexible flat feet are classified into two categories based on the tightness of the tendo-Achilles, and they claimed that the flexible flat feet with tight tendo-Achilles is the pathological one and the category which needs treatment. Moreover they postulated -based on the long term follow up- that the children without tight tendo-Achilles will live a normal life with no activity limitation and they does not need any kind of treatment or follow up. This categorization is supported by Staheli et al[7]; they also suggested only documentation and observation for such children.

Tight tendo-Achilles testing is obvious to be crucial in the decision making as regarding to treat or not to treat flexible flat feet. A great care should be directed to evaluate the tendo-Achilles tightness in a proper way. In our study we included only cases with tight tendo-Achilles as proved with Silfverskiöld's test [8].

There are many surgical techniques in the literature described to treat flexible flat feet. Calcaneostop screw method and anterior calcaneal lengthening osteotomy are the most popular methods. To our knowledge there is no study in the literature comparing both procedures in the treatment of flexible flat feet. In this study we compared the results of the anterior calcaneal osteotomy and the calcaneostop screw in the treatment of idiopathic flexible flat feet in children.

Patients and Methods

In the period between March 2009 and December 2012 a prospective study was carried out on 16 children with painful idiopathic flexible flatfeet. All cases were selected from the pediatric orthopaedic outpatient clinic of Mansoura University.

The cases included in the study were:

1) Idiopathic flatfeet: none of our cases had any known etiological causes (neurological, muscular, genetic or generalized ligamentous laxity).
2) Flexible flatfeet: rigid flat feet were excluded (e.g. congenital vertical talus, tarsal coalition, peroneal spastic flat foot).
3) Pain and/or fatigue not relieved by conservative treatment.
4) Age: range from 6- 13 years.
5) All patients had tight tendo-Achilles.

We have used two operative techniques for the selected cases; anterior calcaneal lengthening osteotomy and calcaneostop screw. The method of treatment have been selected by systematic random sampling; so that the patients with odd numbers were treated with calcaneostop screw and those with even numbers by anterior calcaneal lengthening osteotomy.

There were 6 males and 10 females. Of which 14 children were bilateral and 2 were unilateral. The age at surgery ranged from 6 years to 13 years with a mean of 11.51 years.

All patients were subjected to both history taking (the presence of pain and history of conservative treatment). Short tendo-Achilles is

detected with Slifverskiold test[42]. Plain antero-posterior and lateral standing radiograph of the feet and ankle were assessed for:

- Lateral talocalcaneal angle (TCA) (25°-50°)
- Antroposterior talocalcaneal angle (TCA) (30°-50°)
- Antroposterior talo-first metatarsal angle (25°-20°)
- Presence of accessory navicular bones.

The follow up of each group after surgery goes as follow:

a) Anterior calcaneal lengthening group: Below knee cast was applied for 8 weeks and changed at 6 weeks to remove the wire.

b) Calcaneostop group: Below knee cast for 6 weeks giving time for the tendo-Achilles to heal.

After removal of the cast all patients started active mobilization as tolerated. Weight bearing with protective arch support is used for 4 month. In the Calcaneostop group screws were removed under general anesthesia after 18 months and patients were allowed full weight bearing on the same day.

The preoperative radiological angles were compared with the same postoperative angles. The radiographic evaluation scale was based on Vanderwilde et al [9] statistical study on normal feet as in table [1].

Data was analyzed using SPSS program (Statistical Package for Social Sciences) version 17. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Quantitative data was tested for normality by Kolmorgrov-Smirnov test. Paired t-test was used for comparison within groups.

	Mean value	SD 1 (68%) 3points	SD 2 (95%) 2 points	SD 3(99%) 1 point	>or < SD 3 0 points
AP talo- calcaneal angle	39.0	33-45	27-32 ,46-51	21-26,52-57	<20 ,>58
AP talo-first metarsal angle	-15.5	-9 to -21	-3 to -8, -22 to -28	4 to -2, -29 to -34	<-35, >5
Lateral talo-calcaneal angle	44.5	39-50	34-38, 51-58	28-33, 57-61	<27, >62

*(-) Values show abduction in the AP plane and dorsal angulation in the lateral plane, (+) Values show adduction in the AP plane and planter angulation in the lateral plane.
** 36- 30 points, excellent; 29- 24 points, good; 23-18 points, fair; < 18 points, poor.
*** Values in Degrees.

Results

The mean age at surgery was 11.56 years in calcaneostop group and 11.85 years in calcaneal osteotomy group. The median age at surgery was 11.32 years in calcaneostop group and 11.43 years in calcaneal osteotomy groups. There were 6 male children and 10 female children. In the calcaneal osteotomy group there were 4 male children and 6 female children. On the other hand in the calcaneostop group there were 2 male children and 4 female children. All the cases were bilateral except one case in the calcaneal osteotomy and one case in the calcaneostop group. The mean follow up period was 27.43 months for the calcaneal osteotomy group and 27.53 months for the calcaneostop group. The mean follow up for both groups was 27.48 months and the range 24-34 months.

In the anterior calcaneal lengthening group the time at which radiological union at the osteotomy site mean time was 6.26 weeks and the range was 6-7 weeks. In the calcaneostop screw group the mean time for the screw removal was 17.2 months. We removed the screw 18 months after surgery in 13 feet and 12 months in 2 feet.

The last follow up radiological angles measurement in both group is shown in table (2,3,4).

The last follow up radiological angles measurement in both group is shown in table (2,3,4).

Radiological measurements	Calcaneal osteotomy group		Calcaneostop group		P Value
	Mean	Median	Mean	Median	
Lateral talo-calcaneal angle	36.6	36	38.2	38	0.432 Not significant
Anterior talo-calcaneal angle	23.1	22	22.8	22	0.324 Not significant
Anterior talo-first metatarsal angle	- 3.2	- 3	- 18.2	- 18	0.005 Highly significant

Calcaneal osteotomy group radiological measurements		Mean	P Value
Lateral talo-calcaneal angle	Pre	53.5	0.05
	Last	36.6	
Anterior talo-calcaneal angle	Pre	50.5	0.05
	Last	23.1	
Anterior talo-first metatarsal angle	Pre	- 24.5	0.003
	Last	- 3.2	

Calanneostop group radiological measurements		Mean	P Value
Lateral talo-calcaneal angle	Pre	52.5	0.05
	Last	38.2	
Anterior talo-calcaneal angle	Pre	51.5	0.05
	Last	22.8	
Anterior talo-first metatarsal angle	Pre	- 24.5	0.341
	Last	- 8.2	

Discussion

Till now there is no universal agreement about the indications of surgical treatment of idiopathic flexible flat feet. Flexible flat feet are still the most common cause of seeking pediatric orthopaedic consultation. This is due to the concern of the parents about the shape of the feet of their child. Although most of the time it is only the shape of the feet is the main concern of the parents but sometimes it is the abnormal gait or easily fatigability of the child. Most of those flat feet are the flexible ones; where the arch of the foot is reconstructed in the non-weight bearing position and disappear when the child is standing.

In our study the mean age was 11.56 years and the range was (6 - 13 years). On reviewing the literature the youngest age of intervention was found to be 6 years as this was claimed to be the age at which the arch of foot is well developed and the foot loses its baby fat [10]. Before this age, observation is the recommended line of treatment together with stretching

exercises to the tendo-Achilles. Mosca[7] and Staheli et al[5] stressed that flat foot deformity is a subtalar complex joint deformity at the beginning but after the age of 14, it will be associated with a fixed compensatory forefoot supination in addition to the primary subtalar deformity.

We included only painful idiopathic flexible flat feet. The pain meant here was the pain related to activities and relieved by rest and analgesia. Night pain, diffuse pain and pain at rest are not the type of pain we were addressing and when it is present another cause of pain is searched for. The site of foot pain was either at the lateral side at the sinus tarsi which could be explained by the impingement of the peroneal tendons or at the medial side at the talo-navicular joint where the stretch of the tibialis posterior tendon or the spring ligament could be the cause. The pain ranges from being mild to moderate pain but no severe pain was encountered. In our study 14 feet had pain at both the lateral and medial side (8 feet in the anterior calcaneal lengthening- 6 feet in the calcaneostop screw), 6 feet on the medial side (3 feet in the anterior calcaneal lengthening- 3 feet in the calcaneostop screw), and 10 on the lateral side only (4 feet in the anterior calcaneal lengthening- 6 feet in the calcaneostop screw).

Calcaneostop screw :

In our study we inserted the calcaneostop screw in the clacaneus of 15 feet with a mean follow up period of 27.53 months. We used cancellous cannulated screws 6.5 mm in diameter and 30 mm in length. Having a cannulated body is responsible for the avoidance of malposition and decreased the size of incision. We used the technique described by Jerosch et al(4). We performed percutanous tenoplasty of tendo-Achilles in all feet. All feet were pain free at the last follow up. The mean time for the screw removal was 17.2 months. There were a significant improvement of the lateral and AP talo-calcaneal angles respectively (52.5° to 38.2°) and (51.5° to 22.8 °) p value for both = 0.05 but there was no improvement in the AP talo-first metatarsal angle p value = 0.324. This

was the same result in Giannini[11] and Carranza[12] studies. Jerosch et al[4] evaluated only the lateral talo-navicular angle and it showed an improvement at the last follow up.

In our study we had one patient (2 feet) in the calcaneostop group had complained of persistent pain at the subtalar joint not relieved by medical treatment removal of the screw in both feet was done after 12 months postoperative but the correction was maintained in the last follow up.

Anterior calcaneal lengthening osteotomy:

We operated 15 feet with idiopathic flexible flat feet with a mean follow up period of 27.43 months. We applied the technique described by Mosca[5]. We performed percutanous tenoplasty of tendo-Achilles in all feet. All feet were pain free at the last follow up. Tri-cortical bone graft, such as iliac crest, is ideal for this procedure because the cortical portion provides excellent structural support and the cancellous portion provides osteoconduction. In our study we used autograft form the iliac crest in all our cases. The mean union time was 6.26 weeks with the range of 6-7 weeks. Dolan et al[13] prospectively compared iliac crest autograft with allograft for correction of the adult-acquired flat foot in 33 feet in 31 patients and reported that all patients showed satisfactory bone healing after 12 weeks. Myerson et al[14] reported successful union in calcaneal osteotomy using fresh-frozen structural allograft for 11 feet with average time to union of 10 weeks.

In our study the anterior calcaneal lengthening osteotomy could correct the flat foot deformity in the 3 planes as indicated by significant improvement in the both the lateral and AP talo-calcaneal angles (53.5 $\rightarrow$ 36.6, P value=0.05) (50.5 $\rightarrow$ 23.1, P value=005) respectively and the AP talo-first metatarsal angle (-24.5 $\rightarrow$ 3.2, P value=0.03). Dogan et al[15] treated 22 feet of 11 patients with the anterior calcaneal lengthening and they evaluated the lateral, AP talo-calcaneal angles and AP talo-fist metatarsal angle. They stated significant improvement in all 3 angles at the last follow up with

77.3% perfect results, 17.6% good, and poor in 5.1%.

Mosca[16] mentioned that the mechanism by which the anterior calcaneal lengthening osteotomy corrects the hindfoot valgus is unknown. As it is considered a distraction osteotomy not merely an opening wedge osteotomy. He added that it is his observation that distracting both fragments of the calcaneal osteotomy with intact planter fascia induces a local windlass effect.

When comparing the radiological angles of both groups at the last follow up we found no statistical significance in the AP and lateral talo-calcaneal angles (P value= 0.324, 0.432 respectively). We found that there was a highly statistical significance in the AP talo-first metatarsal angle in both groups (P value= 0.005).

Both techniques have advantages and disadvantages. The advantages of the calcaneostop technique are, it is supposed to be a simple procedure, reversible; meaning that the screw could be removed without change in the anatomy of the subtalar joint and a controlled degree of correction depending on the height of the screw left prominent at the lateral process of the talus. The disadvantages of this technique are using internal fixation material, complications due to these materials such synovitis which occurred only in one case (2 feet) in our series and the need of second surgery to remove the screw earlier. But the biggest disadvantage of this procedure is being able to correct deformities in the saggittal and coronal planes but not at the horizontal plane. This was evidenced by the improvement of the lateral talo-calcaneal angle and the AP talo-calcaneal angle, but not statistically significant improvement of the AP talo-first metatarsal angle in the last follow up X-ray.

The calcaneo-stop procedure is a simple and reliable method for the correction of severe flexible paediatric flat. Our prospective, short-term results following the screw implantation into the clacaneus seem to correlate well with the results of similar or different arthroereisis methods. However,

further investigations are required to evaluate the long-term outcome of the screw calcaneostop method, including the conditions following implant removal.

The anterior calcaneal lengthening osteotomy is a distraction osteotomy capable in correcting of the flat feet deformity in the three planes. The disadvantages of calcaneal lengthening osteotomy range from being a more technically demanding procedure in comparison to the calcaneostop to the incidence of Mild or moderate subluxation in the calcaneocuboid joint, although it is usually asymptomatic, increased possibility of insufficient correction or overcorrection directly associated with the graft size, and malposition of the graft when it is not fixed migration of the graft into the subtalar joint

Summery

Children presented with idiopathic painful flexible flatfeet not responding to conservative management are the scope of this study. Surgical treatment by either the Calcaneostop method or the anterior calcaneal osteotomy are considered different as regard the principle by which each of them will correct the foot deformity. The Calcaneostop method utilizes a biological and a mechanical principle. Stimulating the propioceptive receptors at the sinus tarsi and mechanical block of the heel eversion is how the stop screw works. On the other hand the anterior calcaneal osteotomy utilize the ligamentotaxis effect gained by the distraction osteotomy; not simply an opening wedge osteotomy. It is capable of correcting the hindfoot and forefoot deformity by inducing a windlass effect on the intact planter fascia.

There were a statistically significance in the radiological improvement in the anterior calcaneal lengthening group at the last follow-up. But in the Calcaneostop group the statistically significant improvement was at the lateral talo-calcaneal angle and the AP talo-calcaneal angle. There was no statistically significant improvement at the AP talo-first metatrasl angles.

Conclusion

The calcaneostop method could

correct the frontal and the sagittal plane deformities but not the transverse plane deformity. The anterior calcaneal lengthening could correct the three planes of deformities.

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ESTROGEN RECEPTORS IN DIFFERENT TYPES OF HUMAN THYROID CARCINOMA

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Abstract

Introduction: *The incidences of thyroid malignancies are three to four times higher in women, suggesting the possible involvement of estrogen. Based on this observed sex bias, we hypothesize that estrogen modulates the growth and metastatic propensity of thyroid cancer cells. The most significant finding is possibly of immense clinical significance is providing evidence for a link between estrogen and adhesion, invasion, and migration of thyroid cells*

Aim of work: *Study of expression of estrogen receptors with correlation to types of carcinoma of thyroid.*

Methods: *From March 2008 to March 2011, 30 patients with thyroid carcinoma were examined in the out patient clinic of endocrine surgery unit. All patients were subjected to: Total thyroidectomy ± LN dissection, small snips were taken from the removed thyroid specimens, all collected samples were processed in the microbiology diagnosis and infection control unit and subjected to molecular identification of estrogen receptor alpha & beta.*

Results: *ERα immunoreactivity was detected in the nuclei of parenchymal cells in thyroid carcinoma. The number of ERα positive cases were 8/30 (26.7% 6/21 [28.6%] females, 2/9 [22.2%] male in thyroid carcinoma). The six females patients were 4 papillary and one follicular and one anaplastic carcinoma, the two male patients were one papillary thyroid carcinoma and one anaplastic thyroid carcinoma. ERβ nuclear immunoreactivity was detected in the nuclei of parenchymal cells in thyroid carcinoma. The number of ERβ positive cases were 9/30 (30%; 7/21 [33.3%] females, 2/9 [22.2%] male in thyroid carcinoma).*

The seven females patients were 5 papillary and one follicular and one anaplastic thyroid carcinoma. The two males patients were both follicular thyroid carcinoma.

Conclusion: *From this study, it is shown that the incidence of thyroid carcinoma was more common in female. Estrogen receptor sites were detected in most of our patients. We hypothesize that estrogen modulate the growth and progress of thyroid cancer cells. Further studies are recommended to prove that anti-estrogen may be of value as an adjuvant therapy to guard against recurrence after thyriodectomy in cancer thyroid patients and also it may be of value to guard against thyroid carcinoma in high risk patients.*

Introduction

Over 200 million people worldwide are affected by thyroid proliferative diseases, including cancer, adenoma, and goiter, annually. The incidences of thyroid malignancies are three to four times higher in women, suggesting the possible involvement of estrogen. Based on this observed sex bias, we hypothesize that estrogen modulates the growth and metastatic propensity of thyroid cancer cells [1].

According to American Thyroid Association, the incidences of thyroid proliferative diseases (TPD) are four to five times more in women than in men. The risk of developing thyroid disorders in women is one in eight, which is comparable to that of sporadic breast cancer in women [2]. Pregnancy and early menopause increases the risk of TPD with a decrease in the incidences of thyroid malignancies after menopause [3]. Abortions and notably recurrent abortions, reproductive challenges, and infertility have all been associated with thyroid hormone abnormalities[4]. The higher incidence of thyroid disorders in women and several lines of correlative evidence for thyroid disorders with estrogen in the etiology of TPD warrant an examination of its precise role in laboratory-based experimental models [2].

Estrogens consist of a group of three biochemically distinct hormones, estrone, estradiol (E2), and estriol, which are produced naturally by the body and are

metabolized into estrogen metabolites such as 2-hydroxyestrone (2-OHE1) and 16-αhydroxyestrone (16-OHE1) [5].

These estrogen metabolites have stronger (6-OHE1) or weaker (2-OHE1) estrogenic ability, and their relative concentration in a female body can influence the risk of a woman for breast, uterine, and other cancers [6].

Estrogen signaling is mediated primarily by two isoforms of the estrogen receptor (ER), ER-α and ER-β, which intersperse with the pro-survival mitogen - activated protein kinase and extracellular signal-regulated kinases signal transduction pathway, presumably leading to cell growth and proliferation [7].

Aim of the Work

Study of expression of estrogen receptors with correlation to types of thyroid carcinoma.

Patients and Methods

From March 2008 to March 2011, patients with thyroid enlargement were examined in the out patient clinic of endocrine surgery unit. From these patients, patients with clinically suspicious malignant goiter were admitted and subjected to further investigations in the Endocrine Surgery Unit at Mansoura University Hospital.

These investigations included:

1) Laboratory investigations:

- Thyroid function tests (T3 / T4 & TSH).
- Routine laboratory investigations in the form of:
- CBC.
- Random blood sugar.
- Serum creatinine.
- Liver function tests.
- Bleeding profile.

2) Thyroid imaging: that included.

a- Neck ultrasound.
b- Colour duplex on neck vessels.
c- Thyroid scan: using Tc99m pertechnecate.
d- CT scan neck.
e- MRI (in some patients).

3) Tissue biopsy: Tru-cut biopsy needle.

These 30 patients were 21

female (70%) and 9 male (30%). Their age ranged from 10 years to 65 years old, with a mean age of ~36 years.

These patients (30 patients) were subjected to total thyroidectomy.

1- Total thyroidectomy ± L.N dissection.

- Small snips were taken from the removed thyroid specimens and stored in deep-freeze (-30^{o}C) then were subjected to Rt-PCR to detect ER (total RNA expression from ER gene).
- The Removed thyroid tissue was cut into slices 0.3 - 0.5 cm and were fixed in neutral buffered formalin, embedded in paraffin.
- Paraffin block section 5 um was obtained for:
  (1) Hx & E stained slides.
  (2) Immunohistochemical for ER.

2- Processing of specimens:

All collected samples were processed in the Microbiology Diagnostics and Infection Control Unit (MDICU) in Medical Microbiology and Immunology department, Mansoura Faculty of Medicine.

3- Molecular identification:

1- RNA extraction [8].
2- RNA purification:
3- RTPCR .

4- Nucleic acid amplification:

PCR amplification of ERα and ERα complementary DNAs [8]:

a) Primers:

The following sense and anti-sense primers were used:

for ERα	5'-GGG TGA AGT GGG GTC TGC TG-3' and 5'-TGC CTC CCC CGT GAT GTA AT-3
for ERβ	5'-CCC TGC TGT GAT GAA TTA CAG-3α and 5'-CTT CTC TGT CTC CGC ACA AG-3'

b) Constituents of the PCR reaction (Life Technologies Inc.):

c) PCR Program:

5- Agarose gel electrophoresis of the amplified DNA[8].

Follow up:

Our patients were followed up

every three months over a period of one year postoperatively.

In each visit the following was performed:

- Clinical examination (for local recurrence or distant metastasis).
- Hormonal assay.
- Serum calcium level.
- Whole body scan was done after a period of one month from the date of operation.

Results

In our study:

Our studied patients (30 patients) were 21 females (70%) and 9 males (30%). Table (1), Figure (1).

The age of the studied patients (30 patients) ranged from 10-65 years old (mean age 36 years). Female age ranged from 10-65 years old (mean age 37 years). Male age ranged from 17-50 years old (mean age 33.5 years). Table (1), Figure (1).

Final pathological examination of the tumor revealed 18 patients (60%) was papillary thyroid carcinoma (figure 3, 4, 5), 8 patients (26.7%) was follicular thyroid carcinoma (figure 7) and 4 (13.3%) patients was anaplastic carcinoma (figure 6).

ERα immunoreactivity was detected in the nuclei of parenchymal cells in thyroid carcinoma (Table 2, figure 2).

The number of ERα positive cases were as follows 8/30 (26.7% 6/21 [28.6%] females, 2/9 [22.2%] male in thyroid carcinoma).

The six females patients were four papillary and one follicular and one anaplastic carcinoma, the two male patients were one papillary and one anaplastic thyroid carcinoma.

ERβ nuclear immunoreactivity was detected in the nuclei of parenchymal cells in thyroid carcinoma (Table 3, figure 2).

The number of ERβ positive cases were as follow 9/30 (30%; 7/21 [33.3%] females, 2/9 [22.2%] male in thyroid carcinoma).

The seven females patients

were five papillary and one follicular and one anaplastic thyroid carcinoma.

The two males patients were both follicular thyroid carcinoma.

Pathological study of removed thyroid specimens proved that 18 patients were papillary thyroid carcinoma, 8 patients were follicular thyroid carcinoma, 4 patients were anaplastic (figure 8, 9, 10).

Table (1): **Sex and age distribution for patients with thyroid carcinoma (30 patients).**

	Female	Male
No. of patients	21	9
Percentage	70%	30%
Age range	10 – 65 years	17 – 50 years
Mean age	~ 37	~ 33.5

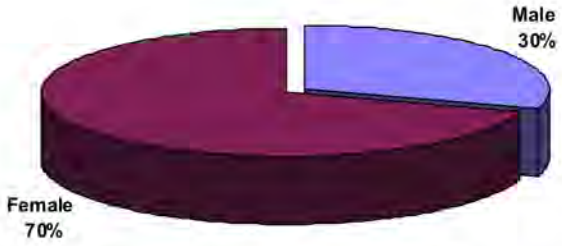

Figure (1): Sex distribution.

Table (2): **Ratio of ERα +ve patients.**

	Total cases	+ve ERα cases	%
Total cases	**30**	**8**	**26.7**
Female	**21**	**6**	**28.6**
Male	**9**	**2**	**22.2**

Type	Total cases	+ve ERα cases	%
Papillary	**18**	**5**	**27.8**
Follicular	**8**	**1**	**12.5**
Anaplastic	**4**	**2**	**50**

Table (3): **Ratio of ERβ +ve patients.**

	No	+ve ERβ cases	%
Total cases	**30**	**9**	**30**
Female	**21**	**7**	**33.3**
Male	**9**	**2**	**22.2**

	Total cases	+ve ERβ cases	%
Papillary	**18**	**5**	**27.8**
Follicular	**8**	**3**	**37.5**
Anaplastic	**4**	**1**	**25**

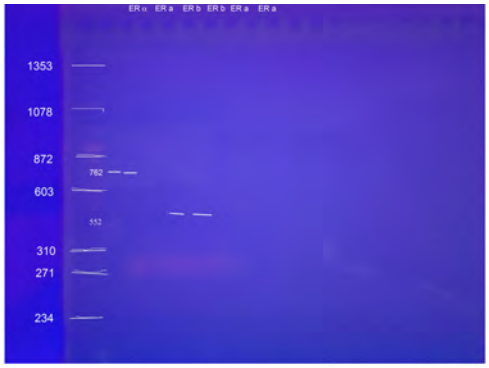

Figure (2): **PCR product of gene of Er α (762 bp)**

PCR product of gene of Er β (552 bp)

Figure (3): Male patient aged 50 years presented with thyroid swelling in the left lobe proved to be papillary thyroid carcinoma.

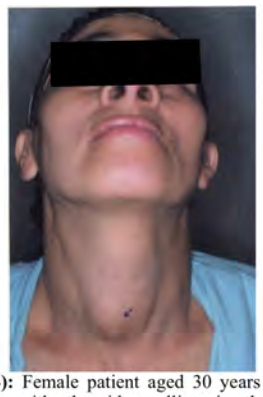

Figure (4): Female patient aged 30 years presented with thyroid swelling in the isthmus proved to be papillary thyroid carcinoma.

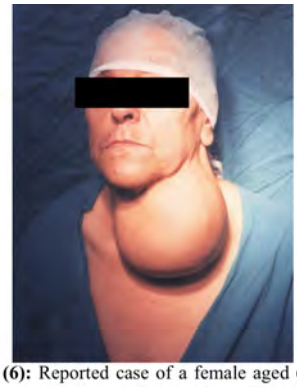

Figure (6): Reported case of a female aged 65 years with huge left thyroid swelling proved to be anaplastic thyroid carcinoma.

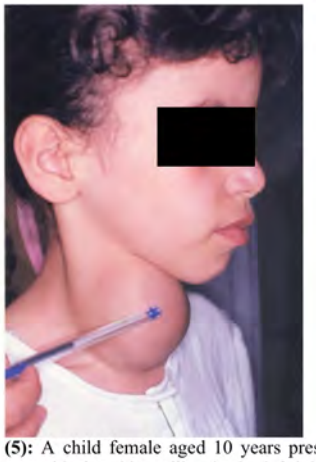

Figure (5): A child female aged 10 years presented with thyroid swelling in the left lobe and isthmus proved to be papillary thyroid carcinoma.

Figure (7): Female patient, 31 years old with thyroid swelling: Proved to be follicular thyroid carcinoma.

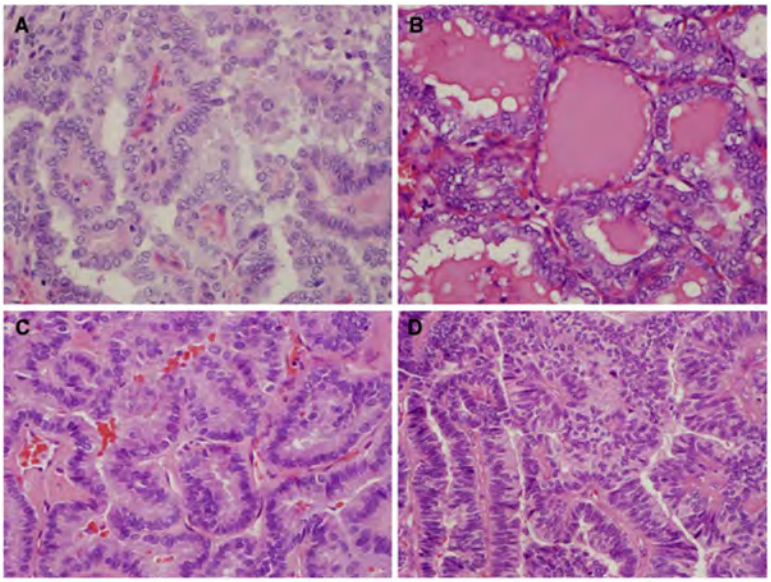

Figure (8): Conventional papillary thyroid carcinoma and papillary thyroid carcinoma variants.

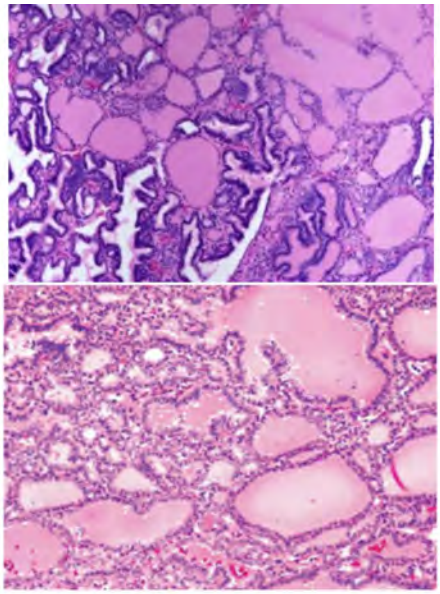

Figure (9): Follicular thyroid carcinoma.

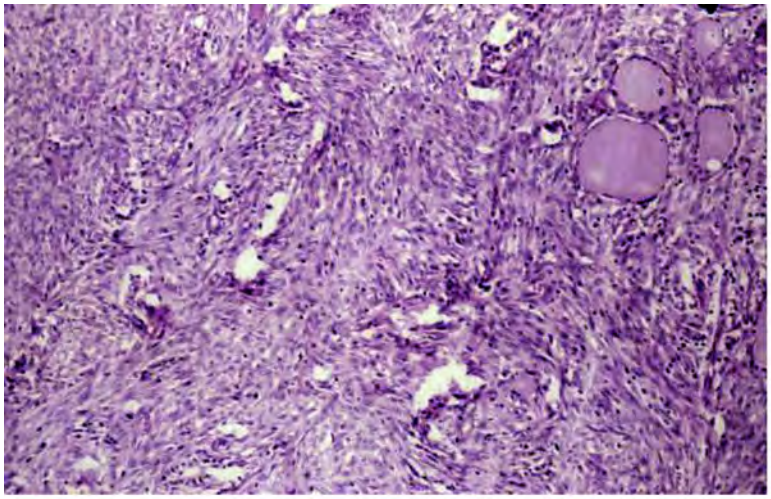

Figure (10): Undifferentiated (Anaplastic) cell carcinoma.

Discussion

In our study, the number of ERα positive cases were 8 patients (26.7%). They were six females and two males. The six female patients were four papillary, one follicular and one anaplastic thyroid carcinoma. The two male patients were one papillary thyroid carcinoma and one anaplastic thyroid carcinoma.

Kawabata et al.[9] found that ERα immunoreactivity was detected in the nuclei of invasive ductal carcinoma employed as positive control of immunostain. Very few follicular cells were positive for ERα in the normal thyroid gland of both women and men. ERα immunoreactivity was not detected in the nuclei of follicular cells in developing fetal thyroid glands. However, ERα immunoreactivity was detected in the nuclei of parenchymal cells in thyroid disorders. The numbers of ERα-positive cases were as follows: 9/24 (37.5%: 6/18 [33.3%] females, 3/6 [50%] males) in adenomatous goiters, 6/23 (26.1%: 4/17 [23.5%] females, 2/6 [33.3%] males) in follicular adenomas, and 74/159 (46.5%: 58/116 [50.0%] females, 16/43 [37.2%] males) in carcinomas. ERα labeling index or

the percentage of ERα-positive cells was as follows: 14.2 ± 6.4 in adenomatous goiter, 13.4 ± 5.1 in follicular adenoma, 17.8 ± 2.7 in papillary carcinoma, 13.3 ± 6.3 in follicular carcinoma, 18.8 ± 5.7 in anaplastic carcinoma, and 5.5 ± 3.8 in medullary carcinoma.

ERα labeling index was significantly higher in adenomatous goiter (14.2 ± 6.4), follicular adenoma (13.4 ± 5.1), and thyroid carcinoma (16.4 ± 2.1%) than in normal thyroid gland (0; P < .05). In papillary carcinoma, ERα labeling index was significantly higher in premenopausal women (28.1 ± 4.5) than in postmenopausal women (14.2 ± 2.9) and men of various ages (7.6 ± 2.7; P < .05). With respect to anaplastic carcinoma, ERα labeling index tends to be higher in men of various ages (34.7 ± 10.1) than in postmenopausal women (8.2 ± 5.4), but the differences did not reach statistical significance. No significant correlations were detected in other histological types. Clinical follow-up data of these patients with thyroid carcinoma also were not available for present study.

In our study, the number of ERβ positive cases were nine cases (30%). They were seven females and two male patients. The seven female patients were five papillary, one follicular thyroid carcinoma and one anaplastic thyroid carcinoma. The two male patients were both follicular thyroid carcinoma.

Kawabata et al. [9] found that ERβ nuclear immunoreactivity was detected widely in both follicular or parenchymal and C-cells of normal thyroid glands, including those in the developing fetal thyroid glands. In addition, ERβ immunoreactivity was detected in the nuclei of various thyroid lesions. The number of ERβ-positive cases or percentages of ER?-positive cases were as follows: 9/24 (37.5%: 6/18 [33.3%] females, 3/6 [50%] males) in adenomatous goiters, 6/23 (26.1%: 4/17 [23.5%] females, 2/6 [33.3%] males) in follicular adenomas and 74/159 (46.5%: 70/116 [60.3%] females, 25/43 [58.1%] males) in carcinomas. ERβ labeling index was as follows: 17.7 ± 4.6 in normal thyroid gland, 15.2 ± 7.3 in adenomatous goiter, 29.2 ± 8.2 in

follicular adenoma, 17.0 ± 2.9 in papillary carcinoma, 15.5 ± 5.3 in follicular carcinoma, 39.9 ± 5.6 in anaplastic carcinoma, and 24.0 ± 7.9 in medullary carcinoma.

ERβ labeling index tended to be higher in anaplastic carcinoma (39.9 ± 5.6) than in other histological types, but the differences did not reach statistical significance. There were no significant correlations between ERβ labeling index and patient age, menopausal status, gender, benign and malignant tumors, histological type and follow-up data (data not shown).

Conclusion

From our study, it is shown that the incidence of thyroid carcinoma was more common in female. Estrogen receptor sites were detected in most of our patients.

We hypothesize that estrogen modulate the growth and progress of thyroid cancer cells. This was also mentioned by Hoelting et al.[10].

Further studies are recommended to prove that anti-estrogen may be of value as an adjuvant therapy to guard against recurrence after thyriodectomy in cancer thyroid patients and also it may be of value to guard against thyroid carcinoma in high risk patients.

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BENHA MEDICAL JOURNAL

ESTROGEN RECEPTORS IN DIFFERENT TYPES OF HUMAN THYROID CARCINOMA

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INTERSTITIAL PULMONARY AFFECTION IN PATIENTS WITH CHRONIC HCV

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Abstract

Background: *Hepatitis C virus (HCV) is the most common cause of chronic liver disease that causes hepatic and extrahepatic manifestations. Emerging clinical data suggest that chronic HCV infection can lead to many direct and indirect effects on the lung. So our study aimed to confirm this effect (s) or association of HCV infection in asymptomatic patients on pulmonary function tests (PFTs), interstitial changes and cellular changes in bronchoalveolar lavage (BAL).* ***Patients and Methods:*** *This cross-sectional prospective study was conducted on 100 patients with proved HCV infection from the Tropical Medicine Unit, Mansoura University Hospitals. High resolution computed tomography (HRCT) chest, pulmonary function tests, arterial blood gases, Real Time PCR for HCV-RNA and liver biopsy were done for all patients while BAL was performed for 10 patients only.* ***Results:*** *The spirometric abnormalities were detected in 43% of patients and 57% of patients were normal. BAL fluid showed increased neutrophils, eosinophils in all patients and increased by lymphocytes in 80% of patients. Mocrophages decreaced in all patietnts. statistical significant difference between asymptomatic and symptomatic pulmonary groups as regard BAL cellular pattern, HRCT chest abnormalities, PCR-HCV RNA and Ishak scores.* ***Conclusion:*** *Chronic hepatitis c may be associated with the development of airway obstruction, interstitial pulmonary affection and changes in the normal cellular patterns in the alveoli despite the abscence of symptoms. We suggest that chronic HCV infection patients might be carefully evaluated by HRCT and BAL analysis even though they have normal*

PFT parameters for early diagnosis interstitial pulmonary fibrosis.

Key words: *Arterial blood gases (ABG), bronchoalveolar lavage (BAL), Histology Activity Index (HAI) and pulmonary function tests (PFTs).*

Introduction

Hepatitis C virus (HCV), a small, single-stranded RNA virus classified in the Flaviviridae family, is the most common cause of chronic liver disease[1]. HCV affects approximately 170 million people worldwide, chronic hepatitis C occurs in 80% of these cases and can lead to cirrhosis and hepatocellular carcinoma[2]. Egypt has the highest HCV prevalence in the world. An estimated 10%-20% of the Egyptian population is chronically HCV infected, a seroprevalence that is 10 to 20-fold higher than in the United States. More than 90% of HCV infections in Egypt are genotype 4, which is found primarily in the Middle East and Central Africa and has a worldwide prevalence of 2%[3].

The liver biopsy is an important diagnostic tool and helps to make important therapeutic decisions in acute and chronic liver disease. It is usually the most specific test to assess the nature and severity of liver diseases; and has been considered the "gold standard" [4].

In attempt to standardize assessment of liver histology by pathologists, several scoring systems have been developed. Among of these, modified Histology Activity Index (HAI) developed by Ishak, et al[5], is used most widely to assess liver histology in chronic hepatitis. The Ishak Modified HAI system provides reliable scores, with relatively little intra- and inter-observer variations [6].

Bronchoalveolar lavage (BAL) is an established diagnostic tool in interstitial lung and infectious bronchopulmonary diseases. In interstitial lung diseases, the diagnosis can be rarely based on BAL findings only, but BAL often provides diagnostically relevant results[7].

Over the last decade, an increasing number of reports have suggested that chronic HCV infection is also associated with both direct and indirect effects on pulmonary tissue. The direct effects of HCV on the lung may present as worsening of lung func-

tion in some patients with preexisting asthma and/or COPD. In other patients, HCV may present with an interstitial pneumonitis and/or pulmonary fibrosis. Cirrhosis of the liver (due to HCV) with the added complications of portopulmonary hypertension (PPHTN) and hepatopulmonary syndrome (HPS), cryoglobulinemia, Sicca-like syndrome, lymphomas, autoimmune thyroid disease, polymyositis, and hypocomplementemic urticarial vasculitis have all been reported in response to HCV infection and may indirectly affect the lung [8].

HCV is well known to induce chronic inflammation and fibrosis in the liver, it was thought that HCV may play a similar role in the lung and be involved in the pathogenesis of pulmonary fibrosis. This idea confirmed the study of Ueda et al[9] and Meliconi et al[10]. They tested the presence of HCV antibodies in a cohort of patients with interstitial pulmonary fibrosis; they found a higher prevalence of serum antibodies to HCV in patients with IPF than in age-matched control subjects, suggesting that chronic HCV infection might affect the lungs through different mechanisms and lead to a spectrum of clinical presentations.

Several lines of evidence support a pathogenic role for chronic HCV infection in interstitial lung disease, but all are limited by sample size and the association remains controversial. This study was to investigate the relationship between HCV infection and interstitial pulmonary involvement, and to reveal the relationship among involvement and age, sex, severity of hepatitis, and respiratory functions.

Material and Methods

This study was carried out on 100 hepatitis C patients confirmed with PCR : 48 males and 52 females. Their age ranged from 19 to 60 years with a mean of 40.11 ± 9.64 years, in the period from October 2008 to January 2010. The patients were selected from patients attending inpatient and outpatient clinic of Tropical Medicine Unit, Mansoura University Hospital.

The followings were the exclusion criteria:

- Patients with lung disease (oth-

er than interstitial pulmonary disease),
- Smokers.
- Patients with previous systemic diseases (as renal failure, congestive heart failure and connective tissue disorders).
- Patients who developed liver cirrhosis or hepatocellular carcinoma, current or past history of anti-viral treatment for HCV.
- Patients with autoimmune liver diseases or any other causes of liver disease.

All patients were subjected to the following:
- Thorough history taking and clinical examination.
- Serologic assays for HCV infection:

a) Serologic assay for HCV antibodies by the third-generation enzyme-linked immunosorbent assay (ELISA).

b) HCV RNA is detected by polymerase chain reaction (PCR).

Interpretation of quantitative PCR as follows:

o Low viraemia: up to 10^5 IU / ml of blood.

o Moderate viraemia: from 10^5 - 10^6 IU / ml of blood.

o High viraemia: >10^6 IU / ml of blood.
- Liver function tests: ALT, AST, serum alkaline phosphatase, serum albumin, serum bilirubin, prothrombin time, and international normalization ratio (INR).
- Alpha foeto- protein (AFP) and abdominal ultrasound.

Ultrasound guided liver biopsy :

Liver biopsy was performed to assess the degree of necroinflammatory activity (grade) and degree of fibrosis (stage) of disease according to Ishak et al score (1995), and ruling out other causes of liver disease.

Ultrasound Biopsy technique :
- Prothrombin time and international normalization ratio (INR) were done.
- Ultrasonography was done prior to a liver biopsy identifies silent mass lesions (such as haemangioma) and defines the anatomy of the liver and the relative positions of the gallbladder, lung, and kidney.
- Ultrasound-guided tru cut biopsy (1.5 cm length or contained

at least 10 portal areas if shorter) was taken from the right lobe of the liver.

- The specimen was fixed, paraffin-embedded and cut at 5 um. Slides were prepared, stained with Hematoxylin-eosin (H&E), Masson's trichrome, Periodic Acid-Schiff (PAS) stains.
- Inflammation in portal areas, interface hepatitis at periportal areas and parenchymal necro-inflammatory activity, and degree of fibrosis were assessed according to Ishak modification of histology activity index (HAI)[5] for scoring of necroinflammatory activity in chronic hepatitis with maximum score is 18 and fibrosis was graded on 0-6 scales (figure 1).

High resolution computed tomography (HRCT) of the chest:

HRCT chest with contrast was done on Toshiba Asteion with collimation 2 mm at 10 mm intervals from lung apices to the costophrenic angles with the patient breath-holding at full inspiration, in a supine position then at the end of expiration, using high spatial frequency algorithm and a 512 x 512 matrix. The scan time was 0.75 seconds, kv 120, mAs 150.

HRCT scans were evaluated for the presence of the following signs:

A. Airway changes: defined as follow [11].

- Bronchial dilatation:
- Bronchial Wall Thickness:
- The "tree -in-bud" pattern:
- Mosaic attenuation.

B. Interstitial changes: defined as follow [12].

- Ground-glass attenuation.
- Nodular areas of high attenuation.
- Septal lines.
- Nonseptal lines.
- Honeycomb cysts.

Pulmonary function testing (PFT):

Pulmonary function tests were done by Spirometry; Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1 / FVC ratio and forced mid-expiratory flow rate FEF 25-75% were measured with standard spirometric technique (Spiro-Jaeger, Germany). The highest value from at least three spirometric maneuvers were selected and done in accordance of American

Thoracic Society criteria [13].

Normal Values of Pulmonary Function Tests [14]:

- FEV1 %: 80% to 120%.
- FVC %: 80% to 120%.
- FEF 25- 75 %: ≥ 60%.

Arterial blood gases:

Arterial partial pressures of oxygen and carbon dioxide (PaO2 and PaCO2) were analyzed with subjects in sitting position and breathing room air using blood gas analyzer (Radiometer, ABL, 50, Denmark).

Alveolar - arterial oxygen gradient, as defined by the following equation:

Alveolo-arterial oxygen gradient: $P_AO_2 - P_a O_2$

$P_AO_2 = (F_iO_2 * (760 - 47)) - (P_aCO_2 / 0.8)$

Normal Alveolar - arterial oxygen gradient is: 8- 15 mmHg [15].

Bronchoalveolar lavage (BAL):

BAL was done for ten cases (10% of patients) who accepted to do fiberoptic bronchoscopy as volunteers after informed consents.

Fiberoptic bronchoscope was introduced through the oral route with the patient in supine position into the middle lobe. Local instillation of 120 ml of sterile normal saline in six aliquots through the bronchoscope and then aspirated through the suction channel and collected in clean containers.

The retrieved BAL fluid was processed by its passage through a loose nylon gauze mesh to trap mucous aggregates then immediately centrifuged at 1,500 r/min for 10 minutes to obtain a cell pellet. The specimen were smeared, fixed immediately in 95% alcohol and stained with H & E and Papanicolaou stains. Manual classification of cell types was carried out for the differential cell count. The percent distribution of cell types (alveolar macrophages, lymphocytes, neutrophils and eosinophils) was determined by a microscopic count of 500 cells/slide. Bronchoepithelial cells were not considered in the cell count.

The following percentages were considered as normal values according to Costabel and Guzma[16]: macrophages >80%, lymphocytes <15%, neutrophils <3%

and eosinophils < 0.5%

Statistical Analysis:

Data was analyzed using SPSS (Statistical Package for Social Sciences) version 10. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Normally distributed data was presented as mean ± SD. Paired t-test was used for comparison within groups. Student t-test was used to compare between two groups. P < 0.05 was considered to be statistically significant.

Results

One hundred patients (48males and 52 females) with mean age 40.11 ± 9.64 years were enrolled in the study. 61% of patients have no pulmonary symptoms, while 34%, 3% and 2% have dyspnea, productive cough and dry cough respectively. HRCT chest was normal in 58% of patients. Non septal and septal lines were the commonest findings followed by the ground glass attenuation (17%, 10% and 5% respectively). Each of the nodular pattern, reticular pattern, and bronchial wall thickening were found in 2 patients. Honey combing was found in one patient and a cyst was found in one patient. Atelectatic band was found in one patient only (table 1).

The spirometric abnormalities were detected in 43% of patients and 57% of patients had normal spirometry. Alveolar-arterial O2 gradient was normal in 65% and increased in 35% of patients. FEV1%, FVC% and FEF 25- 75% were abnormal in 11%, 23% and 23% of patients respectively (tables, 2).

Bronchoalveolar lavage analysis shows that : macrophages % decreased accompanied by increased lymphocytes % in 8 cases (80%), neutrophils and eosinophils % in all patients (table, 3) and (figure 2).

The comparison between asymptomatic and symptomatic pulmonary groups as regard BAL cellular pattern reveals no statistically significant difference between them (p=0.342, 0.682, 0.683 and 0.738 for macrophages, lymphocytes, neutrophils and eosinophils respectively) (table, 4).

There was no statistically significant difference between normal and interstitial changes in HRCT chest groups as regard to PCR for HCV RNA and Ishak scores (activity score and fibrosis score) (p value was 0.872, 0.767and 0.249 respectively) (table, 5).

There were high statistically significant positive correlations between liver enzymes and AFP and Ishak scores (p value < 0.01) but not for alkaline phosphatase (table, 6).

No statistically significant correlation between age, PCR-HCV RNA and Ishak scores with pulmonary function tests in the studied patients (table, 7). Also there was no statistically significant correlation between BAL cellular pattern with PCR-HCV RNA and Ishak scores (table 8).

Table (1): Clinical characteristics of the studied patients (n:100).

Age year (mean±SD)	40.11±9.64
Sex	
Male/female (%)	48/52
Pulmonary symptoms	
Asymptomatic	61%
Dyspnea	34%
Productive cough	3%
Dry cough	2%
HRCT	
• **Normal**	58 %
• **Abnormal:**	42%
Airway changes	
- Bronchial wall thickening	3%
- Atelectatic band	1%
Interstitial changes	
- Non septal lines	17 %
- Septal lines	10 %
- Ground glass opacity	5 %
- Nodular pattern	2 %
- Reticular pattern	2 %
- Honey combing	1 %
- Cyst	1%
Hepatic parameter (mean±SD)	
• GOT U/L	54.720±27.9621
• GPT U/L	57.200±34.9461
• Alkaline phosphatase U/L	26.480±15.8002
• Alpha fetoprotein ng/ml	3.579±5.7117
• PCR-HCV RNA IU / ml	830425.9±1288340.9
• Activity score	6.070±2.1985
• Fibrosis score	2.610±1.2135
Pulmonary function tests:	
• Normal/Abnormal (%)	57/43%
Alveolar-arterial O2 gradient:	
• Normal/Increased (%)	65/35%

Table (2): Pulmonary function tests abnormalities in the studied patients.

No=100	Mean ±SD	Range	Normal/Abnormal (%)
FEV1 %	96.45 ± 12.44	70 – 119	89/11
FVC %	86.59 ± 12.32	60 – 113	77/23
FEF 25- 75 %	80.50 ± 22	35 - 119	77/23

Table (3): BAL cellular pattern interpretation in the studied patients(n=10).

No = 10	Normal No (%)	Increased No (%)	Decreased No (%)
Macrophages %			10 (100 %)
Lymphocytes %	2 (20 %)	8 (80 %)	
Neutrophils %		10 (100 %)	
Eosinophils %		10 (100 %)	

Table (4): Comparison between asymptomatic and asymptomatic group as regard BAL cellular pattern:

	Asymptomatic Mean±SD	Symptomatic Mean±SD	t	P
Macrophages %	62.95±8.71	55.77±12.20	1.010	0.342
Lymphocytes %	19.77±6.18	22.47±11.43	0.426	0.682
Neutrophils %	13.75±10.65	18.50±20.34	0.424	0.683
Eosinophils %	3.30±1.63	2.90±1.88	0.346	0.738

Table (5): Comparison between normal and interstitial changes in HRCT chest as regard to Ishak scores and PCR for HCV:

	HRCT		Test of significance	P value
	Normal (n=58)	Interstitial changes (n=42)		
PCR for HCV*	317474.5	365755.5	U = 01195.0	0.872
Activity score*	6 (2-10)	6 (2-12)	U = 1176.0	0.767
Fibrosis score*	3 (1-6)	2 (0-4)	U = 1057.0	0.249

- Median

Table (6): Correlation between liver enzymes, viremia levels and Ishak scores.

		SGOT	SGPT	Alkaline ph	AFP
PCR for HCV	r	-0.144	-0.192	0.007	-0.045
	P	0.1518	0.0555	0.9418	0.6532
Activity score	r	0.516	0.465	0.087	0.239
	P	0.0000	0.0000	0.3871	0.0166
Fibrosis score	r	0.413	0.337	0.018	0.200
	P	0.0000	0.0006	0.8607	0.0456

r= Pearson correlation coefficient P= significant p value

Table (7): Correlation between PFTs with age, PCR-HCV and Ishak scores.

		FEV1	FVC	FEV1/FVC%	FEF 25-75%
Age	r	0.132	0.104	0.020	0.022
	P	0.192	0.304	0.843	0.828
PCR for HCV	r	0.021	– 0.045	– 0.075	0.114
	P	0.837	0.660	0.461	0.258
Activity score	r	0.027	0.009	0.006	– 0.069
	P	0.791	0.927	0.955	0.495
Fibrosis score	r	0.101	0.132	– 0.113	– 0.078
	P	0.318	0.191	0.264	0.438

Table (8): Correlation between BAL cellular pattern with PCR-HCV and Ishak scores.

		Macrophages %	Lymphocytes %	Neutrophils %	Eosinophils %
PCR for HCV	r	– 0.537	0.254	0.200	– 0.212
	P	0.110	0.478	0.580	0.557
Activity Score	r	– 0.282	– 0.427	0.424	0.308
	P	0.430	0.218	0.222	0.386
Fibrosis Score	r	– 0.009	– 0.316	0.191	0.130
	P	0.981	0.374	0.598	0.720

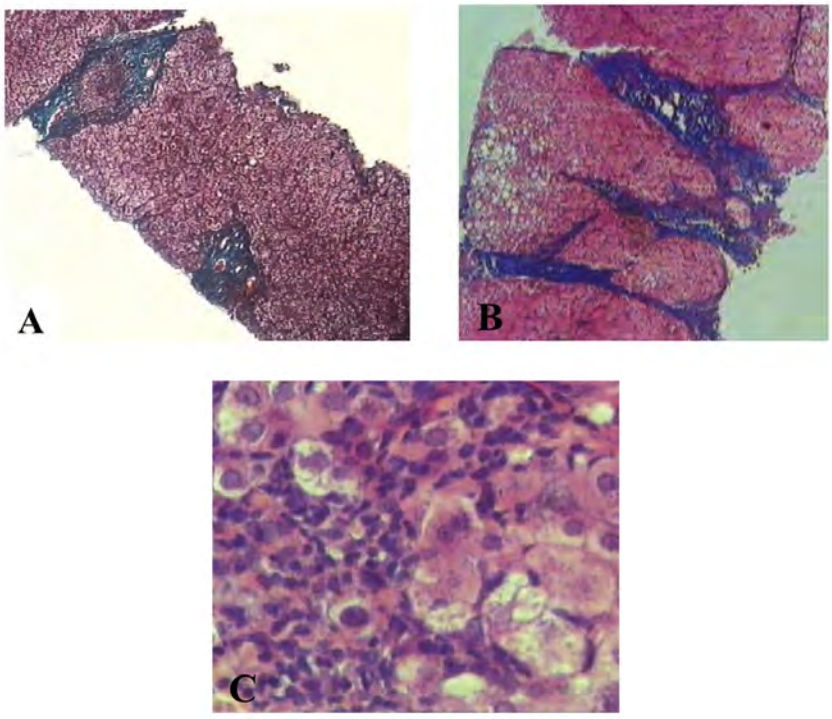

Fig. 1 : Portal tract expansion by fibrosis (A) and with marked bridging (B). Masson trichrom stain x40. (C): Interface hepatitis, H&E stain x 400.

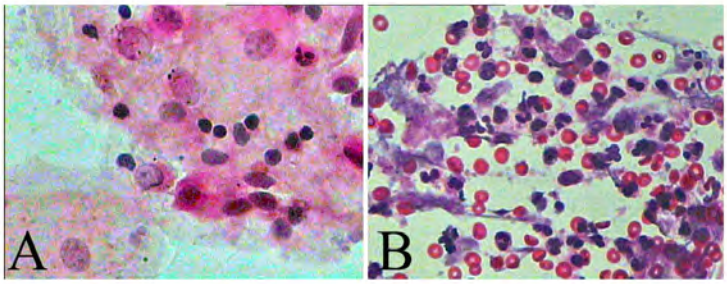

Fig. 2 : Cellular changes in bronchoalveolar lavage : (A) increased lymphocytes and (B) increased neutrophils, H & E stain x400 .

Discussion

HCV causes acute infection, which can be characterized by mild to severe illness but is usually asymptomatic. In approximately 75%-85% of persons, HCV persists as a chronic infection, placing infected persons at risk for liver cirrhosis, hepatocellular carcinoma (HCC), and extrahepatic complications that develop over the decades following onset of infection [17].

Individuals infected with HCV are often asymptomatic and not aware of their illness until severe and irreversible liver diseases occur[18]. So, duration of the disease was not investigated among factors that would possibly affect the development of pulmonary involvement. PFT parameters were not related with age. In our opinion, because duration of HCV infection may not be accurately determined, age may indirectly be a sign of the duration of the disease.

In our study, 57 % of patients with HCV infection had normal PFTs while 43% have decreased PFTs. We determined that the alveolar-arterial O2 gradient was normal in 65% and increased in 35% of patients. FEV1 %, FVC % and FEF 25- 75 % were abnormal in 11%, 23% and 23% of patients respectively.

Bronchoalveolar lavage analysis shows that : macrophages % decreased accompanied by increased neutrophils and eosinophils % in all patients and there was an increase in lymphocytes % in 8 cases (80%). This results explained by HCV infection might be associated with an occult pulmonary inflammatory reaction manifested by an increased number of polymorphonuclear neutrophils in BAL fluid. This finding suggests that HCV may have the potential to induce an alveolitis leading to fibrotic changes in the lung. There are other mechanisms such as accumulation to lung tissue of immunoglobulin and/or immune complex or direct involvement of HCV-RNA. These mechanisms may be mutually related [19].

Although, BAL is an established diagnostic tool in interstitial lung and infectious bronchopulmonary diseases [7], our results revealed no statistically significant

differences between symptomatic and asymptomatic pulmonary groups as regard BAL cellular pattern (p=0.342, 0.682, 0.683 and 0.738 for macrophages, lymphocytes, neutrophils and eosinophils respectively). So in interstitial lung diseases, the diagnosis can be rarely based on BAL findings only, but BAL often provides diagnostically relevant results. BAL measures cellular and non-cellular constituents of the bronchoalveolar space. This space is anatomically inhomogeneous and consists of bronchi, bronchioles and alveoli. Therefore, it is possible that the method may affect the results of BAL [20].

Little is known about the relationship between the incidence of IPF and HCV. Conflicting studies on the incidence of HCV infection in patients with IPF have been published. Ueda et al.,[9] and Meliconi et al.,[10] reported a higher prevalence of HCV-antibody in patients with IPF compared with the general population. However, Irving et al.,[21] could not confirm the hypothesis that HCV may be a cause of IPF. We found no statistically significant difference between normal and interstitial changes in HRCT chest groups as regard to PCR-HCV RNA and Ishak scores (activity score and fibrosis score) (p value was 0.872, 0.767and 0.249 respectively). This was in agree with Ferri et al., [22] study, which investigated 300 patients with clinically evident HCV infection for the presence of pulmonary fibrosis, HRCT assessments revealed a moderate degree of pulmonary fibrosis in 4 cases and severe pulmonary fibrosis in another 4 cases. In all of these 8 cases, there were various degrees of decreases in diffusion capacity that correlated with HRCT findings and less frequently restriction in PFT parameters.

In a retrospective study by Al-Moamary et al.,[23] on 81 liver transplant candidates with hepatitis C-induced cirrhosis, the results of echocardiography, arterial blood gas analysis, and PFTs were reviewed. Pulmonary changes were found to be frequent in this cohort, with reduced PFT being the most common (found in 43% of patients), followed by restrictive lung impairment (17%) and obstructive airway disease (11%).

Further evidence of interstitial involvement with chronic HCV infection was provided by a study done by Kula et al.,[24] that assessed lung function by measurement of epithelial permeability with 99mTc-labeled diethylenetriamin-epentaacetic acid aerosol scintigraphy. In this study, significantly increased epithelial permeability was found in HCV-positive patients compared to control subjects, a finding that generally suggests early interstitial lung disease [8].

Although liver biopsy is an invasive procedure and includes a risk of complications, such as pain, pneumothorax, puncture of other viscera and hemorrhage, it is still the gold standard for grading the severity of necroinflammation and staging the extent of liver fibrosis in patients with chronic HCV infection[25]. The presence of HCV RNA in the serum is the most reliable way to diagnose hepatitis C[26]. Our results revealed that, there were high statistically significant positive correlations between liver enzymes and Ishak scores (p value <0.01) and significant positive correlation between alkaline phosphatase and AFP with Ishak scores (p value < 0.05). This was in agreement with Fouad et al.[25] study, they found that AST, ALT, HCV-RNA and Göteborg University Cirrhosis Index (GUCI) (normalized AST x INR x 100/ platelet count (x 10^9/L) showed good accuracy, moderate sensitivity, and high specificity for the diagnosis of early cirrhosis. In this study, these measures demonstrated significant correlation with both the stage of liver fibrosis and the grade of activity.

In addition to hepatic diseases, HCV infection has also been found to be involved in a variety of extrahepatic diseases. Several clinical manifestations have been reported to be linked with HCV infection[27]. However few studies about its effect on the lung showed that there was an uncertain relationship between HCV infection and pulmonary involvement [28, 29].

We found no statistically significant correlation between PCR-HCV RNA and Ishak scores with pulmonary function tests in the studied patients. Also there was

no statistically significant correlation between BAL cellular pattern with PCR-HCV RNA and Ishak scores. Against our study, Okutan et al. [30] found a negative correlation between HRCT score and PFTs in chronic HCV patients.

Chronic hepatitis C virus infections may cause mild pulmonary involvement without any pulmonary symptoms resulting in a minimal decrease in PFT. In our study, we found that pulmonary involvement was not related with the degree of liver pathology. However, we suggest that chronic HCV infection patients might be carefully evaluated by HRCT and BAL analysis even though they have normal PFT parameters.

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INTERSTITIAL PULMONARY AFFECTION IN PATIENTS WITH CHRONIC HCV

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EFFICACY OF TONSILLECTOMY AS A TREATMENT OF OBSTRUCTIVE SLEEP APNEA IN ADULTS WITH TONSILLAR HYPERTROPHY

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Abstract

Background: *High surgical success rates for tonsillectomy or adenotonsillectomy in children with obstructive sleep apnea (OSA) have been confirmed in various studies. The situation in adults is not that clear since many other factors seem to influence the outcome of isolated tonsillectomy in adult patients suffering from OSA and tonsillar hypertrophy. The present study was, therefore, geared at exploring the efficacy of isolated tonsillectomy as a treatment of OSA in adults with tonsillar hypertrophy with special emphasis on the effect of the position of the palate in relation to the tongue base on the outcome of the operation.*

Study design: *20 adult patients with obstructive sleep apnea and tonsillar hypertrophy (Friedman grades 3 or 4) were included in the study. Patients with body mass index > 40, or other obvious causes of OSA were excluded from the study. The patients were randomly allocated to two equal groups according to Friedman palatal position score. Conventional extracapsular tonsillectomy and standard preoperative and postoperative polysomnography were performed for all patients. The criteria for successful surgical response were defined as a 50% drop and below 20 of postoperative AHI.*

Results: *The mean apnea hypopnea index (AHI) of group A was 31.6 preoperatively and 10.7 postoperatively and the difference between pre- and postoperative indices was statistically significant. The mean apnea hypopnea index (AHI) of group B was 50.3 preoperatively and 44.7 postoperatively and the difference between pre- and postoperative indices was not statistically significant. The outcome of isolated tonsillectomy of group A was significantly better than the outcome of the procedure of group B.*

Conclusions: *Adult patients with OSA who are likely to benefit from isolated tonsillectomy, in the absence of other causes of OSA, are those with mild OSA, Friedman tonsillar grades 3 or 4, and Friedman palatal scores 1 or 2.*

Keywords: *Tonsillectomy, Obstructive sleep apnea, Tonsils, Polysomnography.*

Introduction

Several studies repeatedly showed that tonsillar hypertrophy is a major risk factor for OSA in Children, and that tonsillectomy is highly successful in treating those children[1.2]. The situation in adults is not that clear. While some studies reported a high success rate approximating 100%, other studies reported disappointing results [3.4.5.6.7].

Clearly, several factors, including anatomical characteristics of the patients, might have attributed to this obvious discrepancy. The position of the palate with respect to tongue base is one of the key clinical factors in predicting the outcome of treatment of OSA[8]. Theoretically, the position of the palate may be one of the anatomical features that should be considered when we select adult patients with OSA who are likely to benefit from an isolated tonsillectomy operation[9].

The present study was, therefore, geared at exploring the efficacy of isolated tonsillectomy as a treatment of OSA in adults with tonsillar hypertrophy with special emphasis on the effect of the position of the palate in relation to the tongue base on the outcome of the operation.

Patients and Methods

The study was performed on 20 adult patients with obstructive sleep apnea and tonsillar hypertrophy that were gathered from monoufiya gavrnorate from octobar 2011 to septemper 2012.

The clinical staging system proposed by Friedman et al[8,9] for the size of the tonsil and the position of the palate was used in the

study. Tonsil size was rated as follows:

Tonsil size 1: Tonsils are barely seen behind the anterior pillars.

Tonsil size 2: tonsils are clearly visible behind the anterior pillars.

Tonsil size 3: Tonsils extend three quarters the way to the midline.

Tonsil size 4: Tonsils extend to the midline (kissing tonsils).

The Position of the palate (relationship between the palate and tongue base) was rated as follows:

Friedman palate position 1: The entire uvula, tonsils, and pillars are visible.

Friedman palate position 2: The uvula is visible but the tonsils are not completely visible.

Friedman palate position 3: The uvula is not visible but the soft palate is visible.

Friedman palate position 4: Only the hard palate is visible.

The classification of OSA was performed according to Viner et al,[10] the severity was rated mild with an AHI of 10 to 29, moderate with an AHI of 30 to 49, and severe with an AHI equal to or greater than 50.

Inclusion Criteria:

1. Tonsil size grades 3 or 4 [8].
2. Apnea/Hypopnea index > 10.

Exclusion criteria:

1. Tonsil size Friedman grades 0, 1, 2.
2. Body mass index (BMI) > 40.
3. Bilateral persistent nasal obstruction.
4. Evident collapse of the hypopharynx and/or retro-lingual area.
5. Obvious skeletal or anatomic deformities such as micrognathia.

Patients were divided into two equal groups:

1. **Group A:** Tonsil size Friedman grades 3 or 4 and Friedman palatal position grades 1 or 2.[8]
2. **Group B:** Tonsil size Friedman grades 3 or 4 and Friedman palatal position grades 3 or 4.

Surgical technique:

All patients underwent conventional extracapsular tonsillectomy under general anesthesia without any palatal procedure or stitching of the tonsillar pillars.

Polysomnographic method:

Each patient underwent a fully attended standard polysomnography.[7] The practice parameters for the indications for polysomnography and related procedures[11] and the definitions for syndromes and events and diagnostic criteria for the different sleep related breathing disorders were followed[12].

Apnea was defined as cessation of airflow at the nose and mouth for ≥ 10 seconds. Hypopnea was defined as a reduction of airflow ≥ 50 for ≥ 10 seconds. The apnea index (AI) was defined as the number of apneic episodes per hour. The apnea hyperpnoea index (AHI) was defined as the number of apneic and hypopneic episodes per hour,

Follow up:

The patients were followed up weekly until complete healing of the tonsillar bed has occurred. Postoperative polysomnography was performed in the same laboratory 3 months after surgery.

Criteria for successful surgical response:

The criteria for successful surgical response were defined as a 50% drop of preoperative AHI and a postoperative AHI below 20 [13]. The paired Student t-test and the Chi Square test with Yates correction were used for statistical analysis. A (P) value less than 0.05 were considered statistically significant.

All patients signed informed consents after the nature of the research has been fully explained to them.

Results

Twenty adult patients, 17 males and 3 females, with obstructive sleep apnea and tonsillar hypertrophy were included in the study. The mean age of the patients of group A and B was 38.8 years and 42.2 years respectively.

A summary of the characteristics of the patients is shown in

table 1. Group A included 7 patients with mild OSA and 3 patients with moderate OSA. Group B included 8 patients with moderate OSA and two patients with severe OSA.

A summary of the polysomnographic results is shown in table 2. The apnea hypopnea index (AHI) of group A decreased from 31.6 preoperatively to 10.7 postoperatively and this decrease was statistically significant (t = 16.583, P< 0.001). The same significant change was observed regarding the apnea index (AI), which decreased from 20.4 preoperatively to 6.4 postoperatively (t= 9.910, P<0.001), and the lowest SpO_2 that increased from 80.8 preoperatively to 90.7 postoperatively (t = 6.631, P<0.001).

On the other hand, the apnea hypopnea (AHI) of group B decreased from 50.3 preoperatively to 44.7 postoperatively. This decrease was not statistically significant. (t=2.109, P>0.005). The same insignificant change was observed in the apnea index (AI), which decreased from 40.1 preoperatively to 36.0 postoperatively (t = 1.982, P>0.05), and the lowest SpO2 that increased from 73.9 preoperatively to 76.1 postoperatively (t =1.753, P> 0.05).

The overall outcome of isolated tonsillectomy is shown in table 2. The operation was successful, according to the AHI 50% reduction criteria, in 9 of group A patients and only 2 of group B patients. The outcome of the operation of group A was significantly better than the outcome of group B (Chi squared equals 7.273 with 1 degree of freedom, P < 0.05).

All patients with mild OSA (AHI < 30) improved after surgery. On the other hand, none of the patients with severe OSA (AHI > 50) responded favorably, according to the criteria for successful surgical response, although they showed some improvement in their respiratory parameters. The study included 11 patients with moderate OSA (AHI = 30-49) in both groups (3 in group A and 8 in group B. Tonsillectomy was successful in 4 of them (2 in each group). The remaining 7 patients showed variable improvement in their postoperative polysomnographic data.

Table 1: Characteristics of the patients' groups.

Groups	Age (Years)	Sex		Mild OSA (AHI=10-29)	Moderate OSA (AHI= 30-49)	Severe OSA (AHI > 50)
		Males	Females			
A	38.8	8	2	7	3	0
B	42.2	9	1	0	8	2

Table 2 : Polysomnographic data before and after surgery.

Group	AHI		AI		Lowest SpO_2		Overall Success	
	Preoperative Mean (Min - Max) SD	Postoperative Mean (Min - Max) SD	Preoperative Mean (Min -Max) SD	Postoperative Mean (Min- Max) SD	Preoperative Mean (Min-Max) SD	Postoperative Mean (Min- Max) SD	Successful	Unsuccessful
A	31.6 (21-47) 8.157	10.7* (2-25) 6.616	20.4 (10-37) 8.934	6.4* (0-21) 7.134	80.8 (77-85) 2.394	90.7* 80-99 5.533	9	1
B	50.3 (39-72) 10.338	44.7 (18-70) 17.107	40.1 (29-64) 11.742	36.0 (13-63) 11.742	73.9 (70-77) 2.282	76.1 (70-85) 5.006	2	8

* The difference between pre- and postoperative values was statistically significant ($p < 0.001$)

Discussion

There are several treatment options for OSA. Patients usually turn to surgical treatment when nonsurgical options fail. Uvulopalatophayngoplasty (UPPP), with its numerous modifications, remains the most common first-step surgical procedure for OSA. However, the success rates of the procedure vary considerably[8.14.15]. A recent meta-analysis of the available data indicates UPPP success rate of about 40%[16]. Another study even mentioned that some the patients who fail to improve actually become worse after UPPP[17]. Furthermore UPPP has its well known immediate and long-term complications[18].

While the usefulness of tonsillectomy in treating children with OSA has been firmly established, its role in adults is not clear or reasonably defined. Although some earlier investigations have shown a reasonable success rate

of tonsillectomy in the treatment of OSA in adults who exhibited tonsillar hypertrophy, they pointed to this success only as a trend without providing any individual polysomnographic data[19.20.21]. In fact, to date, the choice between UPPP and tonsillectomy remains a crucial decision to select when the tonsils are large in OSA patients. This encouraged us to study the efficacy of isolated tonsillectomy as a treatment of adults with OSA due to tonsillar hypertrophy and to characterize the patients who are more likely to benefit from the procedure.

Palate position had been previously studied and found to be an important clinical indicator of OSA. Friedman et al[9] found that the key clinical findings in predicting the presence of OSA were found to be palate position in relation to tongue base, tonsil size, and body mass index (BMI). In the present study, twenty patients were divided into 2 groups according to the relationship between the palate and tongue base. The tonsils in both groups were graded 3 or 4 according to Friedman's grading system of tonsils size.[8]

In the present series, the success rate of tonsillectomy, defined as a 50% drop of preoperative AHI and to a postoperative AHI below 20[13], was 90% for group A, and 20% for group B. The difference between the two groups was statistically significant. Further analysis of the results revealed statistically significant differences between pre- and postoperative AHI, AI, and lowest SpO_2 in group A only. On the other hand, postoperative changes of group B patients were quite insignificant.

The high success rate of group A agrees with the findings of Nakata et al[13] who reported a success rate of 88.9% in their series and without any serious complications. Verse et al also reported similar findings[22] supporting our results and pointing to the efficiency of the operation in some selected patients. The poor results of group B, on the other hand, highlighted the important role of the palate in the pathogenesis of the disease as well as in predicting the expected outcome of the operation.

The present study showed that

tonsillectomy had a positive influence on AHI, AI, as well as SpO2 in adult patients with OSA and tonsillar hypertrophy. There was also an inverse relationship between the response rate and the severity of OSA, the operation being more successful in mild OSA. Therefore, based on the results of the study, we can conclude that isolated tonsillectomy may be indicated, as a curative measure, in patients with mild OSA, Friedman tonsil size grades 3 or 4, and Friedman palatal position grades 1 or 2. For other patients, the operation may be done in conjunction with other procedures or to help the patients to use their continuous positive airway pressure (CPAP) devices.

Summary

Isolated tonsillectomy can be an effective treatment in carefully selected adults with obstructive sleep apnea due to tonsillar hypertrophy.

1. The patients who are likely to benefit from isolated tonsillectomy are those with:
   i. Friedman tonsil size grades 3 or 4.
   ii. Friedman palatal position grades 1 or 2.
   iii. Mild, and to a less degree, moderate obstructive sleep apnea.
2. Isolated tonsillectomy is not suitable in patients with
   a. Friedman palatal scores 3 or 4.
   b. Severe obstructive sleep apnea.
   c. Airway obstruction at other levels such as the hypopharynx.
3. The operation, however, may be used in conjunction with other surgical procedures or to facilitate the use of CPAP.

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EFFICACY OF TONSILLECTOMY AS A TREATMENT OF OBSTRUCTIVE SLEEP APNEA IN ADULTS WITH TONSILLAR HYPERTROPHY

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OBESTATIN AND SMALL INTESTINAL MOTILITY IN VITRO

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Abstract

Obestatin is a 23 amino acid peptide, was identified through sequence analysis of the ghrelin precursor gene, proghrelin and originally purified from the rat stomach mucosa. In spite of established stimulatory effect of ghrelin on gastrointestinal motility, the effect of obestatin remains controversial. So our study aims to investigate the effect of obestatin on basal and ghrelin stimulated rat jejunal motility in vitro and to study the possible mechanisms of its action. Albino Wister rats of both sexes were classified into 5 groups each group consists of 6 experiments. Segments from the jejunum were mounted in 5 ml organ bath chambers containing oxygenated Krebs solution and the motility was continuously recorded. Obestatin showed a significant decrease in both the basal and the ghrelin stimulated jejuna motility. This inhibitory effect of obestatin was prevented by the pre addition of L-NAME. While after adding propranolol hydrochloride, phentolamine and naltrexone, obestatin was still able to produce its inhibitory effect on the motility. All these findings could explain the involvement of nitregeric neurons in mediating the effects of obestatin on jejunal motility.

Keywords: *Obestatin, GPR39, Ghrelin, L-NAME (N^G – nitro – L – arginine methyl ester).*

Introduction

The word obestatin is a contraction of obese, and derives from Latin 'obedere', meaning 'to devour' and 'statin', denoting suppression. It is a 23 amino acid peptide, was identified through sequence analysis of the ghrelin precursor gene, proghrelin and originally purified from the rat stomach mucosa [1]. It was detected in the circulation suggesting

that the following release from the stomach mucosa might act as endocrine regulator [2]. Obestatin secretion is pulsatile and displays an ultradian rhythmicity, in a similar way to ghrelin and GH secretion, but plasma ghrelin and obestatin levels are not strictly correlated and the number of obestatin pulsatile episodes may seem slightly lower than that observed for ghrelin and GH secretion [3].

Obestatin binds to the orphan G-protein-coupled receptor GPR39 which is distinct from the ghrelin receptor. GPR39 was discovered in relatively large amounts in the jejunum and duodenum. Besides the intestinal tissues, it was found in the stomach, pituitary gland, and in smaller amounts in the ileum, liver and hypothalamus. The smallest amount of GPR39 was found in heart, pancreas, cerebellum, cerebrum, kidney, colon and lung tissues [4].

It was named obestatin to reflect its ability to counteract the effects of its sister peptide, ghrelin, on food intake and gastrointestinal motility and suppress weight gain in mic [1]. The significant effect was found within the range of doses between 1 and 5 nM[2]. The first study of obestatin by Zhang et al.[1] indicated that intraperitoneal (IP) injection of obestatin suppressed cumulative food intake, decreased body weight gain, and inhibited gastric emptying and jejunal muscle contraction in mice. Subsequently, Moechars et al. [5] suggested that the rate of gastric emptying is increased in GPR39 knockout mice, an observation which may be consistent with a gastric regulatory function for obestatin. However, Gourcerol et al.[6] and Nogueiras et al[7] showed that intraperitoneal (IP) injection of obestatin had no effect on cumulative food intake, gastric emptying, or cholecystokinin (CCK)-induced satiety signaling in rats and mice. Due to this controversial effect of obestatin on gastrointestinal motility, this study aims to clear the exact effect of obestatin on spontaneous and ghrelin stimulated small intestinal motility in vitro and its possible mechanism of action.

Material and Methods

Thirty Albino Wister rats of both sexes weighing 100-180 gm were obtained from the Mansoura University animal House, adapted to room and cage environments for 2 weeks. They were randomly divided into 5 groups using a computer-assisted randomization procedure to ensure similar distribution of body weights. They were caged in a temperature controlled room (22℃) with a 12 hour light – dark cycle and were maintained during this period on commercial chow diet. They had free access to tap water. These animals were not exposed to any chemical substances.

Procedure:

After cervical dislocation of the rats, a midline incision were performed and the small intestine were blunt-dissected and placed immediately in Krebs solution (NaCl 121.5, CaCl2 2.5, KH2PO4 1.2, KCl 4.7, MgSO4 1.2, NaHCO3 25.0, glucose 5.6mM) previously equilibrated with 5% CO2 in O2 at room temperature. Segments from the jejunum were mounted in 5 ml organ bath chambers containing Krebs solution bubbled with 5% CO2/95% O2 and maintained at 37℃. The lower ends of the tissue segment were anchored to the bottom of the chamber and the other ends were connected to an isotonic transducer .The motility were continuously recorded with a Power Lab recording unit and further analyzed with chart 7 software. In this system isotonic contractions were recorded throughout the experiments. The segments were allowed to equilibrate for 60 min; during this time the solution was changed every 15 min. Following the equilibration period of the stabilization, control contractions were recorded for 10 min and chemicals were added to the tissue bath. The amplitude (in mm) and tone of contractions were recorded. All chemicals were purchased from Sigma. All sacrificed animals had been disposed by safety cabinet in MERC (Medical experimental research centre) in Mansoura University.

The experiments were classified into 5 groups each group consists of 6 experiments:-

Group I: to study the effects of different doses of obestatin (10-9M), (5x10-9M), and (10-6M)

dissolved in distilled water on the basal jejunal motility [2].

Group II: to study the effect of obestatin after adding ghrelin (10-6M) dissolved in 1% acetic acid by 4 minutes [8].

Group III: to study the effect of obestatin on the motility after 4 minutes of adding propranolol hydrochloride 10-6M (β adrenergic blocker) dissolved in ethanol (10 mg/ ml) [9], and phentolamine 10-6M (α adrenergic blocker) dissolved in distilled water [10].

Group IV: to study the effect of obestatin after adding naltrexone (opoid receptors blocker) 10-7M by 4 minutes,its crystalline compound dissolved in distilled water (100mg/ml) [11].

Group V: to study the effect of obestatin on the motility after adding NG - nitro - L - arginine methyl ester (L-NAME)(NOS inhibitor) by 4 minutes in a dose of 10-4M dissolved in distilled water [12].

Statistical analysis

All of the intestinal segments included in the analyses showed spontaneous contractions. For each segment, the mean amplitude of contractions was calculated as the average of maximum to minimum value of each contraction over 4 min and was expressed in mm. The tone of contractions was calculated as the mean of the tension values (mg) recorded in 4 min.

Statistical analysis was done by using SPSS (statistical package for social science) program version10, 1999. Data was expressed as means±S.D. The statistical significance of any differences between unpaired data was determined using Student's two-tailed t-test. . $P<0.05$ was considered statistically significant.

Results

(1) Effects of obestatin:

In table (1) and figure (1) obestatin (10-9M) show significant decrease in the amplitude (from 4±0.3 to 1±0.02mm) and the tone (from 3±0.02 to 1.5±0.04mg) ($P<0.05$), obestatin (5x10-9M) show also significant decrease in the amplitude (from 1±0.1 to 0.5±0.05mm) and the tone (from

1.5±0.04 to 1±0.1mg) of the jejunal motility ($P<0.05$), while obestatin (10-6 M) show insignificant change in the inhibitory effects of obestatin (5x 10-9 M) both on amplitude and tone of the jejunal motility. So, the maximal inhibitory effect of obestatin was observed at the concentration of (5x10-9M) with a 90% reduction in the amplitude of intestinal contractility. No significant effect with increasing the concentration of obestatin. The inhibitory effects of obestatin are confirmed by the stimulatory effects of acetylcholine after obestatin as shown in figure (2).

(2) Effects of Obestatin on the jejunal motility after Ghrelin:

Table (2) and Figure (3) Ghrelin shows significant increase in the amplitude (from 1±0.015 to 10±0.5mm) and the tone (from 3± 0.5 to 7±0.8mg) of the jejunal motility. However, addition of obestatin significantly reduces this ghrelin increasing effects either in amplitude (from 10±0.5 to 1±0.05mm) or in tone (from7±0.8 to 2±0.2mg).

(3) Effects of obestatin on the jejunal motility after phentolamine (β adrenergic blocker) and propranolol HCL (α adrenergic blocker) administration:-

In Table (2) and figure (4) obestatin shows also a signific.ant decrease in the amplitude (from4±0.9 to 2±0.1mm) and the tone (from5.4±0.5 to 1±0.15mg) of the jejunal contraction after addition of phentolamine and propranolol HCL.

(4) Effects of obestatin in the jejunum after Naltrexone (opoid receptor blocker):-

Table (2) and Figure (5) show significant decrease in both amplitude (from4±0.1 to 2±0.27mm) and tone (from 5.8±0.9 to 2.3±0.1mg) of jejunal contractility after addition of Naltrexone.

(5) Effects of obestatin on the jejunal motility after L-NAME (NOS inhibitor):-

Table (2) and Figure (6) L-NAME shows significant increase in the tone and the amplitude of the jejunal motility, while addition of obestatin after L-NAME is unable to produce its relaxing effect

Table (1): Dose dependant effects of Obestatin on basal jejunal motility

	Amplitude (mm)		Tone (mg)	
	Basal	**Effect**	**Basal**	**Effect**
Obestatin(10^{-9} M) Mean **±SD** % of change P1	5 ±0.3	1 ±0.02 -80% < 0.05	3 ±0.02	1.5 ±0.04 -50% < 0.05
Obestatin (5 X 10^{-9} M) Mean SD % of change P1	1 ±0.1	0.5 ±0.05 -50% < 0.05	1.5 ±0.04	1 ±0.1 -33.3% < 0.05
Obestatin (10^{-9} M) Mean ±SD % of change P1	0.5 ±0.01	0.5 ±0.01 0% NS	1 ±0.1	1 ±0.15 0% NS

%: percent change as compared with the basal contractions.
P1: values as compared with the basal contractions.

Table (2): Effects of obestatin on the basal jejunal motility after adding ghrelin phentolamine, propranolol hydrochloride, naltrexone and L-NAME

	Amplitude (mm)		Tone (mg)	
	Basal	**Effect**	**Basal**	**Effect**
Obestatin after ghrelin Mean SD % of change P2	10 ±0.5	1 ±0.05 -90% < 0.05	7 ±0.8	2 ±0.2 -71.4% < 0.05
Obestatin after phentolamine and propranolol hydrochloride Mean ±SD % of change P3	4 ±0.9	2 ±0.1 -50% <0.05	5.4 ±0.5	1 ±0.15 -81.5% < 0.001
Obestatin after naltrexone Mean ±SD % of change P4	4 ±0.1	2 ±0.27 -50% <0.05	5.8 ±0.9	2.3 ±0.1 -60.3% <0.05
Obestatin after L-NAME Mean ±SD % of change P5	3 ±0.2	3 ±0.1 0% NS	5 ±0.21	5 ±0.25 0% NS

%: percent change as compared with the basal contractions.
P2: values as compared with the contractions after adding ghrelin .
P3: values as compared with the contractions after adding phentolamine and propranolol Hcl.
P4: values as compared with the contractions after adding naltrexone.
P5: values as compared with the contractions after adding L-NAME.

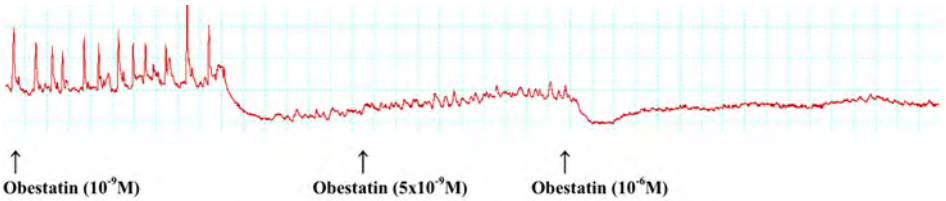

Figure (1): Dose dependant effects of Obestatin on basal jejunal contractility

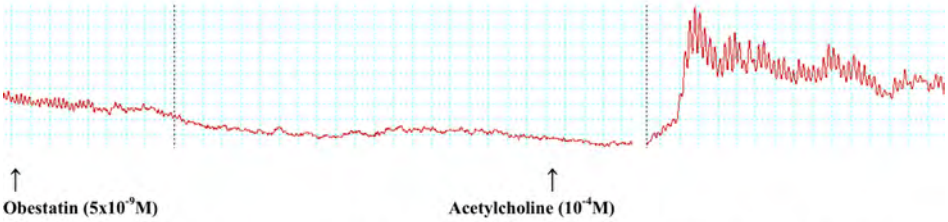

Figure (2): Effect of Acetylcholine (10^{-4}M) on jejunal contractility after adding Obestatin ($5x10^{-9}$M).

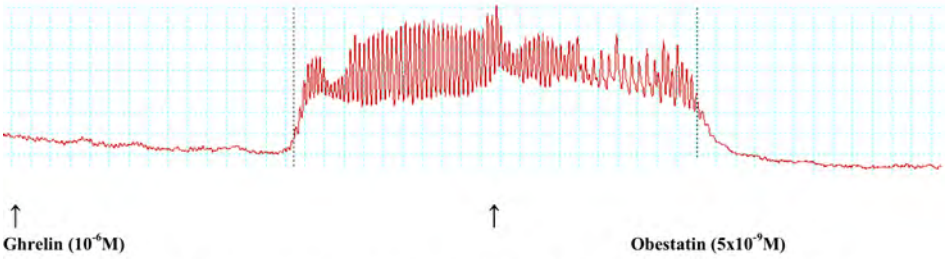

Figure (3): Effect of Obestatin ($5x10^{-9}$M) on jejunal contractility after adding Ghrelin (10^{-6}M).

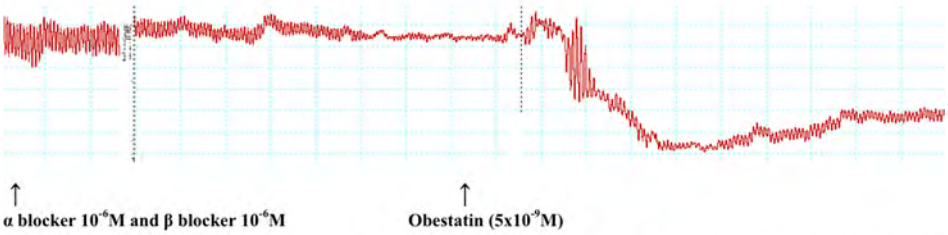

Figure (4): Effect of Obestatin ($5x10^{-9}$M) on jejunal contractility after adding propranolol hydrochloride (10^{-6}M) (β blocker), and phentolamine (10^{-6}M) (α blocker).

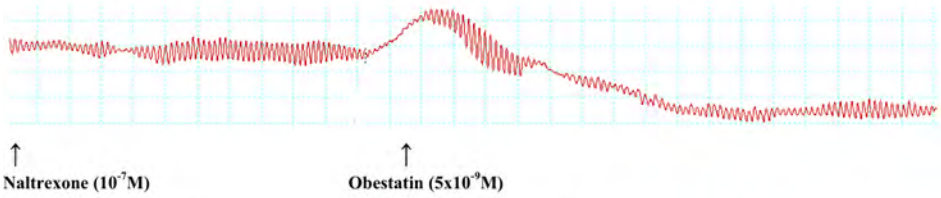

Figure (5): Effect of Obestatin ($5x10^{-9}$M) on jejunal contractility after adding naltrexone (10^{-7}M)

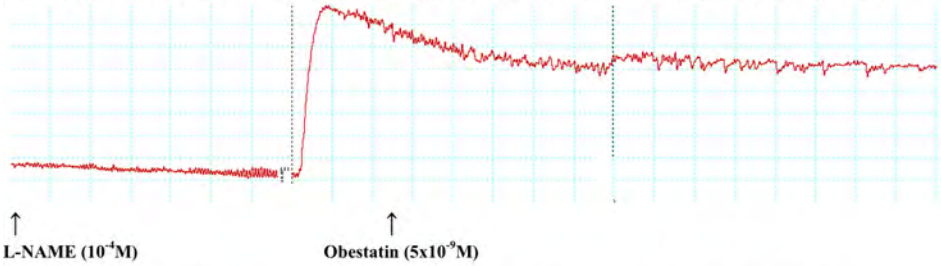

Figure (6): Effect of Obestatin ($5x10^{-9}$M) on jejunal contractility after adding L-NAME (10^{-4}M)

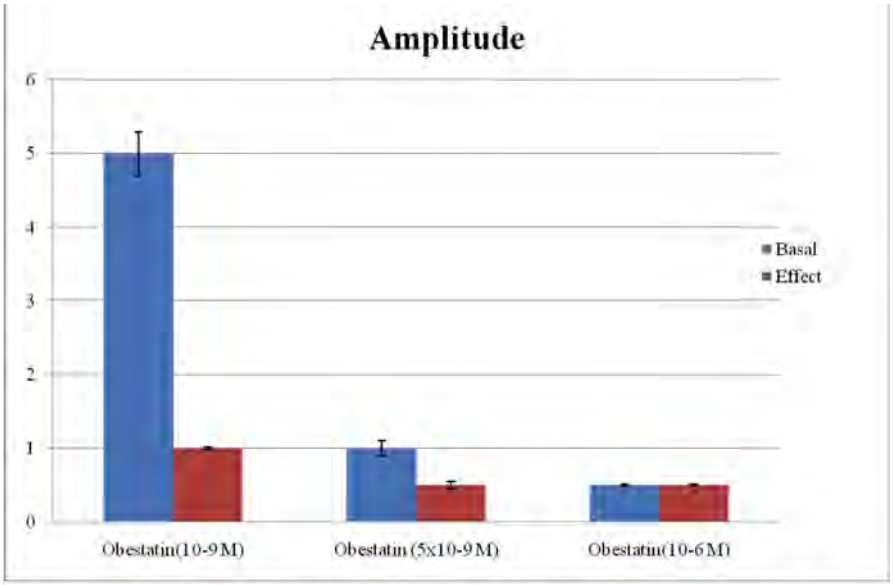

Chart (1): Dose dependant effects of Obestatin on basal jejunal motility amplitude .

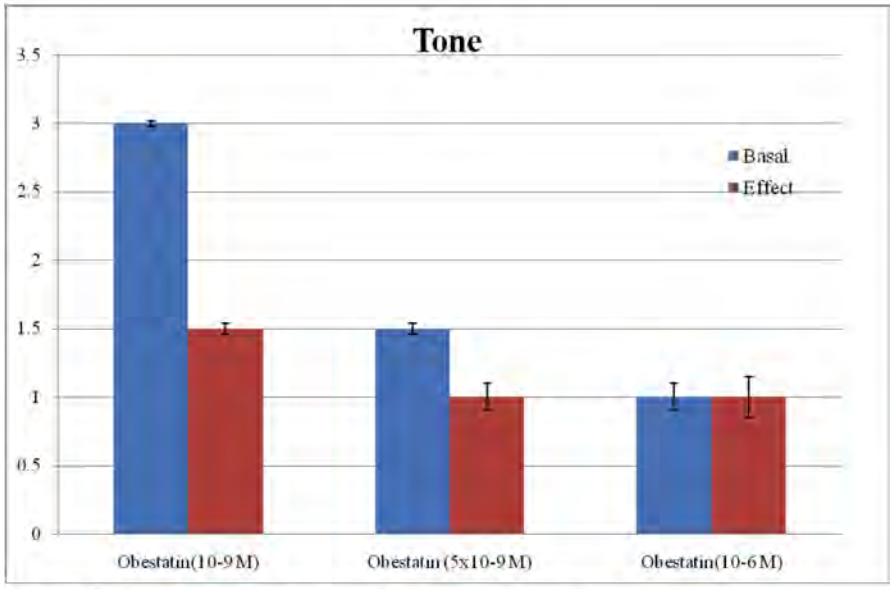

Chart (2): Dose dependant effects of Obestatin on basal jejunal motility tone .

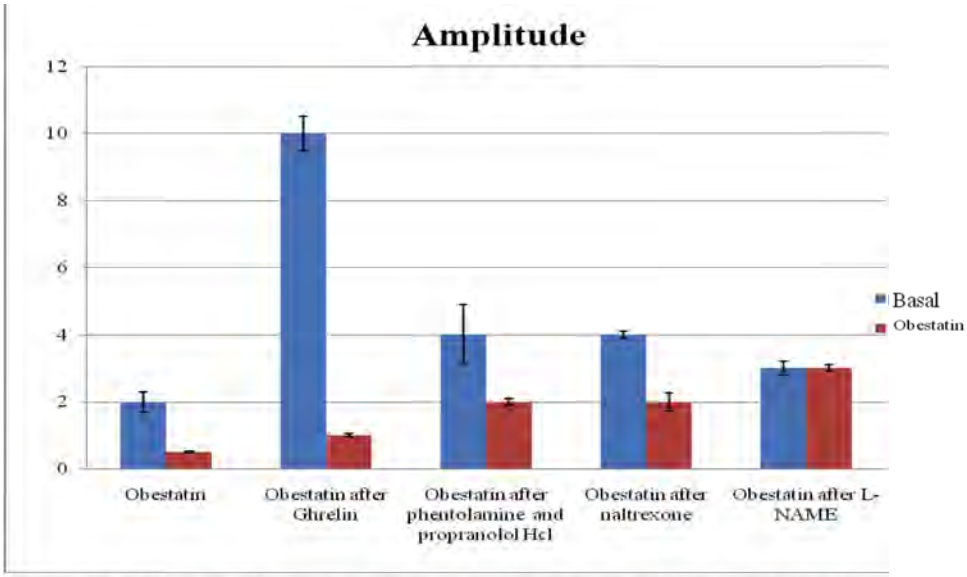

Chart (3): Effects of obestatin on the basal jejunal motility amplitude and its effects on motility after adding ghrelin and L-NAME

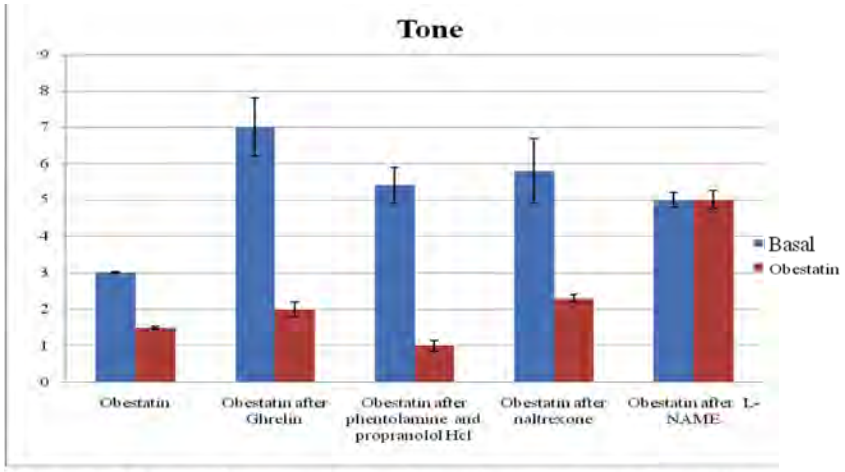

Chart (4): Effects of obestatin on the basal jejunal motility tone and its effects on motility after adding ghrelin and L-NAME.

Discussion

Our knowledge concerning obestatin and its biological role is growing fast, but it is still scanty as compared that of ghrelin. In brief, the circulating ghrelin level increase considerably before each meal and decrease rapidly after food intake[13], and exogenous ghrelin administration stimulates appetite and food intake both in rodents and humans [14,15], suggesting that ghrelin is an important factor in meal initiation. In addition, ghrelin stimulates gastrointestinal motility [16], gastric acid secretion[17], and pancreatic exocrine secretion[18], and control the post natal development of the gastrointestinal tract[19]. it is somehow surprising that obestatin, though derived from the same peptide precursor, suppressed food intake, decreased body-weight gain and antagonized the actions of ghrelin when both peptides were co-administered [1].

Some reports indicate that obestatin is unlikely to be the endogenous ligand for GPR39[20]. Granata et al.[21] reported that obestatin promotes beta cell and human islet survival by binding to glucagon-like peptide-1 receptor (GLP-1R), the receptor through which incretins act. Similarly, incretins also reduce gastrointestinal motility with a paracrine peripheral action and

induce satiety and reduce food intake. To date, the receptor for obestatin remains unknown and further studies are required to reveal the exact relationship between obestatin, GPR39 and GLP-1R.

The effects of obestatin were studied in models of rat GI motility that were sensitive to the prokinetic activity of ghrelin. The ability of ghrelin to induce a small facilitation of cholinergically mediated contractions in rat isolated forestomach has been observed previously [22,23]. Similarly, our experiments confirm Edholm et al. [8] results on the ability of ghrelin to increase rat small intestinal motility.

In the present study, obestatin, in contrast to ghrelin, had the ability to inhibit small intestinal motility, in the isolated jejunum in a dose dependent manner with maximum inhibition was at the dose of (5x10-9M) and also it functionally antagonize the excitatory effects of ghrelin in the same preparation. These data confirm the observations of Zhang et al.[1], who found that obestatin could provoke a sustained inhibition of rat gastric emptying, inhibit spontaneous contractile activity in the isolated rat small intestine. Also, the present results agree with Ataka et al. [24] who found that i.v. administration of obestatin inhibits the motor activity in the antrum and duodenum in conscious rats in the fed state but not in the fasted state, and they found that the inhibitory action of obestatin appeared 30-90 minutes after i.v. injection, which is consistent with the timing of the effects of i.v. injection of ghrelin (30 minutes) on gastroduodenal motility.

Since the alimentary tract is equipped with the largest collection of neurons outside the central nervous system, so it can be considered as a neurological organ. The communication network of the enteric nervous system (ENS) involves acetylcholine, tachykinins, nitric oxide (NO), adenosine triphosphate (ATP), vasoactive intestinal polypeptide (VIP) and opioid peptides as major transmitters[25]. Studies show that NO neurons are wide spread in the mammalian gut wall[26]. Also Krantis et al[27] found that

either ATP or VIP gastrointestinal relaxant effect can be mediated through NO inhibitory motor neurons.

By a trial to search for the mechanism by which obestatin exerts its relaxing effect, we blocked the adrenergic receptors by administration of phentolamine and propranolol hydrochloride simultaneously. But after adrenergic receptor blocking, obestatin was still able to reduce the jejunal motility significantly. This result proved that the inhibitory effect of obestatin is not mediated through adrenergic receptors. We also blocked the opoid receptors by administration of Naltrexone, but obestatin showed also significant decrease in jejunal motility after naltrexone administration. This excludes the role of opoid receptors in the inhibitory effect of obestatin.

However, addition of nitric oxide synthase inhibitor (L-NAME) before obestatin is found to block its effect, this observation means that the relaxing effect of obestatin may be most probably mediated through NO release. So, it is suggested that obestatin acts on nitrergic neurons and stimulates NO release which is leading to relaxation of the jejunal smooth muscles. The possible mechanisms involved in NO-induced relaxation are 1) cyclic GMP-dependent reduction of cellular free Ca^{2+} via activation of soluble guanylyl cyclase in smooth muscle cells changing the membrane potential [28], 2) cyclic GMP-dependent opening of apamin-sensitive K- channels or other types of ion channels to produce hyperpolarization and relaxation [29], and 3) cyclic GMP-independent mechanisms, such as actions of NO on ion channels involved in muscle contractility, either directly or via membrane hyperpolarization[30]. Also NO can mediating relaxation indirectly by decreasing the acetylcholine release from the enteric cholinergic neurons[31].

Conclusion

The present study shows that obestatin, in contrast to ghrelin, has the ability to inhibit small intestinal motility, in the isolated jejunum and also it functionally antagonizes the excitatory effects of ghrelin in the same preparation.

The relaxing effect of obestatin may be most probably mediated through its action on nitrergic neurons and NO release which acts both directly on smooth muscle and indirectly through inhibition of acetylcholine release.

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Author Contribution:

Shereen M. Samir and Abeer F. Mostafa designed & performed research, analyzed data and wrote the paper

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EFFECT OF DIFFERENT DOSES OF DEXMEDETOMIDINE COMPARED WITH KETAMINE ON THE OUTCOME OF BURN WOUND DRESSING

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Abstract

Objective: *The aim of this study is to evaluate the effects of different doses of dexmedetomidine compared with the traditional used ketamine on the stress and inflammatory responses in patients with burn wound dressing besides monitoring of the hemodynamic, analgesia and sedation scores or possible complications.* ***Methods:*** *Following Ethics Committee approval and informed patient consent, 88 ASA physical statuses I -III adult burn patients were included in the study. Patients were randomly divided into four groups (22 patients in each group). Ten minutes before burn dressing change, the dexmedetomidine groups (D) received a continuous infusion of dexmedetomidine at different doses either 0.5 -1 - 1.5 ug/kg^{-1}/min^{-1} till the end of procedures. Patients in ketamine group (K) received ketamine 1.25mg/kg^{-1}/min^{-1} till the end of the procedures. Serum level of cortisone, C-reactive protein, glucose level and WBCs count were assessed on admission before burn dressing (baseline values), 6 hours, 12 hours and 24 hours post-dressing. Hemodynamic variables were monitored during dressing, post-dressing pain and sedation scores were assessed.* ***Results:*** *Demographic data (age, weight, sex), and time for dressing changes were comparable among the studied groups. Heart rate and mean arterial blood pressure were significantly reduced in D1 and D2 groups and highly significant reduced in D3 group compared with K group. One case of hypotension was reported in D3 group and treated with intravenous infusion of saline 0.9%. There was statistically significant increase in post - dressing*

pain score in D1group than k group. Moreover, sedation score at one hour and six hours after dressing was significantly higher in D3 group than k group. There was statistically significant reduction in inflammatory markers (WBCs count, C- reactive protein, and blood glucose and morning sample of serum cortisone level) in D2 group and D3 group compared with K group at 6hour, 12 hours and 24 hours post dressing. In k group; three patients experienced nausea and vomiting while hallucination was reported in two patients with no recorded cases of hypoxia and apnea in any of the studied patients. Conclusion: The use of Dexmedetomidine at a doses of 1ug/kg^{-1}/min^{-1} and 1.5ug/kg^{-1}/min^{-1} during burn wound dressing had a dose dependent anti-stress and anti inflammatory effects beside its sedoanalgesia and hemodynamic stability without significant side effects compared with intravenous ketamine 1.25mg/kg^{-1}/min^{-1}.

Keywords: *Dexmedetomidine, ketamine, burn, Glucose , WBCs , cortisone, C-reactive protein.*

Introduction

Burn is the most intense stress that human body can suffer. This stress can alter the immune system and resulting in changes of the metabolic and physiologic processes specially when the burn is sever and in more than 20% of the total body surface area[1,2,3]. Cortisone and catecholamine's levels are increased following massive burn that contribute to the hypermetabolic response and catabolic state [4] A burn dressing has three principal functions, comfort: a dressing shields the wound surface from air current and absorbs wound secretions. Metabolic: a dressing reduces evaporative heat loss, minimizes cold stress and shivering. Protective: a dressing should isolate the wound so that few of any organisms are ever recoverable. [1]

Ketamine is one of the famous drugs which has been widely used in burn dressing for its sedative properties[5,6]. Although, Ketamine remains a relatively safe drug, monitoring of these patients is essential, as there are reported cases of respiratory and cardiovascular depression[7].

Dexmedetomidine is a specific α2-receptor agonist, used to reduce anxiety, tension, and to promote relaxation and sedation with hemodynamic stability[6,8]. Dexmedetomidine was used to induce adequate analgesia and also, to reduce the use of morphine or other opioids avoiding its numerous side effects [9]. Previous studies had investigated the anti-inflammatory effects of dexmedetomidine. However, it does not clarify the definitive mechanism by which dexmedetomidine decreases inflammatory mediators[10,11]. This randomized, prospective and double blind study was designed to evaluate the anti-inflammatory effects of different doses of dexmedetomidine in comparison with the traditional used ketamine in patients with burn dressing changes. As the hypothesis of our study based on the anti-inflammatory property of dexmedetomidine as the first outcome, we used the Serum level of cortisone, C-reactive protein, WBC count and glucose as indicators. The second outcome of our study is monitoring of the hemodynamic variables, analgesia and sedation scores in burnt patients undergoing dressing.

Patients and Methods

The study was conducted at the Centre for burn and plastic surgery, Mansoura University Principal Hospital, Egypt. A common approach to burn wound care consists of wound cleaning and debridment daily or twice daily and application of the dressing. Silver sheets and meshes have broad spectrum antimicrobial properties. They are painless with no significant toxicity or antimicrobial resistance. A variety of proteolytic enzymes have been utilized to debride wounds including protease, collagenase and papain-urea [7].

After receipt of Ethics Committee approval and informed written patient consent' 88 ASA physical statuses I and III adult patients aged 18-45 years, suffering from 2^{nd}-3^{rd} degree burn of TBSA (20-50%) who admitted for dressing; were included in the study. In the operating room, routine monitors were applied for recording heart rate (HR), mean arterial blood pressure (MBP), and peripheral

oxygen saturation (SpO2). Baseline values before dressing change were recorded then patients were randomly allocated to receive one of the four study protocols via sealed envelope assignment. All patients in dexmedetomidine Groups (D1-D2-D3) received intravenous dexmedetomidine start ten minutes before dressing but at different doses. The patients in 1st group D1 (n=22) received dexmedetomidine at dose of 0.5 $ug/kg^{-1}/min^{-1}$, While patients in the 2nd group D2 (n=22) received $1ug/kg^{-1}/min^{-1}$. Patients in the 3rd group D3 (n=22) received $1.5ug/kg^{-1}/min^{-1}$. Patients in the fourth group (K) (n=22) received ketamine 1.25 $mg/kg^{-1}/min^{-1}$ start ten minutes before dressing.

Patients were instructed on the use of Visual Analogous Scale (VAS) self-rating method as (0, no pain; 10, worst possible pain). Sedation was assessed on a five-point scale ('0' = no sedation-patient wide awake and alert; 4' = deep sleep, difficult to rouse). Pain and sedation were assessed by an assistant at 2, 6,12, 24 hours postdressing . Pain score <5 was considered adequate analgesia. As, sedo-analgesia was defined primarily as VAS <5 and sedation scores >2 [6]. Serum level of cortisone(morning sample), C-reactive protein, WBC count and glucose level, were assessed on admission before burn dressing (baseline values)and at 6 hours ,12 hours and 24 hours post-dressing. Recording complications and its management such as bradycardia was treated by atropine 0.5mg, hypotension was treated by intravenous saline 0.9% at rate of 10 $ml/kg^{-1}/min^{-1}$, if persist ephedrine 5mg was given, nausea, vomiting were treated by metclopropmide 10mg.

Sample size was calculated by using one way ANOVA test in G *power 3.1.5 program. According to pilot study 20 patients (5 patients in each group) we calculated that 22 patients per group were sufficient to give $p<0.05$ significant with confidence interval 95% with an actual power of 86% when mean of cortisone level at 4 hours post-dressing in k group (36.4µg/dl), D1 group (34.3µg/dl), D2 (33 µg/dl)and D3 group (29.6µg/dl) Statistical analyses were performed using SPSS 16

(SPSS Inc., Chicago, IL, USA). Parametric data were analyzed using the independent t-test and repeated measures ANOVA followed by post hoc test. Non-parametric data were compared using Mann-Whitney test. All values were expressed as mean (SD), or the number of patient. P-value ≤ 0.05 was considered as statistically significant.

Results

The characteristics of the 88 patients who completed the study are summarized in table 1. Demographic data (age, weight, sex), and time to dressing changes were comparable among the studied groups . Heart rate and mean arterial blood pressure were significantly reduced in D1 and D2 groups ($p \leq 0.05$) and highly significant reduced in D3 group ($p \leq 0.001$) compared with K group. One case of hypotension was reported in D3 group and treated with intravenous infusion of saline 0.9% 10ml/kg^{-1} /min^{-1}. There was statistically significant increase in postdressing pain score in D1 group than k group. Moreover sedation score at one hour and six hours after dressing was significantly higher in D3 group than k group ($p<0.05$). There was statistically significant reduction in inflammatory markers includes; WBCs count, C-reactive protein level ,blood glucose level, and serum cortisone level in D2 group and D3 group at 6hour, 12 hours and 24 hours post dressing compared with K group. In k group; three patients experienced nausea and vomiting while hallucination was reported in two patients with no recorded cases of hypoxia or apnoea in any of the studied patients

Heart rate and mean arterial blood pressure were significantly reduced in D1 and D2 groups($p \leq 0.05$) and highly significant reduced in D3 group ($p \leq 0.001$) compared with K group .

There was statistically significant increase in post dressing pain score in D1group than k group. Moreover sedation score at one hour and six hours after dressing was significantly increase in D3 group than k group ($p<0.05$).

Table 1. Demographic characteristics of patients in the study groups . data was expressed as mean ± SD and numbers.

	Kgroup (n=22)	**D1group (n=22)**	**D2group (n=22)**	**D3group (n=22)**
Age(years)	27.9±4.9	29.1±2.1	27.1±3.3	28.5±3.2
Sex male/ female	10/12	13/9	8/14	11/11
Weight(Kg)	67.5±5.2	66.8±4.6	67.8± 2.4	68.1±2.1
Time of dressing change(min)	22.3± 2.5	19.6± 5.5	20.7 ± 3.7	21.5±3.1

K group: ketamine group D1group: Dexmedetomidine 0.5ug group

D2group: Dexmedetomidine 1ug group D3group: Dexmedetomidine 1.5ug group

Table 2. Level of inflammatory markers in ketamine and three different doses of dexmedetomidine groups, data expressed in mean ±SD.

		Preinduction	**6hours post dressing**	**12hours post dressing**	**24hours post dressing**
WBC $\times 10^3$/uI	K group	15.4± 4.4	17.1±4.1	16.5± 3.6	15.6±3.8
	D1 group	16.4± 3.9	16.4±3.6	15.4±3.1	14.3± 3.3
	D2 group	16.7± 4.1	14.4±3.3* *(p 0.4)*	13.1± 2.6 * *(p 0.002)*	12.5± 2.3 * *(p 0.003)*
	D3 group	16.5± 3.9	13.9± 3.6* *(p 0.03)*	12.3±2.7* *(p 0.001)*	11.4±2.7* *(p 0.001)*
C RP mg/dl	K group	31.9± 6.4	33.9±5.5	32.4±6.1	33.1± 5.4
	D1 group	32.3±6.6	34± 6.1	32.3± 5.2	32.7± 6.5
	D2 group	31±9.2	26.5±6.02* *(p 0.001)*	22.5±5.9* *(p 0.000)*	22.2± 6.2 * *(p 0.000)*
	D3 group	31.1± 6.7	24.5±7.5 *(p 0.000)*	21.3±6.1* *(p 0.000)*	20.5± 8.1* *(p 0.000)*
Cortisone level µg/dl	K group	41.1± 7.9	40.9± 8.3	-	39.7± 7.6
	D1 group	39.5± 5.6	38.9±3.1	-	38.1±4.2
	D2 group	39.2± 8.9	36±8.7* *(p 0.03)*	-	32.9± 2.9 *(p 0.003)*
	D3 group	38.9± 7.5	29.2± 4.3* *(p 0.000)*	-	26.5± 2.2* *(p 0.000)*
Glucose level mg/dl	Kgroup	100.4± 11.2	109.5±15.5	111.3±17.7	106.3± 10.4
	D1group	99.6±11.1	108.7± 19.1	110.9± 19.4	109.9± 12.9
	D2group	100.9±10.9	100± 11.4* *(p 0.02)*	99.4± 10.6* (p<0.001)	100.1± 9.8* (p< 0.005)
	D3group	100.1± 9.6	99.2±9.8* (p<0.000)	100.3± 9.8* (p< 0.000)	98.9± 10.1* (p<0.000)

CRP :c reactive protein WBC: white blood cells K group: ketamine group

D1group: Dexmedetomidine 0.5ug group D2group: Dexmedetomidine 1ug group

D3group: Dexmedetomidine 1.5ug group p significant <0.05 P highly significant <0.001

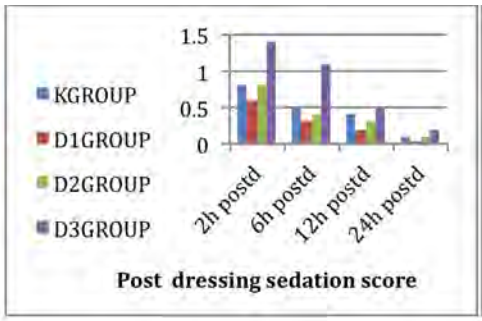

Figure1: Sedation score
K group: ketamine group
D2group: Dexmedetomidine 1ug group

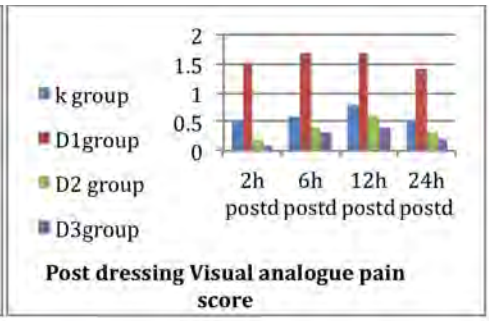

Figure 2: Visual analogue pain score
D1group: Dexmedetomidine 0.5ug group
D3group: Dexmedetomidine 1.5ug group

Discussion

Burn injury initiate a biological cascade of events that is associated with the release of different inflammatory, anti-inflammatory mediators and stress hormones.[12] Interestingly, this is the first study that compared the anti-inflammatory effects of ketamine and dexmedetomidine at different doses.

The main results of our study that the use of Dexmedetomidine at a doses of 1ug/kg^{-1}/min^{-1} and 1.5ug/kg^{-1}/min^{-1} during burn wound dressing had a dose dependent sedoanalgesic, anti-stress and anti- inflammatory effects compared with ketamine at a dose of 1.25mg/kg^{-1} /min^{-1}, Akhlag and coworkers[13] also, reported that ketamine at dose of 1.25mg/kg^{-1}/m^{-1} did not cause any change in C-reactive protein, cortisone, White blood cell count, and glucose level during and after coronary artery bypass graft surgery.

The possible anti-inflammatory effects of a2-adrenoceptor agonists : peripherally through enhancement of innate immunity[14] and centrally through its sympatholytic actions that enhance parasympathetic tone to control inflammation.[15] Thus, peripherally α2-adrenoceptor stimulation may induce pro-inflammatory effects and central anti-inflammatory action. Moreover, inflammation itself may shift response of α 2-adrenoceptor stimulation from pro- to anti-

inflammation.[16]. Also, substance P a nociceptive mediator was inhibited by activation of ?2-adrenergic receptors in the dorsal horn of the spinal cord , leading to primary analgesic effects and potentiation of opioid-induced analgesia [17]

Zhong-Ling et al [18] reported that Dexmetomidine infusion can effectively inhibit the perioperative stress response as it reduced the level of blood glucose ,plasma B-endorphin ,tumour necrosis factor ?(TNF-?) and interleukine -6(IL-6) in patients undergoing radical esophagectomy. Uyar and coworkers' (19) recorded that the significant increase in plasma concentration of cortisone and glucose in placebo group, than in the dexmedetomidine group. Additionally, Mukhar et al (20) demonstrated that dexmedetomidine was inhibit the hyperglycaemic response to surgery that may be attributed to inhibition of the sympatho-adrenal response. However, previous research demonstrated that dexmedetomidine was increased cortisone level in patients undergoing minor gynaecologic surgery.[21] Dexmedetomidine produce dose -dependent sedation and analgesia (involving spinal and supraspinal sites) without respiratory depression[22]. Dexmedetomidine provide adequate sedation even with preoperative single dose of 0.5 μg/kg^{-1} / min^{-1} [23,24. Present work showed three patients were experienced vomiting and two patients developed hallucination in ketamine group. Hallucination and emesis which are common complications with intravenous ketamine [11,25,26].

Moreover ,current study reported that there was a dose dependent significant reduction in the heart rate and mean arterial blood pressure in the three dexmedetomidine groups compared with ketamine group and only one case of hypotension was reported with dexmedetomid ine at a dose of 1.5ug/kg-1 /min-1 and treated with intravenous infusion of saline 0.9% 10ml/kg-1/min-1 .Previous researchers found that Dexmedetomidine causes dose-dependent decreases in heart rate and blood pressure, in parallel with decreasing plasma catecholamines level(22,27) . Also,the per-

ioperative hemodynamic stability associated with Dexmedetomidine due to effective attenuation of the sympathoadrenal stress responses .It reduce sympathetic nervous system activity centrally and decrease plasma catecholamine release in response to stressful events(28)

Conclusion

The use of Dexmedetomidine at doses of 1ug/kg -1/min-1 and 1.5ug/kg -1 /min-1 during burn wound dressing had a dose dependent anti-stress and anti inflammatory effects beside its sedoanalgesia without significant side effects compared with intravenous ketamine 1.25mg/kg-1/min-1

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EFFECT OF DIFFERENT DOSES OF DEXMEDETOMIDINE COMPARED WITH KETAMINE ON THE OUTCOME OF BURN WOUND DRESSING

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LEFT VENTRICULAR FUNCTIONAL AND STRUCTURAL ABNORMALITIES IN CIRRHOTIC PATIENTS ASSOCIATED WITH HCV INFECTION

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Abstract

Introduction: *Hepatitis C virus (HCV) becomes chronic in 55 - 85% of the person infected with HCV. Immunological studies demonstrated the presence of HCV in extrahepatic organs, including the heart. HCV infection has been largely considered as an important cause of myocarditis and heart failure, however, fewer studies investigated cardiac functional and structural abnormalities in HCV positive cirrhotics without clinical evidence of cardiac problem.*

Aim of study: *The study was designed to study the functional and structural changes among the HCV positive cirrhotics without clinical evidence of cardiac problems.*

Subject and Methods: *The study comprised of 63 HCV +ve cirrhotics; 34 HCV negative cirrhotics and 22 control subjects. Control subjects were clinically free with no regular medications. Exclusion criteria included very obese, elderly subjects, patients with ischemic heart disease or organic heart disease, autoimmune disorders including autoimmune liver disease, endocrinal disorders and history of medication affecting the heart. All subjects had history taking, clinical examination, routine laboratory and radiologic investigations, anti HCV antibodies, HCV PCR and complete echo Doppler study.*

Results: *There was no statistically significant difference between the three main patient and control groups regarding age, sex, body mass index, systolic and diastolic blood pressure. However, HCV +ve cirrhotics revealed significant lower ejection fraction and fractional shortening*

than the other groups. Also they revealed higher myocardial performance index (MPI), lower mitral E/A ratio, higher mitral E/Ea TDI ratio, higher LV mass and mass index and LV dimensions. ***Conclusion:*** *HCV infection could be an additional factor in the pathogenesis of left ventricular systolic and diastolic dysfunctions as well as increased LV wall thickness and dimensions in cirrhotics.*

Introduction

The association between chronic hepatitis C viral (HCV) infection and extrahepatic manifestations (EHMs) of disease was first reported in the early 1990s [1].

Hepatitis C virus (HCV) causes not only liver disease but also a broad clinical spectrum of extrahepatic manifestations. Immunological studies demonstrated the presence of HCV in extrahepatic organs including the heart [2,3].

The importance of HCV infection in patients with dilated and hypertrophic cardiomyopathy and myocarditis has been noted [4-10].

HCV infection becomes chronic in 55-85% of the persons infected with HCV. HCV infection is generally asymptomatic. It is known that chronic HCV infection triggers autoimmune disorders. A strong relationship has been found with essential mixed cryoglobulinemia, glomerulonephritis and porphyria tarda. Additionally HCV infection has been associated with extrahepatic involvement such as Sjogren's syndrome, lichen planus and Hashimoto thyroiditis [11].

It is known that, the hyperdynamic circulation in portal hypertension begins in the portal venous bed. As liver disease progresses and liver function deteriorates. The systemic hyperdynamic circulation becomes more manifest with activation of the renin-angiotensin-aldosterone system (RAAS)[12].

It has been widely reported that HCV antibodies were more prevalent in patients with myocarditis and heart failure, also, HCV infection has been largely considered as an important cause of myocarditis and heart failure[13].

However, fewer studies investigated left ventricular systolic and diastolic functions abnormalities in cirrhotic patients with HCV infection without clinical evidence of heart failure.

This study was designed to investigate the left ventricular functional and structural abnormalities in a group of Egyptian patients with liver cirrhosis without clinical evidence of myocarditis or cardiomyopathy as well as heart failure.

Subjects and Methods

The present study comprised 3 main groups:

Sixty three cirrhotic patients (based on clinical and ultrasound data) with positive anti-HCV and HCV RNA by PCR.

Thirty four cirrhotic patients (based on clinical and ultrasound data) with negative HCV and HCV RNA.

Twenty two control subjects who were non-cirrhotic with no clinical, laboratory or ultrasonographic evidence of liver disease, kidney disease, cardiac disease as well as endocrinal disease.

Patients and control were selected and evaluated at Mansoura University Hospitals (MUH), from January 2010 to December 2011.

The two patient groups and the control group were matched regarding age, sex and systolic blood pressure (SBP).

Patients and controls exclusion criteria included:

Very obese and old ages more 65 years, ages less than 39 years, technically difficult cases for echo Doppler study ischemic heart disease (IHD), rheumatic heart disease, atherosclerotic heart disease, moderate or severe hypertensives or subjects on antihypertensive medications, endocrinal diseases including diabetes mellitus, renal failure or dialysis, autoimmune liver disease, alcohol intake, patients with history of sclerotherapy, pregnant and lactating women and subjects refusing enrollment to the study. Patients with positive HBSAg were also excluded.

All subjects were subjected to:

Thorough history taking, full clinical examination, routine laboratory investigation to exclude considerable renal disease, endocrinal disorders as well as autoimmune liver disease.

Liver function tests, hepatitis C virus antibody, hepatitis B surface antigen, Quantitative PCR for hepatitis C virus infection.

Electrocardiography (standard resting 12 leads ECG), all of the patients were in sinus rhythm and none of them were on cardioactive medications.

Abdominal ultrasound examination of the abdomen using 3.5 MHz transducer connected to the Aloka SSD 680 device; portal hypertension was diagnosed taking the criteria reported previously by Yong[14] as a guide.

Echocardiographic examination was performed using general electric vivid 5, equipped with 3.5MHz transducer. All examinations were performed with the subject in left lateral decubitus in accordance to the recommendation of American Society of Echoardiography [15].

During echocardiography, a one lead electrocardiogram was recorded continuously, internal left ventricular end systolic (LVES), left ventricular end diastolic (LVED), interventricular septal thickness during diastole and systole (IVSTD, IVSTS) and posterior wall thickness during diastole and systole were all measured, standard methods were used to calculate LV ejection fraction (LVEF) and left ventricular fractional shortening (LVFS). Pulsed-wave mitral flow velocities were measured from the apical four-chamber view. Mitral early and late diastolic velocities were recorded. Mitral Early (E) deceleration time and isovolumetric relaxation time (IRT, ms) were recorded as well isovolumic contraction time (IRT, ms) and left ventricular ejection time (ET, ms).

Lt ventricular myocardial performance index (MPI) was calculated using (ICT + IRT) / ET formula[16]. Each representative value was obtained from the average of the three consecutive measurements.

Tissue Doppler imaging measurements:

The apical four-chambers view was used to obtain tissue Doppler imaging of the mitral annulus. A sample volume of the pulsed wave Doppler was positioned at the septal side of the mitral and tricuspid annulus and then the spectral signal of the mitral and tricuspid annular velocity was recorded. Peak Ea was measured and E/Ea was calculated as an index of left ventricular filling pressure[17,18]. The average of five consecutive Doppler signals was used [19].

Statistical analysis:

Statistics were run on an IBM compatible computer system using the statistical package for Social Scientists (SPSS) for windows 15 (SPSS Inc, Chicago, IL, USA). Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Mean and standard deviations were used to describe data. Student t-test was used to compare between two groups. F-test (One Way Anova) was used to compare between more than two groups. P value was considered significant if less than 0.05.

Results

There was no statistically significant difference between HCV +ve cirrhotics, HCV -ve cirrhotics and control groups regarding age, body mass index, SBP, DBP, dyspnea and palpitation by one way Anova (Table 1).

Portal vein diameter (PVD), superior mesenteric vein diameter (SMV) and splenic vein (SV) diameters were statistically higher in patient groups than the control group (Table 2)

Also, PV and SMV and SV maximum flow rates were statistically significant lower in patient groups than the control group (table 2)

HCV +ve cirrhotics showed statistically significant higher LVED, LVES diameters, as well as significant higher LV cardiac output and cardiac index, significant higher LVM, LVMI, aortic root diameter and left atrial diameter . They also showed statistically significant lower LVEF and LVFS as well as significant lower LV PWT in dias-

tole and systole when compared with HCV -ve cirrhotics. Statistically significant higher MPI of left ventricle is found in HCV +ve cirrhotics than other groups, (Table 3).

HCV +ve cirrhotics showed significant higher E wave deceleration time and E/Ea MV-TDI ratio and significant lower mitral peak E wave velocity, mitral E/A ratio and MV-TDI Ea wave velocity when compared with HCV -ve cirrhotics (table 4)

HCV +ve cirrhotic showed significant positive correlation of HCV viraemia with interventricular septal thickness during diastole as well as near positive correlation with MPI of left ventricle (table 5).

LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; IVST_d, interventricular septal thickness in diastole; IVST s, interventricular septal thickness in systole; LVPWT_d, left ventricular posterior wall thickness in diastole; LVPWT s, left ventricular posterior wall thickness in systole; LVEF, left ventricular ejection fraction; HR, heart rate; COP, cardiac output; LVM, left ventricular mass; LVMI, left ventricular mass index; LVMPI, left ventricular myocardial performance index.

Table (1): Comparison of clinical features among HCV +ve cirrhotics; HCV –ve cirrhotics and control group.

Clinical parameters	HCV +ve cirrhotics (n = 63)	HCV –ve cirrhotics (n = 34)	Control (n = 22)	P of the groups	P*
Age (y)	46.94 ± 4.0	47.91 ± 2.76	46.95 ± 2.01	NS	NS
BSA (m^2)	1.93 ± 0.13	1.89 ± 0.08	1.89 ± 0.16	NS	NS
BMI (kg/m^2)	25.57 ± 3.35	24.46 ± 1.14	25.19 ± 3.82	NS	0.019
SBP (mmHg)	121.98 ± 13.43	118.47 ± 7.35	118.18 ± 2.46	NS	NS
DBP (mmHg)	77.54 ± 9.99	73.97 ± 5.33	75.0 ± 5.12	NS	0.024
Dyspnea	26 (41.3%)	11 (32.4%)	3 (13.6%)	NS	NS
Paplitation	24 (38.1%)	11 (32.4%)	3 (13.6%)	NS	NS

BSA, body surface area ; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, not statistically significant; n, number
P = comparison of all groups
P* = comparison of HCV +ve cirrhotics and HCV –ve cirrhotics

Table (2): Comparison of some Doupplex abdominal ultrasound parameters among the studied groups.

	HCV +ve cirrhotics (n = 63)	HCV –ve cirrhotics (n = 34)	Control (n = 22)	P value	P *
PVD (cm)	1.42 ± 0.37	1.79 ± 0.17	1.02 ± 0.10	< 0.001	< 0.001
PV maximum flow rate (cm/sec)	14.35 ± 4.85	12.35 ± 1.61	22.0 ± 2.96	< 0.001	0.004
SMVD	1.00 ± 0.21	1.41 ± 0.30	0.75 ± 0.17	< 0.001	< 0.001
SMV maximum flow rate (cm/sec)	14.60 ± 4.61	15.85 ± 2.39	22.2 ± 5.24	< 0.001	0.081
Splenic vein diameter (cm)	1.24 ± 0.37	1.70 ± 0.26	0.76 ± 0.14	< 0.001	< 0.001
Splenic vein maximum flow rate (cm/sec)	17.18 ± 5.35	19.32 ± 1.93	23.72 ± 5.15	< 0.001	0.005

PVD, portal vein diameter; PV, portal vein; SMVD, superior mesenteric vein diameter; SMV, superior mesenteric vein. P = comparison of all groups

P* = comparison of HCV +ve cirrhotics and HCV –ve cirrhotics

Table (3): Comparison of some M-mode echocardiographic parameters among the studied groups.

M- mode echo. data	HCV +ve cirrhotics (n = 63)	HCV –ve cirrhotics (n = 34)	Control (n=22)	P value
LV EDD (cm)	5.24 ± 0.49	4.74 ± 0.53	4.50 ± 0.39	P1 < 0.001; P2 < 0.001
LV ESD (cm)	3.63 ± 0.30	3.08 ± 0.38	2.98 ± 0.25	P1 < 0.001; P2 < 0.001
LV PWT d (cm)	0.96 ± 0.18	1.07 ± 0.08	0.85 ± 0.10	P1 < 0.001; P2 < 0.001
LV PWT s (cm)	1.31 ± 0.17	1.41 ± 0.11	1.27 ± 0.13	P1 < 0.05; P2 < 0.05
IVST d (cm)	1.09 ± 0.17	1.07 ± 0.12	0.94 ± 0.12	P1 < 0.001; P2 > 0.05
IVST s (cm)	1.49 ± 0.20	1.47 ± 0.14	1.34 ± 0.20	P1 < 0.05; P2 > 0.05
Ejection fraction (EF) (ratio)	65.94 ± 7.25	72.04 ± 6.44	70.61 ± 6.00	P1 < 0.001; P2 < 0.001
HR (b/m)	73.02 ± 6.81	76.15 ± 2.83	70.41 ± 2.89	P1 < 0.05; P2 < 0.05
COP (L/min)	7.21 ± 2.41	6.05 ± 2.03	4.70 ± 1.46	P1 < 0.001; P2 < 0.05
Cardiac index (CI) (L/minute/m^2)	3.74 ± 1.26	3.17 ± 0.96	2.49 ± 0.73	P1 < 0.001; P2 < 0.05
LVM (gm)	242.75 ± 55.17	216.03 ± 39.26	150.22 ± 23.55	P1 < 0.001; P2 < 0.05
LVMI (gm/m2)	125.72 ± 27.71	113.92 ± 17.81	80.16 ± 15.92	P1 < 0.001; P2 < 0.05
LV MPI	0.51 ± 0.11	0.47 ± 0.04	0.42 ± 0.02	P1 < 0.001; P2 < 0.05
Aortic root diameter(ARD) (cm)	3.66 ± 0.36	3.43 ± 0.20	3.17 ± 0.21	P1 < 0.001; P2 < 0.001
Left atrial diameter (LAD) (cm)	4.01 ± 0.51	3.80 ± 0.28	3.36 ± 0.26	P1 < 0.001; P2 < 0.05

P1= Comparison betweeen all groups

P2= Comparison betweeen HCV –ve cirrhotics and HCV +ve cirrhotics groups

Table (4): Comparative analysis of some mitral valve Doppler echocardiographic parameters among the HCV –ve; HCV +ve and control groups.

MV Doppler parameter	HCV +ve cirrhotics (n = 63)	HCV –ve cirrhotics (n = 34)	Control (n=22)	P value
Peak_E wave velocity (cm/sec)	0.58 ± 0.12	0.75 ± 0.11	0.77 ± 0.14	P1 < 0.001; P2 < 0.001
Peak_A wave velocity (cm/sec)	0.65 ± 0.14	0.64 ± 0.11	0.52 ± 0.10	P1 < 0.001; P2 > 0.05
E/A_Ratio	0.89 ± 0.26	1.19 ± 0.13	1.52 ± 0.44	P1 < 0.001; P2 < 0.001
E wave dec time (m sec)	138.89 ± 33.18	124.26 ± 9.09	118.36 ± 13.12	P1 < 0.001; P2 < 0.05
IVRT (m sec)	93.59 ± 12.75	90.09 ± 7.27	88.27 ± 13.70	P1 > 0.05; P2 > 0.05
MV- TDI Ea wave velocity (m/s)	0.05 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	P1 < 0.001; P2 < 0.001
E/Ea MV- TDI ratio	11.02 ± 1.30	8.99 ± 0.85	7.94 ± 0.86	P1 < 0.001; P2 < 0.001
MV- TDI Aa wave velocity (m/s)	0.09 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	P1 < 0.001; P2 > 0.05

P1= Comparison ( ) all groups
P2= Comparison ( ) HCV –ve cirrhotics and HCV +ve cirrhotics groups
MV,mitral valve; IVRT, isovolumetric relaxation time; MV-TDI, mitral valve tissue Doppler imaging

Table (5): Correlation between quantitative HCV PCR and some M-mode echocardiographic parameters in anti-HCV +ve patients.

	r	P
LVEDD (cm)	-0.045	0.724
LVESD (cm)	0.059	0.644
IVST_d (cm)	0.423	0.001
LV PWT_d (cm)	0.111	0.387
LV EF (%)	-0.127	0.323
LVFS	-0.130	0.309
LV MPI	0.237	0.061

LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; IVST_d, interventricular systole thickness in diameter; LVPWT_d, left ventricular posterior wall thickness in diastole; LVEF, left ventricular ejection fraction; LVFS, left ventricular fraction shortening; LVMPI, left ventricular myocardial performance index.

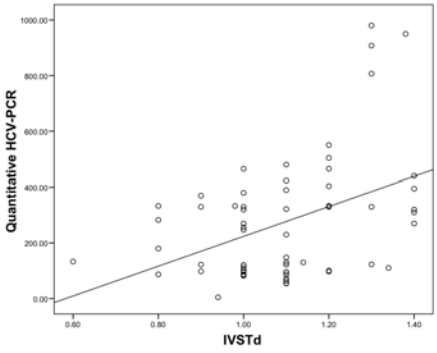

Fig. 1 : Correlation between quantitative HCV-PCR and IVSTd.

Discussion

Heart in patients with cirrhosis presents with structural and functional abnormalities that have been termed cirrhotic cardiomyopathy [20].

The cause of these cardiac alteration in portal hypertension is not clear and probably both continuous mechanical stress and neurohumoral factors play a role in the condition [21,22].

Many studies reported diastolic dysfunction that worsen with the presence of ascites and physical stress, less numerous studies reported abnormal systolic response to stress[23]. Some authors reported that their cirrhotic patients, in response to stress were unable to adequately increase their heart rate, stroke volume and cardiac index. As a consequence, cirrhotic patients present a reduced exercise capacity and lower maximal workload. Other data suggested that cirrhotic patients have reduced maximal oxygen consumption with an early anaerobic threshold supporting the hypothesis of impaired cardiac response to stress as an important contributor to the reduced exercise capacity [24].

Although some authors reported that cardiac alterations in cirrhotics are mild and independent of the etiology of cirrhosis;links between hepatitis C virus and dilated cardiomyopathy were strongly reported [25,26]. Moreover, findings and new diagnostic tests resulted in increased interest in inflammatory cardiomyopathies and a better understanding of its pathophysiology [27]. Impaired autonomic function is caused by chronic HCV infection [28]. It was reported that neither cryoglobulinaemia, nor the serum HCV RNA level correlated with impaired autonomic function. They also reported that cardiovagal autonomic dysfunction in patients with chronic HCV infection independently correlates with ALT level.

This study of patients two main groups and control subjects showed no statstically significant differences regarding age and sex distribution as well as systolic blood pressure. A significant decrease in the maximum flow rate of PV, SMV and splenic vein in the

HCV +ve cirrhotic patients than the HCV -ve cirrhotics and control subjects was obvious in this study. These findings had been noticed by several authors ion patients with cirrhosis.[29,30]. Also, Yi-Hong Chou, 2003[31] documented significant decrease in PV flow velocity rate in cirrhotics than both patients with hepatic schistosoma Japonicum and control subjects.

In this study, it was found that HCV +ve cirrhotics had significantly higher LVED, LVES, LAD, ARD, cardiac output and cardiac index as well as significant higher LV mass and LV mass index than HCV -ve cirrhotics and control.

HCV +ve cirrhotics also showed tendency to diastolic function abnormalities when compared with HCV -ve cirrhotics as evidenced by higher E/A ratio, higher E/Ea-TDI ratio and longer IVRT.

Moreover, they exhibited significantly lower ejection fraction, these findings were more or less similar to previous reports documented by Demir et al.; Kawai et al. and Demir & Demir[32-34], as they reported an association between HCV infection and left ventricular hypertrophy in terms of left ventricular systolic dysfunction could exist

Moreover, this study also revealed that HCV +ve cirrhotic showed significant positive correlation of HCV viraemia with interventricular septal thickness during diastole as well as near positive correlation with MPI of left ventricle.

Although, portal hypertension itself can be an important factors in the pathogenesis of the cardiac diastolic dysfunction, and other factors like aldosterone may play some role particularly as the liver dysfunction progresses[35]. Also, a significant increase in aldosterone levels among the cirrhotics and they suggested a role of activated RAAS in the pathogenesis of cardiac contractile dysfunction is to be considered.

It is not exactly known which mechanisms were involved in cardiac damage due to HCV infection, however, that HCV - RNA was demonstrated in the myocardium

suggests that the virus may have a direct cytopathic effect on the myocardium and may induce cell growth [2,36].

HCV core protein is supposed to play a direct important role in the development of ventricular systolic abnormalities associated with HCV infection. Omura et al. (2005)[37] reported histological abnormalities in mice transgenic for HCV-core gene including: hypertrophy of cardiomyocytes, cardiac fibrosis, disarray and scarcity of myofibrils, vacuolization and deformity of nuclei, myofibrillar lysis, streaming of Z-bands and increased number of bizarre-shaped mitochondria. Mice transgenic for HCV-core showed showed activation of activator protein-1 (AP-1) induced cardiomyocyte hypertrophy, such activation was observed in progressive ventricular remodeling [38,39].

Also, inflammatory cytokines as interleukin-1, -6 or TNF released secondary to HCV infection could account for cardiac involvement in patients infected with HCV. However, Some authors considered HCV infection/replication itself to be not cytopathic, both liver and neurological pathologies in chronic HCV infection are immune mediated [40,41].

Conclusion

HCV infection could be additional factor in the pathogenesis of left ventricular systolic and diastolic dysfunction in cirrhotics. Cardiac involvement should be considered during follow up of a patient with HCV infection especially if liver transplantation is planned.

Limitation of the study:

- A small number of cirrhotic patients and control subjects in this study.
- Lacking of determination of plasma aldosterone levels as well as RAAS and serum levels of cryoglobulins that should be added to variables investigated in such studies.
- Lacking of HLA typing inspite of its higher as not all the HCV infected patients exhibited abnormal cardiac findings.
- Lacking of autonomic dysfunction evaluation.

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LEFT VENTRICULAR FUNCTIONAL AND STRUCTURAL ABNORMALITIES IN CIRRHOTIC PATIENTS ASSOCIATED WITH HCV INFECTION

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MINIMAL INVASIVE APPROACH FOR TREATMENT OF ACUTE GRADE III ACROMIO-CLAVICULAR DISLOCATION

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Abstract

Objective: *To determine the efficacy of minimally invasive surgery (MIS) in the treatment of type III acromio-clavicular dislocations.*

Methods: *Between June 2009 and July 2011, coraco-clavicular fixation was performed using the modified Bosworth technique (minimally invasive surgery) in 14 patients (12 males, 2 females; mean age 31 years; range 19 to 52 years) with Rockwood grade III pure acromio-clavicular dislocations. MIS consisting of small incision, percutaneous reduction, and placement of a partial thread 3.5 mm screw with a metal washer. The screws were removed under local anesthesia after eight weeks postoperatively. The patients were evaluated for cosmetic appearance, functional status, pain, localized tenderness, articular range of motion, and with the functional Constant scoring system.*

Results: *The mean constant score was 91.6 (range 83 to 100). The results were excellent in 12 patients (85.7%) and good in two patients (14.2%). None had a poor result.No intraoperative complications except rupture of a drill bit.There was subluxation of the acromio-clavicular joint in one patient (7.1%). The alignment of the acromio-clavicular joint was normal in the remaining patients. None of the patients showed joint degeneration. All patients were achieved full range of motion.*

Conclusions: *With ease of application, low complication rate, and low rate of acromio-clavicular joint arthrosis, the minimally invasive surgery (MIS) is an effective surgical method in providing satisfactory*

shoulder function in type III acromio-clavicular dislocations.

Key words: *type III acromio-clavicular dislocations, shoulder, surgery, evaluation.*

Introduction

The acromio-clavicular joint (ACJ) dislocation was described since the Egyptian papyri. Hippocrates was the first to distinguish the ACJ injuries from the glenohumeral ones, as well as the mechanism of injury[1,2].

ACJ is an arthrodial joint composed of a fibro-cartilaginous meniscus and surrounded by a thin capsule preventing its horizontal displacement. It is reinforced by the AC ligaments: anterior, posterior and superior, with the latter being the strongest one. Its dynamic stabilizers are the deltoid and trapezius muscles; its passive stabilizers are the coraco-clavicular(CC) ligaments (conoid and trapezoid)[3].

The conoid ligament prevents the upward displacement of the clavicle relative to the coracoid, and the trapezoid prevents the medial translation of the clavicle relative to the acromion when supporting axial loads. The normal AC space is 6mm, a greater articular space is considered as pathological. The normal coracoclavicular interval is from 1.1 to 1.3 cm approximately. Half of all the normal AC joints have a certain degree of saddling on the clavicle, which contributes to explain the mechanism of injury that may lead to a clavicular fracture or an ACJ dislocation[3].

The incidence of complete ACJ dislocation is 3 per 100,000 in Germany, with up to 50% occurring during sports activities and represent 10% of all dislocations around the shoulder[4]. ACJ dislocations are most often incomplete than complete, with a ratio of 2:1. This is because the delto-trapezoidal fascia provides a passive stabilization of the lateral clavicle, even after a complete tear of the CC and AC ligaments. The complete dislocation of the AC joint requires the tear of both the CC and the AC ligaments, as well as of the acromio-clavicular articular capsule and the delto-trapezoidal fascia. Recent cadaver research has shown that the AC joint can

stand high forces of as much as 500 to 700 N[3,5,6].

The most common mechanism of injury is usually a direct blow to the upper edge of the acromion with the arm in an adducted position leading to an inferior and anterior translation of the scapula relative to the clavicle. The indirect mechanism, especially due to falls on an arm detached from the body, produce both shearing and compression of the joint[1,6].

In 1960s, Tossy et al. described three types of AC dislocation (I, II and III)[7,8]. This classification was modified in 1984 by Rockwood et al. with addition of types IV, V, and VI [1]. The classification is based on the extent of the disruption of the AC and CC ligaments, using radiologic types of displacement of the clavicle relative to the acromion. In type I dislocations, the AC and CC ligaments are intact. In type II dislocations, the AC ligaments are completely ruptured, whereas the CC ligaments are intact. Type I and II dislocations are treated conservatively[9].

In type III dislocations, the CC ligaments are also ruptured. Although there is no established treatment method for type III injuries, surgical treatment is preferred particularly in younger, active patients with physically demanding work[9-12]. Type IV dislocations are rare injuries, where the distal end of the clavicle is displaced posteriorly into the trapezius muscle. Type V injuries are more severe than type III and IV injuries. The lateral clavicle is detached from the muscles. Pain is the prominent symptom. Loss of support to the arm may lead to tension in the brachial plexus and neurogenic pain. Type VI injuries are also rare. The distal end of the clavicle is displaced inferiorly towards the sub-acromial space. Surgery is the treatment of choice for type IV, V, and VI injuries[9].

Treatment of complete ACJ disruption remains controversial and ranges from rehabilitation to an extensive surgical reconstruction using tendon grafts[13-15]. The indications for surgical or conservative management are based on the patient's characteristics, i.e., age, activity level and demand for cos-

metic results. Concerning type III repairs, different studies have shown that surgery offers a clear advantage compared with conservative treatment[16-18]. More than 60 surgical techniques for ACJ reconstruction have been described indicates that the optimum procedure for this condition has not been found yet. [17,18]

Although more than 60 procedures have been described for surgical treatment of acromioclavicular joint dislocations, there is no gold standard for management of these dislocations[19-21]. These procedures can be grouped in five main categories: (1) fixation of the acromio-clavicular joint with a K-wire or hook plate, (2) dynamic muscle transfer, (3) fixation between the clavicle and the coracoid (with the use of a Bosworth screw or synthetic augmentation), (4) ligament reconstruction, and (5) distal clavicle resection[6,13-15] The major causes for the variation among the current techniques may be summarized into four categories: the time when the surgery was performed, the choice of the surgical approach, the choice of ligament reconstruction, and the technique used to stabilize the reconstruction[16].

The main problems in the clinical routine of rigid repairs using metal implants are implant breakages and implant migration due to the complex multidirectional forces to which the ACJ is subjected. Ligament reconstructions with tendon grafts are associated with donor site morbidity and require an extensive surgical approach. Augmentation of the torn CC ligaments with a single polydioxanone (PDS) cerclage has disadvantage of anterior subluxation of the clavicle with malreduction of the ACJ if the cerclage is placed at the anterior aspect of the coracoid. Furthermore, PDS banding still requires an extensive surgical approach, and able to saw through the coracoid owing to rotational motion of the clavicle[22,23].

Recently, minimally invasive or arthroscopic techniques have been developed with the aim of reducing morbidity during the rehabilitation period and improving surgical outcome. One advantage

of suture anchors is that they can be placed at or close to the insertion site of the CC ligaments. Therefore, the augmentation can imitate the course of the CC ligaments more closely than can a PDS circulage that is placed around the coracoid. However,this augmentation differing from rotator cuff or labral repair, in which a soft tissue is fixed to bone, CC augmentation means anchoring bone to bone. Furthermore, the coracoid suture anchor has to withstand up to 500 N in a physiological loading situation[24] and the anchors in the CC augmentation are loaded with tensile loads which does not fit the "tension reduction. These experimental and clinical findings indicate that a well-defined operative standard is still missing [25].

Minimal incision surgery (MIS) is a surgical approach that allows performing interventions through small incisions without the direct exposure of the surgical planes, leading to minimum trauma of the neighboruing tissues, and uses radiologic or fluoroscopic control during the intervention as a guide [3].

The objective of this paper is to determine the efficacy of the MIS technique in the treatment of G III acromio-clavicular dislocations using the Imatani evaluation(26).

Patients and Methods

Between June 2009 and July 2011, 14 patients with Rockwood grade III pure acromio-clavicular dislocations were underwent MIS consisting of percutaneous reduction, placement of a partial thread 3.5 mm screw with a metal washer. There were 12 males and 2 females, with a mean age of 31 years (19 to 52). All patients had a history of trauma and presented at the Mansoura Emergency Hospital on the day of the injury. There were then diagnosed clinically and radiologically (shoulder AP, shoulder stress AP, and shoulder oblique X-rays) (Fig 1) (table 1) and immobilized with a sling.

All patients were offered the possibility of undergoing surgery versus conservative treatment; the risks and benefits of each option were explained to them. The patients underwent surgery with the MIS technique within 2 days of the injury after the anesthetic assessment.

Operative technique:

Percutaneous reduction is performed with patients under general anesthesia. They are placed in dorsal decubitus in the beach chair position on a radiolucent table with the arm at 0°, and the forearm bent at 90° on the abdomen. After performing asepsis and antisepsis of the shoulder, a 1cm-transverse incision is made with a No. 14 scalpel on the lateral third of the clavicle and a 2cm- long incision is made on coracoid.

Using fluoroscopic control, a 2.5 mm drill bit is used to drill both cortices of the clavicle through transverse incision and guided by the index to drill 75% of the coracoid process through the long incision. Partial thread 3.5 mm screw with a metal washer is inserted from the clavicle to the coracoid (Figure 2). Note: the washer is used to increase the contact surface, avoid micro-fractures and facilitate extraction.

Postoperative Care :

The operated limb was immobilized with a sling; X-ray was done in the first 24-48 hours (Figure 3), and patients were discharged with a prescription of non-steroidal anti-inflammatory agents and oral antibiotic for 7 days.

The follow-up was performed after 2, 6, 8, 12 weeks. At the second week; the stitches were removed and the patients were instructed to begin pendular exercises and not to elevate the arm more than 90°.

At the six week; the patients were allowed to use their operated limb for daily requirements and to do active mobilization. At the eighth week; the screw was removed with local anesthesia in the operation room. Rehabilitation therapy was begun at this moment to improve the ranges of motion and for muscle strengthening purposes.

Three types of criteria were used for the evaluation of results: radiological, functional and esthetic, and those related with personal satisfaction.

Functional criteria were assessed using the Imatani test (26), shown in table 2, at the outpatient clinic between 6 weeks and 6

months after surgery. This functional scoring system attributes 40 points to pain; 30 to function and 30 to motion. A score of 90 to 100 points is considered to be an excellent result; 80 to 89 good; 70 to 79 fair and less than 70 poor.

Results

A total of 14 patients were included, 12 males (85.7%) and 2 females (14.3%), with a clinical and radiographic diagnosis of Tossy-Rockwood Type III acute acromioclavicular dislocation, treated with percutaneous reduction, placement of a 3.5 mm partial threaded screw with a metal washer and followed-up for at least 18 weeks (table 3).

The mean age is 31 years (19 to 52). The main mechanism of injury was direct contusion of the shoulder in 78.6% of cases (11/14). Indirect mechanism included fall from a height in 21.4% (3/14) of cases. Four (28.6%) patients were hard workers, four (28.6%) were students, three (21.4%) were farmers, two teachers (14.3%), and one (7.1%) housewife.The predominant side of injury was the right side, which accounted for 78.5% (11/14) of the cases. No added lesions were seen upon admission in 85.7% (12/14) and 14.3% (2/14) had fracture femurs.

The surgical procedure was performed under general anesthesia, with a mean operative time of 60 minutes, and a fluoroscopy time of 10 seconds. After postoperative 8 weeks, patients were seen again to remove the screw.

Although no elevations above the shoulder level were allowed for the first eight weeks, none of the patients had any restriction in shoulder movements, and all achieved full range of motion within a short period of time (12-14 weeks). The mean constant score was 91.6 (range 83 to 100). The results were excellent in 12 patients (85.7%) and good in two patients (14.2%). None had a poor result (Table 3).

No intraoperative complications except rupture of a drill bit. Postoperative complications observed before week 8 were subluxation in one patient 7.1% (1/14). Although the fixation was made with a cancellous screw, this patient had an

almost osteoporotic bone structure. No shoulder complication or pain was observed in this patient despite acromio-clavicular joint subluxation.

Radiographically, the alignment of the acromio-clavicular joint was normal in all but one patient with subluxation. None of the patients showed joint degeneration or notable ossification. Cosmetically, no patient had a poor scar tissue and the acromio-clavicular joint was barely visible. Each patient had full shoulder movements. All the patients returned to preinjury jobs or sports activities.

Table 1 : Clinical data.

Case No	Sex	Age	Mechanism	Occupation	Side	Trauma
1	M	20	Direct	Student	R	Isolated
2	F	24	Direct	Student	R	Isolated
3	M	40	Direct	Hard worker	R	Isolated
4	M	19	Direct	Student	R	Isolated
5	M	35	Direct	Hard worker	R	Polytrauma
6	M	28	Indirect	Farmer	L	Isolated
7	M	42	Direct	Farmer	R	Isolated
8	M	52	Indirect	Hard worker	R	Isolated
9	F	22	Direct	House wife	R	Polytrauma
10	M	26	Indirect	Farmer	R	Isolated
11	M	24	Direct	Student	L	Isolated
12	M	35	Direct	Teacher	L	Isolated
13	M	28	Direct	Teacher	R	Isolated
14	M	39	Direct	Student	R	Isolated

Table 2 : The Imatani evaluation system for acromioclavicular dislocation

Pain (40 points)	None	40
	Slight, occasional	25
	Moderate, tolerable, limits activities	10
	Severe, constant, disabling	5
Function (30 points)	Weakness (proportion of pre-injury)	20
	Use of shoulder	5
	Change of occupation	5
Movement (30 points)	Abduction	10
	Flexion	0
	Adduction	10
Result	Excellent	91 to 100
	Good	81 to 90
	Fair	61 to 80
	Poor	< 61

Table 3 : Results.

Case No	Function				Movement			Total
	Pain	Weakness	Use shoulder	Change in activity	Abd.	Flex.	Add.	
1	30	20	5	5	10	10	10	90
2	35	20	5	5	10	10	10	95
3	40	18	5	5	10	10	10	98
4	40	20	5	5	10	10	10	100
5	35	15	5	5	10	10	10	90
6	40	20	5	5	10	10	10	100
7	40	20	5	4	10	10	10	99
8	35	15	4	5	10	10	10	89
9	40	20	5	5	10	10	10	100
10	40	20	5	5	10	10	10	100
11	25	18	5	5	10	10	10	83
12	40	20	5	5	10	10	10	100
13	40	20	5	5	10	10	10	100
14	40	20	5	5	10	10	10	100

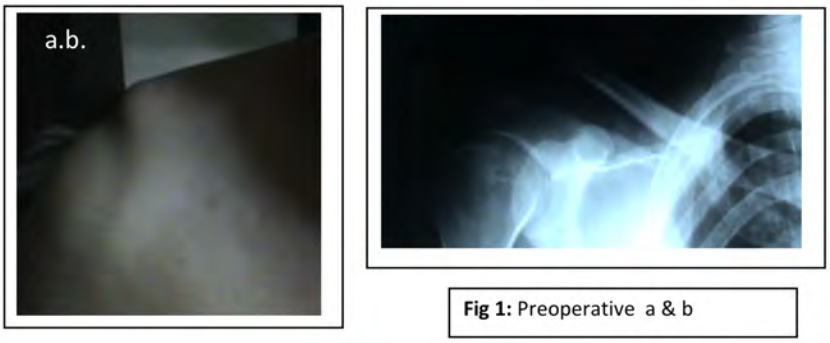

Fig 1: Preoperative a & b

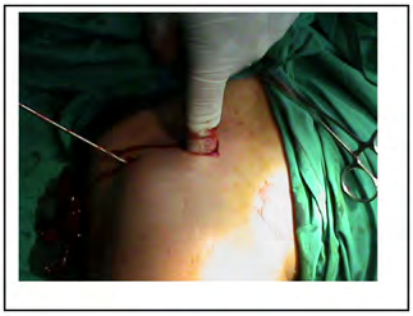

Fig. 2: Intraoperative picture (finger guide the drill to the coracoid)

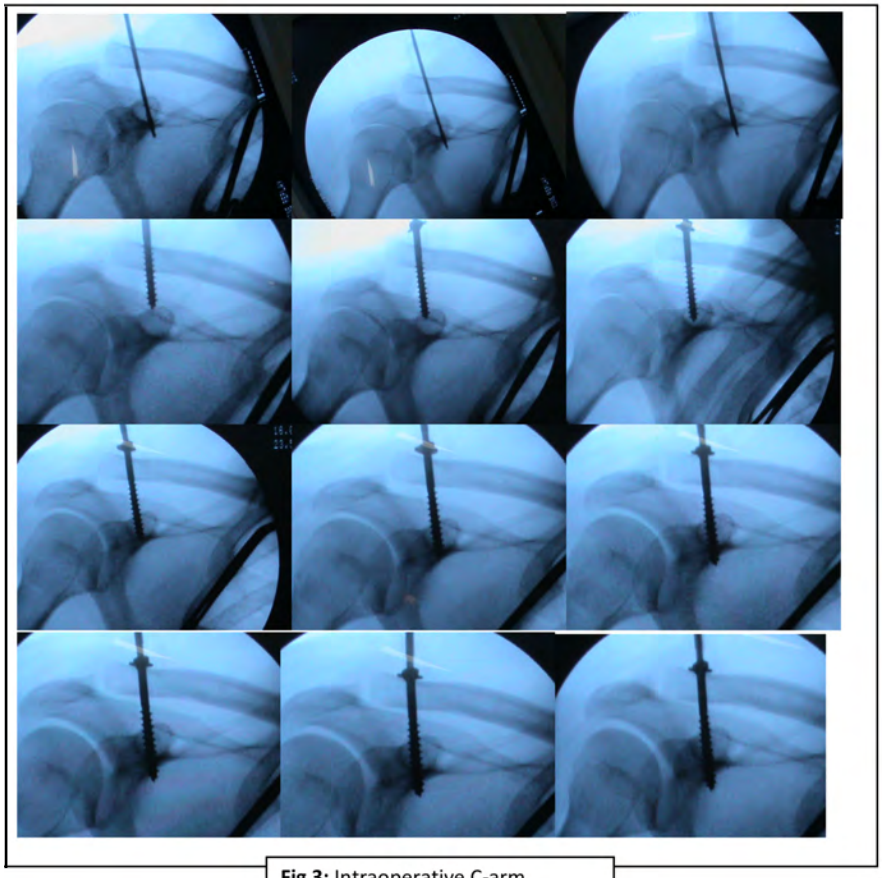

Fig 3: Intraoperative C-arm

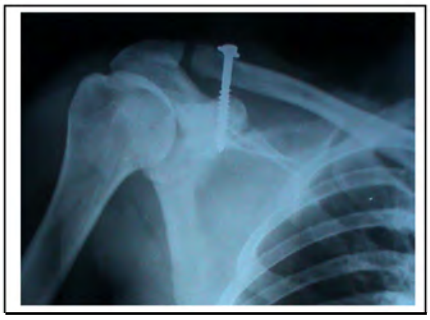

Fig 4: Postoperative X-ry.

Discussion

The acromio-clavicular joint represents a surgical conflict due to its multiple motion axes and its big lever arm. These are the reasons why many acromio-clavicular fixation methods fail. Although there are more than 60 procedures, modifications and approaches, as well as techniques described for surgical treatment of CCJ dislocations, there is still no gold standard. The wealth of surgical options reflects the difficulties involved in the successful restoration of the very complex balance of forces that pass through the joint[9,14,27].

CC stabilization with a lag screw was described by Bosworth in 1940s. No repair or reconstruction of the ligament was described in the original technique. In 1990s, Rockwood and Young recommended repair of the ligament in acute cases, and reconstruction of the ligament in chronic cases, along with the use of a Bosworth screw[9]. Arthroscopy-aided fixation with a CC screw described by Rolla et al.[28] in 2004 improved this treatment method one step further,minimizing surgical trauma.

Biomechanical studies on the coracoacromial ligament have shown that the strength and stiffness of the coracoacromial ligament are only about half of the CC ligament. Repair by polyester slings and suture hooks has similar strength, but 30% less stiffness[29]. Despite satisfactory clinical outcomes, coracoid and clavicular erosions and infections have been reported following surgeries using non-absorbable materials[30]. The mechanical performance of the CC screw is closest to that of the original ligament.

The strength of the CC screw is 80% greater than that of the original ligament when it is placed bicortically, compared to only half in unicortical placement[29], which indicates the critical importance of correct screw placement. In our cases, the screws were placed bicortically and efficiency of the fixation was always verified by fluoroscopy following the procedure. Only one of the patients developed subluxation. Loosening of the screw and recurrence of

dislocation are among the most common complications of this treatment[31]. Bektaer et al..[32] reported a recurrence rate of 8.8% in patients treated with the Bosworth technique. In addition, loss of coracoacromial fixation has been reported to be 16%[33]. Only 60-87% of the cases with recurrent dislocations had satisfactory results[33]. In cases with subluxation, no relationship has been reported between the amount of subluxation and the outcome. Although some patients with subluxation may have complaints of mild pain and discomfort during follow-up, most patients have been reported to have good results without any adverse effect of subluxation[12]. Similarly,no pain and restriction in movements were observed during the follow-up of our patient with subluxation.

Drilling the clavicle and the coracoid through the disrupted CC ligaments enhances the biological response, i.e. biological fixation. It has been reported that residual bone dust which is produced during drilling of the clavicle and deposits between the clavicle and the coracoid during the placement of the screw contributes to bone healing. Furthermore, approximation of the ends of ruptured acromio-clavicular ligaments enhances healing of these ligaments[32]. Clayer et al. [33] demonstrated the development of fibrosis in the CC distance by magnetic resonance studies. So in our cases, we not repaired the ruptured CC ligaments.

Despite biomechanical advantages, screw cut out,infection, and irritation under the head of the screw have been reported with CC screw fixation[27]. However, no infection and irritation were observed in our series.

The failure of the fixation system, to the extent of causing a high complication rate, may lead to challenge the need for or the indication of a certain surgical technique, especially when the conservative treatment of the injury has produced good results. The current techniques are overshadowed by complications such as: infection, anesthetic risk, hematomas, non-cosmetic scars, recurrence of the deformity, rupture, material migration or loosening, erosion or

fracture of the distal end of the clavicle, pain and postoperative limitation of motion, a second procedure needed to remove the fixation, late acromio-clavicular arthrosis, and soft tissue calcification[7,36].

Minimally invasive surgery has evolved through the years as a medical or surgical procedure aimed at decreasing the degree of invasion of the body or, in other words, avoiding the unnecessary harms resulting from the approaches that stem from the incision itself. Traditionally, an important number of orthopedic surgical procedures were performed openly. The incisions were of a considerable size to dissect the different structures of each joint and repair them if necessary.

It is easy to understand that the approach to the joint it self led to harming healthy structures, and that the incision size and the abruptness in tissue management led to longer recovery times, postoperative pain and complications. The current aim of the orthopedic treatment is to preserve circulation, early mobility, and patient satisfaction with minimal aggression. Thus the need to improve the above described treatments is invaluable. That is why the MIS technique was used for acromioclavicular dislocations. This technique is characterized by providing treatment of acute injuries with minimal dissection and placement of a 3.5 mm partially threaded screw with a metal washer.

The main mechanism of injury of our patients was direct contusion of the shoulder in 78.6%. 85.7% of our patients were working males, so this condition represented a socioeconomic problem.

Unlike other techniques, the treatment-related complication rate for the MIS technique used for the treatment of acromioclavicular dislocation was 7.1%. No infections, hematomas, noncosmetic scars, osteolysis of the distal clavicle or paresthesias of the surgical site were observed.

Another complication is the need for a second intervention under local anesthesia to remove the CC screw. This surgical procedure

undoubtedly increases the costs and patient discomfort, and may put the cosmetic scar at risk if there is the need to broadn the surgical area due to difficulties for the removal. The biodegradable screw will be an option once its cost starts coming down.

Conclusion

In conclusion, MIS and fixation with a cancellous screw in the surgical treatment of acromio-clavicular joint dislocations is an effective method in achieving satisfactory shoulder functions, with ease of use, lower complication rate, and lower rate of acromio-clavicular joint arthritis. It provides a new treatment option when surgery of the AC joint is being considered.The following recommendations can be put forward: (1) approximation of the ends of ruptured acromio-clavicular ligaments enhances healing of these ligaments in acute cases (2) a cancellous screw should be used and it must be placed bicortically in the coracoid; (3)the screw should be removed at week 8; (4) a shoulder sling should be used in overweight and portly patients for a period of three weeks, while slim patients can be allowed to move their arms up to the shoulder level.

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MINIMAL INVASIVE APPROACH FOR TREATMENT OF ACUTE GRADE III ACROMIO-CLAVICULAR DISLOCATION

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INSULIN RESISTANCE IN RHEUMATOID ARTHRITIS PATIENTS ASSOCIATION WITH DISEASE PARAMETERS AND SUBCLINICAL VASCULAR PATHOLOGY

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Abstract

Aim: *to determine the prevalence of insulin resistance (IR) in a group of rheumatoid arthritis (RA) patients and to clarify the association between IR and the various disease parameters and the subclinical vascular pathology in those patients.*

Subjects and Methods: *The present study included 40 patients with RA in addition to 20 healthy age and sex matched controls. The study participants were subjected to careful history taking, thorough clinical examination and laboratory investigations including CRP, RF, ESR and (IR). VAS and Disease activity score were calculated for all patients. Ultrasound assessment of carotid arteries was also performed.*

Results: *The study included 40 RA patients. They comprised 31 females (77.5 %) and 9 males (22.5%) with a mean age of 42.7 ± 8.3 years and BMI of 28.6 ± 3.2 Kg/m^2. Patients had significantly higher CIMT when compared with controls. Also, it was found that patients had significantly higher HOMA-IR when compared with control. HOMA-IR was significantly correlated with BMI and CIMT.*

Conclusions: *Insulin resistance is prevalent in RA patients. It significantly contributes to the development of subclinical atherosclerosis in those patients.*

Key words: *Rheumatoid arthritis, Insulin resistance, carotid atherosclerosis.*

Introduction

Rheumatoid arthritis (RA) is the most common arthritic disorder characterized by immunologic dysfunction and systemic inflammation.[1] It was declared

that patients with RA have a 2-fold higher risk of cardiovascular events than the general population and, in those with RA for 10 years or more, the risk is increased to > 3-fold [2]. The mechanisms behind this higher incidence of cardiovascular disease in RA patients are not fully understood but may be related to vascular inflammation and vascular endothelial injury which is common in RA patients.[3]

Moreover, it has been suggested that RA patients had higher risk for the development of subclinical vascular pathology when compared with healthy controls.[4]

On the other hand, many studies had shown that hyperinsulinemia is associated with both atherogenic (in pathological concentrations) and anti-atherogenic (in physiological concentrations) effects on the vessels. The latter vasodilatory action might be lost or down-regulated in the insulin resistant state, where increased insulin secretion in combination with hyperglycemia leads to smooth muscle cell hypertrophy and hyperplasia, and excess synthesis of extra-cellular matrix proteins.[5]

Increased prevalence of insulin resistance (IR) has been observed in patients with RA. High-grade systemic inflammation is implicated in the development of insulin resistance in these patients.[6]

Aim of the Study

The present study aims to determine the prevalence of insulin resistance in a group of RA patients and to clarify the association between IR and the various disease parameters and the subclinical vascular pathology in those patients.

Subjects and Methods

The present study included 40 patients with RA diagnosed according to the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) classification criteria for RA.[7] In addition, there were 20 healthy age and sex matched controls. Patients were excluded if they had experienced atherosclerotic cardiovascular events before.

RA patients were subjected to

careful history taking, thorough clinical examination and laboratory investigations including CRP, RF and ESR. For all patients visual analog scale (VAS)[8], Disease activity score (DAS28ESR)[9], tender and swollen joints count were calculated. Assessment of functional status was performed using health assessment questionnaire (HAQ)[10]. Ultrasound assessment of vascular subclinical atherosclerosis in the form of carotid intima media thickness (CIMT) of the carotid arteries was achieved according to the technique described by Rundek et al.[11]

All subjects were evaluated for serum insulin and glucose levels. Serum insulin was determined by electrochemiluminescence immunoassay. IR was then investigated in all patients at baseline by the homeostasis model for the assessment of IR (HOMA-IR) using the standard formula: fasting Glucose (mg/dl) x fasting Insulin(μU/mL) / 405. Patients were considered insulin resistant if HOMA-IR ≥ 2.[12]

Statistical analysis was performed using SPSS 17. Numerical variables were represented in the form of mean ± SD where categorical ones are represented the form of number and percent. Comparison between numerical variables was achieved using student t test while Chi-square test was used to compare categorical variables. Correlations were performed using Pearson's correlation coefficient. P value less than 0.05 was considered statistically significant.

Results

Basic demographic, clinical and laboratory characteristics of the studied patients were shown in table 1. The study included 40 RA patients. They comprised 31 females (77.5%) and 9 males (22.5%) with a mean age of 42.7 ± 8.3 years and BMI of 28.6 ± 3.2 Kg/m^2. The studied patients had a mean disease duration of 5.1 ± 2.9 years, a tender joints count of 2.1 ± 2.5 and a swollen joints count of 1.4 ± 1.3. The VAS score was 41.1 ± 15.1 while DAS28 ESR was 4.6 ± 0.9 and HAQ was 13.1 ± 3.0. The laboratory findings in the studied patients included ESR of 39.6 ± 10.6 (mm/h).

Comparison between patients and controls regarding HOMA-IR,

insulin resistance and CIMT thickness was demonstrated in table-2. Patients had significantly higher CIMT when compared with controls (0.8 ± 0.14 vs 0.64 ± 0.04; p=0.0001). Also, it was found that patients had significantly higher HOMA-IR when compared with control (1.89 ± 0.51 vs 1.02 ± 0.34; p=0.0001). The frequency of IR in RA patients was 40.0% compared to 0 % in controls (p=0.001).

Table-3 illustrated the correlations between HOMA-IR, RA parameters and CMIT. HOMA-IR was significantly correlated with BMI and CIMT.

Table 1 : Demographic, clinical and laboratory findings in the studied RA patients (n=40).

Age (years)	Range	29.0 - 59.0
	Mean ± SD	42.7 ± 8.3
Sex	Male	9 (22.5 %)
	Female	31 (77.5 %)
BMI (Kg/m^2)	Range	22.9 - 35.7
	Mean ± SD	28.6 ± 3.2
Disease duration (years)	Range	4.0 - 16.0
	Mean ± SD	9.1 ± 2.9
Tender joints	Range	1.0 - 10.0
	Mean ± SD	5.1 ± 2.5
Swollen joints	Range	0.0 - 4.0
	Mean ± SD	1.4 ± 1.3
VAS	Range	10 - 62
	Mean ± SD	41.1 ± 15.1
DAS28 ^{ESR}	Range	2.8 - 5.9
	Mean ± SD	4.6 ± 0.9
HAQ	Range	3.0 - 20.0
	Mean ± SD	13.1 ± 3.0
ESR (mm/h)	Range	2.0 - 58
	Mean ± SD	39.6 ±10.6

Table 2 : Comparison between patients and control regarding CMIT, HOMA-IR and insulin resistance.

	Patients (n=40)	**Controls (n=20)**	**P**
CMIT*	0.8 ± 0.14	0.64 ± 0.04	0.0001
HOMA-IR*	1.89 ± 0.51	1.02 ± 0.34	0.0001
Insulin resistance**	16 (40.0 %)	0	0.001

* Student t test ** Chi-square test

Table 3 : Correlations between HOMA-IR, clinical parameters and CIMT in RA patients.

	HOMA-IR	
	R	**p**
Age	0.14	0.38
BMI	0.34	0.031*
Disease Duration	-0.06	0.68
Tender joints	-0.11	0.46
Swollen joints	0.05	0.75
VAS	-0.09	0.54
DAS-28	-0.17	0.26
HAQ	-0.12	0.43
CIMT	0.4	0.009*

Discussion

Rheumatoid arthritis (RA) has long been known to be associated with increased mortality, which is mostly attributable to an increased prevalence of atherosclerosis, particularly ischemic heart disease.[13] Impaired insulin sensitivity has been shown to be an independent cardiovascular risk factor in individuals with[14] and without [15] diabetes.

Accordingly, it has been hypothesized that insulin resistance (IR) may have a role in promoting atherosclerosis in RA[16] and IR has been reported previously among patients with RA.[17]

Despite this, the association between IR and atherosclerosis remains under debate. So, the present study aimed to contribute to this issue by determining the prevalence of insulin resistance in a group of RA patients and to clarify the association between IR and the various disease parameters and the subclinical vascular pathology in those patients.

In the present study, patients had significantly higher CIMT when compared with controls (0.8 ± 0.14 vs 0.64 ± 0.04; p=0.0001). This is in agreement with the findings of Södergren et al.[18] who found that patients with RA had significantly higher CIMT when compared with controls. Also, Stamatelopoulos et al.[4] found that CIMT in both femoral and carotid arteries are significantly higher in patients when compared with controls.

In addition, the present study found that RA patients had a significantly higher HOMA-IR when compared with controls. It was also shown that RA patients had IR frequency of 40% compared to 0% in controls. These findings are consistent with the conclusions of La Montagna et al.[19] who found a significantly higher frequency of IR in RA patients when compared with healthy controls. However, in the study of Pamuk et al.[20], no significant differences were found between RA patients and controls regarding IR or HOMA-IR. This is attributed to the high cut-off value this study used to determine IR.

The current study found a significant correlation between HOMA-IR and BMI. This is in accordance with the findings of Chen et al.[21] who noted that BMI was significantly and independently associated with HOMA-IR. Also, it was found that HOMA-IR is

significantly associated with CIMT in agreement with the results of La Montagna et al.[19] In their study, Spearman correlation showed a significant positive relationship between IMT and HOMA-IR ($p<0.001$).

Conclusions

Insulin resistance is prevalent in RA patients. It significantly contributes to the development of subclinical atherosclerosis in those patients.

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EVALUATION OF ANALYTICAL PERFORMANCE OF HCV CORE ANTIGEN FOR HCV SCREENING IN BLOOD DONORS

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Abstract

Hepatites C Virus (HCV) remains a major cause of post-transfusion hepatitis despite of routine screening of the donors. This fact denotes that the current screening schedule is defective, as anti-HCV may not appear in acute infection up to 2 months (window period). In this study we aimed to evaluate the analytical performance of this new test as a screening test for HCV infection in blood donors. A total of 120 serum samples of which 60 anti HCV – Ab positive and 60 anti-HCV-Ab negative were analyzed with HCV-RNA and HCV-Ag test. According to HCV-RNA detection , sensitivity and specificity of HCV-Ag Test was measured as 98.2% and 100% respectively and of anti HCV-Ab were measured as 98.2% and 87.1% orderly An excellent positive predictive value for HCV-Ag test was detected as 100% whereas 90.8% for anti HCV-Ab Test. There was significantly positive correlation between HCV-Ag and HCV-RNA quantification analysis.

In conclusion, HCVcAg is shown to be useful in screening for HCV infection and in discrimination between active and past infections. So, it a better screening test for safe blood transfusion, and an alternative to the quantitation of HCV RNA levels using qRT-PCR in confirmation of infection .

Introduction

HCV is a positive - strand RNA virus that belongs to the family Flaviviridae[1,2]. HCV is a global health care problem, and the World Health Organization (WHO)

estimates that at least 170 million people (3% of the world's population) are infected with HCV worldwide[3]. HCV infection often occurs as acute hepatitis of which 75% are asymptomatic and 70%of those patients can evolve into chronic infection, it is the second most common cause of cirrhosis and hepatocellular carcinoma all over the world[4]. The Egyptian Demographic Health Survey in 2009 estimated an overall anti-HCV antibody prevalence of 18.5% reaching 45% in males over 40 years and 30% in females over 50 years[5]. The number of Egyptians estimated to be chronically infected was 9.8%, and approximately, 20% of Egyptian blood donors are anti-HCV positive [6]. Considering that HCV is the major cause of transfusion-associated hepatitis, so, effectively screening of the donors' blood samples plays an important role in preventing HCV transmission [7].

Diagnosis and monitoring of HCV infection are commonly based on anti-HCV assays, recombinant immunoblotting, and HCV RNA viral load in current clinical practices(8). However, anti-HCV assays have limitations, including a lack of detection sensitivity in the early window periods (45 to 68 days after infection)[9], although second- and third-generation anti-HCV assays have improved the diagnostic accuracy. Real-time quantitative reverse transcription-PCR (qRT-PCR) analyses for measuring viral loads also have some drawbacks. These techniques are not completely free from contamination and false-positive results. Furthermore, some PCR assays require technical skills and expensive[10].

An ELISA assay to detect HCV core antigen in peripheral blood of patients with HCV has been developed after the anti-HCV core antigen monoclonal antibody is available[11]. The purpose of this study to evaluate the analytical performance of this new test as a screening test for HCV infection in blood donors

Material and Methods

Serum samples were taken from blood donors from blood bank of zagazig university hospital (from January to june 2011). The

donors were divided into 2 groups; G1 comprised 60 blood donors with +ve anti-HCV, their ages in years (42.9±8.4), it included 12 females and48 males and G2 comprised 60 blood donors with -ve anti-HCV, their ages in years (39.2±7.8). it included 10 females and50 males.

The following cases were excluded :

1- Any blood donors of diabetes collagen disease, kidney disease or any one under corticosteroid therapy.
2- Blood donor with HBs-Ag positive
3- Blood donor with AIDS antibody positive.

Both groups were subjected to the following :

1- full medical history and thorough clinical examination
2- Routine laboratory investigations including :
   - blood picture .
   - liver function tests (Bilirubin, ALT, AST, alkaline phosphatase; total protein and albumin, Gama- glutamyl transpeptidas).

3- Serological assay

ARGHITECT HCV antigen assay :

The ARCHITECR HCV ag test is a two-step immunoassay that uses chemiluminescent microparticle technology to quantify the HCV core Ag in serum or plasma samples. The HCV Ag is detected in the presence or absence of anti-HCV antibodies since samples are automatically treated to dissociate the antigen - antibodies complex. The processing time is around 40 min. The manufacturer's specifications indicate a cut-off 0.06 pg\ml (3.00 fmol/L). The upper limit of linearity is 400 pg\ml,

4- Molecular analysis :

The COBAS AmpliPrep/COBAS taqMan HCV Quantitative test, v2.0 is a nucleic acid amplification test for the quantitation of hepatitis C Virus (HCV) RNA in human serum or EDTA plasma. Specimen preparation is automated using the COBAS TaqMan Analyzer or the COBAS TaqMan 48 Analyzer.

The COBAS AmpliPrep\COBAS TaqMan HCV Quantitative test, v2.0 is based on three major

processes: (1) Specimens preparation to isolate HCV RNA (2) reverse transcription of the target RNA to generate complementary DNA (cDNA) and (3) simultaneous PCR amplifications of target cDNA and detection of cleaved dual-labeled oligo-nucleotide detection probes specific to the target.

Statistical analysis :

Data were analyzed with SPSS for version 15.0 (statistical package for the Social Science, Chicago, IL). Patients ages were expressed as mean±standard deviation and analysed by independent sample t test while other quanitative data was expressed as median (range) and data analysis was done using Mann Whitney T-est. Qualitative data was expressed as number and percentage and was analyzed by Chi square (X^2) test. Pearson correlation coefficient was used to measure the correlation between HCV core Ag and HCV RNA. P-value was considered significant if <0.05 and highly significant if <0.001. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined.

Results

The present study included 2 groups of patients; G1comprised 60 blood donors with +ve HCV Abs, their ages in years (42.9±8.4), it included 12 females and48 males and G2comprised 60 blood donors with -ve HCV Abs, their ages in years (39.2±7.8). it included 10 females and50 males. The actual status of HCV infection as judged by HCV RNA and HCV core Ag is described in table (1). As regard the Quantitative disrtribution of HCV RNA and HCV core Ag among studied patients, out of 55 patients with +ve PCR in both groups we found 54 (98.2%) patients with +ve core Ag. Group1 showed rang of HCV RNA by PCR (1,000-3,000,000 Iu/ml) with median value 450,000 Iu/ml, while, the rang of HCV core Ag was (2-91 pg/ml) with median value 29 pg/ml. In this group 54 out of 60 patients was positive HCV-RNA, of 54 HCV-RNA positive samples, 53 were positive with HCV-Ag test. HCV-Ag test was negative in 1 patient whose anti-HCV-AB and HCV-RNA were positive (Viral load was 1000 Iu\ml). On the other hand group 2 showed only one patients with +ve PCR (35,000 Iu/

ml), their HCV core Ag was (2.5 pg/ml). The distribution of HCV core Ag among different categories of viremia is shown in table (2). The Diagnostic validity of HCV core Ag versus HCV Ab as a screening test for HCV is shown in table (3). HCV core Ag showed a significantly positive correlation with HCV RNA.

Table (1) : Qualitative disrtribution of HCV RNA and HCV core Ag among patients with and without HCV AB.

Parameter	HCV AB +ve N=60	HCV AB -ve N=60	x^2	P
HCV PCR Positive	54(90%)	1(1.6%)	94.3	<0.001
HCV Core Ag positive	53(88.3%)	1(1.6%)	91.03	<0.001

Table (2): Distribution of HCV core Ag among different categories of viremia .

Viral Load	< 100000 N=9	100000 to 1000000 N=44	>1000000 N=2
HCV Core Ag positive	8	44	2

Table (3): Diagnostic validity of HCV core Ag versus HCV Ab as a screening test for HCV with PCR as standard for existing HCV infection

	SENSitivITY	SPECificITY	ppv	npv
HCV Core Ag	98.2%	100%	100%	98.5%
HCV Ab	98.2%	87.1%	90.8%	98.3%

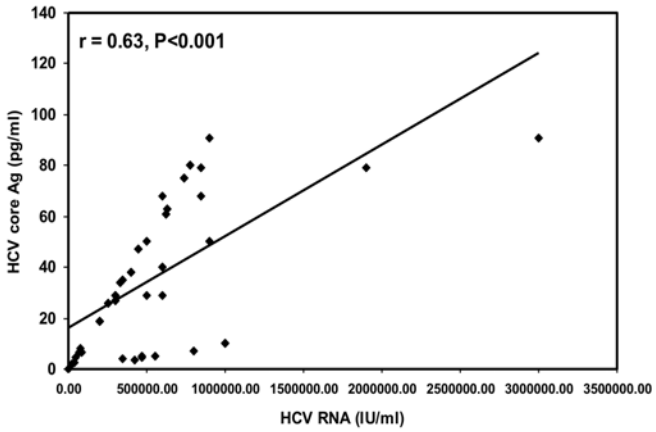

Figure (1) : The correlation between HCV-RNA and HCV-Core Ag.

Discussion

Diagnosis of HCV infection is mainly based on the detection of anti-HCV antibodies by the enzyme immunoassay (EIA) or chemiluminescence immunoassay (CLIA) of serum samples. The anti-HCV assay is used as a screening test. Recombinant immunoblot assays are used as supplemental tests and for the final detection and quantification of HCV RNA is used as a confirmatory test[12]. Anti-HCV assays have several disadvantages, such as the inability to distinguish between acute (viremic), past (recovered), and persistent infections, and a possibility of false negativity with samples from immunocompromised patients, who may not have an adequate antibody response [13].

The most common problem in the laboratory-screening anti-HCV assay is the false positive infection, the proportion of false-positive results is approximately 35% (range, 15% to 60%). Recombinant immunoblot assays, types of EIAs, also have several disadvantages, such as being difficult to perform and having a high percentage of indeterminate results and a high cost. Therefore, these anti-HCV assays are not often used in developing countries or in routine diagnostic laboratory procedures [14].

The HCV RNA assay is a reliable method but The HCV RNA test is extensively used to confirm anti - body - based screening test results. Amplification methods (target amplification by RT - PCR, transmission-mediated mplification [TMA], and signal amplification by branched DNA) are the most expensive methods compared with anti-HCV and HCV Ag tests and require sophisticated technical equipment and highly trained personnel [15].

In this study 6 patient (In group 1) out of 60 patients were positive anti HCV-Ab, negative HCV-RNA, negative HCV-Ag , this results can be due to non-viremic stage of the disease or recovery from infection. One specific problem with the HCV RNA assay is that HCV RNA can be temporarily undetectable because of the transient, partial control of viral replication by the immune response. Patients in a period of non viremia may be found to be anti-HCV positive and HCV RNA negative. In this situation, the HCV RNA test should be repeated a few weeks later with a new sample. This need for retesting is a disadvantage of the HCV RNA test. In addition, nucleic acid amplifications are labor-intensive and time-consuming methods and have the risk of laboratory contamination; for these reasons, amplification methods are not suitable for widespread use in most laboratories, especially in developing countries[16].

In present study, the sensitivity, specificity, and positive and negative predictive values of the HCV Ag Abbott CLIA were found to be 98.2%, 100%, 100%, and 98.5%, respectively. In different studies performed with HCV Ag Abbott CLIA gave comparable result to ours [17,18,19,20,21], apart from slightly lower diagnostic sensitivity (90.2%) reported by Park et al.[17].

In this study, the diagnostic specificity and positive predictive value of 100% means that there were no false-positive results: all 54 positive cases found by the HCV Ag test were also positive with the HCV RNA test (54/54 subjects). It can be concluded that the positive results of the HCV Ag

test can be reported as positive. As regard, the diagnostic sensitvity and negative predictive value of both HCV AG and HCV Ab was nearly the same as couldn't evaluate the onset of infection. However, there was one HCV Ag-reactive and anti-HCV-negative case (In group 2), one month later this patient developed anti-HCV antibodies, so the early HCV infection without antibodies can be detected using the HCV Ag assay. Leary et al, 2006[22] demonstrated that the HCV Ag was detected prior to the appearance of anti-HCV in the patients' sera and this phenomenon may have resulted in a reduction of the window period by 23 days or even longer. Therefore, the HCV Ag assay is needed as a supplemental or pre-confirmatory test to confirm anti-HCV results and distinguish false-positive results from the accurate ones because it is easy to perform and reliable, has high specificity and sensitivity rates, is cost-effective, is able to shorten the duration of the time to diagnosis of infection in patients during the window period, and has a lower risk of laboratory contamination than assays based on nucleic acid amplification technology [23].

Figure 1 illustrates that , HCV Ag concentrations were positively correlated with HCV RNA levels. So The newly developed automated HCV Ag assay could be used as an alternative (qualitative and quantitative) test for HCV RNA assays. Soffredini et al [24], reported that the HCV RNA-to-Ag ratio was not constant but increased as the RNA level was increased. These data suggest that more HCV core Ag is released into the blood as more viral replication occurs, but not as much as the increment of viral load. This is reasonable, since viral core Ag is not a direct marker of replication. Meanwhile, others reported there is a determined ratio between HCV RNA and HCV Ag [25,26]. This disagreement might be due to the difference between the assays adopted and would hinder the exact conversion between RNA and Ag concentrations. A more precise RNA-to-Ag ratio and the exact nature of its variation need to be assessed in further studies.

The limitation of our study is that we have no data regarding

the effect of HCV genotypes on the correlation. Some results suggest that the HCV genotype does not influence the correlation. However, a previous study reported that the correlation coefficients were different from each other according to the genotype [27]. In another study, HCV Ag and RNA levels were better correlated before therapy than after therapy (28). Further research on these subjects with the automated HCV Ag assay might be needed to clarify the relationship between HCV Ag and RNA, depending on the genotypes and therapies.

In conclusion, the automated HCV Ag assay showed results comparable to those of the HCV RNA viral load assay, and it has the advantages of easy testing and rapid reporting. This assay would be useful in screening for HCV infection .

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EVALUATION OF ANALYTICAL PERFORMANCE OF HCV CORE ANTIGEN FOR HCV SCREENING IN BLOOD DONORS

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URINARY TRACT INFECTION AMONG NEWBORNS WITH INDIRECT HYPERBILIRUBINEMIA

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Abstract

Hyperbilirubinemia is one of the most common causes for hospital admission in neonatal period. The aim of this study was to evaluate the presence of urinary tract infection in newborns with unexplained indirect hyperbilirubinemia in the first two weeks of life. ***Patients and Methods:*** *one hundred twenty full term newborns with jaundice, less than two weeks of age were included in this study from Mars 2011 to January 2012. They were selected from NICU, Benha Children Hospital and Kafr Shukr Hospital (Qalubia governate). Full medical history was taken and complete examination for all cases. Work up was performed including: complete blood count, CRP, reticulocyte count, serum level of bilirubin (unconjugated and conjugated), Comb's test, blood group of the neonate and the mother, urine analysis and urine culture.* ***Results:*** *a total of 120 neonates (73 males and 47 females) were enrolled. Among them 9 cases (7.5%) had positive urine culture (positive group). In positive UTI group; 7(77.8%) were males and 2(22.2%) females. E.coli was the most common cultured bacteria (6 cases) followed by klebsiella. There was no statistical significant difference between cases with and without UTI regarding age, body weight, gestational age and duration of admission.* ***Conclusion:*** *performing urinary tests to exclude the possibility of coincidental UTI may be necessary for admitted neonates with unexplained hyperbilirubinemia in the 1st two weeks of life, especially in neonates with maternal history of infections during pregnancy. We should not rely on simple urine analysis only for detection UTI in jaundiced newborns.*

Introduction

Jaundice is a frequently encountered problem during the neonatal period. Although up to 60% of term newborns have clinical jaundice in the first week of life, few have a significant underlying disease. However, it can be associated with severe illnesses such as hemolytic disease, metabolic and endocrine disorders, enzymatic deficiencies of the liver and infections [1].

Urinary tract infections (UTIs) are a common clinical problem in febrile infants younger than 8 weeks old, with prevalence between 5% and 11%[2].

Bacteremia and sepsis have been well- documented in previous studies a cause of neonatal jaundice in seriously ill newborns. Multiple studies have described patients with proven bacterial infection, who developed jaundice during the course of their illness. Other studies, have noted that jaundice may be one of the first signs of bacterial sepsis in neonates in the first few days of life [2].

Subject and Method

This study was conducted on 120 full term jaundice neonates (73 males and 47 females) below 2 weeks of age with indirect hyperbilirubinemia (serum bilirubin ≥ 15mg/dl). They were selected from NICU in Benha Children Hospital and Kafr Shukr Hospital; cases were recruited during the study period from Mars 2011 to January 2012. Full medical history was taken from all cases including name, sex, date of admission, age at presentation, mode of delivery, birth weight, age at onset of jaundice, and type of feeding, bilirubin level at presentation and duration of hospital stay.

Excluded cases : newborns more than two weeks of age, cases with serum bilirubin <15, neonates jaundiced in 1st 24hrs with signs of hemolysis and cases with fever or signs of sepsis.

Blood samples were taken for the following :

- EDTA tube for CBC by sysmex K_x-21,

- Serum CRP was measured by turbidity assay against control. reticulocytic count, blood group of the baby and the mother and RH, direct and indirect Comb's test to exclude hemolytic diseases and infection.
- Serum level of bilirubin (unconjugated and conjugated) by Bs-300 autamated chemistry analuzer.

Urine analysis: urine was collected in a sterile way using a urine catheter. All samples were sent to the laboratory for microscopically analysis and culturing. Culture on was CLED agar considered positive when a single pathogen with more than 10^5 colony forming units/ml. Media used were blood agar for gram positive bacteria, and Mac-conckey medium for gram negative bacteria. For further identification of gram negative bacteria, biochemical reaction was done. Mollar-hetton Agar was used for sensitivity.

Urine culture was repeated if more than one pathogen was discovered or if the number of colonies did not match the above criteria.

Statistical analysis :

Data were analyzed using statistical package of social sciences (SPSS) version 16 program using whole numbers, frequencies, mean (±SD), median (minimum - maximum). Chi-squared tests were used to test differences between participants. P values <0.05 were considered significant.

Results

One hundred and twenty jaundiced newborns were included in this study. Urine culture was done for all cases and it was positive in 9 of 120 neonates (7.5%) {positive group}.

Sixty nine (62.2%) of newborns in the negative group were males and 42(37.8%) females. But in the UTI group 7(77.8%) were males and 2(22.2%) females' fig (1). The mean age at admission was 5.11 days in negative group and 4.9 days in positive group. The mean gestational age was 38.5 ± 1.08 in negative group and 38.2 ±1.09 in UTI group.

According to feeding of neonates 97(80.8%) neonates were

breast feed (7 of them had UTI group) and 10 (8.3%) neonates were artificially fed (1 had UTI) and 13(10.8%) received mixed feeding (one in the positive group). There was no statistically significant difference between both groups as regard type of feeding.

We found that 3cases from the positive group had past history of premature rupture of membrane and 4 had positive history of maternal UTI.

Our study showed that 15 (12.5%) neonates out of 120 had positive history of jaundice in previous sibling, only one of them had UTI. All male cases were uncircumcised.

As regards hematological findings our study showed that reticulocytes were statistically higher among positive group but still keeping normal range. On other hand, no significant difference could be detected in other blood components.

Only 6 out of 9 neonates (positive group) in our study had pyuria by urine analysis.

Table (1) Shows that there was no statistical significant difference between positive cases for UTI and cases without UTI regarding age, body weight, gestational age and duration of admission.

E.coli was the most common cultured bacteria (6 cases) followed by klebsiella (3 cases) (Table 2).

Table (1): Characters of the studied cases

Group	Negative group (N=111)		Positive group (N=9)		"t"	P
Parameter	Mean	±SD	Mean	±SD		
Age on admission(days)	5.11	2.14	4.89	2.571	0.29	0.77
Weight (kg)	2.99	0.313	2.9	0.16	0.53	0.6
Gestational age (week)	38.5	1.08	38.2	1.09	0.83	0.41
Duration of admission	4.68	1.36	4.8	0.83	0.21	0.84

Table (2): Culture results among the studied sample.

Variable		(N=120)	%
Microbial growth	Non	111	92.5
	E.coli	6	5.0
	Klebsiella	3	2.5

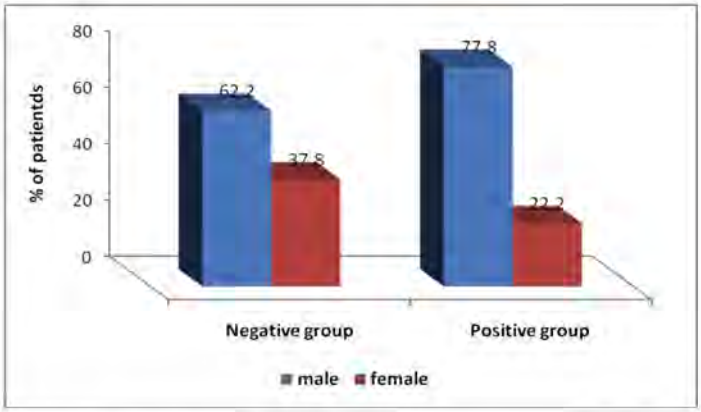

Fig (1): Comparison between studied groups according to sex.

Discussion

The majority of the studied groups were males 73 (60.8%) while 47 (39.2%) were females. In positive group males were 3.5 times that of girls (7 males and 2 females).

Our study demonstrated that 9 neonates (7.5%) of studied neonates with hyperbilirubinemia had UTI, this is in agreement with the study by Garcia and Nager (2002)[2] who reported that incidence of UTI was (7.5%), and study of Bilgen et al., (2006)[1] in Turkey, which found 8 cases out of 100 (8%) had UTI. The incidence reported in a study by Eslami and Sheikhh,[3] in Iran was 11%. Also it was 8.2% in study by Jafarzadeh and Mohammadzadeh, (2009)[4] and 6.97% in Boskabadi et al.,[5] study. More high incidence was found by Nejad et al., [6] 18% of jaundiced neonates had UTI in Tehran and it was 21% in study by Omar et al., 2011[7] but

lower incidence (5.5%) in Chen et al.,[8].

E.coli was the most common cultured bacteria (6 cases) followed by klebsiella (3 cases), this in agreement with study of Jafarzadeh and MohammadZadeh, (2009)[4] who reported that the most common isolated organism from urine culture is E.coli. But in recent study in Tehran klebsiella and E-coli are equally causative organisms of UTI. Also, the study by Chen et al., (2011) [8] reported that most of cultured bacterium in urine was E-coli.

There was no statistically significant difference between the positive and negative groups as regards gestational age, post conception age or mode of delivery. All neonates included in the study were uncircumcised as circumcision is an important protective factor against UTI. Also Ghaemis, (2007)[9] found that nearly 60% of male infants with UTI were uncircumcised.

Only 6 out of 9 neonates (positive group) in the present study had pyuria by urine analysis. This agrees with the study of Crain and Gershel, (1990)[10] as 50% of the urine analysis was normal among 32 infants with UTI. Similarly only 5 infants among 12 with UTI had abnormal urine analysis in the study by Carcia and Nager, (2002)[2].

A number of possible mechanisms for UTI to cause jaundice have been documented including: (i) haemolysis, (ii) direct invasion of the liver parenchyma by blood-borne or lymph borne microorganisms, (iii) hepatocellular injury by circulating endotoxins, (iv) non-specific injury to the liver related to hyperpyrexia, malnutrition and anoxia. On other hand jaundice can lead to UTI by altering bactericidal activity in the sera of jaundiced newborns making them more prone to infections. Some cases of haemolysis have been reported to contribute to jaundice in patients with infections caused by E-coli, as certain strains of E-coli produce a haemolysin[11].

As regards hematological findings our study showed that reticulocytes were higher among

positive group but still keeping normal range of reticulocytes, with statistically significant difference between them (p-value <0.05). On other hand, no significant difference could be detected as regard other components of blood.

From the previous discussion, we can come to the conclusion that UTI may occur in jaundiced newborns and point to be considered in such neonates with maternal history of infections during pregnancy, circumcision is considered as an important protective factor against UTI and should be performed as early as possible and we should not rely on simple urine analysis only for detection UTI in jaundiced newborns.

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URINARY TRACT INFECTION AMONG NEWBORNS WITH INDIRECT HYPERBILIRUBINEMIA

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STUDY OF CHRONIC INFLAMMATORY ARTHRITIS IN EGYPTIAN PATIENTS WITH HEPATITIS C VIRUS INFECTION

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Abstract

Background and Aim: *Autoimmunity and viral infections are closely related fields, and viruses have been proposed as possible etiological or triggering agents of auto-immune diseases. The aim of this work is to study the prevalence and describe the characteristics of rheumatoid arthritis (RA) in Egyptian patients associated with HCV infection with recent available laboratory and radiological investigations and to compare them with the HCV related arthritis.*

Patients and Methods: *1100 hepatitis C virus infected patients participated in this study. Among these patients, 93 (8.45%) patients had arthritis (34 patients had HCV related arthritis and 59 patients had HCV with RA, SLE or SS). Those patients represented the target population for this study.*

Results: *The mean age of the patients with HCV related arthritis was 50.3 ±12.7 years compared to 46.7 ±13.4 years in the patients having HCV associated with RA, SLE or SS (p>0.05). The female/male ratio in the patients had HCV related arthritis was 26/8 while in patients of HCV associated arthritis was 55/4 (p>0.05). Sleep disturbance was more frequent in the HCV related arthritis group of patients. Fever, hepatomegally and spleenomegally were more frequent also in the HCV related arthritis group. Pattern of arthritis, tenosnovitis, myalgia and fibrimyalgia are significantly different between the 2 groups. Also HAQ, quality of life and VAS are significantly different between HCV related arthritis and HCV with RA patients. As regards the laboratory findings,*

the hemoglobin concentration, WBCs count, platelets, albumin and bilirubin are significantly different between the 2 groups of patients. Anti-CCP and cyroglobulinemia are significantly different between the 2 groups. Erosions and deformity are present only in HCV patients with RA.

Conclusion : *HCV associated arthritis can be misdiagnosed as RA, especially as 50% of patients fulfill the ACR criteria of diagnosis of RA. Patients represented with: arthritis which may be oligoarticular affecting big joints, arthritis similar to RA but non erosive or non-deforming or elevated liver enzymes should be screened for HCV infection. Anti-CCP antibodies are one of the markers that help to differentiate HCV associated arthritis from RA. Cryoglobulins together with CRP, ESR, and RF are considered as markers of disease activity in HCV related arthritis.*

Keywords HCV. *Rheumatoid arthritis.*

Introduction

Hepatitis C virus (HCV) was 1st identified in 1989 as a lymphotropic and hepatotropic single stranded ribonucleic acid (RNA) virus that infects peripheral blood lymphocytes as well as hepatocytes [(1)]. HCV is a rapidly mutating virus, with several subtypes that differ according to geographic distribution [(2)].

HCV is a global health problem affecting 3% of the world's population (about 180 millions)[(3)]. Egypt has the highest prevalence of HCV infection in the world ranging from 12% to 24% in the general population[(4)]. More than 90% of HCV infections in Egypt are due to genotype 4[(5)]. The majority of infected individuals are unable to eliminate the virus. Around 20% of them will go on to develop hepatic fibrosis and eventually cirrhosis and there is a 3-5% annual risk of the development of hepatocellular carcinoma. Infection with the HCV has become the principal indication for liver transplantation[(6)].

In addition to its hepatic effects, chronic infection with the HCV is responsible for numerous extrahepatic manifestations[(7)], of

which the rheumatological manifestations are amongst the most frequent. Recently, there is outgrwoing interest in the relationship between chronic HCV infection and systemic autoimmune diseases (SAD) such as Sjogren's syndrome (SS), rheumatoid arthritis (RA), polyarteritis nodosa, systemic lupus erythematosus (SLE) and the antiphospholipid syndrome. However, the bulk of the data are based on small sample groups and case studies.

The aim of this work is to determine the prevalence and to study the characteristics of chronic inflammatory arthritis in Egyptian patients with HCV infection in order to reach the proper diagnosis with recent available laboratory and radiological investigations, with subsequent modificat-ion of future therapeutic regimens

Another suggested aim

The aims of this study were to describe the patterns of disease expression in a large series of patients with SAD associated with chronic HCV infection and to analyse the epidemiological, clinical and immunological implications of this association.

Patients and Methods

Patients recruitment

This study comprised 1100 (684 males and 416 females) patients who had serological evidence of chronic HCV infection: at least two positive determinations by ELISA[8] and/or positive HCV-RNA by polymerase chain reaction (PCR)[9]. Their ages ranged between 22-69 years. They were collected from either in-patient or out-patient Hepatology clinic and from the Rheumatology and Rehabilitation outpatient clinic of Mansoura University Hospitals between June 2008 and October 2009. All patients are screened for evidence of arthritis. Those patients with arthritis represented the target population for this study.

We excluded patients with other possible causes of arthropathy. So, none of the 93 HCV infected patients had any endocrinal abnormalities including diabetes mellitus (DM) (by blood sugar level) and thyroid function (by thyroid stimulating

hormone level), recent history of systemic infection, hepatitis B virus infection (by hepatitis B surface antigen), hepatocellular carcinoma (by Alfa Fetoprotein level), amyloidosis, sarcoidosis, hemochromatosis, haemophilia, haemoglobinopathies., malignancies, familial auto-inflammatory disorders, crystal induced arthropathies. None of our female patients were pregnant nor lactating (pregnancy test was done to exclude pregnancy).

Clinical Assessment.

The eligible participants patients with arthritis completed preliminary questionnaires inquiring into patient demographics, the history of the hepatitis, cumulative hepatic and extrahepatic manifestations during disease evolution (from diagnosis until inclusion) and treatments received, the rheumatological manifestations (arthralgia, arthritis, myalgia, deformities, tenosynovitis) and the effects of treatments on these manifestations. The questions were precise enough to distinguish between inflammatory and mechanical joint disease.

Pain severity was assessed using visual analogue scale (VAS)[10]. Joint tenderness was assessed using Ritchie articular index (RAI)[11]. Functional assessment or ability is measured by using Stanford Health Assessment Questionnaire (HAQ) which covers nine general component categories (dressing and grooming, arising, eating, walking, hygiene, reach, grip, outside activity, and sexual activity)[12]. Quality of life (qol) was assessed using questionnaire composed of 30 items with a yes / no response[13]. The overall score is the sum of the individual item scores (range 0-30), with a lower score indicating better Qol.

Laboratory assessment

Immunological parameters such as rheumatoid factor (RF)[14], anti-cyclic citrullinated peptides antibodies (Anti-CCP Ab)[15], antinuclear antibodies (ANA) (specifically anti - SSA / SSB)[16], cryoglobulin[17] and complement levels were measured for nearly all patients with arthritis, as well as erythrocyte sedimentation rate (ESR)[18] and C-reactive protein (CRP) values[19]. Serum creatinine and liver

enzymes were also evaluated. Anti HCV antibodies were assessed[8], viral activity of the hepatitis was assessed by the transaminase level and the viral load measured by PCR[9]. For a proportion of patients a liver biopsy was performed with an evaluation of the degree of histological activity and fibrosis with the 'Metavir' score (20), which measures the activity score (A) from 0-3 and the fibrosis score (F) from 0-4.

Radiological assessment

Plain radiographs were done by the standard views comprising antero-posterior, lateral and oblique projections of both hands and feet. Magnetic resonance imaging (MRI) was requested when image cuts were needed to establish a diagnosis. Liver and spleen states are evaluated using abdominal ultrasound.

Statistical analysis :

The data were entered into a computer and analyzed using SPSS version 17.0 (SPSS Inc, Chicago, IL). Descriptive statistics consisted of numbers (percentages) for qualitative variables and means (±SD) for quantitative variables. Qualitative variables were compared using the chi-square test. To compare quantitative variables, we used independent-sample Student's t test. Pearson correlation test was used to determine the correlation between variables. P values smaller than 0.05 were considered significant.

Results

1100 HCV infected patients participated in this study. Among these patients, 93 (8.45%) patients had arthritis. Eighty one (87%) patients were females and 12 (13%) males with a mean age of 48.36 ± 11.61 years and BMI 31.38 ± 3.51 kmg/m^2. At entry of study, the duration of HCV infection was 4.54 ±4.33 years while the duration of arthritis was 29.04±14.4 months.

These 93 patients were classified into 2 groups: (i) Group I: Patients with HCV associated arthritis (n=59) distributed in three subgroups: Rheumatoid arthritis (RA) associated with HCV infection (RA-HCV) (n=42, 45.2%), Systemic lupus erythematosus (SLE) associated with HCV infection (SLE-HCV) (n=12, 12.9%) and Sjogren's

syndrome (SS) associated with HCV infection (SS-HCV) (n=5, 5.4%) and (ii) Group II: Patients with HCV related arthritis (n=34).

Arthritis was diagnosed in older ages in HCV related arthritis than in HCV associated arthritis (46.7 ±13.4 vs. 50.3 ±12.7 respectively). This difference was insignificant. The female : male ratio of HCV associated arthritis was 55:4 compared to 26:8 in HCV related arthritis. This difference was also insignificant.

Table 1 shows no significant difference between HCV associated arthritis group and HCV related arthritis group as regarding; fatigue, malaise, morning stiffness, Raynaud's phenomenon, oral ulcers, sicca symptoms, DM, or hypertension (P>0.05). Significant differences were found between the two groups as regarding; myalgia (P<0.001), sleep disturbance (P<0.001), and photosensitivity (P<0.05).

Table 2 showed significant difference (P<0.05) as regarding fever, subcutaneous nodules, and malar rash. There was insignificant difference (P>0.05) as regarding jaundice when comparing the two groups.

Table 3 showed significant difference as regarding hepatomegally (P<0.001) and splenomegally (P<0.01) when comparing the two groups.

Musculoskeletal deformities was found in 42.9% of the HCV associated arthritis group while none of the HCV related arthritis group had deformities (P<0.001). Fibromyalgia was significantly higher in the HCV related arthritis group (61.8%) when compared to HCV associated arthritis group (10.2%) (P<0.001). No significant difference was found between the two groups as regarding tenosynovitis and cutaneous vasculitis.

Also, no significant difference was found between the two groups as regarding the pattern of arthritis; however, involvement of proximal interphalangeal joints (PIPs) metacarpophalangeal joints (MCPs), wrist joints and ankles is significantly higher in the HCV associated arthritis group when compared to HCV related arthritis

group. Elbows, shoulders, hips, knees, and metatarsophalangeal joints are affected in both groups with no significant differences (Table 4).

Table 5 shows that there is significant difference between the two groups as regarding HAQ, Qol, VAS, and RAI.

As regarding hemoglobin concentration, white blood cells count, and platelets counts, there were significant differences in HCV associated arthritis group compared to HCV related arthritis group ($P<0.05$, $P<0.001$, and $P<0.05$ respectively). ESR and CRP were elevated in both groups but it was statistically insignificant ($P>0.05$). Hepatic transaminases are elevated with no significant difference ($P>0.05$) and elevated bilirubin in both groups ($P<0.01$) (Table 6).

Table 7 shows that ANA was found in 18 patients out of 59 patients (30.5%), while ANA was found in 7 patients out of 34 patients (20.6%) in HCV related arthritis group ($P>0.05$). Anti-ds DNA was found in 9 patients out of 59 patients (15.3%), -ve in all patients with HCV related arthritis ($P<0.05$). RF was found in 53 patients out of 59 patients (89.8%), while RF was found in 28 patients out of 34 patients (82.4%) in HCV related arthritis group ($P>0.05$). Anti-CCP Ab was found in 38 patients out of 59 patients (64.4%) and these patients were all diagnosed as RA associated with HCV, while Anti-CCP Ab was -ve in all patients with HCV related arthritis ($P<0.001$), which may help to differentiate the two conditions.

Table 8 shows that erosions were found only in RA subgroup of HCV associated arthritis group & it was statistically significant ($P<0.001$).

Table 9 shows a significant increase of CRP in cryoglobulin +ve patients when compared to cryoglobulin -ve group (<0.05), and also a significant increase of RF in cryoglobulin +ve patients when compared to cryoglobulin -ve group ($P<0.01$). ESR was higher in cryoglobulin +ve when compared to cryoglobulin -ve patients but it was statistically insignificant ($P>0.05$) in both 1^{st} and 2^{nd} hours.

From table 10 we can detect a positive correlation between cryoglobulins and CRP (r=0.588, p=0.027) and a positive correlation between cryoglobulins and RF (r=0.820, p=0.000).

Table 1 : Comparison between HCV associated arthritis and HCV related arthritis as regarding history findings (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Fatigue	36	61	18	52.9	NS
Malaise	22	37.3	12	35.3	NS
Myalgia	7	11.9	17	50	<0.001
Sleep disturbance	20	33.9	24	70.6	<0.001
Morning stiffness	26	44	9	26.5	NS
Raynaud's	13	22	5	14.7	NS
Oral ulcers	4	6.8	0	0	NS
Photosensitivity	8	13.6	0	0	0.0247
Sicca symptoms	5	8.5	0	0	NS
DM	9	15.3	7	20.6	NS
Hypertension	10	16.9	8	23.5	NS

Table 2. Comparison between HCV associated arthritis and HCV related arthritis as regarding general examination (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Fever	8	13.6	12	35.3	0.014
Jaundice	4	6.8	6	17.6	NS
Subcutaneous nodules	10	16.9	0	0	0.011
Malar rash	10	16.9	0	0	0.011

Table 3. Comparison between HCV associated arthritis and HCV related arthritis as regarding abdominal examination (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Hepatomegally	8	13.6	17	50	<0.001
Spleenomegally	3	2.1	8	23.6	0.0079
Ascitis	1	1.7	2	5.9	NS

Table 4: Comparison between HCV associated arthritis & HCV related arthritis as regarding musculoskeletal manifestations (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Arthritis	59	100	34	100	NA
Pattern of arthritis					
Mono-oligoarthritis	17	28.8	12	35.3	NS
Polyarthritis	42	71.2	22	64.7	NS
Deformities	18	42.9	0	0	<0.001
Distribution of arthritis					
PIPj	39	66.1	3	8.8	<0.001
MCPj	39	66.1	4	11.8	<0.001
Wrists	43	72.9	5	14.7	<0.001
Elbows	9	15.3	2	5.9	NS
Shoulders	2	3.4	4	11.8	NS
Hips	1	1.7	2	5.9	NS
Knees	15	25.	6	11.1	NS
Ankles	9	15.3	5	14.7	0.0194
MTPj	4	6.8	2	5.9	NS
Tenosynovitis	9	15.3	3	8.8	NS
Cutaneous vasculilits	2	3.4	0	0	NS
Fibromyalgia	6	10.2	21	61.8	<0.001

Table 5. Comparison between HCV associated arthritis & HCV related arthritis as regarding disease activity indices (student's t test)

	HCV associated arthritis		HCV related arthritis		P
	mean	±SD	mean	±SD	
HAQ	16.54	±3.95	14.12	±3.33	0.0034
Qol	21.48	±3.10	18.81	±3.85	<0.001
VAS	3.14	±0.15	2.01	±0.11	<0.001
RAI	10.54	±3.98	8.12	±3.53	0.003

Table 6. Comparison between HCV associated arthritis & HCV related arthritis as regarding laboratory findings (student's t test)

	HCV associated arthritis		HCV related arthritis		P
	mean	±SD	mean	±SD	
Hemoglobin	11.12	1.37	10.51	1.36	0.041
Red blood cells.	4.11	0.54	4.33	0.64	NS
White blood cells.	5.62	1.62	4.37	±1.62	<0.001
Platelets	205.41	50.21	231.34	±61.12	0.0294
ESR (1st hour)	44.34	17.64	48.88	±22.01	NS
ESR (2nd hour)	72.13	14.51	77.11	±16.13	NS
CRP (n, %)	48	81.4	22	64.7	NS
Bilirubin (n, %)	12	20.3	17	50	0.0029
SGOT	44.23	16.27	48.33	±16.11	NS
SGPT	41.21	20.33	45.02	±13.08	NS
Albumin	3.31	1.01	3.78	±0.25	0.0092
Creatinine	0.81	0.1	0.85	±0.15	NS

Table 7. Comparison between HCV associated arthritis & HCV related arthritis as regarding immunological findings (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
ANA	18	30.5	7	20.6	NS
Anti-ds DNA	9	15.3	0	0	0.0165
Anti HCV Ab	59	100	34	100	NA
RF	53	89.8	28	82.4	NS
Anti-CCP Ab	38	64.4	0	0	<0.001
Cryoglobulins (n=22)	-	-	14	63.6	-
Anti-Ro/La (n=5)	2	40	-	-	-

Table 8. Comparison between HCV associated arthritis and HCV related arthritis as regarding radiological findings (chi square test).

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Erosion	38	64.4	0	0	<0.001

Table 9. Comparison between cryoglobulin +ve cryoglobulin –ve patients in the group of HCV related arthritis (n=22)

	Cryoglobulin -ve HCV patients (n= 8)	Cryoglobulin +ve HCV Patients (n=14)	P
CRP *(n, %)*	3 (37.5%)	11 (78.6%)	0.0139*
RF *(n, %)*	1 (12.5%)	10 (71.4%)	0.0078*
ESR 1st hour *(mean ±SD)*	42.7±9.1	49.3±10.5	NS**
ESR 2nd hour *(mean ±SD)*	65.1±8.4	73.1±12.4	NS**
** Chi square test, ** Student's t test.*			

Table 10 : Correlation between cryoglobulin +ve patients, CRP, and RF in the group of HCV related arthritis

	CRP		RF	
	r	P	r	P
Cryoglobulins	0.588	0.027	0.820	0.000
CRP	-	-	0.577	0.031
RF	0.577	0.031	-	-

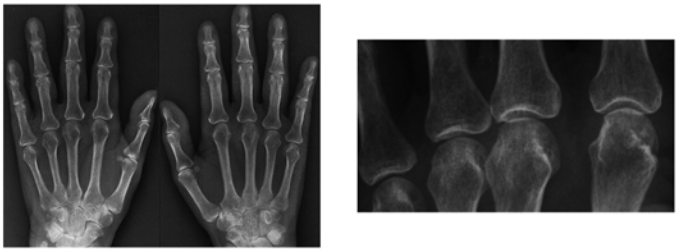

Figure 1. X-ray features of rheumatoid arthritis including evidence of soft tissue swelling of the MCP and PIP joints, periarticular osteoporosis, symmetric joint space narrowing of several MCP and PIP joints of both hands, and erosions of the right 3rd and right and left 4th MCP joints

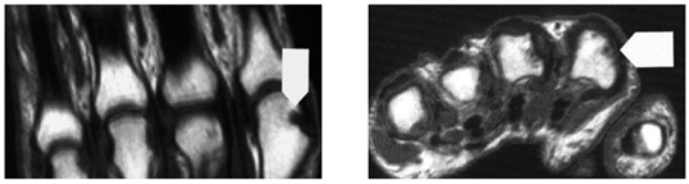

Figure (4): Coronal and axial T1-weighted spin echo MRI scans show bony erosions at the radial aspect of the second metacarpal bone (arrow).

Discussion

The current study included 1100 HCV infected patients as proven by presence of anti HCV antibodies and +ve PCR. Among these patients, 93 patients (8.45%) had arthritis. Those patients represented the target population for this study.

Garcia-Monforte and associates[21] described only 2% incidence of frank arthritis, and reported 52% incidence of arthralgia. Buskila and co-workers[22] found arthritis in 4% of HCV infected patients. Lee et al.[23] found a higher prevalence of arthralgia and arthritis (35%) in patients with HCV infection. This high prevalence value may be attributed to studying the prevalence of both arthralgia and arthritis. In another study conducted in the Middle East region by Al-Awadi and others in 1998[24] on 40 patients with chronic HCV whose age group ranged between 30 - 50 years, polyarthralgia and polyarthritis were the most common rheumatic manifestations (35%). On the other hand, Rivera et al.[25] have reported a frequency of HCV infection (7.6%) among 303 patients with RA. Zuckerman et al.[26] diagnosed arthritis in 28 (11.4%) patients who were recruited from 245 patients with

HCV infection attending the liver clinic.

Cacoub et al.,[27] found that the rheumatologic manifestations represented 38% of the patients, 19% suffered from arthralgia and arthritis. Ferri et al.,[28] found that arthralgia was present in 72% of patients at baseline, while arthritis was detected in 8% of patients. Arthritis was manifested as oligo-arthritis predominating in the large joints, with no erosions or distinctive clinical features. Lormeau et al.,[29] found that the prevalence of chronic inflammatory arthritis across different studies ranged from 4% to 11%. Palazzi et al.,[30] found that although asymptomatic joint involvement and arthralgias are frequent in patients with HCV chronic infection, a true arthritis affects only 4% of the patients.

In our study the number of patients fulfilling the criteria for diagnosis of HCV associated RA, SLE, or SS was 59 patients (5.4% out of 1100 HCV patients and 36.4% out of 93 patients with arthritis). This was nearly similar to that found by Ramos-Casals et al.[31] who found that the prevalence of patients fulfilling the criteria for diagnosis of HCV associated RA, SLE, and SS among 1020 HCV-infected patients was 2% - 6%.

In our study, the 93 patients with arthritis were diagnosed by clinical examination, laboratory investigations and radiological evaluation and classified into 2 groups; HCV associated arthritis group (59 patients) [RA in 42 patients (45.2%), SLE in 12 patients (12.9%), and Sicca syndrome in 5 patients (5.4%)] and HCV related arthritis group (34 patients).

Cacoub et al.,[27] screened a total of 411 HCV patients, and found that the most frequently identified SAD were SS (n=10) and SLE (n=6). That of SLE-HCV is similar to that found in our study. Ramos-Casals and Font [32] found that prevalence of HCV associated SAD (RA, SLE, and SS) varies widely according to geographic area. The best example is SS: Studies from Europe describe a prevalence of 10%-20%, while studies from USA found a prevalence of <1%, probably due to the lower prevalence of HCV infection

in these countries. Sene et al., [33] specifically investigated SS in a total of 216 HCV patients, and found 11 (5%) fulfilled classification criteria for SS, a prevalence 5 to 10 folds greater than that found in general population.

In this study, the age of our patients ranged between 22 years to 69 years (mean 48.36±11.61). They were 81 females (87.1%) and 12 males (12.9%). Their BMI ranged from 24.16 to 41.79 kgm/m^2 (mean 31.38±3.51). The duration of HCV infection ranged from 1 to 15 years (mean 4.54±4.33) and the duration of arthritis ranged from 6 months to 4 years (mean 2.42±1.2). In our study, the prevalence of arthritis in our patients was higher in females than males in all studied groups and that arthritis was diagnosed in older ages in HCV related arthritis than in HCV associated arthritis. But these differences were insignificant.

Ramos-Casals et al.[32] studied 180 SAD associated with HCV cases; 130 (72%) patients were females and 50 (28%) were males. The mean age at diagnosis of arthritis was 50.3±1.3 years and at HCV infection diagnosis was 53.7±1.2 years. These differences may be due to geographical distribution as the patients in this study were from Spain.

The majority of studies reporting the prevalence of chronic HCV infection in patients with SAD found a higher prevalence than that in the general population. This can be explained on the basis that viruses are often proposed as etiologic or triggering agents of SAD. About 70% of all reported cases of SAD in patients with HCV infection came from Mediterranean countries [31].

As regarding history findings in our patients, fatigue was found in 88 (94.6%) patients, photosensitivity in 8 (8.6%) patients, Raynaud's phenomenon was found in 18 (19.4%) patients, sicca symptoms in 5 (5.4%) patients, and oral ulcers in 4 (4.3%) patients. Lee and colleagues[23] conducted a study on 49 Korean patients with HCV and observed that cutaneous manifestations were found in 37% of patients, Raynaud's phenomenon in 8%, dry eyes in 22%, dry

mouth in 10%, and oral ulcers in 33%. The prevalence of their manifestations was slightly higher than that in our study. This may be due to geographic distribution and genetic basis.

In our study, myalgia was found in 34 (36.6%) patients. Buskila and co-workers[22] reported myalgia in 24 % of patients, while Cacoub et al.,[27] found Myalgia in 2% of patients. Morning stiffness lasting more than1 hour is present in 35 patients (37.6%). Zuckerman et al.,[34] also found that morning stiffness affects two thirds of HCV infected patients and may be severe lasting for more than one hour.

As regarding liver involvement there was; hepatomegally in 25 (26.9%), splenomegally in 11 (11.8%), and jaundice in 10 (10.8%). Ascitis was found in 3 (3.2%) patients. In agreement with our study, Ramos-Casals et al.[32] demonstrated hepatomegally in 26%, spleenomegally in 12%, jaundice in 6% of patients, and only 9% had other manifestations of hepatic decompensation (ascitis, encephalopathy, and gastro-intestinal bleeding).

In our study, fibromyalgia was found in 27 (29%) patients. Fibromyalgia was significantly higher in the HCV related arthritis group (61.8%) when compared to HCV associated arthritis group (10.2%). 12 patients (12.9%) of our patients had evidence of tenosynovitis, 2 patients (2.2%) from SLE-HCV subgroup had cutaneous vasculitis and 18 patients (19.4%) from RA-HCV subgroup had musculoskeletal deformities. Al-Awadi and others in 1998[24] studied 40 patients with chronic HCV and found cutanous vasculitis in 50% of their patients. Buskila and co-workers[22] found sicca symptoms in 8%, cutaneous vasculitis in 2%, polymyositis in 1%, and fibromyalgia was diagnosed in 16% of patients. Goulding et al.[35] found fibromyalgia in 5% of the studied patients. Cacoub et al.[27] found Myalgia in 2%, which is lower than in our study, may be due to that their patients came exclusively from Internal Medicine and Infectious Diseases Departments while in our study they came also from Rheumatology clinic, and so the main complaint was

rheumatologic symptoms.

Zuckerman et al.[34] found that HCV related arthritis usually runs a relatively benign course that, in contrast to RA, is typically non-deforming. Mahmoud et al.[36] found that HCV related arthritis was frequently associated with non-deforming arthritis, which is similar to that found in our study.

In our study, the pattern of arthritis were mono-oligoarthritis in 29 patients (31.2%) and polyarthritis in 64 patients (68.8%). Euller-Ziegler et al.[37] found that there is a high incidence of arthritis in patients with HCV infection: 6.4% had oligoarthritis, and 20.6% had polyarthritis. Fadda et al.[38] observed that there is no uniform clinical picture of arthritis in patients with chronic HCV infection; but two different patterns of inflammatory arthritis are found, one mimicking RA and another oligoarticular type affecting large joints. While, Sene et al.[33] stated that there are two types of articular involvement in the course of HCV infection: a polyarthritis involving small joints that resembles RA, usually milder and frequently meets the ACR classification criteria for RA, and a non erosive oligoarthritis involving the medium-sized and large joints. Also, Liu et al.[39] found that HCV infection may induce extra hepatic manifestations, including polyarthritis that mimic RA. Kaptanoglu et al.[40] found that HCV infection induces EHMs including arthritis which can mimic RA, and concluded that the discrimination between HCV related arthritis and RA is difficult.

In our study, involvement of PIPs MCPs, wrist and ankle joints are significantly higher in the HCV associated arthritis group when compared to HCV related arthritis group. Elbows, shoulders, hips, knees, and metatarsophalangeal joints are affected in both groups with no significant differences. Sawada et al.[41] found that in most HCV patients, soft tissue swelling and synovitis of large joints are present, most commonly involving the wrists, shoulders, ankles and knees. Zuckerman et al.[26] found that the joints most commonly involved in HCV related arthritis are the MCPs, PIPs,

wrists and ankles. Arthritis related to HCV infection most often affects PIPs, MCPs, and knees[29]. While musculoskeletal deformities were significantly higher in the HCV associated arthritis group when compared to HCV related arthritis group. No significant difference was found between the 2 groups as regarding tenosynovitis and cutanous vasculitis.

Our study revealed a significant difference between the two groups as regarding HAQ, Qol, VAS, and RAI. Similarly, Kozanoglu et al.[42] found that pain intensity scored on a VAS and sleep disturbance were higher in the HCV group. Mohamed et al.[43] did not find a significant reduction of Qol and VAS of arthritis in patients with chronic HCV infection. Also, Thein et al.[44] found no statistically significant difference in grades of VAS and Qol in HCV infected patients. Ferri et al.[45] found high values of VAS and RAI in HCV associated arthritis. Basseri et al.[46] found that HCV poses an increasing healthcare burden associated with maladaptive lifestyle habits and poor Qol. Similarly, Sinakos et al.[47] showed that Qol in HCV related arthritis patients was worse than that of HCV associated arthritis patients. Pain intensity measured by VAS was higher in HCV associated arthritis group[48].

As regarding hemoglobin, white blood cells, and platelets, there were significant differences in HCV associated arthritis group compared to HCV related arthritis group. This is similar to that found by Ramos-Casals et al.[32] who found cytopenias in 26% of patients with HCV associated with autoimmune diseases. And also similar to that found by Ufearo et al.[49]

Our results shows elevated hepatic transaminases and elevated bilirubin in both groups with no significant difference, which is similar to that found by Ramos-Casals et al.[32]. ESR was elevated in both group but it was statistically insignificant which is similar to that found by Ufearo et al.[49]. Zuckerman et al.,[26] found that unlike true RA, ESR is elevated only in about half of the patients with HCV related arthritis. Bombardieri et al.[15] found higher

ESR level in the HCV associated arthritis group, which is similar to our results.

In our study, ANA was +ve in 25 patients (26.9%), anti ds-DNA in 9 patients (9.7%), RF in 81 patients (87.1%) and anti-CCP Ab in 38 patients (40.9%). Pawlotsky et al.[50] found that 50% - 70% of HCV- infected patients are positive for RF. In our study, RF positivity was comparable to that reported by Pawlotsky and others[51,52] who found that RF was positive in 71% of their patients. While, Clifford and his colleagues[52] reported a higher prevalence of RF positivity (76%). Al-Awadi et al.[24] found RF +ve in 47.5%, Buskila et al.[22] found RF +ve in 44%, and Cacoub et al.[27] found RF +ve in 36% of patients. Agarwal et al. (2001) in a study on 25 patients with chronic HCV infected patients found that 72% demonstrated autoimmune markers. RF and cryoglobulins were the most frequent detected markers in 32% of patients. Ramos-Casals et al.[32] in a study of 180 HCV infected patients with arthritis found; +ve ANA in 69%, RF in 56%, and anti-dsDNA in 15% of patients. Lormeau et al.[29] found RF in 50-80% of cases. Similarly, Sene et al.[33] also found RF positive in 50-80% of HCV infected cases.

Galossi et al.[53] found that autoimmune antibodies such as ANA, RF, anticardiolipin antibodies, cryoglobulins, anti-smooth muscle antibodies, anti-liver / kidney microsomal antibodies, and anti-thyroid antibodies are detected in 40-65% of patients with HCV infection. These antibodies are usually detected in the course of other autoimmune disease (i.e. autoimmune hepatitis, RA) which should be considered in the differential diagnosis.

As regarding Anti-Ro (SS-A) and anti-La (SS-B) antibodies, they were found positive in 2 out of 5 patients with sicca syndrome associated with HCV (40%). Ramos-Casals et al.[54] found positive anti-Ro (SS-A) and anti-La (SS-B) antibodies in 25% of patients with SS-HCV, a prevalence that is half that found in primary SS. This suggests that the main differential aspect between primary SS and SS-HCV is the immunological pattern, with a pre-

dominance of cryoglobulin related markers (mixed croglobulins, RF, and hypocomplementemia) over SS-related markers (anti-Ro and anti La auto-antibodies)[31].

In our study, ANA was found in 18 patients out of 59 patients with HCV associated arthritis (30.5%), while ANA was found in 7 patients out of 34 patients (20.6%) in HCV related arthritis group which is statistically insignificant. Anti ds-DNA was found in 9 patients out of 59 patients with HCV associated arthritis (15.3%), and it was found -ve in all patients with HCV related arthritis. Wilson et al.[55] found that ANA activity may be present with HCV related arthritis, while anti ds-DNA is not seen in those patients and this is similar to our results. Riccio et al.[56] found no significant difference regarding ANA between RA associated with HCV and HCV related arthritis, which is also similar to our results..

Our results also shows that RF was found in 53 patients out of 59 patients (89.8%), while RF was found in 28 patients out of 34 patients (82.4%) in HCV related arthritis group. Maillefert et al.[57] concluded that the high frequency of RF positivity in patients with HCV infection usually makes it difficult to make the differential diagnosis between RA associated with HCV and HCV related arthritis. In contrast to these results, Kaptanoglu et al.[40] found that there were significant differences between HCV related arthritis and RA regarding RF positivity.

As regarding Anti-CCP antibodies, it was found in 38 patients out of 59 patients (64.4%) and these patients were all diagnosed as RA associated with HCV, while it was -ve in all patients with HCV related arthritis, which can be useful in discriminating patients with RA from patients with HCV related arthritis. In agreement with our study, Schellekens et al., (58) have postulated that anti-CCP antibodies appear to be very specific for RA associated with HCV (96% - 98%). Similar to our results, Wener et al.[59] found that anti-CCP antibodies were always negative in patients with HCV related arthritis and they reported that some false positive results were observed in patients with

Cryoglobulinemia. Also, Bombardieri et al.[15] found anti-CCP antibodies in 60% of patients with RA associated HCV, but found none in HCV related arthritis patients, which is also similar to our results. Sene et al.[33] investigated the diagnostic reliability of anti-CCP antibodies in distinguishing HCV-related arthritis from RA and found that anti-CCP antibodies were the most specific biological marker for RA, and found -ve Anti-CP antibodies in more than 94% of patients with HCV related arthritis.

Liu et al.[39] observed that patients with RA were more likely to have higher incidence of anti-CCP antibodies (84%) than those with HCV related arthritis (8.8%). The difference between HCV related arthritis group and the RA group were statistically significant. The mechanism underlying presence of low titer of anti-CCP antibodies in patients with HCV related arthritis is unknown and may be related to a cross-reaction with CCP of some immunoglobulins (Ig) induced by HCV infection. Rebeski et al.[60] explained the false +ve ELISA results of Anti-CCP Ab by the non-specific binding of Ig to plastic micro titer plates. Kaptanoglu et al.[40] found that there were significant differences between HCV related arthritis and RA regarding Anti-CCP Ab, which is similar to our results.

Erosions were found in 38 patients who represent the RA subgroup of HCV associated arthritis (64.4%) diagnosed by X–ray and in some patients by MRI, while erosions were not found in HCV related arthritis group. Lovy et al.[61] found that HCV related arthritis, in contrast to RA, is not associated with articular bony erosions. Zuckerman et al.[34] also found that HCV related arthritis usually runs a relatively benign course that, in contrast to RA, is typically non-deforming and not associated with articular bony erosions. These findings were similar to that observed by Vassilopoulos and Calabrese[62] who found that patients with HCV related arthritis had rheumatoid like picture without erosive changes. Ferri et al.[28] found that arthritis was manifested as oligoarthritis predominating in the large joints, with no erosions. Mahmoud et

al.[36] found that HCV related arthritis was frequently associated with non-erosive arthritis. Also, Ramos-Casals et al.[31] found that RA associated with HCV patients had radiographic erosions in 89% of patients, but no erosions in HCV related arthritis group. Kaptanoglu et al.[40] found that the discrimination between HCV related arthritis and RA associated with HCV is by observing erosions in the RA associated arthritis group.

Cryoglobulins were done in 22 patients of the HCV related arthritis group. Cryoglobulins was +ve in 14 (63.6%) patients with HCV related arthritis and -ve in 8 (36.4%) patients. This is similar to that found by Ramos-Casals et al.[31] who found +ve cryoglobulins in 52% of HCV patients. Dammacco and Sansonno[63] found cryoglobulins in 41% to 84% of HCV infected patients. This is nearly similar to our results. Pawlotsky et al.[50] found cryoglobulins in 30%-50% of HCV patients. Lee et al.[23] found that the prevalence of cryoglobulins is around 50% in HCV patients. Anis et al.[64] found a significantly higher prevalence of cryoglobulins in HCV patients. Habib et al.[65] detected a lower figure of cryoglobulins (30%) in their HCV patients.

RF was +ve in 71.4% of the cryoglobulin +ve group and +ve in 12.5% of the cryoglobulin -ve group which is statistically significant when comparing the two groups. This can be explained by the fact that cryoglobulinemia produces colonal B lymphocyte expansion responsible for RF activity[66]. Pawlotsky et al.[50] reported +ve RF in 70% of HCV related arthritis patients which is nearly similar to our results and this was attributed to the presence of cryoglobulinemia, as RF is considered as one of the components of cryoglobulins. Wener et al.[59] demonstrated RF in 76% of cryoglobulin +ve patients compared to 44% in cryoglobulin -ve patients, which was statistically significant. Habib et al.[65] found a significant increase of +ve RF in cryoglobulin +ve patients when compared to cryoglobulin -ve patients. Iannuzzella and Garini[67] found that patients with mixed cryoglobulinemia have increased RF activity.

Ramos-Casals et al.[31] found +ve RF in 57% of HCV patients.

CRP was +ve in 78.6% of the cryoglobulin +ve group and +ve in 37.5% of the cryoglobulin -ve group which is statistically significant when comparing the two groups. These results were similar to that found by Weiner et al.[68] who provided the first evidence for the frequent occurrence of CRP in cryoglobulins of all three types according to Brouet's classification. ESR was higher in cryoglobulin +ve when compared to cryoglobulin -ve patients but it was statistically insignificant in both 1st and 2nd hours which is similar to that reported by Habib et al.[65] who found high ESR in cryoglobulin +ve patients when compared to cryoglobulin -ve patients.

A positive correlation between cryoglobulins and CRP (r=0.588, p=0.027) as well as between cryoglobulins and RF. Very similar to our results, Kassel et al.[69] found a positive correlation between CRP with the presence of RF and cryoglobulinemia. Habib et al.[65] found a positive correlation between cryoglobulins and RF.

Since RF, CRP and ESR are markers of disease activity as that stated by Lane and Gravel[70] and Breda et al.[71]. And since, Zuckerman et al.,[72] found that improvement of joint inflammation in HCV patients with +ve cryoglobulins was correlated with the reduction of cryoglobulin level in the serum indicating its role in disease activity. He found also that cryoglobulins become undetectable in 75% of patients, although a complete biochemical and virological response at the end of treatment was achieved in only 36% and 20% respectively. Elevated ESR, CRP, cryoglobulins and RF were found in 60 HCV infected patients with no correlation with aminotransferases activity[73].

Our results confirm that there is an inter - relation between Cryoglobulinemia and RF, CRP, ESR, indicating its role in disease activity and may be useful for monitoring therapeutic response.

From this study we can conclude that HCV associated arthritis can be misdiagnosed as RA, especially as 50% of patients fulfill the ACR criteria of diagnosis of

RA. Patients represented with: arthritis which may be oligoarticular affecting big joints, arthritis similar to RA but non erosive or non deforming or elevated liver enzymes should be screened for HCV infection. Anti-CCP antibodies are one of the markers that help to differentiate HCV associated arthritis from RA. Cryoglobulins together with CRP, ESR, and RF are considered as markers of disease activity in HCV related arthritis.

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STUDY OF CHRONIC INFLAMMATORY ARTHRITIS IN EGYPTIAN PATIENTS WITH HEPATITIS C VIRUS INFECTION

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Abstract

Background and Aim: *Autoimmunity and viral infections are closely related fields, and viruses have been proposed as possible etiological or triggering agents of auto-immune diseases. The aim of this work is to study the prevalence and describe the characteristics of rheumatoid arthritis (RA) in Egyptian patients associated with HCV infection with recent available laboratory and radiological investigations and to compare them with the HCV related arthritis.*

Patients and Methods: *1100 hepatitis C virus infected patients participated in this study. Among these patients, 93 (8.45%) patients had arthritis (34 patients had HCV related arthritis and 59 patients had HCV with RA, SLE or SS). Those patients represented the target population for this study.*

Results: *The mean age of the patients with HCV related arthritis was 50.3 ±12.7 years compared to 46.7 ±13.4 years in the patients having HCV associated with RA, SLE or SS (p>0.05). The female/male ratio in the patients had HCV related arthritis was 26/8 while in patients of HCV associated arthritis was 55/4 (p>0.05). Sleep disturbance was more frequent in the HCV related arthritis group of patients. Fever, hepatomegally and spleenomegally were more frequent also in the HCV related arthritis group. Pattern of arthritis, tenosnovitis, myalgia and fibrimyalgia are significantly different between the 2 groups. Also HAQ, quality of life and VAS are significantly different between HCV related arthritis and HCV with RA patients. As regards the laboratory findings,*

the hemoglobin concentration, WBCs count, platelets, albumin and bilirubin are significantly different between the 2 groups of patients. Anti-CCP and cyroglobulinemia are significantly different between the 2 groups. Erosions and deformity are present only in HCV patients with RA.

Conclusion : *HCV associated arthritis can be misdiagnosed as RA, especially as 50% of patients fulfill the ACR criteria of diagnosis of RA. Patients represented with: arthritis which may be oligoarticular affecting big joints, arthritis similar to RA but non erosive or non-deforming or elevated liver enzymes should be screened for HCV infection. Anti-CCP antibodies are one of the markers that help to differentiate HCV associated arthritis from RA. Cryoglobulins together with CRP, ESR, and RF are considered as markers of disease activity in HCV related arthritis.*

Keywords HCV. *Rheumatoid arthritis.*

Introduction

Hepatitis C virus (HCV) was 1st identified in 1989 as a lymphotropic and hepatotropic single stranded ribonucleic acid (RNA) virus that infects peripheral blood lymphocytes as well as hepatocytes [(1)]. HCV is a rapidly mutating virus, with several subtypes that differ according to geographic distribution [(2)].

HCV is a global health problem affecting 3% of the world's population (about 180 millions)[(3)]. Egypt has the highest prevalence of HCV infection in the world ranging from 12% to 24% in the general population[(4)]. More than 90% of HCV infections in Egypt are due to genotype 4[(5)]. The majority of infected individuals are unable to eliminate the virus. Around 20% of them will go on to develop hepatic fibrosis and eventually cirrhosis and there is a 3-5% annual risk of the development of hepatocellular carcinoma. Infection with the HCV has become the principal indication for liver transplantation[(6)].

In addition to its hepatic effects, chronic infection with the HCV is responsible for numerous extrahepatic manifestations[(7)], of

which the rheumatological manifestations are amongst the most frequent. Recently, there is outgrwoing interest in the relationship between chronic HCV infection and systemic autoimmune diseases (SAD) such as Sjogren's syndrome (SS), rheumatoid arthritis (RA), polyarteritis nodosa, systemic lupus erythematosus (SLE) and the antiphospholipid syndrome. However, the bulk of the data are based on small sample groups and case studies.

The aim of this work is to determine the prevalence and to study the characteristics of chronic inflammatory arthritis in Egyptian patients with HCV infection in order to reach the proper diagnosis with recent available laboratory and radiological investigations, with subsequent modificat-ion of future therapeutic regimens

Another suggested aim

The aims of this study were to describe the patterns of disease expression in a large series of patients with SAD associated with chronic HCV infection and to analyse the epidemiological, clinical and immunological implications of this association.

Patients and Methods

Patients recruitment

This study comprised 1100 (684 males and 416 females) patients who had serological evidence of chronic HCV infection: at least two positive determinations by ELISA[8] and/or positive HCV-RNA by polymerase chain reaction (PCR)[9]. Their ages ranged between 22-69 years. They were collected from either in-patient or out-patient Hepatology clinic and from the Rheumatology and Rehabilitation outpatient clinic of Mansoura University Hospitals between June 2008 and October 2009. All patients are screened for evidence of arthritis. Those patients with arthritis represented the target population for this study.

We excluded patients with other possible causes of arthropathy. So, none of the 93 HCV infected patients had any endocrinal abnormalities including diabetes mellitus (DM) (by blood sugar level) and thyroid function (by thyroid stimulating

hormone level), recent history of systemic infection, hepatitis B virus infection (by hepatitis B surface antigen), hepatocellular carcinoma (by Alfa Fetoprotein level), amyloidosis, sarcoidosis, hemochromatosis, haemophilia, haemoglobinopathies., malignancies, familial auto-inflammatory disorders, crystal induced arthropathies. None of our female patients were pregnant nor lactating (pregnancy test was done to exclude pregnancy).

Clinical Assessment.

The eligible participants patients with arthritis completed preliminary questionnaires inquiring into patient demographics, the history of the hepatitis, cumulative hepatic and extrahepatic manifestations during disease evolution (from diagnosis until inclusion) and treatments received, the rheumatological manifestations (arthralgia, arthritis, myalgia, deformities, tenosynovitis) and the effects of treatments on these manifestations. The questions were precise enough to distinguish between inflammatory and mechanical joint disease.

Pain severity was assessed using visual analogue scale (VAS)[10]. Joint tenderness was assessed using Ritchie articular index (RAI)[11]. Functional assessment or ability is measured by using Stanford Health Assessment Questionnaire (HAQ) which covers nine general component categories (dressing and grooming, arising, eating, walking, hygiene, reach, grip, outside activity, and sexual activity)[12]. Quality of life (qol) was assessed using questionnaire composed of 30 items with a yes / no response[13]. The overall score is the sum of the individual item scores (range 0-30), with a lower score indicating better Qol.

Laboratory assessment

Immunological parameters such as rheumatoid factor (RF)[14], anti-cyclic citrullinated peptides antibodies (Anti-CCP Ab)[15], antinuclear antibodies (ANA) (specifically anti - SSA / SSB)[16], cryoglobulin[17] and complement levels were measured for nearly all patients with arthritis, as well as erythrocyte sedimentation rate (ESR)[18] and C-reactive protein (CRP) values[19]. Serum creatinine and liver

enzymes were also evaluated. Anti HCV antibodies were assessed[8], viral activity of the hepatitis was assessed by the transaminase level and the viral load measured by PCR[9]. For a proportion of patients a liver biopsy was performed with an evaluation of the degree of histological activity and fibrosis with the 'Metavir' score (20), which measures the activity score (A) from 0-3 and the fibrosis score (F) from 0-4.

Radiological assessment

Plain radiographs were done by the standard views comprising antero-posterior, lateral and oblique projections of both hands and feet. Magnetic resonance imaging (MRI) was requested when image cuts were needed to establish a diagnosis. Liver and spleen states are evaluated using abdominal ultrasound.

Statistical analysis :

The data were entered into a computer and analyzed using SPSS version 17.0 (SPSS Inc, Chicago, IL). Descriptive statistics consisted of numbers (percentages) for qualitative variables and means (±SD) for quantitative variables. Qualitative variables were compared using the chi-square test. To compare quantitative variables, we used independent-sample Student's t test. Pearson correlation test was used to determine the correlation between variables. P values smaller than 0.05 were considered significant.

Results

1100 HCV infected patients participated in this study. Among these patients, 93 (8.45%) patients had arthritis. Eighty one (87%) patients were females and 12 (13%) males with a mean age of 48.36 ± 11.61 years and BMI 31.38 ± 3.51 kmg/m^2. At entry of study, the duration of HCV infection was 4.54 ±4.33 years while the duration of arthritis was 29.04±14.4 months.

These 93 patients were classified into 2 groups: (i) Group I: Patients with HCV associated arthritis (n=59) distributed in three subgroups: Rheumatoid arthritis (RA) associated with HCV infection (RA-HCV) (n=42, 45.2%), Systemic lupus erythematosus (SLE) associated with HCV infection (SLE-HCV) (n=12, 12.9%) and Sjogren's

syndrome (SS) associated with HCV infection (SS-HCV) (n=5, 5.4%) and (ii) Group II: Patients with HCV related arthritis (n=34).

Arthritis was diagnosed in older ages in HCV related arthritis than in HCV associated arthritis (46.7 ±13.4 vs. 50.3 ±12.7 respectively). This difference was insignificant. The female : male ratio of HCV associated arthritis was 55:4 compared to 26:8 in HCV related arthritis. This difference was also insignificant.

Table 1 shows no significant difference between HCV associated arthritis group and HCV related arthritis group as regarding; fatigue, malaise, morning stiffness, Raynaud's phenomenon, oral ulcers, sicca symptoms, DM, or hypertension (P>0.05). Significant differences were found between the two groups as regarding; myalgia (P<0.001), sleep disturbance (P<0.001), and photosensitivity (P<0.05).

Table 2 showed significant difference (P<0.05) as regarding fever, subcutaneous nodules, and malar rash. There was insignificant difference (P>0.05) as regarding jaundice when comparing the two groups.

Table 3 showed significant difference as regarding hepatomegally (P<0.001) and splenomegally (P<0.01) when comparing the two groups.

Musculoskeletal deformities was found in 42.9% of the HCV associated arthritis group while none of the HCV related arthritis group had deformities (P<0.001). Fibromyalgia was significantly higher in the HCV related arthritis group (61.8%) when compared to HCV associated arthritis group (10.2%) (P<0.001). No significant difference was found between the two groups as regarding tenosynovitis and cutaneous vasculitis.

Also, no significant difference was found between the two groups as regarding the pattern of arthritis; however, involvement of proximal interphalangeal joints (PIPs) metacarpophalangeal joints (MCPs), wrist joints and ankles is significantly higher in the HCV associated arthritis group when compared to HCV related arthritis

group. Elbows, shoulders, hips, knees, and metatarsophalangeal joints are affected in both groups with no significant differences (Table 4).

Table 5 shows that there is significant difference between the two groups as regarding HAQ, Qol, VAS, and RAI.

As regarding hemoglobin concentration, white blood cells count, and platelets counts, there were significant differences in HCV associated arthritis group compared to HCV related arthritis group ($P<0.05$, $P<0.001$, and $P<0.05$ respectively). ESR and CRP were elevated in both groups but it was statistically insignificant ($P>0.05$). Hepatic transaminases are elevated with no significant difference ($P>0.05$) and elevated bilirubin in both groups ($P<0.01$) (Table 6).

Table 7 shows that ANA was found in 18 patients out of 59 patients (30.5%), while ANA was found in 7 patients out of 34 patients (20.6%) in HCV related arthritis group ($P>0.05$). Anti-ds DNA was found in 9 patients out of 59 patients (15.3%), -ve in all patients with HCV related arthritis ($P<0.05$). RF was found in 53 patients out of 59 patients (89.8%), while RF was found in 28 patients out of 34 patients (82.4%) in HCV related arthritis group ($P>0.05$). Anti-CCP Ab was found in 38 patients out of 59 patients (64.4%) and these patients were all diagnosed as RA associated with HCV, while Anti-CCP Ab was -ve in all patients with HCV related arthritis ($P<0.001$), which may help to differentiate the two conditions.

Table 8 shows that erosions were found only in RA subgroup of HCV associated arthritis group & it was statistically significant ($P<0.001$).

Table 9 shows a significant increase of CRP in cryoglobulin +ve patients when compared to cryoglobulin -ve group (<0.05), and also a significant increase of RF in cryoglobulin +ve patients when compared to cryoglobulin -ve group ($P<0.01$). ESR was higher in cryoglobulin +ve when compared to cryoglobulin -ve patients but it was statistically insignificant ($P>0.05$) in both 1^{st} and 2^{nd} hours.

From table 10 we can detect a positive correlation between cryoglobulins and CRP (r=0.588, p=0.027) and a positive correlation between cryoglobulins and RF (r=0.820, p=0.000).

Table 1 : Comparison between HCV associated arthritis and HCV related arthritis as regarding history findings (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Fatigue	36	61	18	52.9	NS
Malaise	22	37.3	12	35.3	NS
Myalgia	7	11.9	17	50	<0.001
Sleep disturbance	20	33.9	24	70.6	<0.001
Morning stiffness	26	44	9	26.5	NS
Raynaud's	13	22	5	14.7	NS
Oral ulcers	4	6.8	0	0	NS
Photosensitivity	8	13.6	0	0	0.0247
Sicca symptoms	5	8.5	0	0	NS
DM	9	15.3	7	20.6	NS
Hypertension	10	16.9	8	23.5	NS

Table 2. Comparison between HCV associated arthritis and HCV related arthritis as regarding general examination (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Fever	8	13.6	12	35.3	0.014
Jaundice	4	6.8	6	17.6	NS
Subcutaneous nodules	10	16.9	0	0	0.011
Malar rash	10	16.9	0	0	0.011

Table 3. Comparison between HCV associated arthritis and HCV related arthritis as regarding abdominal examination (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Hepatomegally	8	13.6	17	50	<0.001
Spleenomegally	3	2.1	8	23.6	0.0079
Ascitis	1	1.7	2	5.9	NS

Table 4: Comparison between HCV associated arthritis & HCV related arthritis as regarding musculoskeletal manifestations (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Arthritis	59	100	34	100	NA
Pattern of arthritis					
Mono-oligoarthritis	17	28.8	12	35.3	NS
Polyarthritis	42	71.2	22	64.7	NS
Deformities	18	42.9	0	0	<0.001
Distribution of arthritis					
PIPj	39	66.1	3	8.8	<0.001
MCPj	39	66.1	4	11.8	<0.001
Wrists	43	72.9	5	14.7	<0.001
Elbows	9	15.3	2	5.9	NS
Shoulders	2	3.4	4	11.8	NS
Hips	1	1.7	2	5.9	NS
Knees	15	25.	6	11.1	NS
Ankles	9	15.3	5	14.7	0.0194
MTPj	4	6.8	2	5.9	NS
Tenosynovitis	9	15.3	3	8.8	NS
Cutaneous vasculilits	2	3.4	0	0	NS
Fibromyalgia	6	10.2	21	61.8	<0.001

Table 5. Comparison between HCV associated arthritis & HCV related arthritis as regarding disease activity indices (student's t test)

	HCV associated arthritis		HCV related arthritis		P
	mean	±SD	mean	±SD	
HAQ	16.54	±3.95	14.12	±3.33	0.0034
Qol	21.48	±3.10	18.81	±3.85	<0.001
VAS	3.14	±0.15	2.01	±0.11	<0.001
RAI	10.54	±3.98	8.12	±3.53	0.003

Table 6. Comparison between HCV associated arthritis & HCV related arthritis as regarding laboratory findings (student's t test)

	HCV associated arthritis		HCV related arthritis		P
	mean	±SD	mean	±SD	
Hemoglobin	11.12	1.37	10.51	1.36	0.041
Red blood cells.	4.11	0.54	4.33	0.64	NS
White blood cells.	5.62	1.62	4.37	±1.62	<0.001
Platelets	205.41	50.21	231.34	±61.12	0.0294
ESR (1st hour)	44.34	17.64	48.88	±22.01	NS
ESR (2nd hour)	72.13	14.51	77.11	±16.13	NS
CRP (n, %)	48	81.4	22	64.7	NS
Bilirubin (n, %)	12	20.3	17	50	0.0029
SGOT	44.23	16.27	48.33	±16.11	NS
SGPT	41.21	20.33	45.02	±13.08	NS
Albumin	3.31	1.01	3.78	±0.25	0.0092
Creatinine	0.81	0.1	0.85	±0.15	NS

Table 7. Comparison between HCV associated arthritis & HCV related arthritis as regarding immunological findings (chi square test)

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
ANA	18	30.5	7	20.6	NS
Anti-ds DNA	9	15.3	0	0	0.0165
Anti HCV Ab	59	100	34	100	NA
RF	53	89.8	28	82.4	NS
Anti-CCP Ab	38	64.4	0	0	<0.001
Cryoglobulins (n=22)	-	-	14	63.6	-
Anti-Ro/La (n=5)	2	40	-	-	-

Table 8. Comparison between HCV associated arthritis and HCV related arthritis as regarding radiological findings (chi square test).

	HCV associated arthritis		HCV related arthritis		P
	n	%	n	%	
Erosion	38	64.4	0	0	<0.001

Table 9. Comparison between cryoglobulin +ve cryoglobulin –ve patients in the group of HCV related arthritis (n=22)

	Cryoglobulin -ve HCV patients (n= 8)	Cryoglobulin +ve HCV Patients (n=14)	P
CRP *(n, %)*	3 (37.5%)	11 (78.6%)	0.0139*
RF *(n, %)*	1 (12.5%)	10 (71.4%)	0.0078*
ESR 1st hour *(mean ±SD)*	42.7±9.1	49.3±10.5	NS**
ESR 2nd hour *(mean ±SD)*	65.1±8.4	73.1±12.4	NS**
** Chi square test, ** Student's t test.*			

Table 10 : Correlation between cryoglobulin +ve patients, CRP, and RF in the group of HCV related arthritis

	CRP		RF	
	r	P	r	P
Cryoglobulins	0.588	0.027	0.820	0.000
CRP	-	-	0.577	0.031
RF	0.577	0.031	-	-

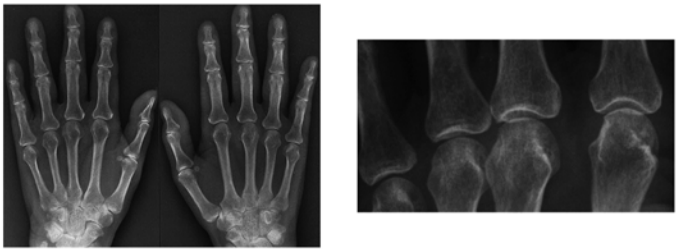

Figure 1. X-ray features of rheumatoid arthritis including evidence of soft tissue swelling of the MCP and PIP joints, periarticular osteoporosis, symmetric joint space narrowing of several MCP and PIP joints of both hands, and erosions of the right 3rd and right and left 4th MCP joints

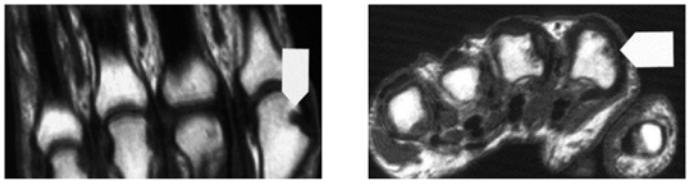

Figure (4): Coronal and axial T1-weighted spin echo MRI scans show bony erosions at the radial aspect of the second metacarpal bone (arrow).

Discussion

The current study included 1100 HCV infected patients as proven by presence of anti HCV antibodies and +ve PCR. Among these patients, 93 patients (8.45%) had arthritis. Those patients represented the target population for this study.

Garcia-Monforte and associates[21] described only 2% incidence of frank arthritis, and reported 52% incidence of arthralgia. Buskila and co-workers[22] found arthritis in 4% of HCV infected patients. Lee et al.[23] found a higher prevalence of arthralgia and arthritis (35%) in patients with HCV infection. This high prevalence value may be attributed to studying the prevalence of both arthralgia and arthritis. In another study conducted in the Middle East region by Al-Awadi and others in 1998[24] on 40 patients with chronic HCV whose age group ranged between 30 - 50 years, polyarthralgia and polyarthritis were the most common rheumatic manifestations (35%). On the other hand, Rivera et al.[25] have reported a frequency of HCV infection (7.6%) among 303 patients with RA. Zuckerman et al.[26] diagnosed arthritis in 28 (11.4%) patients who were recruited from 245 patients with

HCV infection attending the liver clinic.

Cacoub et al.,[27] found that the rheumatologic manifestations represented 38% of the patients, 19% suffered from arthralgia and arthritis. Ferri et al.,[28] found that arthralgia was present in 72% of patients at baseline, while arthritis was detected in 8% of patients. Arthritis was manifested as oligo-arthritis predominating in the large joints, with no erosions or distinctive clinical features. Lormeau et al.,[29] found that the prevalence of chronic inflammatory arthritis across different studies ranged from 4% to 11%. Palazzi et al.,[30] found that although asymptomatic joint involvement and arthralgias are frequent in patients with HCV chronic infection, a true arthritis affects only 4% of the patients.

In our study the number of patients fulfilling the criteria for diagnosis of HCV associated RA, SLE, or SS was 59 patients (5.4% out of 1100 HCV patients and 36.4% out of 93 patients with arthritis). This was nearly similar to that found by Ramos-Casals et al.[31] who found that the prevalence of patients fulfilling the criteria for diagnosis of HCV associated RA, SLE, and SS among 1020 HCV-infected patients was 2% - 6%.

In our study, the 93 patients with arthritis were diagnosed by clinical examination, laboratory investigations and radiological evaluation and classified into 2 groups; HCV associated arthritis group (59 patients) [RA in 42 patients (45.2%), SLE in 12 patients (12.9%), and Sicca syndrome in 5 patients (5.4%)] and HCV related arthritis group (34 patients).

Cacoub et al.,[27] screened a total of 411 HCV patients, and found that the most frequently identified SAD were SS (n=10) and SLE (n=6). That of SLE-HCV is similar to that found in our study. Ramos-Casals and Font [32] found that prevalence of HCV associated SAD (RA, SLE, and SS) varies widely according to geographic area. The best example is SS: Studies from Europe describe a prevalence of 10%-20%, while studies from USA found a prevalence of <1%, probably due to the lower prevalence of HCV infection

in these countries. Sene et al., [33] specifically investigated SS in a total of 216 HCV patients, and found 11 (5%) fulfilled classification criteria for SS, a prevalence 5 to 10 folds greater than that found in general population.

In this study, the age of our patients ranged between 22 years to 69 years (mean 48.36±11.61). They were 81 females (87.1%) and 12 males (12.9%). Their BMI ranged from 24.16 to 41.79 kgm/m^2 (mean 31.38±3.51). The duration of HCV infection ranged from 1 to 15 years (mean 4.54±4.33) and the duration of arthritis ranged from 6 months to 4 years (mean 2.42±1.2). In our study, the prevalence of arthritis in our patients was higher in females than males in all studied groups and that arthritis was diagnosed in older ages in HCV related arthritis than in HCV associated arthritis. But these differences were insignificant.

Ramos-Casals et al.[32] studied 180 SAD associated with HCV cases; 130 (72%) patients were females and 50 (28%) were males. The mean age at diagnosis of arthritis was 50.3±1.3 years and at HCV infection diagnosis was 53.7±1.2 years. These differences may be due to geographical distribution as the patients in this study were from Spain.

The majority of studies reporting the prevalence of chronic HCV infection in patients with SAD found a higher prevalence than that in the general population. This can be explained on the basis that viruses are often proposed as etiologic or triggering agents of SAD. About 70% of all reported cases of SAD in patients with HCV infection came from Mediterranean countries [31].

As regarding history findings in our patients, fatigue was found in 88 (94.6%) patients, photosensitivity in 8 (8.6%) patients, Raynaud's phenomenon was found in 18 (19.4%) patients, sicca symptoms in 5 (5.4%) patients, and oral ulcers in 4 (4.3%) patients. Lee and colleagues[23] conducted a study on 49 Korean patients with HCV and observed that cutaneous manifestations were found in 37% of patients, Raynaud's phenomenon in 8%, dry eyes in 22%, dry

mouth in 10%, and oral ulcers in 33%. The prevalence of their manifestations was slightly higher than that in our study. This may be due to geographic distribution and genetic basis.

In our study, myalgia was found in 34 (36.6%) patients. Buskila and co-workers[22] reported myalgia in 24 % of patients, while Cacoub et al.,[27] found Myalgia in 2% of patients. Morning stiffness lasting more than1 hour is present in 35 patients (37.6%). Zuckerman et al.,[34] also found that morning stiffness affects two thirds of HCV infected patients and may be severe lasting for more than one hour.

As regarding liver involvement there was; hepatomegally in 25 (26.9%), splenomegally in 11 (11.8%), and jaundice in 10 (10.8%). Ascitis was found in 3 (3.2%) patients. In agreement with our study, Ramos-Casals et al.[32] demonstrated hepatomegally in 26%, spleenomegally in 12%, jaundice in 6% of patients, and only 9% had other manifestations of hepatic decompensation (ascitis, encephalopathy, and gastro-intestinal bleeding).

In our study, fibromyalgia was found in 27 (29%) patients. Fibromyalgia was significantly higher in the HCV related arthritis group (61.8%) when compared to HCV associated arthritis group (10.2%). 12 patients (12.9%) of our patients had evidence of tenosynovitis, 2 patients (2.2%) from SLE-HCV subgroup had cutaneous vasculitis and 18 patients (19.4%) from RA-HCV subgroup had musculoskeletal deformities. Al-Awadi and others in 1998[24] studied 40 patients with chronic HCV and found cutanous vasculitis in 50% of their patients. Buskila and co-workers[22] found sicca symptoms in 8%, cutaneous vasculitis in 2%, polymyositis in 1%, and fibromyalgia was diagnosed in 16% of patients. Goulding et al.[35] found fibromyalgia in 5% of the studied patients. Cacoub et al.[27] found Myalgia in 2%, which is lower than in our study, may be due to that their patients came exclusively from Internal Medicine and Infectious Diseases Departments while in our study they came also from Rheumatology clinic, and so the main complaint was

rheumatologic symptoms.

Zuckerman et al.[34] found that HCV related arthritis usually runs a relatively benign course that, in contrast to RA, is typically non-deforming. Mahmoud et al.[36] found that HCV related arthritis was frequently associated with non-deforming arthritis, which is similar to that found in our study.

In our study, the pattern of arthritis were mono-oligoarthritis in 29 patients (31.2%) and polyarthritis in 64 patients (68.8%). Euller-Ziegler et al.[37] found that there is a high incidence of arthritis in patients with HCV infection: 6.4% had oligoarthritis, and 20.6% had polyarthritis. Fadda et al.[38] observed that there is no uniform clinical picture of arthritis in patients with chronic HCV infection; but two different patterns of inflammatory arthritis are found, one mimicking RA and another oligoarticular type affecting large joints. While, Sene et al.[33] stated that there are two types of articular involvement in the course of HCV infection: a polyarthritis involving small joints that resembles RA, usually milder and frequently meets the ACR classification criteria for RA, and a non erosive oligoarthritis involving the medium-sized and large joints. Also, Liu et al.[39] found that HCV infection may induce extra hepatic manifestations, including polyarthritis that mimic RA. Kaptanoglu et al.[40] found that HCV infection induces EHMs including arthritis which can mimic RA, and concluded that the discrimination between HCV related arthritis and RA is difficult.

In our study, involvement of PIPs MCPs, wrist and ankle joints are significantly higher in the HCV associated arthritis group when compared to HCV related arthritis group. Elbows, shoulders, hips, knees, and metatarsophalangeal joints are affected in both groups with no significant differences. Sawada et al.[41] found that in most HCV patients, soft tissue swelling and synovitis of large joints are present, most commonly involving the wrists, shoulders, ankles and knees. Zuckerman et al.[26] found that the joints most commonly involved in HCV related arthritis are the MCPs, PIPs,

wrists and ankles. Arthritis related to HCV infection most often affects PIPs, MCPs, and knees[29]. While musculoskeletal deformities were significantly higher in the HCV associated arthritis group when compared to HCV related arthritis group. No significant difference was found between the 2 groups as regarding tenosynovitis and cutanous vasculitis.

Our study revealed a significant difference between the two groups as regarding HAQ, Qol, VAS, and RAI. Similarly, Kozanoglu et al.[42] found that pain intensity scored on a VAS and sleep disturbance were higher in the HCV group. Mohamed et al.[43] did not find a significant reduction of Qol and VAS of arthritis in patients with chronic HCV infection. Also, Thein et al.[44] found no statistically significant difference in grades of VAS and Qol in HCV infected patients. Ferri et al.[45] found high values of VAS and RAI in HCV associated arthritis. Basseri et al.[46] found that HCV poses an increasing healthcare burden associated with maladaptive lifestyle habits and poor Qol. Similarly, Sinakos et al.[47] showed that Qol in HCV related arthritis patients was worse than that of HCV associated arthritis patients. Pain intensity measured by VAS was higher in HCV associated arthritis group[48].

As regarding hemoglobin, white blood cells, and platelets, there were significant differences in HCV associated arthritis group compared to HCV related arthritis group. This is similar to that found by Ramos-Casals et al.[32] who found cytopenias in 26% of patients with HCV associated with autoimmune diseases. And also similar to that found by Ufearo et al.[49]

Our results shows elevated hepatic transaminases and elevated bilirubin in both groups with no significant difference, which is similar to that found by Ramos-Casals et al.[32]. ESR was elevated in both group but it was statistically insignificant which is similar to that found by Ufearo et al.[49]. Zuckerman et al.,[26] found that unlike true RA, ESR is elevated only in about half of the patients with HCV related arthritis. Bombardieri et al.[15] found higher

ESR level in the HCV associated arthritis group, which is similar to our results.

In our study, ANA was +ve in 25 patients (26.9%), anti ds-DNA in 9 patients (9.7%), RF in 81 patients (87.1%) and anti-CCP Ab in 38 patients (40.9%). Pawlotsky et al.[50] found that 50% - 70% of HCV- infected patients are positive for RF. In our study, RF positivity was comparable to that reported by Pawlotsky and others[51,52] who found that RF was positive in 71% of their patients. While, Clifford and his colleagues[52] reported a higher prevalence of RF positivity (76%). Al-Awadi et al.[24] found RF +ve in 47.5%, Buskila et al.[22] found RF +ve in 44%, and Cacoub et al.[27] found RF +ve in 36% of patients. Agarwal et al. (2001) in a study on 25 patients with chronic HCV infected patients found that 72% demonstrated autoimmune markers. RF and cryoglobulins were the most frequent detected markers in 32% of patients. Ramos-Casals et al.[32] in a study of 180 HCV infected patients with arthritis found; +ve ANA in 69%, RF in 56%, and anti-dsDNA in 15% of patients. Lormeau et al.[29] found RF in 50-80% of cases. Similarly, Sene et al.[33] also found RF positive in 50-80% of HCV infected cases.

Galossi et al.[53] found that autoimmune antibodies such as ANA, RF, anticardiolipin antibodies, cryoglobulins, anti-smooth muscle antibodies, anti-liver / kidney microsomal antibodies, and anti-thyroid antibodies are detected in 40-65% of patients with HCV infection. These antibodies are usually detected in the course of other autoimmune disease (i.e. autoimmune hepatitis, RA) which should be considered in the differential diagnosis.

As regarding Anti-Ro (SS-A) and anti-La (SS-B) antibodies, they were found positive in 2 out of 5 patients with sicca syndrome associated with HCV (40%). Ramos-Casals et al.[54] found positive anti-Ro (SS-A) and anti-La (SS-B) antibodies in 25% of patients with SS-HCV, a prevalence that is half that found in primary SS. This suggests that the main differential aspect between primary SS and SS-HCV is the immunological pattern, with a pre-

dominance of cryoglobulin related markers (mixed croglobulins, RF, and hypocomplementemia) over SS-related markers (anti-Ro and anti La auto-antibodies)[31].

In our study, ANA was found in 18 patients out of 59 patients with HCV associated arthritis (30.5%), while ANA was found in 7 patients out of 34 patients (20.6%) in HCV related arthritis group which is statistically insignificant. Anti ds-DNA was found in 9 patients out of 59 patients with HCV associated arthritis (15.3%), and it was found -ve in all patients with HCV related arthritis. Wilson et al.[55] found that ANA activity may be present with HCV related arthritis, while anti ds-DNA is not seen in those patients and this is similar to our results. Riccio et al.[56] found no significant difference regarding ANA between RA associated with HCV and HCV related arthritis, which is also similar to our results..

Our results also shows that RF was found in 53 patients out of 59 patients (89.8%), while RF was found in 28 patients out of 34 patients (82.4%) in HCV related arthritis group. Maillefert et al.[57] concluded that the high frequency of RF positivity in patients with HCV infection usually makes it difficult to make the differential diagnosis between RA associated with HCV and HCV related arthritis. In contrast to these results, Kaptanoglu et al.[40] found that there were significant differences between HCV related arthritis and RA regarding RF positivity.

As regarding Anti-CCP antibodies, it was found in 38 patients out of 59 patients (64.4%) and these patients were all diagnosed as RA associated with HCV, while it was -ve in all patients with HCV related arthritis, which can be useful in discriminating patients with RA from patients with HCV related arthritis. In agreement with our study, Schellekens et al., (58) have postulated that anti-CCP antibodies appear to be very specific for RA associated with HCV (96% - 98%). Similar to our results, Wener et al.[59] found that anti-CCP antibodies were always negative in patients with HCV related arthritis and they reported that some false positive results were observed in patients with

Cryoglobulinemia. Also, Bombardieri et al.[15] found anti-CCP antibodies in 60% of patients with RA associated HCV, but found none in HCV related arthritis patients, which is also similar to our results. Sene et al.[33] investigated the diagnostic reliability of anti-CCP antibodies in distinguishing HCV-related arthritis from RA and found that anti-CCP antibodies were the most specific biological marker for RA, and found -ve Anti-CP antibodies in more than 94% of patients with HCV related arthritis.

Liu et al.[39] observed that patients with RA were more likely to have higher incidence of anti-CCP antibodies (84%) than those with HCV related arthritis (8.8%). The difference between HCV related arthritis group and the RA group were statistically significant. The mechanism underlying presence of low titer of anti-CCP antibodies in patients with HCV related arthritis is unknown and may be related to a cross-reaction with CCP of some immunoglobulins (Ig) induced by HCV infection. Rebeski et al.[60] explained the false +ve ELISA results of Anti-CCP Ab by the non-specific binding of Ig to plastic micro titer plates. Kaptanoglu et al.[40] found that there were significant differences between HCV related arthritis and RA regarding Anti-CCP Ab, which is similar to our results.

Erosions were found in 38 patients who represent the RA subgroup of HCV associated arthritis (64.4%) diagnosed by X–ray and in some patients by MRI, while erosions were not found in HCV related arthritis group. Lovy et al.[61] found that HCV related arthritis, in contrast to RA, is not associated with articular bony erosions. Zuckerman et al.[34] also found that HCV related arthritis usually runs a relatively benign course that, in contrast to RA, is typically non-deforming and not associated with articular bony erosions. These findings were similar to that observed by Vassilopoulos and Calabrese[62] who found that patients with HCV related arthritis had rheumatoid like picture without erosive changes. Ferri et al.[28] found that arthritis was manifested as oligoarthritis predominating in the large joints, with no erosions. Mahmoud et

al.[36] found that HCV related arthritis was frequently associated with non-erosive arthritis. Also, Ramos-Casals et al.[31] found that RA associated with HCV patients had radiographic erosions in 89% of patients, but no erosions in HCV related arthritis group. Kaptanoglu et al.[40] found that the discrimination between HCV related arthritis and RA associated with HCV is by observing erosions in the RA associated arthritis group.

Cryoglobulins were done in 22 patients of the HCV related arthritis group. Cryoglobulins was +ve in 14 (63.6%) patients with HCV related arthritis and -ve in 8 (36.4%) patients. This is similar to that found by Ramos-Casals et al.[31] who found +ve cryoglobulins in 52% of HCV patients. Dammacco and Sansonno[63] found cryoglobulins in 41% to 84% of HCV infected patients. This is nearly similar to our results. Pawlotsky et al.[50] found cryoglobulins in 30%-50% of HCV patients. Lee et al.[23] found that the prevalence of cryoglobulins is around 50% in HCV patients. Anis et al.[64] found a significantly higher prevalence of cryoglobulins in HCV patients. Habib et al.[65] detected a lower figure of cryoglobulins (30%) in their HCV patients.

RF was +ve in 71.4% of the cryoglobulin +ve group and +ve in 12.5% of the cryoglobulin -ve group which is statistically significant when comparing the two groups. This can be explained by the fact that cryoglobulinemia produces colonal B lymphocyte expansion responsible for RF activity[66]. Pawlotsky et al.[50] reported +ve RF in 70% of HCV related arthritis patients which is nearly similar to our results and this was attributed to the presence of cryoglobulinemia, as RF is considered as one of the components of cryoglobulins. Wener et al.[59] demonstrated RF in 76% of cryoglobulin +ve patients compared to 44% in cryoglobulin -ve patients, which was statistically significant. Habib et al.[65] found a significant increase of +ve RF in cryoglobulin +ve patients when compared to cryoglobulin -ve patients. Iannuzzella and Garini[67] found that patients with mixed cryoglobulinemia have increased RF activity.

Ramos-Casals et al.[31] found +ve RF in 57% of HCV patients.

CRP was +ve in 78.6% of the cryoglobulin +ve group and +ve in 37.5% of the cryoglobulin -ve group which is statistically significant when comparing the two groups. These results were similar to that found by Weiner et al.[68] who provided the first evidence for the frequent occurrence of CRP in cryoglobulins of all three types according to Brouet's classification. ESR was higher in cryoglobulin +ve when compared to cryoglobulin -ve patients but it was statistically insignificant in both 1st and 2nd hours which is similar to that reported by Habib et al.[65] who found high ESR in cryoglobulin +ve patients when compared to cryoglobulin -ve patients.

A positive correlation between cryoglobulins and CRP (r=0.588, p=0.027) as well as between cryoglobulins and RF. Very similar to our results, Kassel et al.[69] found a positive correlation between CRP with the presence of RF and cryoglobulinemia. Habib et al.[65] found a positive correlation between cryoglobulins and RF.

Since RF, CRP and ESR are markers of disease activity as that stated by Lane and Gravel[70] and Breda et al.[71]. And since, Zuckerman et al.,[72] found that improvement of joint inflammation in HCV patients with +ve cryoglobulins was correlated with the reduction of cryoglobulin level in the serum indicating its role in disease activity. He found also that cryoglobulins become undetectable in 75% of patients, although a complete biochemical and virological response at the end of treatment was achieved in only 36% and 20% respectively. Elevated ESR, CRP, cryoglobulins and RF were found in 60 HCV infected patients with no correlation with aminotransferases activity[73].

Our results confirm that there is an inter - relation between Cryoglobulinemia and RF, CRP, ESR, indicating its role in disease activity and may be useful for monitoring therapeutic response.

From this study we can conclude that HCV associated arthritis can be misdiagnosed as RA, especially as 50% of patients fulfill the ACR criteria of diagnosis of

RA. Patients represented with: arthritis which may be oligoarticular affecting big joints, arthritis similar to RA but non erosive or non deforming or elevated liver enzymes should be screened for HCV infection. Anti-CCP antibodies are one of the markers that help to differentiate HCV associated arthritis from RA. Cryoglobulins together with CRP, ESR, and RF are considered as markers of disease activity in HCV related arthritis.

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STUDY OF CHRONIC INFLAMMATORY ARTHRITIS IN EGYPTIAN PATIENTS WITH HEPATITIS C VIRUS INFECTION

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SERUM FERRITIN LEVEL AS A PREDICTOR OF RESPONSE TO PEGYLATED INTERFERON/RIBAVIRIN THERAPY IN EGYPTIAN CHRONIC HEPATITIS C PATIENTS

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Abstract

Increased serum ferritin (SF) level has been encountered in various conditions, such as inflammatory syndromes and iron deposits in hepatic mesenchymal cell. Interferon alpha is one of the stimulants of ferritin production; serum iron level may affect the treatment response of chronic hepatitis C (CHC) .The aim of the study was to find out the relationship between SF level and response to treatment of CHC by peg interferon and ribavirin. Methods: Data from 200 consecutive patients with CHC, fulfilling the criteria for treatment, were collected. Baseline SF level was measured and correlated to the therapeutic outcome. Results: Early virological response (EVR) was achieved in 84% of the patients, while Sustained Virological Response (SVR) was achieved in 53% of patients. EVR was significantly related to low viral load, fibrosis stage, alfa fetoprotein and low serum ferritin level (P=<0.001). SF was not significantly related to SVR. Age of the patients and serum alkaline phosphatase have a significant relationship to SVR. Conclusion: in CHC, pretreatment SF level is a useful marker for predicting EVR, while its value in predicting SVR needs further evaluation.

Introduction

Hepatitis C virus (HCV) infection is a serious worldwide problem. It has been estimated that there are 170 - 210 million HCV infection worldwide with an increasing incidence of new infections (3 - 4 million every year)[1],[2]. The prevalence varies markedly from one geographic area to another, the burden of disease is greatest in developing

countries, in Egypt, the estimated adjusted national prevalence rate of chronic hepatitis C virus infection is 9.8% in 2008 [3].

Chronic hepatitis C (CHC) is an important cause of chronic liver disease which may progress to cirrhosis or hepatocellular carcinoma[4]. The combination of pegylated interferon (IFN) and ribavirin is the approved and well accepted standard-of-care for chronic hepatitis C. The primary goal of HCV therapy is to eliminate circulating HCV after cessation of treatment that could be checked by early virological response (EVR) which is defined as undetectable HCV RNA in the blood at week 12 of treatment (complete EVR); or HCV RNA drops by more than 2 logs from the baseline level by week 12 (partial EVR)[5], while sustained virological response (SVR), is defined as an undetectable HCV RNA level 24 weeks after treatment withdrawal.[5],[6],[7],[8]. Relapsers are defined as patients who achieved an end-of-treatment response (undetectable HCV RNA at the end of treatment) but subsequently relapsed and did not achieve an SVR.[9]. The above mentioned parameters are referred to the baseline viral load which is defined as the amount of hepatitis C virus (HCV RNA) in the blood before treatment starts.[5].

Therapy for hepatitis C virus (HCV) is expensive, of long duration, and challenging for the patient and the health care professional. The side effects need careful follow up and good selection of patients.[10],[11], so, defining predictors of response will help to avoid these obstacles.

Baseline viral load is one of the important factors affecting treatment outcome, lower viral load levels showed increased probability of SVR[12],[13],[14]. Ferritin is the major iron storage protein which is considered as a marker for liver iron deposition[15]. Serum ferritin (SF) is an acute phase reactant, and is affected by chronic hepatitis C, it may affect the treatment response. [16],[17], [18] [19].

This study aimed at evaluating the baseline serum ferritin (SF) level and other predictors of Early and Sustained Virological

Response (EVR & SVR) in CHC patients treated with PEG-IFN and Ribavirin.

Patients and Methods

This is a prospective study, conducted on 200 nieve Chronic Hepatitis C (CHC) patients seen in the Hepatology Outpatient Clinics; Al Mansoura New General Hospital between March 2010 and December 2011. Inclusion criteria were, age of 18 years or more, chronic hepatitis C diagnosed by positive HCV antibody for more than 6 months confirmed by positive HCV RNA PCR and liver biopsy, BMI ≤ 30 Kg/m^2. Exclusion criteria include: decompensated liver disease, other causes of chronic liver diseases and iron disorders as hemolytic anemia, hemochromatosis, iron therapy or iron depletion therapy[20].

All patients were treated by pegylated interferon and ribavirin and HCV PCR was checked at 12 weeks, 48 weeks (end of treatment) and 72 weeks (6 months after the end of treatment). Patients who failed to clear the virus or to drop the viral load by two logs at 12 weeks will be considered as failure to achieve early virological response and will stop treatment. Patients who achieved end of therapy (EOT) response were identified by negative HCV RNA PCR at 48 weeks of treatment. Follow up of those patients by HCV PCR at 72 weeks to identify sustained virological responders (PCR negative); and relapsers (PCR positive). Patients who discontinued treatment due to side effects were not included in the study.Serum ferritin (SF) was measured before treatment and haematological and biochemical parameters were monitored. Written informed consent was obtained from all patients before treatment.

Statistical analysis:

Baseline and follow up demographic, and laboratory data were collected. SPSS Statistics 19 program was used for data analysis. Dichotomous or categorical variables were presented as number and percentage. Continuous variables were presented as mean ± standard deviation. Univariate analysis by using Pearson Chi square test was used to compare dichotomous or categorical vari-

ables, and two tailed t test for continuous variables.Significance was considered at P value 0.05 or less.

Results

Two hundred patients were included in this study; all patients received PEG-INF and ribavirin. HCV RNA PCR was checked at 12, 48 and 72 weeks as mentioned before. Table 1 and 2 showed the comparison between patients responding and not responding to treatment at 12 weeks (EVR) and 72 weeks (SVR) as regards demographic, laboratory and histopathological parameters in the form of (Mean ± SD) for continuous variables and (no. &%) for the dichotomous or categorical variables.

Out of 200 patients, 168 (84%) achieved an Early Virological Response at 12 weeks, while Sustained Virological Response was achieved in 106 patients (53%) 6 months after the end of treatment. When comparing SVR in patients younger and older than 44 years, 58/97(59.7 %) of patients younger than 44 years and 47/103 (48.4%) of patients aged ≥44 years achieved SVR.

In table (1): there was no significant difference between patients showed EVR and patients did not show as regards age, sex, smoking, DM, BMI and all laboratory test except serum α fetoprotein > 5 ng/ml (P = 0.02), serum ferritin (p value = 0.001) and low viral load (P value 0.006), also, there was a significant difference between early virological responders and non responders as regards the fibrosis score where 57.8% of responders have low fibrosis score (F1 and F2) compared to 31.3% for the non responders.. There was no significant difference between the studied groups as regards the modified HAI. Histological evaluation of the grade of inflammation and the stage of fibrosis were evaluated according to the Metavir scoring system[21].

Baseline serum ferritin level was significantly lower in Early Virological Responding patients but no significant difference between the sustained virological responding and non responding patients. Pretreatment serum ferritin as a predictor of EVR is highly significant with

area under ROC curve (AUROC) 71% (CI 95% = 61% - 81%). Correlation analysis revealed that pretreatment serum ferritin levels are linked with serum cholesterol (pearson correlation = 0.16, P = 0.03).

In table 2, there was statistically significant difference between SVR and non SVR groups as regard age and serum alkaline phosphatase, while no difference between the 2 groups as regards alpha feto protein, low viral load, fibrosis score and baseline serum ferritin which were significantly different in the EVR responders and non responders.

Table (1): Comparison between patients responding and not responding to treatment at 12 weeks (EVR) as regards demographic laboratory and histopathological parameters. (mean ± SD or no. %).

Parameter	EVR (168 pt, 84%)	No EVR (32 pt, 16%)	P- value
Age (yr)	44.5 ± 6.9	45.2 ± 6.2	0.6
Sex (male)	137 (81.5%)	29 (90.6%)	0.3
Smoking	44 (26.2%)	7 (21.9%)	0.6
DM	12 (7.1%)	3 (9.4%)	0.7
BMI (kg/m^2)	27.2 ± 3.5	27.1 ± 3.4	0.8
Cholesterol (mg/dl)	189.7 ± 51.4	183.3 ± 50.9	0.5
Triglyceride (mg/dl)	113.3 ± 22.8	117.9 ± 121.9	0.3
Glucose (mg/dl)	120.7 ±29.2	120.5 ± 30.6	0.9
Creatinine (mg/dl)	1.03 ± 0.2	1.03 ± 0.2	0.9
Albumin (g/dl)	4.2 ± 0.6	4.2 ± 0.6	0.7
alkaline Phosphatase (IU/dl)	74.7 ±23.2	76.8 ± 72.9	0.6
AST (IU/dl)	85.9 ± 77.3	83 ± 24.6	0.9
ALT (IU/dl)	95.2 ± 31.2	91.9± 29.3	0.9
T Bilirubin (mg/dl)	1.9 ± 0.9	1.7 ± 1	0.5
WBS (10^3/cmm)	7.3 ± 1.8	7.2 ± 1.9	0.9
Hb (gm/cmm)	12.6 ± 0.9	12.7 ± 0.7	0.8
Platelet (10^3/cmm)	158.3 ± 26.3	160.1 ± 27.9	0.7
AFP > 5 (ng/dl)	75 (44.6%)	22 (68.8%)	0.02*
Serum Ferritin (mg/dl)	188.1 ± 59.9	233.7 ± 58.2	< 0.001*
Low viral load (< 600.000 copy/cmm)	88 (52.4%)	8 (25%)	0.006*
Fibrosis score			0.05*
F1	50 (29.8)	4 (12.5%)	
F2	47 (28%)	6 (18.7%)	
F3	45 (26.8%)	14 (43.8%)	
F4	26 (15.4%)	8 (25%)	
Modified HAI	9.6 ± 1.6	9.4 ± 1.6	0.5

Significance level * P < 0.05, BMI body mass index, ALT alanine aminotransferase, AST Aspartate amino transferase, Hb haemoglobin, DM diabetes mellitus, HAI modified Histological index, EVR early virologic response, F1 = portal fibrosis without septa, F2 = portal fibrosis with rare septa, F3 = numerous septa without cirrhosis, F4 = Girrhosis

Table (2): Comparison between patients responding and not responding to treatment at 72 weeks (SVR) as regards demographic laboratory and histopathological parameters. (mean ± SD or no. %).

Parameter	SVR (106 pt, 53%)	No SVR (94 pt, 47%)	P- value
Age (yr)	43.7 ± 7.2	45.6 ± 6.2	0.05**
Sex (male)	89 (84%)	77 (81.9%)	0.7
Smoking	29 (27.4%)	22 (23.4%)	0.6
DM	10 (9.4%)	5 (5.3%)	0.3
BMI(kg/m^2)	27.1 ± 3.3	27.3 ± 3.7	0.5
Cholesterol (mg/dl)	186.6 ± 51.6	190.9 ± 50.9	0.5
Triglyceride (mg/dl)	114.6 ± 22.3	113.3 ± 23.3	0.7
Glucose (mg/dl)	119.6 ± 30.7	121.9 ± 27.9	0.6
Creatinine (mg/dl)	1.03 ± 0.19	1.03 ± 0.21	0.8
Albumin (g/dl)	4.2 ± 0.6	4.1 ± 0.6	0.4
Alkaline Phosphatase (IU/dl)	71.7 ±24.1	78.2 ± 21.7	0.05**
AST (IU/dl)	81.2 ± 29.4	79.8 ± 26.9	0.2
ALT (IU/dl)	75.5 ± 28.9	77.6 ± 32.2	0.7
T Bilirubin (mg/dl)	1.7 ± 1	1.6 ± 1	0.8
WBS (10^3/cmm)	7.4 ± 1.8	7.1 ± 1.7	0.2
Hb (gm/cmm)	12.7 ± 0.9	12.6 ± 0.9	0.9
Platelet (10^3/cmm)	158 ± 27	159 ± 26	0.8
AFP > 5 (ng/dl)	48 (45.3%)	49 (52.1%)	0.4
Low viral load (< 600.000 copy/cmm)	45 (42.5%)	51 (54.3%)	0.1
Fibrosis score			0.2
F1	34 (32.1%)	20 (21.3%)	
F2	27 (25.5%)	20 (27.7%)	
F3	31 (29.2%)	28 (29.7%)	
F4	14 (13.2%)	20 (21.3%)	
Modified HAI	9.7 ± 1.6	9.5 ± 1.7	0.5
Serum Ferritin (mg/dl)	196.6 ± 61.9	194 ± 61.9	0.8

Discussion

The gold standard treatment of chronic hepatitis C (CHC) is PEG-IFN and ribavirin. Treatment of CHC is of long duration, expensive and of heterogeneous response; so, accurate prediction of response to therapeutic regimen is of great interest[10],[22],[23]. We therefore investigated factors that help to predict response to PEG-IFN plus ribavirin therapy especially serum ferritin.

In this study, EVR to therapy was 84% while SVR was 53%, which is not much different from other international reports [24], [25]. When stratifying patients according to age group; 59.7% of patients younger than 44 years and 48.4% of patients aged ≥44 years achieved SVR, this indicates that patients with increasing age has

reduced chances of cure and this is in agreement with Vutien et al [26] and Foster et al[14] who had shown that SVR rates were 70%, 52%, and 36% in patients of age 20, 43, and 60 years respectively. However there are some studies that didn't show a significant relation to age [12].

In this study, there is no effect of BMI on the response rate, also, the lower the degree of fibrosis (F1 and F2), the higher the EVR (57.8% in responders compared to 31.2% in non responders), this effect was lost in SVR. Other studies showed that male gender, increase BMI and presence of advanced fibrosis reduces the response to treatment.[27],[29],[24] and [25]. The policy of Egyptian ministry of health is to exclude obese patients with BMI >30. Also, there is a strict exclusion of those who failed to have early virological response to save treatment for the responders, this may be not the same in western countries where the resources are enough to continue treatment in those who did not fulfill the criteria of EVR but insist to continue treatment. [20]

It is noted that, modified HAI showed no significant difference between responders and non responders in both early and sustained virological response, while the fibrosis score did; this signifies the fibrosis score and nullifies the hepatitis activity score as a predictor of response to treatment.

Patients who had low viral load were found to have significantly higher EVR (52.4% in responders and 25% in non responders, p value 0.006) while there was no significant difference in SVR (p value 0.1). this could be explained by the fact that, those patients who had higher viral load did not complete the course of treatment after 12 weeks, also, high baseline viral load means that the virus is reproducing rapidly and viral cure/SVR may be less likely to occur.[27]

Hyper-ferritinemia in CHC may reflect ongoing necroinflammatory events and iron deposits in hepatic mesenchymal cell [28]. Patients with lower serum levels of ferritin and those after therapeutic phlebotomy may have an improved response to IFN therapy. [30]

In this study, higher base line serum ferritin levels were correlated to lower response rate to antiviral therapy, similar results were reported by other authors [31],[32][33]. Two possible explanations for reduced response rate to antiviral therapy in high serum ferritin patients can be suggested. First high serum ferritin level may be due to chronic activation of endogenous INF system, so, these patients may have a limited response to exogenous IFN [34]. Second, ferritin may have immune-modulatory activities by inhibiting delayed type hypersensitivity response and by inducing myelosuppression [35]. Thus, it is possible that the chronic high serum ferritin levels hampered the response of these patients to antiviral treatment. A lower baseline SF level appears to be associated with a favorable treatment response in chronic hepatitis C, this is in agreement with many studies[36][37],[32][4]. Conclusion : In patients with CHC, pretreatment SF level is a useful marker for predicting EVR, while its value in predicting SVR needs further evaluation.

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SERUM FERRITIN LEVEL AS A PREDICTOR OF RESPONSE TO PEGYLATED INTERFERON/RIBAVIRIN THERAPY IN EGYPTIAN CHRONIC HEPATITIS C PATIENTS

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CORRELATION BETWEEN ANXIETY AND DEPRESSIVE DISORDERS IN PATIENTS WITH FUNCTIONAL DYSPEPSIA : A RANDOMIZED CONTROLLED EGYPTIAN TRIAL

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Abstract

Background and Aim: *Functional dyspepsia (FD), is the most common type of dyspepsia encountered in primary care and gastroenterology practice. Patients with FD would have a greater prevalence of treatable psychiatric disorders. The aim of this trial was to study the prevalence of psychiatric disorders and somatic symptoms in patients with functional dyspepsia and to compare them with those with duodenal ulcer disease(DU).* ***Patients and Methods:*** *In this prospective randomized controlled study, we selected 100 consecutive FD patients and the same number of endoscopically proven DU patients(as a control group). Patients were subjected to full history taking, complete clinical examination, laboratory investigations, esophagogastroduodenoscopy and psychiatric evaluation through General Health Questionnaire (GHQ), Hospital Anxiety Depression Scale (HADS) and Structured Psychiatric Interview (PSE10).* ***Results:*** *Patients with FD were significantly younger, more likely to be females, less tendency to smoke, had more frequent dyspeptic symptoms, a longer disease history and lower incidence of H. pylori infection than duodenal ulcer patients. Psychiatric morbidity was significantly higher in FD patients than DU patients (65% compared to 26%). Prevalence of psychiatric disorders was as follow: somatoform disorders 36%,mixed anxiety depressive disorder 21%,generalized anxiety disorder 17%,and depressive episode 4% in FD patients. As regard DU patients; somatoform disorders 8%, mixed anxiety disorder 10% and generalized disorder 8%. Using stepwise logistic*

regression model, it had been shown that, somatoform autonomic dysfunction and anxiety symptoms of (HADS) were independently associated with functional dyspepsia and the best predictors of diagnosing functional dyspepsia or duodenal ulcer. ***Conclusion:*** *Psychiatric disorders are strongly associated with functional dyspepsia that warrant psychiatric intervention that may benefit FD patients specially those with chronic symptoms.*

Key Words: *Functional dyspepsia, Depression, Anxiety, Peptic ulcer.*

Introduction

Dyspepsia is a common symptom with an extensive differential diagnosis and a heterogeneous pathophysiology. It occurs in approximately 25 percent (range 13 to 40 percent) of the population each year, but most affected people do not seek medical care. Functional (also referred to as idiopathic or nonulcer) dyspepsia, is the most common type of dyspepsia encountered in primary care and gastroenterology practice[1]. Functional dyspepsia (FD), defined as the presence of symptoms thought to originate in the gastro duodenal region in the absence of organic, systemic, or metabolic disease that is likely to explain the symptoms, is one of the most common gastrointestinal disorders[1]. According to the Rome II consensus for diagnosis of FD, At least 12 weeks, which need not be consecutive, in the preceding 12 months of: persistent or recurrent symptoms (pain or discomfort centered in the upper abdomen); no evidence of organic disease (including at upper endoscopy) that is likely to explain the symptoms; and no evidence that dyspepsia is exclusively relieved by defecation or associated with the onset of a change in stool frequency or stool form. It could be classified into : Ulcer-like dyspepsia (pain centered in the upper abdomen is the predominant, most bothersome symptom); Dysmotility-like dyspepsia(an unpleasant or troublesome nonpainful sensation (discomfort) centered in the upper abdomen is the predominant symptom; this sensation may be characterized by or associated with upper abdominal fullness, early satiety, bloating,or nausea) and Unspecified (nonspecific) dyspepsia (symptomatic patients whose symptoms do not fulfill the criteria for ulcer-like or dysmotility-like dyspepsia) [1]. So, typical

FD symptoms are early satiation, postprandial fullness, epigastric pain, and epigastric burning. Other symptoms such as upper abdominal bloating, belching, and nausea often coexist, and a subset of patients may experience weight loss[2]. At present, there is no treatment with established efficacy for FD. Proton pump inhibitors and prokinetic drugs are most frequently used, although the evidence to support their efficacy is limited [3].

Functional gastrointestinal (GI) disorders such as the irritable bowel syndrome (IBS) and FD, are characterized by chronic symptoms referable to the gastrointestinal tract in the absence of any biochemical or structural explanation[4]. The patients with symptoms indicative of ulcer, but without evidence of ulcer, based on upper endoscopy, are given the diagnosis of non ulcer dyspepsia or FD is considered a heterogeneous disorder with different pathophysiological mechanisms, contributing to the symptom pattern. The Room II committee proposed that subdividing patients FD patients with predominant pain versus discomfort might identify subgroups with homogenous clinical properties (Karamanolis et al., 2006)[5]. The term non ulcer dyspepsia reflects the previously dominant viewpoint that dyspepsia without ulcer, represent a special more peaceful course of ulcer disease. However, repeated observation of functional dyspepsia patients show a low propensity of ulcer development[6]. Hyper secretion of gastric acid and the bacteria Helicobacter pylori which seem to be major etiological factors in DU ,do not seem to be important in functional dyspepsia[7]. The cause of FD remains essentially unknown, but psychological factors have been implicated. For example, patients with FD reported more life stress and psychological distress than healthy controls in clinic-based studies [8]. German investigators reviewed, with meta analytic methods, observational studies on the association of FD with anxiety and depression. This association was established, with more patients with FD having major depression or any anxiety disorder than either healthy controls or patients with similar upper abdominal symptoms of known or-

ganic origin. The amount of self-reported depressive symptoms was not different between patients seeking medical care for their complaints and those who did not consult, but the degree of anxiety was higher in consulters than in non consulters[9]. In support of these data are the results of a nested case-control study in a random community sample in the United States, showing that psychosocial factors, particularly somatization, interpersonal sensitivity, and total life event stress, were associated with FD[10]. Also, depressive symptoms were found to be quite common in patients with FD[11].

Hypothesis and Aim of the Work:

Patients with functional dyspepsia would have a greater prevalence of treatable psychiatric disorders, higher scores on measures of psychiatric symptomatology and characteristic somatic symptoms distinguishing them from patients with duodenal ulcer.

Aim of the work

The aim of this work was to study the prevalence of psychiatric disorders and somatic symptoms in patients with nonulcer dyspepsia in comparison to those of duodenal ulcer disease.

Subjects & Methods

Population Study : In this prospective randomized controlled double blind (subject, investigator) study, We recruited one hundred consecutive patients with FD and the same number with those of duodenal ulcer (DU). They were studied at their routine consultation at the outpatient's clinic of internal medicine department, Benha University Hospitals from May 2009 to June 2011. Patients were directly invited by the authors to participate in the study; they were informed that the objective was to assess the possible relationship between psychiatric disorders and upper gastrointestinal complaints. All patients provided informed consent after receiving a full explanation of the nature and protocol of the study. The study protocol was approved by the ethics committee of the hospital. Consecutive patients with endoscopically verified duodenal ulcer(by the same endoscopist, the investigator) were included in the study as a comparative group because of

similarities of symptoms. Dyspeptic patients without any positive endoscopic findings and fulfill the Rome III criteria for FD were recruited[12].

Inclusion Criteria: Patients aged more than 18 years and of both sexes; patients who had in the prior 5 years, a normal esophagogastroduodenoscopy (EGD) (no esophagitis, Barrett's esophagus, cancer, erosions, or ulcer disease), and would had been diagnosed with functional dyspepsia after specialist consultation and patients who failed to adequately respond to antisecretory therapy in the past for functional dyspepsia to be suitable. Exclusion Criteria : Any documented history of endoscopic esophagitis, or predominant heartburn or acid regurgitation, or these symptoms two or more times per week in the prior year, to exclude GERD; those who had an adequate response to antisecretory therapy according to the physician interview, to exclude patients with disease easy to control with first line therapy or misdiagnosed GERD; any documented peptic ulcer disease; regular use of non-steroidal anti-inflammatory drugs or proton pump inhibitors or antibiotics; subjects had serious mental disorder or undergone psychiatric treatment, any history of drug abuse, or currently taking psychotropic medication; a history of abdominal surgery or planned surgical treatment (proximal gastric vagotomy or gastric resection) except appendectomy, cholecystectomy or hysterectomy more than one year previously; subjects with concurrent major physical illness (including cardiac or liver disease, diabetes, inflammatory bowel disease, urinary retention, active thyroid disease, vasculitis, lactose intolerance explaining symptoms), psychotic illness or eating disorder; subjects whose literacy skills are insufficient to complete self report questionnaires; Pregnancy, or refusal to apply adequate contraceptive measures during the trial. Also, patients with indigestion due to heart, liver, pancreatic diseases or gastrointestinal malignancy were excluded.

Tools and Methods of Assessment :

Medical Examination: The functional dyspepsia and duoden-

al ulcer patients were medically examined; the medical examination included a detailed medical history including dyspeptic symptoms, smoking and alcohol., coffee, tea and use of medications; clinical examination; abdominal ultrasonography; bed side laboratory investigations and H.pylori rapid urea breathe test[13] and Olympus video upper gastrointestinal endoscopy.

Psychiatric Examination:

All patients were interviewed by a psychiatrist blinded to the result of upper gastrointestinal endoscopy. During this interview, the following information was elicited: 1) demographic data 2) data on past psychiatric history including; previous psychiatric hospitalizations or visits to professionals; use, kinds and efficacy of psychotropic drugs and suicide attempts 3) information from a formal psychiatric examination, performed to make a DSM-IV or ICD10 diagnosis and 4) information on recent psychiatric symptoms, obtained through General Health Questionnaire (GHQ), Hospital Anxiety Depression Scale (HAD). Psychiatric diagnoses were assessed according to ICD10 through structured psychiatric interview: present state Examination (PSE10).

General Health Questionnaire (GHQ) : The general health questionnaire is the most widely used screening test to detect psychiatric disorders in medical practice and measures possible prevalence of non psychotic psychiatric disturbances, especially anxiety and depressive disorders. We made use of the short GHQ 12 version in which each of the 12 items with a 4 answering categories was scored on a bimodal response scale resulting in a score ranging from(0 to 12). A cut-off score of(5-6) was used to discriminate possible psychiatric cases (score ≥ 6) and non cases (scores ≤5), which according to a GHQ evaluating study shows a positive and negative predictive value of 71% and 78% respectively. All patients completed the Arabic version [14].

Hospital Anxiety and Depression Scale (HADS):

The Hospital Anxiety and Depression Scale (HADS) were originally developed for use in hospital settings, as the name suggests. It

was designed as a self-completed questionnaire to assess patients' anxiety and depression whilst in in-patient care according to two sub-scales. The Anxiety and Depression scales, both comprise 7 questions rated from a score of (0 to 3) depending on the severity of the problem described in each question. The two sub-scales can also be aggregated to provide an overall anxiety and depression score. The anxiety and depression scores are categorized as below: Aggregate Score: (0-7) Normal, (8-10) Mild, (11-14) Moderate and (15-21) Severe (15). All patients completed the Arabic version of HADS (16).

Present State Examination 10th revision (PSE10) (WHO, 1992): It is a part of the SCAN system (Schedule for Clinical Assessment in Neuropsychiatry), which is a set of instruments and manuals aiming at assessing, measuring and classifying the psychopathology and behavior associated with the major psychiatric disorders of adult life. The SCAN text has 3 components; the tenth edition of the Present State Examination (PSE10), the Item Group Checklist (IGC) and the Clinical History Schedule (CHS). PSE 10 itself has two parts: part I covers somatoform, dissociative, anxiety, depression, and bipolar disorders and problems associated with eating, alcohol and other substance use disorders. Part II covers psychotic and cognitive disorders and observed abnormalities of speech, affect and behavior. The central Principle of the PSE is that the interview, although substantially structured, retains the features of a clinical examination. The aim of the interviewer is to discover which of a comprehensive list of phenomena have been present during a designated period of time and with what degree of severity. The examination is therefore based on a process of matching the respondent's behavior and description of subjective experiences against the clinical definitions provided in the glossary of the Scale(17).

Statistical analysis: All the data were recorded in an investigative report form. These data were transferred to IBM card, using IBM compatible computer with statistical program (Statistical

package for Social Sciences): SPSS software version 16 to obtain the results. Data were presented as mean ± standard error of the mean for parametric and nonparametric data. Symptoms were compared by paired t test. A multivariate analysis using a stepwise logistic regression was used to assess the variables as best predictors of diagnosing functional dyspepsia or duodenal ulcer which was found significant on univariate analysis. Differences were considered significant, with P<0 .05.

Results

Sociodemographic factors:

There was an overall highly significant group differences in age (p<0.05)owing to being FD patients younger than DU patients. FD patients were more likely to be females (61%vs 39%), while women /men ratio in DU patients (47%vs 53%). Beyond this, there were no significant differences between the groups in socio demographic factors (Table1).

Life style factors: there was significant group difference in smoking. Patients with FD smoked less than DU-patients (p<0.05). There was also a significant group difference in alcohol consumption (p<0.05), FD patients consumed significantly less alcohol compared to DU-patients.

There was a significant group difference in irregular meals, 39% of FD patients had irregular meals which was significantly more than the DU patients (25%) (p<0.05) (Table 2).

History and symptoms differences: There was a significant difference due to duration of dyspeptic complaints between FD and DU patients, each episode seemed to last longer for the FD patients, the mean duration for the actual episode of dyspeptic complaints being 8 months for FD patients and 5 months for the DU patients (p<0.05). Epigastric pain was reported by 84% of DU compared to 73% of FD patients with no significant difference (p>0.05). It was significantly frequently severe, occurred at night, and in clusters (episodic) in DU than FD patients (p≤0.05). Pain relief by food was significantly more often reported by DU (48%) than by those with FD patients

(32%) (P< 0.05), whereas pain provoked by food was significantly more frequent in FD patients (45%) than in DU patients (31%) (p<0.05). Otherwise, no significant difference in other pain characters. As regards other dyspeptic symptoms: bloating, postprandial fullness and early satiation were significantly more frequent in FD than DU patients (p≤ 0.05). Otherwise no significant difference in other dyspeptic symptoms (Table 3).

As regard the result of H. pylori breath test, it was positive in 48% of DU patients compared to only 12% of FD patients, with highly statistical significant difference (p<001). Also it was found that 12% of FD patients reported history of treatment of anxiety and/or depressive disorders compared to 7% of DU patients and 12% of FD patients was under psychotropic medications (antidepressants and benzodiazepines), compared to 5% of DU patients with no statistical significant difference (p>0.05).

Psychological measures :

According to the GHQ cut-off score, 65% of FD patients were considered as psychiatric cases with a median range GHQ score of 8 (6 - 12), whereas 35% of patients showed no psychiatric disorders, with a median GHQ score of 0 (0 -5). As regard DU patients 26% where considered as psychiatric cases whereas 74% showed no psychiatric disorders.

FD patients scored significantly higher on Hospital anxiety depression scores than DU patients (Table 4).

Table 5: shows the frequency of psychiatric disorders among both groups of patients, where it was found that : among FD patients 36% of them met the criteria for somatoform disorders (somatoform autonomic dysfunction 16%, somatization disorder 8% and hypochondriasis 12%), compared to 8% of DU patients (somatoform autonomic dysfunction 3%; somatization disorder 2% and hypochondriasis 3%) with statistical significant difference (p<0.05); depressive episodes occurred only in 4% of FD patients. This table also shows that, mixed anxiety depression disorder occurred in 21%

of FD patients compared to 10% of DU patients and generalized anxiety disorder occurred in 17% of FD patients, compared to 8% of DU patients with statistical significant difference ($p< 0.05$). Also, it was found that in 13% of FD patients, somatoform disorders were co morbid with mixed anxiety depressive disorders.

The result of logistic regression analysis:

Using stepwise logistic regression analysis, it has been shown that, while the initial model contained 17 variables; including : sex, life style factors, dyspeptic symptoms and psychological measures; the final model included only 2 variables as best predictors of diagnosing functional dyspepsia or duodenal ulcer, the presence or absence of somatoform autonomic dysfunction and anxiety symptoms of HADS. The overall predictability of the model was found to be 87.5% (Table 6).

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

Variables \ Patients	FD Patients (n=100	DU Patients (n=100)	Test	*P*
Age(years):				
Mean	33.55	43.44	t=-14.373	0.001*
± SD	5.8	3.7		
Sex: No (%):				
Males	39(39%)	53(53%)	X^2=	0.05*
Females	61(61%)	47(47%)	3.945	
Marital status:				
Single	22(22%)	16(16%)		
Married	57(57%)	62(62%)	X^2=	0.85
Divorced	10(10%)	9(9%)	1.388	
Widowed	7(7%)	8(8%)		
Separated	4(4%)	5(5%)		
Occupation:				
Active remunerated	60(60%)	56(56%)	X^2=	0.83
Active nonremunerated	19(19%	20(20%)	0.364	
Inactive	21(21%)	24(24%)		
Education:				
Primary	10(10%)	13(13%)		
Preparatory	37(37%)	10(10%)	X^2=	0.927
Secondary	34(34%)	38(38%)	0.886	
University	7(7%)	35(35%)		
Postgraduate	12(12%)	4(4%)		

Active non-remunerated: student & housewife. Inactive: retired & unemployed.
*Significant $P\leq (0.05)$

Table (2): Life style factors:

Patients / Factors	FD Patients (n=100)	DU Patients (n=100)	Test	*P*
Smoking :				
Never	48(48%)	31(31%)	X^2 6.82	0.03*
Formerly	15(15%)	21(21%)		
Current	37(37%)	48(48%)		
Alcohol use	5(5%)	13(13%)	X^2=3.907	0.05*
Irregular meals	39(39%)	25(25%)	X^2=4.504	0.05*

* Significant ($p \leq 0.05$.

Table 3 : Dyspeptic symptoms in FD and DU Patients.

Patients / Symptoms	FD Patients (n=100)	DU Patients (n=100)	test	*P*
Duration of symptoms (in months):				
Mean ±SD	8.14±11.29	5.12±1.83	T=0.264	0.009*
Pain:				
Epigastric	73(73%)	84(84%)	X^2=3.585	0.06
Frequently severe	37(37%)	51(51%)	X^2=3.977	0.05*
Relieved by food	32(32%)	48(48%)	X^2=5.333	0.02*
Relived by antacids	73(73%)	66(66%)	X^2=1.156	0.28
Provoked by food	45(45%)	31(31%)	X^2 4.160	0.04*
Occurs at night	32(32%)	50(50%)	X^2 6.697	0.01*
Cluster(episodic)	34(34%)	48(48%)	X^2 4.051	0.04*
Anorexia	35(35%)	32(32%)	X^2= 0.202	0.7
Vomiting	30(30%)	35(35%)	X^2= 0.57	0. 5
Bloating	50(50%)	35(35%)	$X^{2=}$ 4.604	0.03*
Belching	59(59%)	50(50%)	X^2= 1.633	0.2
Heart burn	28(28%)	40(40%)	X^2= 3.209	0.07
Post prandial fullness	53(53%)	39(39%)	X^2 = 3.945	0.05*
Early satiation	50(50%)	36(36%)	X^2 = 3.998	0.05*

* Significant: ($p \leq 0.05$)

Table (4): Hospital Anxiety Depression Scores (HAD) among both groups of patients.

Patients / Scores	FD Patients (n=100)	DU Patients (n=100)	test	*P*
HAD depressive scores:				
No of Patients (%) Normal	35(35%)	74(74%)		
Mild & Moderate	40(40%)	16(16%)	X^2=30.668	0.002 *
Severe	25(25%)	10(10%)		
HAD anxiety scores:				
Normal	35(35%)	74(74%)		
Mild & Moderate	45(45%)	12(12%)	X^2=34.118	0.0003*
Severe	20(20%)	14(14%)		

* Highly Significant ($p < 0.01$)
Normal (0-7), Mild (8-10), Moderate (11-14), Severe (14-21)

Table (5): Prevalence of ICD10 Psychiatric disorders among both groups of patients.

Disorders \ Patients	FD Patients (n=100)	DU Patients (n=100)	Test X^2	*P*
Depressive episode	4(4%)	0(0%)	4.082	0.04*
Mixed anxiety depressive disorder	21(21%)	10(10%)	4.619	0.03*
Generalized anxiety disorder	17(17%)	8(8%)	3.703	0.05 *
Somatization disorder	8(8%)	2(2%)	3.789	0.05 *
Hypochondriasis	12(12%)	3(3%)	5.838	0.02 *
Somatoform autonomic dysfunction	16(16%)	3(3%)	9.828	0.002 *

* Significant :($p \leq 0.05$).

Table (6): The Results of Logistic Regression Analysis: Final Logistic Regression Model.

Variables	B	SE	Walds't'	*P*	Odds ratio
Somatoform autonomic dysfunction:	- 5.6	1.2	23.4	0.000 *	0.004
Anxiety scores:	- 2.4	0.5	22.8	0.000*	0.09
Heart burn:	-11.2	16.8	0.4	0.51	0.004
Early satiation:	9.8	16.8	0.3	0.56	6.8
Constant:	6.5	1.3	25.2	0.000*	-

* Statistical significant at p<0.01 level Model X^2:114.5 p= 0.000
SE : Standard error of B **B** : Regression coefficient

Discussion

In spite of similar clinical symptomatology of FD and DU patients, they comprised at least two different somatic entities. The patients differ in illness history, life style factors, symptom characteristics and measures of psychiatric symptomatology and prevalence of psychiatric disorders.

Sociodemographic characteristics of FD and DU groups: There was an overall a statistical significant difference between both groups regarding age and gender, as FD patients was younger and more likely to be females than DU patients. These findings were consistent with other previous studies [18,19].

Life style factors:Smoking was found to be less common in FD group than DU group. This result was found to be consistent with

other previous studies[20,21] .Many studies reported the significant role of smoking as a risk factor for peptic ulcer disease[22,23]. FD patients use alcohol significantly less than DU patients and the overall percentage in both groups were found to be small as compared to the percentage in western studies. This could be explained by cultural differences and religious attitude in our culture that alcohol is prohibited. Patients with FD had more irregular meals than DU patient. This can be a causal factor or a result of their dyspepsia.

Dyspeptic complaints:Patients with FD had a longer disease history and reported more frequent dyspeptic symptoms compared to patients with duodenal ulcer. This may reflect the fact that these patients have a low threshold to visceral pain and visceral hypersensitivity that is currently regarded as the mechanism responsible for both motor alteration and abdominal pain in functional bowel disorders including FD[24]. The expression of dyspeptic symptoms may also be influenced by the personality profile as neurotic patients may seek medical advise sooner and more often than those who are emotionally stable[25]. Also, this finding could be also explained by psychological distress that affects illness behavior, where in our study we found a high prevalence of psychiatric morbidity. Cheng[26], examined the difference in behavioral and perceptual characteristics between non consulter and consulter in FD in a Chinese population ,he found that the non consulters were distinguishable by their perceptual style copying behaviors and psychological symptoms. Moreover, high level of anxiety and depression were found to be highest in consulter compared to non consulter and healthy controls.

In our study, FD patients had more bothersome postprandial fullness in upper abdomen that occurs after ordinary sized meals and several times a week as well as early satiation that prevents finishing a regular meal .This finding was found to be consistent with the study done by Camilleri et al.,[27]. Also, it was found that the incidence of H. pylori infection was significantly higher in DU

patients (48%) than FD (12%). this result was consistent with many previous studies [5,28,7].

Psychiatric co morbidity:

Our results revealed that psychiatric morbidity was significantly higher in FD patients than DU patients, where general health questionnaire screening detected 65% of psychiatric cases compared to 26% of DU patients. Also, FD patients scored significantly higher on depression and anxiety scale (HAD) than DU patients ($p<0.001$). Also, FD patients had significantly more psychiatric diagnoses on ICD10 compared to DU patients. The most common psychiatric disorders were somatoform disorders, anxiety disorders and depressive disorders. In our multiple logistic regression models somatoform autonomic dysfunction and anxiety symptoms were independently associated with functional dyspepsia suggesting that these factors may be involved in the etiopathogenesis rather than just driving health care utilization i.e. act as confounding factors.

Our results were consistent with previous studies abroad., Magni et al.,[29] in a clinical based study found that 87% of FD patients, compared to 25% of patients with organic dyspepsia, had a psychiatric diagnosis. A larger study using structured interview and psychometric tools revealed that 34% of FD patients versus 15% of DU patients had a psychiatric diagnosis; the authors also found that the psychometric test for multiple somatic complaints was the most powerful discriminating factor, followed by general psychopathology and anxiety suggesting that involvement of these factors in the causative pathway of FD [21].

In the same direction, Pajala et al.,[19] reported in their study that the prevalence of mental distress among patients with functional dyspepsia and organic dyspepsia was 38% and 36.4% respectively, these findings are not consistent across studies but conflicting results may be explained by use of different sample populations.

In contrast to the latter findings, an earlier study demonstrated no statistically signifi-

cant difference between functional dyspepsia and duodenal ulcer patients on various psychometric tests scores. However, elevated level of neuroticism, anxiety and depression were found in FD patients compared to controls .No explanation was offered for the lack of difference between DU and FD patients[30]. Moreover, another study had demonstrated no psychological difference between people with functional bowel disorders who have not consulted a physician compared to community based health controls[31], this has engendered the association that psychosocial factors are not implicated in the etiopathogenesis of FD and irritable bowel syndrome but rather serve to motivate health care seeking.

More recently, in contrast to this dogma elevated level of psychological distress across all domains(except phobic anxiety) of the SCL-90-R ,a measure of psychological state have been demonstrated in a population based study of subjects with functional gastrointestinal disorders (functional dyspepsia and irritable bowel syndrome), inclusive of both consulters and non consulters suggesting that these factors may be involved in the etiopathogenesis rather than just driving health care utilization [10].

In our Arab culture, Abdulhafeiz et al. [32]; in a case control study investigated the relationship between functional dyspepsia, life events and mental illness, they found that psychiatric illness was significantly more in patients than the controls .Anxiety and depressive disorders dominated the clinical picture (84%) and the symptoms were of mild nature

In our study, somatoform disorders were reported in 36% of FD patients, this could be explained by the suggestion that the psychosomatic patients are assumed to have difficulties in expressing emotions verbally and to have tendency to react with different somatic complaints[33]. Also, it has been suggested that, somatizing symptoms can be a cultural mode of expressing mental distress in ethnic groups including Arab culture. Okasha; reported that depression among Egyptian patients is manifested mainly by agitation,

somatic symptoms, hypochondriasis, physiological changes such as decreased libido, anorexia and insomnia, which is not characterized by early morning awakening. Egyptian patients mask their affect with multiple somatic symptoms, which occupy the foreground and the affective component of their illness recedes to the background. This may be because of the greater social acceptance of physical complaints than of psychological complaints, which are either not taken seriously or are believed to be cured by rest or extra praying. The increase in somatic symptoms can be explained by the seriousness with which people in a given culture view psychological stresses compared with physical illnesses[34].

The association of psychiatric morbidity and FD could be explained through the gut –brain axis it has become more evident that FD is a biopsychosocial disorder[35], in which gastrointestinal motor abnormalities, altered visceral sensation and psychosocial factors interact to generate the symptoms. The motor, sensory and secretory activities of the gut occur through the bidirectional communication between the central nervous system, the autonomic nervous system and the enteric nervous system. FD symptoms may result from deregulated interactions at any level of the brain - gut axis [36].

Though high prevalence of psychiatric disorders in FD patients ,only small percentage (12%) were under psychiatric treatment, and also 5% of those with DU, that could be explained by lack of mental health awareness and stigmata of mental illness in our culture. So efforts should be directed towards identification and management of these obstacles.

The present study had found that FD patients had more or less distinctive clinical characteristics; significantly higher prevalence of somatoform, anxiety and depressive disorders distinguishing them from DU patients. Somatoform autonomic dysfunction and anxiety symptoms were the most predictive of diagnosis and differentiating between FD and DU patients suggesting that these factors may

be involved in the etiopathogenesis of FD rather than just driving health care utilization. It should be clear, however, that the model constructed in this study should by no means replace diagnostic testing, but should be considered a helpful aid for doctors as well as for patients especially when upper gastroendoscopic finding is negative and the symptoms are bothersome chronic.

Co morbid psychiatric illness in FD patients warrants treatment with conventional therapies. Available evidence suggests that psychological therapies may benefit FD patients particularly those with chronic symptoms[3]. The rationale for use of psychotropic medications in FD patients is apparent. Future studies are needed to evaluate the efficacy of antidepressants and psychotherapeutic measures in relieving suffering of FD patients. Primary care physicians and gastroenterologists should be aware of these psychiatric disorders for early identification and management or referral to mental health professionals in some cases for better quality of life. Our results are suggesting that functional dyspepsia and psychopathology share common pathophysiology that warrant further examination.

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CORRELATION BETWEEN ANXIETY AND DEPRESSIVE DISORDERS IN PATIENTS WITH FUNCTIONAL DYSPEPSIA : A RANDOMIZED CONTROLLED EGYPTIAN TRIAL

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IMAGE INTENSIFIER-GUIDED PERCUTANEOUS DRILLING FOR OSTEOID OSTEOMA OF THE PROXIMAL FEMUR

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Abstract

Purpose: *Evaluation of the efficacy of image intensifier- guided percutaneous drilling destruction of the nidus as a minimally invasive surgery for osteoid osteoma of the proximal femur.*

Background: *Osteoid osteoma is a painful, benign, small osteogenic bone tumor. For a long time, surgery was the only treatment for these lesions. Different minimally invasive therapeutic techniques have been proposed.*

Methods: *Between 2009 and 2011, 14 patients (9 males and 5 females) with osteoid osteoma were treated by percutaneous drilling destruction of the nidus under image intensifier.*

Results: *All procedures were technically successful. Clinical success was achieved in 94.5% of patients. Only one patient had incomplete removal of the nidus. There were no other complications.*

Conclusion: *Image intensifier -guided percutaneous drilling destruction of the nidus is a safe, simple and effective minimally invasive technique for the treatment of osteoid osteoma of the proximal femur.*

Keywords: *Osteoid osteoma, Image intensifier, Drilling.*

Introduction

Osteoid osteoma is a benign bone tumor with a nidus of less than 2 cm, surrounded by a zone of reactive bone. This lesion accounts for approximately 10% of all benign bone tumors. It occurs most frequently in the second decade and affects males twice as often as females [1].

The proximal femur is the most common location followed by the tibia, the posterior elements of the spine and finally the humerus. Osteoid osteoma is found more

often in the proximal than in the distal metaphysis or diaphysis [1].

The distinct clinical picture, the classic radiological presentation and the typical pathological findings make the diagnosis and even symptomatic control an easy problem. The main problem and matter of controversy is the control and eradication of the lesion itself [2].

Osteoid osteoma causes an intense and chronic inflammatory response in the surrounding tissues with a periosteal reaction, sclerosis of bone and synovitis because of the production of prostaglandins by the tumor which regresses spontaneously after removal of the nidus [2-5].

Osteoid osteoma can resolve without treatment in an average of 33 months, but if the patient does not wish to endure the pain and the prolonged use of non-steroidal anti-inflammatory medication, surgical excision or percutaneous removal are indicated[6]. Surgical excision has been common place until recently. Many surgeons thought that for surgery to be successful, the tumour including the reactive zone was to be completely removed. Extensive surgery has a greater chance of success, but carries a higher risk of complications and a longer recovery period. It is currently well accepted that removal of the nidus is sufficient[6].

Most of these tumors are situated in weight-bearing bones, so the recovery from surgery may require a long period of limited weight bearing, often with crutches[6].

Several methods have been proposed as an adjunct to surgery in order to reduce the risk of failed surgery and minimize the amount of bone removed[7-10]. In recent years, several techniques of percutaneous treatment of osteoid osteoma under CT control have been proposed: excision through bone trephination[11-13], a combination of partial percutaneous resection with subsequent intralesional ethanol injection[14,15] and destruction of the nidus using radiofrequency electrodes[9,16] or laser photocoagulation[17].

Patient And Methods

In a period of two years between / 2009 and / 2011, 14 patients (9 males, 5 females) with Osteoid Osteoma were treated by percutaneous destruction. The mean age at operation was 19 years (range 12 years to 30 years). The average follow-up period was 20 months (range 7-24 months). The duration of pain before treatment varied from 4 months to one year. The decision concerning treatment was made on the basis of clinical and radiological criteria. Clinical criteria included pain, worse at night and at rest, and relieved by non-steroidal anti-inflammatory drugs. Radiological criteria included four diagnostic features: (A) a sharp round or oval nidus that was (B) less than 2 cm in diameter, (C) had a homogeneous dense center, and (D) a 1-2 mm peripheral radiolucent zone[6]. CT was the preferred method of evaluation, especially if the lesion was obscured by reactive sclerosis. The mean size of the nidus was 8 mm (range 6 mm-12 mm). There were 4 OOs in the femoral neck and 3 in the lesser trochanter, 7 in the isthmus region.

Operative procedure :

The operation was carried out on a standard operating table under a high-resolution image intensifier control. Magnification and adjustment of contrast are often required to see the nidus clearly in both antero-posterior and lateral planes.

Pneumatic drill was used to insert a guide wire (2 mm) under image intensifier into the center of the nidus, passing through its two margins. A skin incision (1-2 cm) was centered on the guide wire. A sleeve was placed over the guide wire. A cannulated drill bit (4.5 mm caliber) was inserted through the sleeve and over the guide wire; it was advanced into the bone with a pneumatic drill (fig 1, 2). Both margins of the nidus were drilled. Subsequently a high speed burr (4 mm caliber) was used to remove the remnants of the nidus. Bone samples obtained from drill bit and speed burr were examined pathologically to confirm the diagnosis. CT-scan was performed postoperatively to assess the excision of the nidus. All patients were mobilized on the first postoperative day, and left the hospital after

3 to 4 days. Oral analgesics were indicated if pain was present after discharge. During follow-up, patients were examined in the 1st two weeks after the procedure and then at 3, 6 and 12 months, evaluating the presence of pain or associated symptoms. After 1 year, follow-up data were obtained by telephone or postal interview. A good response was defined as the disappearance of symptoms.

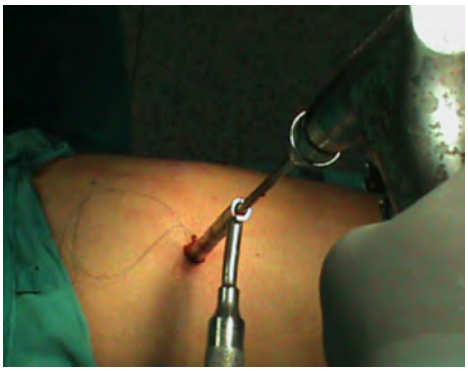


Fig. 1 : Sleeve and 4.5mm cannulated drill bit over a guide wire.

Fig. 2: C-arm picture of the sleeve and the drill.

Results

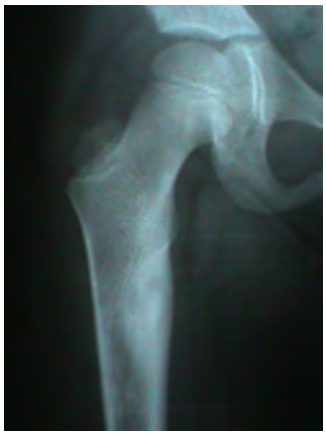
In 13 patients, the postoperative radiographs showed complete removal of the nidus. In these patients, histopathological examination confirmed the presence of the nidus of an osteoid osteoma. Rapid (24-48 hours) relief of pain was observed in these patients. All returned to normal daily activities within 2 weeks. Six months after surgery, complete sclerosis of the nidus was confirmed with plain radiographs.

There was one failure of the primary procedure. In this patient, the nidus was not completely removed and histopathological examination confirmed the presence

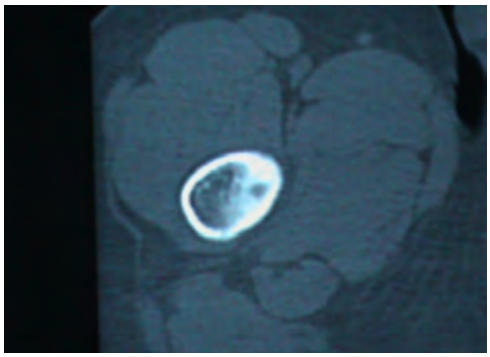
of the reactive bone only. This patient had partial relief of pain for three months. Because of the return of pain a second procedure was performed. In this operation, complete excision of the nidus was confirmed radiologically and histopathology, and pain relief was lasting.

Radiographs showed rapid healing of the operative defects. In the younger patients, the reactive changes in the proximal femur had resolved within 12 to 24 months. In older patients, with lesions at the calcar, reactive sclerosis persists. There were no infections, recurrences or major complications.

Figure 3, 14 years old male, presented with knee pain at night.



A



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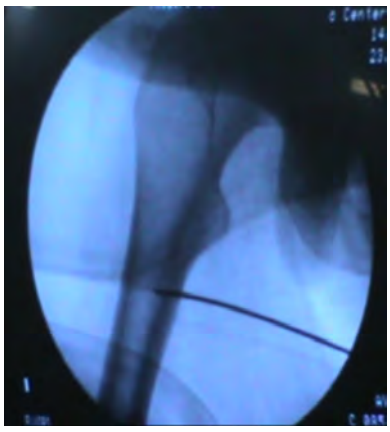

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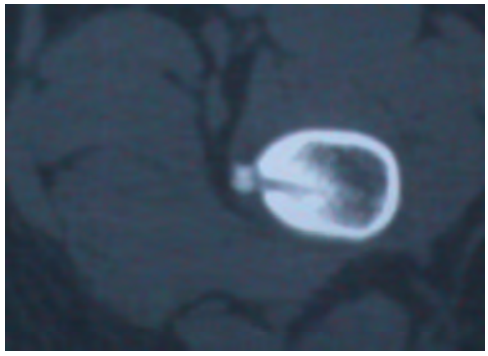

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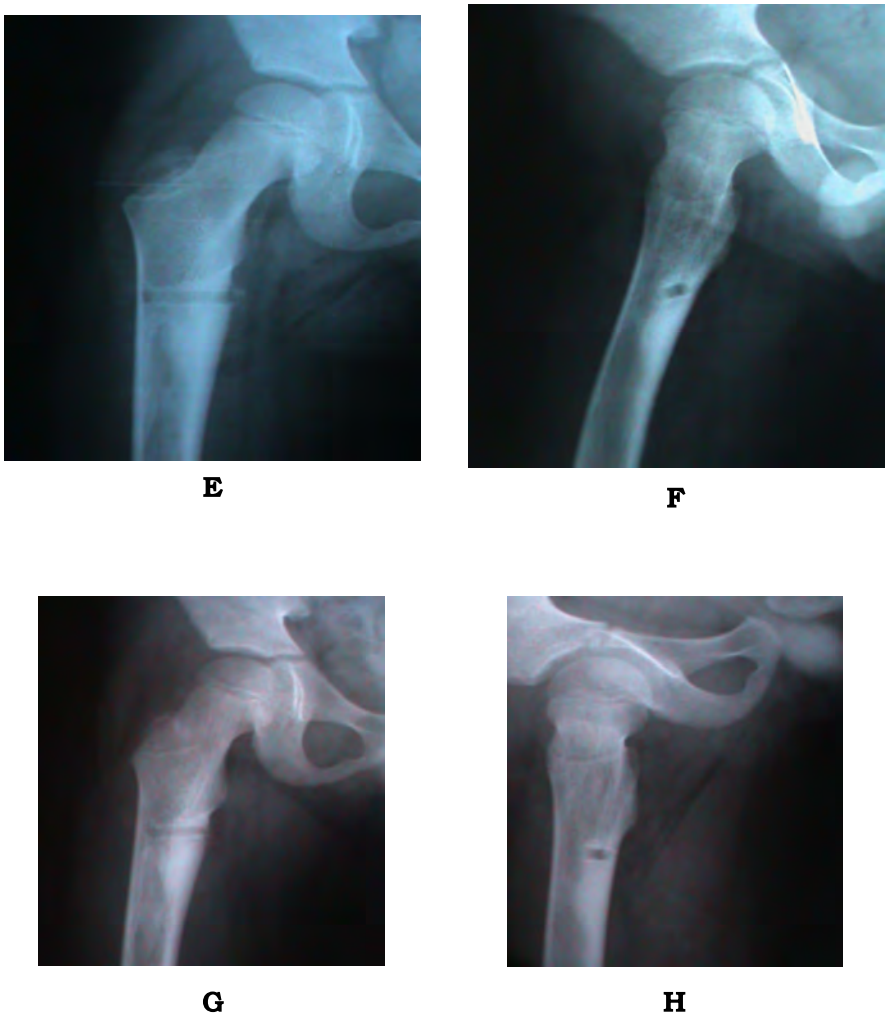
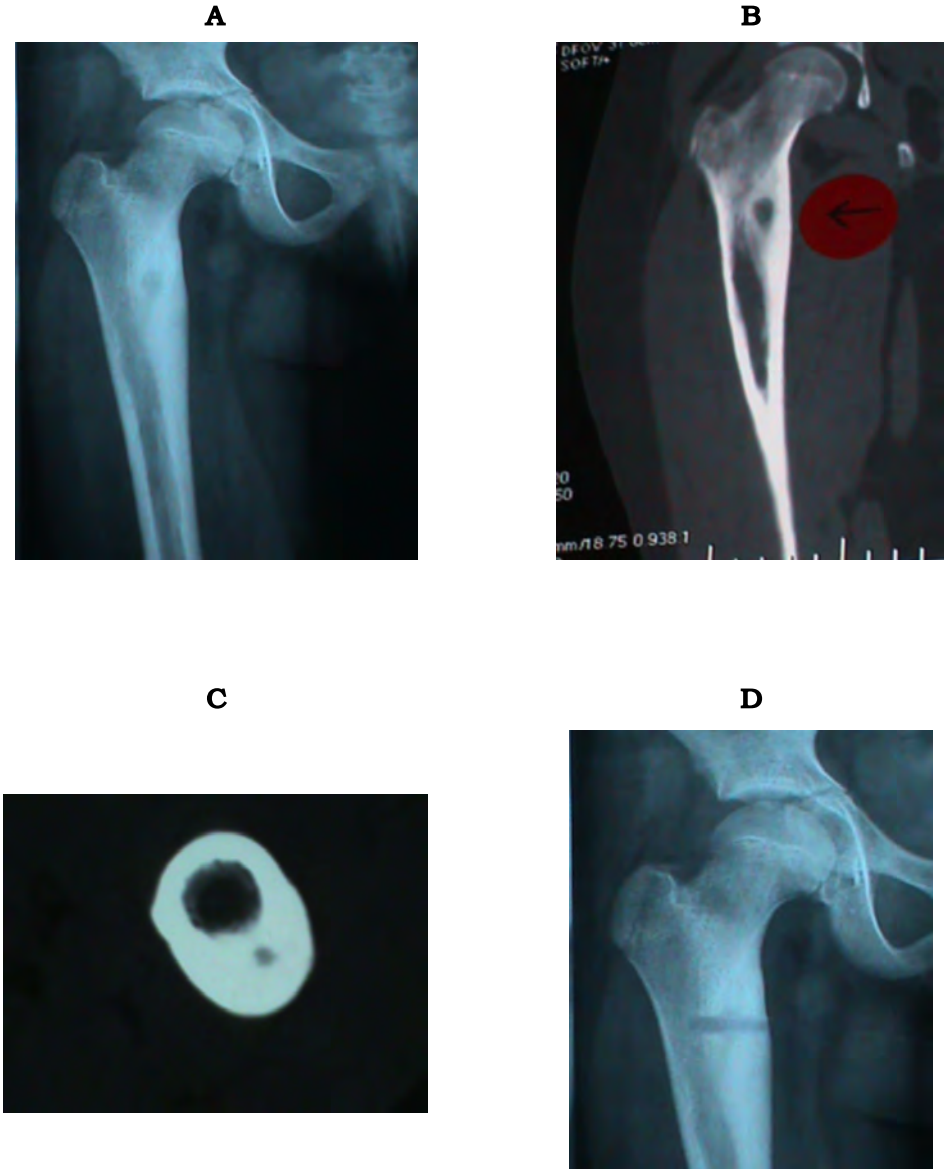


Fig. 3 : - **A & B;** Preoperative X-ray and CT.
- **C;** intraoperative C-arm picture showing the intralesional wire.
- **D,** postoperative CT showing removal of the nidus
- **E & F;** X-ray (Ap & Lat views).
- **G & H;** 3 months postoperative.

Fig. 4, a 16 years female patient, presented with hip pain.



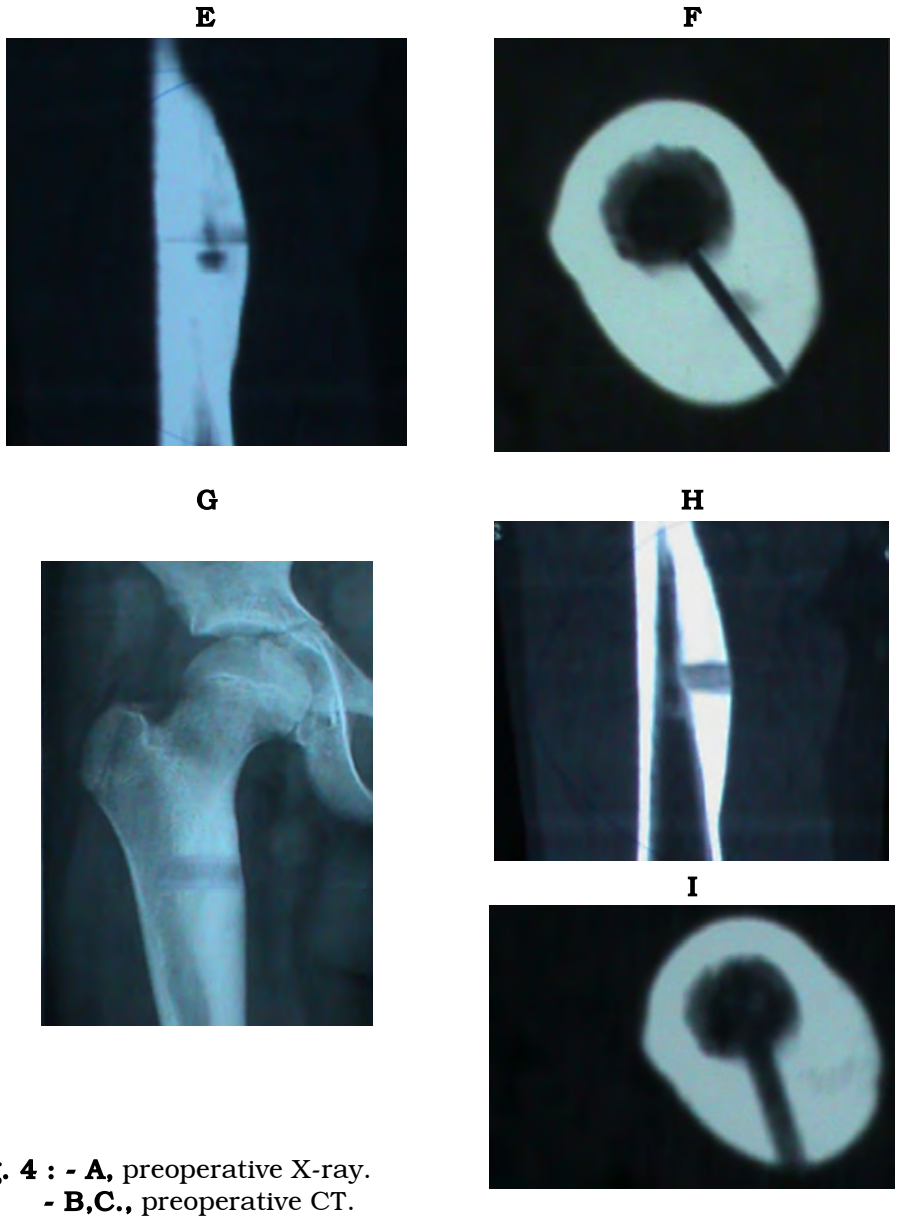


Fig. 4 : - A, preoperative X-ray.
- B,C., preoperative CT.
- D., postoperative X-ray showing incomplete removal of the nidus.
- E.,F., postoperative CT showing incomplete removal of the nidus.
- G. X-ray after rereaming by larger beer.
- H.,I., CT after rereaming by larger beer, showing complete removal of the nidus.

Discussion

Surgery that consists of en bloc resection of the nidus is successful in the majority of cases[18]. However, because OO is a small lesion often surrounded by dense reactive bone sclerosis, preoperative localisation of the nidus may be difficult. In a small percentage of cases, the nidus may be missed during surgery resulting in failure and reoperation [18,19].

Some locations may be problematic. Surgical excision of a paraarticular nidus may require arthrotomy[20], which has its own complications (reflex sympathetic dystrophy, infection, and secondary degenerative changes).

In recent years, several techniques of percutaneous treatment of OO under CT control have been proposed. Excision through bone trephination[11,13], a combination of partial percutaneous resection with subsequent intralesional ethanol injection[14,15], and destruction of the nidus using radiofrequency electrodes[16,21] or laser photocoagulation[17].

Percutaneous treatment of OO has several advantages over surgery; it allows precise localization and complete destruction of the nidus [11,13].

In a series of 38 patients treated by percutaneous resection with a trephine, the overall rate of complications was 24%[22]. These complications included two fractures and two skin burns. Parlier-Cuau et al[23] reported that two out of 32 patients had skin and soft tissue burns. In order to prevent secondary fractures their patients were asked to avoid weight bearing and to use crutches for six weeks when the OO was located in the lower limb [23].

Percutaneous resection with a trephine requires relatively large caliber instruments; therefore subsequent structural weakness of the affected bone can lead to impaired weight bearing for up to six weeks after surgery and even to fracture[23].

In our study, a 4.5-mm bone drill bit, followed by a 4-mm speed burr, through the same track, was sufficient for complete destruction of the nidus. Because of the only a

small amount of bone was removed with percutaneous resection, the patients stay 3 or 4 days in the hospital and quickly able to return to their normal activities[24]. This relatively small caliber of drill bit and burr avoided fractures, but there instruments provided enough material for histopathological examination. Moreover a sufficiently large skin incision (2 to 3 cm) and the use of a sleeve protected the skin and the soft tissues against sepsis and burns.

The use of the fracture table and the C-arm for antero-posterior and lateral radiographs is familiar to all orthopaedic surgeons and the equipment is widely available. We would stress, however, that the image intensifier must give a high-resolution image, have good contrast control and be fitted, preferably, with magnification facilities.

Rosenthal et al [25] described the technique of percutaneous radiofrequency thermo-coagulation in 1992, and later reported their results in 18 patients [16]. Sixteen patients had a successful outcome, while two had residual pain. Linder et al [26] described 58 cases treated with percutaneous radiofrequency ablation. The treatment was immediately successful in 95% of the patients and in 100% after a second ablation. De Berg et al [21] reported 17 patients treated successfully by percutaneous radiofrequency thermocoagulation of the nidus; one patient needed two procedures. In 1997 Gangi et al [17] discussed their experience with laser interstitial photocoagulation of OO.

They had 13 successful results and one failure, due to a technical problem. The main disadvantage of either percutaneous radiofrequency thermocoagulation or laser interstitial photocoagulation is the lack of histological verification [26].

Medical management of osteoid osteoma in surgically difficult sites has recently been advocated as an alternative to high-risk surgery (Kneisl and Simon 1992) [27], but our patients' symptoms were not controlled by non-steroidal drugs, even when combined with other analgesics [27]. We suggest that,

for many children, minimally invasive surgery is preferable to prolonged medication and incomplete control of symptoms. When a child's sleep is regularly disturbed by night pain, family life can be seriously disrupted.

Conclusion

Percutaneous treatment of Osteoid osteoma is minimally invasive, safe and simple. It allows an early return to normal activities. The procedure is particularly useful for a lesion located deep in the skeleton, which requires an extensive approach with conventional surgery. Surgical en bloc excision should be restricted to cases impossible to treat with percutaneous methods. Percutaneous destruction of the nidus is more cost effective than the other percutaneous methods of destruction of the nidus (either by radiofrequency thermo-coagulation or laser interstitial photocoagulation). Moreover, the destruction technique has fewer risks for the structural integrity of the skin and the subcutaneous tissues.

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IMAGE INTENSIFIER-GUIDED PERCUTANEOUS DRILLING FOR OSTEOID OSTEOMA OF THE PROXIMAL FEMUR

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ASSESSMENT OF EPITHELIAL DIFFERENTIATION OF EPENDYMOMA USING CD10, CK AND EMA IN CORRELATION WITH MICROVASCULAR DENSITY AND PROLIFERATION ACTIVITY USING TISSUE MICROARRAY

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Abstract

Background: *Although ependymomas are the third most common type of brain tumor in children (following astrocytoma and medulloblastoma), they are relatively rare. The prognosis remains poor, regadless of introduction of radiotherapy or chemotherapy in the treatment protocols. So, there is considerable interest in the search for new molecular markers of ependymomal neoplasma that may serve as basis for future targeted therapy.*

Material and Methods: *Twenty cases of intracranial ependymoma diagnosed in the pathology department, Mansoura University, Egypt from 2010-2012 were graded according to WHO classification for central nervous system (CNS) tumors 2007. A tissue microarray (TMA) block was prepared and immunohistochemical analysis with CD10, epithelial membrane antigen (EMA), Ki-67, CD34 and cytokeratin (CK) was performed.*

Results: *All 20 cases were intracranial ependymomal tumors.The age group ranged from 13 years to 65 years (median 39 years). They were 16 cases grade II and 4 cases grade III. CD10 and CK showed negative immunoreactivity in all tested ependymomas. The tumor cells showed EMA dot like intracytoplasmic immunoreactivity in only one case. While CD34-MVD immunopositivty was seen in 4 cases (20%) of tested ependymomas grade III. The Ki-67 showed immunopositivity in 7 cases (3 cases diagnosed as gradeII, and 4 cases diagnosed as grade III), and the labeling index is increased from grade II to grade III tumors. The difference was highly significant between grade II and grade III (<2% vs. >5%).*

Conclusion : *There is no detected expression of CD10 or CK in the tested ependymomal neoplasms.High grade ependymomas showed immunopsitivity for CD34 and more prominent labelling indices of Ki-67.*

Key Words : *Ependymoma, TMA (Tissue micro array), IHC (immunohistochemistery).*

Introduction

Brain tumors are the second most frequent cause of childhood malignancy after haematological disorders. Ependymomas account for 2.1% of all primary brain tumors regardless of age.They represent 6.4% when patients between 0 and 14 years, and 5% between the ages of 15 and 19[1]. Ependymomas are glial tumors that exhibit ependymal differentiation. This group of neoplasms includes, subependymoma (WHO grade I), myxopapillary ependymoma (WHO grade1), ependymomas (WHO grade II), and anaplastic ependymomas (WHO gradeIII)[2]. Ependymomas are the most common primary tumors of the spinal cord especially in adults[3]. These tumors are equally distributed between the sexes[4]. An ependymal cell is a cell that has basically dual morphological and functional characteristics as an epithelial cell covering the surface of the ventricular system and as a glial cell that shows alterations closely resembling those of astrocytes under various pathological conditions[5]. These dual characteristics are also expressed to varying degrees in ependymal neoplasms[6]. Such neoplasms exhibit multiple varieties of histologic patterns. The cellularity and architecture of ependymomas vary considerably not only from case to case but also from area to area within the same tumor[7].

Although vascular proliferation is commonly seen in ependymomas, relatively little is known about the angiogenic profile of these tumors. The ependymoma vasculature is relatively mature and has little angiogenic activity compared with malignant gliomas[8]. CD34 is a potential indicator of vascular differentiation. The typical immunohistochemical staining pattern shared by the majority of ependymal neoplasms is positivity for S100, glial fibrillary acidic protein (GFAP) but GFAP is not specific of ependymomal neoplasms as it is expressed in all gliomas. CD99 and Vimentin

are also expressed in most ependymomal tumors [9,10]. The morphological distinction of ependymomas with epithelial cytology from metastatic carcinoma may pose a significant problem in differential diagnosis[11]. Epithelial membrane antigen (EMA) staining often shows a punctuate, dotlike positivity; ringlike EMA staining is less common[12]. Myxopapillary ependymomas are EMA negative[13] Stains for cytokeratin and neural markers are typically negative. Many authors regarded increased mitotic activity or high Ki67 labeling index as the most significant correlate with the prognosis of ependymal neoplasms[14]. As regard proliferation index, Ki67 labeling is lowest in subependymoma and myxopapillary ependymoma, with WHO grades II and III showing higher labeling indices that are correlative with their respective higher grades[15]. The cut off point was 2% (low) 2-5% (moderate) and >5% (high) index[16]. CD10 is a cell surface enzyme with neutral metalloendopeptidase activity which inactivates a variety of biologically active peptides. CD10 is expressed on breast myoepithelial cells, bile canaliculi, fibroblasts, with specially high expression on the brush border of kidney and gut epithelial cells. CD10 is also expressed on the cells of lymphoblastic, Burkitt's, and follicular germinal center lymphomas, and on cells from patients with chronic myelocytic leukemia (CML). It is also expressed on the surface of normal early lymphoid progenitor cells, immature B cells within adult bone marrow and germinal center B cells within lymphoid tissue.Anti CD10 target therapy is now in use for some solid tumors with CD10 immunopositivity as in gastrointestinal stromal tumors (GIST)[17].

This is the first study to assess the utility of CD10 immunostaining as an epithelial marker for identification of ependymal neoplasm differentiation, and whether it can be a useful adjuvant to identify this tumor and may impact the future management of CD10 immunoreactive ependymomas.

The aim of this study was to

evaluate the immunoreactivity of some epithelial markers CD10, CK and EMA in ependymomal neoplasms, correlation of CD34- MVD with the Ki-67 labeling index using TMA in attempt to elucidate the epithelial differentiation in ependymomal tumors to point out the possibility of new adjuvant therapy.

Materials and Methods

This study was performed on the formalin fixed paraffin embedded tissue from 20 ependymomas recruited from the pathology department archives, Mansoura University, Egypt in the period 2010-2012. All of the reports were reviewed for clinical information including age and gender of patients,location of the tumor and use of adjuvant radiation therapy or chemotherapy. A tissue microarray was constructed manually according to[18]. Slides were cut, 4 um thickness and immunohistochemical staining was performed for the following antibodies, CD10, CD34, EMA, CK and Ki67 (MIB-1) according to the streptavidin-biotin-peroxidase technique using anti-ki67 (rabbit monoclonal antibody Thermo Scientific Lab Vision, dilution 1:100). Anti CD34 antibody (Clone QBEnd/10, Thermo Fisher Scientific, Fremont, CA, USA)) anti CD10 (monoclonal mouse anti CD10, Clone 56C6, Dako), anti EMA (monoclonal mouse anti EMA, clone E29 dako) and anti CK (Polyclonal Rabbit Anti-Cytokeratin, DAKO). The slides were deparafinized in xylene before being rehydrated sequentially in ethanol (100%, 90%, and 70%) and placed in a 1%phostate-buffered solution (PBS, pH 7.4). The sections were heated in a pressure cooker for 4 min. The sections were then allowed to cool to room temperature for 40 min before using 2% normal horse serum to block nonspecific protein binding. The sections were incubated with the primary antibodies overnight at 4℃ in a humidified chamber. Detection was done using Avidin-biotin horseradish peroxidase complex with DAB and Mayer's hematoxylin (Fisher Scientific, Fair Lawn, NJ). Cores of colonic carcinoma and tonsillar tissue were used as control and a negative control was used by substituting phosphate buffered saline (PBS) for the primary antibody to evaluate non-specific staining

and better interpretation of specific staining. All controls yielded appropriate results.

Interpretation of staining:

Only the nuclear immunoreactivity for ki67 was considered specific. The result of ki67 was assessed by percentage of positively stained nuclei out of the total cell counted (the labeling index) and categorized as: negative if no nuclear staining in the examined cells, low if there was nuclear staining in 2% of cells, moderate if there was nuclear staining in 2-5% of cells, and high if there was nuclear staining in >5% of cells.All slides were immunocytochemically evaluated without any information of clinical or radiological data.

Ki67 Labeling index: count 1000 nuclei in most immunohistochemically stained fields and calculate the % of the positive cells. Cut off point was 2% (low) 2-5% (moderate) and >5% (high) index [16].

Determination of microvascular density (MVD):

CD34 reactivity was considered positive only in the newly formed small vessels and not in the well-formed vessels and vessels of reossets. MVD was evaluated according to the method described by[19]. Brown-stained endothelial cell or endothelial cell cluster, which was clearly separate from adjacent microvessels and other connective tissue elements, was considered a single,countable blood vessel. Screening of the cores was first performed at a low power (40 X) to identify areasof the highest MVD. Counting was performed in the three highest MVD areas at high power (400 X). The mean value of the counted three fields was considered as the MVD of an individual case.

EMA was considered positive if there is intracytoplasmic dot-like or ring-like staining.

Statical analysis:

Statistical analysis of the individual immunocytochemical stains were calculated using statistical package for social science (SPSS), version 12. Fisher exact test was used to test association of markers expression with the

different grades of tumors.P value of less than 0.0.5 was considered as level of significance.

Results

All 20 cases were intracranial ependymal neoplasms. They were12 males and 8 females, with M:F ratio 3:2. The age group ranged from 13 years to 65 years (median 39 years). They were classified according to the diagnostic criteria devised by the recent WHO classification of tumors, 2007. There were 16 cases grade II and 4 cases grad III.

All tested tumors showed CD10, and CK negative immunoreactivity. Seven out of 20 cases (35%) showed immunopositivity for Ki67 (3 cases diagnosed as grade II, and 4 cases diagnosed as grade III) with variable labeling index as follow (3 cases <2%, 1 case 2-5% and 4 cases >5%). The labeling index is increased from grade II to grade III tumors. The difference was highly significant between grade II and grade III (<2% vs. >5%).

CD34 staining revealed immunopositivity of newly formed small vessels in 4 cases (20%) out of 20 tested tumors. All 4 positive tumors were grade III. There was highly significant correlation (P=0.007) between Ki76 and CD34 immunopositivity in cases of grade III ependymal neoplasms. The tumor cells showed EMA dot like intracytoplasmic immunoreactivity in only one case.

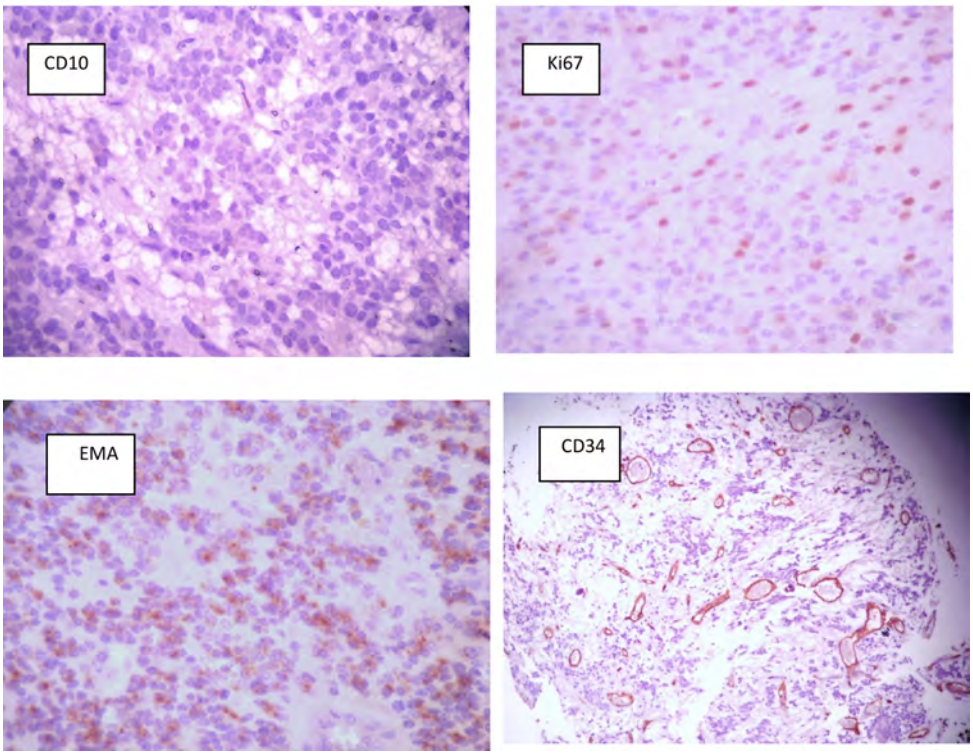

Fig. 1 : **(A)** CD10 negative immunostaining(original magnification x 400). **(B)** Ki67: Positive nuclear staining in high grade ependymoma (original magnificationx400). **(C)** EMA: positive intracytoplasmic dotlike immunostaining in high grade ependymoma(original magnification x 400). **(D)** CD34 : positive immunostaining in newly formed vessels in comparison to vessels of rossets(original magnification x 100).

Discussion

Intracranial ependymomas are the third most common primary brain tumors in children. Although, clinical and histological criteria for ependymoma prognosis are recognized, studies have reported contradictory results. Prognostic significance based on immunohistochemistery of ependymal tumors has been reported in a few studies. Some immunohistochemical variables were found to be the strong predictors of ependymoma recurrence and they seem to be useful for assessing individual tumor prognosis in routinely processed biopsy specimens together with tumor grade.[20].

Targeting the neovasculature of tumors is a promising addition to the current conventional treatment of cancer[10]. Angiogenesis has been increasingly recognized as an important process in the outgrowth of solid tumors. Parameters related to tumor angiogenesis such as MVD-CD34 expression have been shown to be predictive of the prognosis in a host of tumors and correlate with histologic tumor grade in gliomas[21]. Moreover, clinical trials of therapies aimed at the neovasculature have produced promising results[22]. The ependymoma vasculature is relatively mature and has little angiogenic activity compared with malignant gliomas. Therefore, the window for vessel normalization as a therapeutic aim might be considered small. However, the status of the tumor vasculature may not be a reliable predictor of treatment effect. Therefore, possible benefits of antiangiogenic treatment cannot be excluded beforehand in patients with ependymomas.

In the current study, MVD-CD34 immunostaining was only detected in 4 out of the 20 tested cases and all of these cases were anaplastic (grade III) tumours. This result is nearly similar to that obtained by [23], who mentioned that Less is known about the neovasculature in pediatric ependymoma,in which the neovascularization is less prominent.

We found that the Ki-67 positive expression in intracranial ependymoma tissues of grade III

was higher than that of grades II. Ki-67 was not detected in many of the grade II ependymomas by immunohistochemistry, which coincides with the findings of 24and 25. Who reported that Ki67 expression in intracranial ependymoma tissues is related to the increase in pathological grades.

In this study, there was only one case with dotlike intracytoplasmic immunopositivity for EMA, which is another marker that has been proven to be highly selective in ependymoma and is considered a marker for the differentiated forms of these tumors[26]. This result is similar to that obtained by[27] who reported that neither staining intensity or pattern was related to tumor type. Moreover, EMA immunoreactivity did not reveal any differences between grade II and grade III ependymomas, so EMA could not differentiate between different types and grades of ependymoma.

In this study, CK and CD10, immunostaining were negative in all grades of ependymal neoplasms.

Conclusion

Results revealed weak immunoreactivity of ependymal neoplasms for EMA. CD10 and CK were not expressed by ependymal neoplasms. Consequently, Anti CD10 target therapy has no role in the therapeutic intervention of this type of tumours. On the other hand, antiangiogenic therapy can be considered and require future studies with more specific markers as CD105

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ASSESSMENT OF EPITHELIAL DIFFERENTIATION OF EPENDYMOMA USING CD10, CK AND EMA IN CORRELATION WITH MICROVASCULAR DENSITY AND PROLIFERATION ACTIVITY USING TISSUE MICROARRAY

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ANOVESTIBULAR FISTULA IN NEONATES : OUTCOME OF SINGLE STAGE REPAIR

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Abstract

Purpose : *Vestibular fistula is the commonest anorectal malformation in the female child. This article reports the treatment and follow-up of 10 patients of vestibular fistula treated by anterior sagittal anorectoplasty (ASARP) in a single stage in early neonatal age group.*

Material and Methods : *From 2008-2010, ten patients of vestibular fistula admitted and operated on at the Department of pediatric surgery, Madinah, KSA. Age group was between 2-11 days. Single stage ASARP was done in these cases in the lithotomy position, the fistula was freed and a sufficient length of the rectum was obtained after separation of the vagina. The proposed anal position was marked via a midline incision and the freed anorectum was pulled back and anoplasty was done. Patients were evaluated for preoperative time, time to start oral feeds and hospital stay. On follow-up visits, all cases were assessed for continence and need to anal dilatation in addition to cosmetic appearance.*

Results : *Operative time ranged between 70-135 minutes. Patients passed stool in good frequency from the first day post-operatively and oral feeds was allowed at fifth day. Follow-up ranges from 18 months to 24 months. Wound infection was seen in one case (10%), mucosal prolapse in two cases (20%) and constipation in two cases (20%). Anal dilatation was routinely done 1 week postoperatively. Continence results was good (80%).*

Conclusions : *This experience with ASARP showed a good results in a single-stage procedure and we recommend it as the procedure of choice for vestibular fistula in females at early neonatal age. On the other hand, we think that to establish this conclusion, further studies with high number of patients and long term follow-up still required.*

Keywords: *vestibular fistula, anterior sagittal anorectoplasty.*

Introduction

Anorectal malformations (ARM) are one of the most common congenital defects in females, having a wide spectrum of lesions from low types to a very complex cloacal anomalies [1,2]. The reported incidence is between 1 per 1500 to1 per 5000 live births [3,4].

Recto vestibular fistula (RVF) is the most common ARM in females and easily diagnosed by perineal inspection that shows a normal urethral, normal vaginal and another orifice which is the rectal fistula in the vestibule[5].

Management of ARM in the neonatal period is important as it detects the future of child as regarding fecal continence and cosmoses'. Pena recommended a limited posterior sagittal anorecto plasty (PSARP) with a preliminary colostomy for this anomaly[6].

The anterior sagittal approach (ASARP) was described with sparing the levator muscle and pushing the rectum to normal location[7].

Multiple stage surgery for RVF increases the time, cost of treatment and psychological stess for the family. In addition, creation of colostomy carries many comlicactions namely, skin excoriation, prolapse, parastomal hernia and bowel obstruction due to abdominal adhesions. Similarly, colostomy closure is not a simple surgery and carries a significant morbidity and mortality[11].

In our study patients of RVF were treated by primary single stage ASARP in the neonatal age group without through bowel prepapation before surgery and without diverting colostomy. The aim of this series is to show our experience with single stage ASARP in managing RVF in female neonates with special concern about functional and cosmetic outcomes.

Methods

The medical records of 10 patients with RVF admitted at pediatric surgery department, Madinah maternity and children hospital, KSA between February 2008 to March 2010 were reviewed. Ages ranged from 2-11 days.

All patients has a preoperative hemogram, hemoglobin level of 10

gm|dl is the minimum of surgery . Ultrasound of the abdomen was done for all patients to rule out associated renal problems. Echocardiography was done in those patients showing clinical features of cardiac anomaly.

Preoperative preparation consisted of saline washouts, holding of feeds 4-6 hours as required for anesthesia.

Preoperative doses of I.V. antibiotics (cepholosporins and metronidazole) were given. The operation was performed under general anesthesia with end tracheal intubation in lithotomy position.

Surgical Technique :

Proposed anal position was identified by muscle stimulator and skin incision is made in the midline. (Fig. no. 1). The fistula was identified by stay sutures, then freed from all surrounding structures with care not to cause vaginal injury. (Fig. no. 2) Dissection was deepened to provide a sufficient length of the rectum to be mobilized posteriorly. Finally anoplasty was performed with vicryl stitches 4/0 and the perineal body is reconstructed. (Fig. 3).

Patients were passing stool in good frequency from the first day postoperatively and good cleaning was advised by normal saline solution. The wound was kept undressed from the second day postoperatively and inspected daily for infection, wound gaping, anal retraction, anal stenosis and mucosal prolapse. After discharge patients were followed in outpatient clinic for 18-24 months.

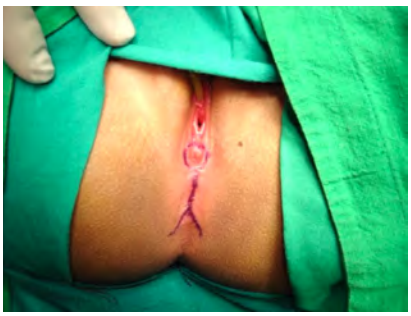

Fig. 1: Skin incision and idetification of the fistula.

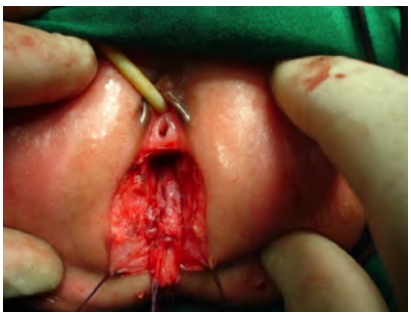

Fig. 2: The anorectum is dissected from the vaginal wall.

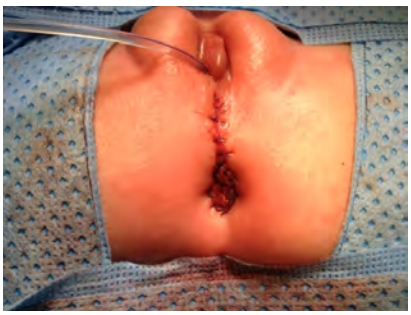

Fig. 3: Anoplasy is comleted.

Oral feeds were allowed at the fifth day postoperatively and patients were discharged after tolerating feeds. Anal dilatation was done regularly once daily one week after the operation. All cases was followed for at least 6 months at our out patients clinics for cosmetic appearance, wound infections, mucosal prolapse and funcional outcome. Functional outcome was assessed by history of soiling noticed by the mother and Kelly"s method mainly after the age of two years.

Results

Mean age at presentation was 3.7 days (range 2-11 days). Associated anomalies were seen in 4 cases (40%), one had sacral anomaly, one had myelomeningocele and 2 had cardiac lesions.

Mean peroperative time was 90 minutes (range 70-135 minutes).

List of complications is demonstrated in table 1.

Wound infection occurred in one case (10%) and responded to frequent dressing and antibiotics .

Two cases developed partial mucosal prolapse that was at one side in the first two cases of our series. They need mucosectomy 6 months later.

Anal stenosis and constipation were observed in 2 cases (20%) and improved after 2 months of regular dilatation and laxatives.

Table 1: Postoperative complications in the postoperative period and on follow up visits.

Complications	Number of cases	Percentage
Vaginal injury	1	10%
Wound infection	1	10%
Mucosal prolapsed	2	20%
Anal stenosis	2	20%
Constipation	2	20%

Table 2 : Postoperative complications of different series of single repair of vestibular fistulas .

Complications	Vijai D. et al (11) (2008)	Dare s. and Shi Ec (2005) (14)	Our series
Vaginal wall injury	2/40 cases (5%)	2/12 cases (16.6%)	1/10cases (10%)
Mucosal prolapse	4/40 (10%)	1/12 (8.3%)	2 (20%)
Wound infection	3/40 (7.5%)	2/12 (16.6%)	1(10%)
Anal stenosis	2/40 (5%)	4/12 (33.3%)	2 (20%)
Constipation (Difference is due to definition of constipation)	6/40 (15%)	4/12 (33.3%)	2(20%)

Discussion

Vestibular fistula is the commonest anorectal anomaly in female children where the bowel opens between the vagina and the fourchette[8]. Most reports classify this anomaly as low type, however, Heinen has considered it as intermediate type[9].

Surgical treatment of AVF involved many techniques starting from cut back operation, anal transposition, Y-V and X-Z plasty and most recently, the limited posterior sagittal anorectoplsty[10]. Okade tal[7] was the first who described anterior sagittal anorectoplasty for vestibular fis-

tula in 1993, where he did the incision anterior to proposed neoanus thus, it is easier to perform this operation in the lithotomy position.

Single stage surgery is quite safe in most of cases of anorectal malformations either low or high types[11]. The value of colostomy is to protect the perineal wound from contamination by stool, thus enhancing good healing, but it carries many complications including prolapse, stenosis, retraction and skin irritations. The option of single stage repair in vestibular fistula in early neonatal period was supported by good bowel preparation that can achieve the colostomy goals. In addition, the meconium formed in early neonatal period is sterile and its effect on wound healing is weak[12]. There is evidence that early restoration of gastro-intestinal continuity is very important to establish the brain defecation reflexes early. Also, the early training of sensory input from the peri-anal skin and synapses are important components of continence, thus enhancing the chance of normal or near normal defecation functions[13].

Dissection of the rectal wall from the vagina can be carried out with safety if the surgeon used his finger to be inserted in the rectum and a feeding tube to identify the fistula [12].

In our study, we have included 10 cases of AVF presented in the neonatal period (less than 15 days of age). Wound infection occurred in one case (10%) and responded to frequent dressing and antibiotics. This rate was lower than Vijai D, et al., (2008)[11] and Dare S, etal., (2005)[14], although they did through bowel preparation. This can be explained by different numbers of cases in each series and multiple policies of antibiotic administrations.

Anal stenosis and constipation were observed in 2 cases (20%) and improved after 2 months of regular dilatation and laxatives. Our rates were higher than Vijai D., (15%) and lower than Dare S., (33.3%). This can be explained by multiple difinitions of constipation in different institutes.

The value of our series is the performance of the repair without a diverting colostomy, thourgh bowel preparation was not required, oral feeds allowed after 5 days and patient went home by the sixth day postoperatively.

Continence results according to Kelley's method were good in 8 cases (80%), fair in one case (10%) and poor in one case (10%). The latter case had meningocele and was repaired one week before our intervention. Cosmetic results were good. Table 2 shows postoperative complications in different series of single stage ASARP for neonatal vestibular fistula.

In conclusion, ASARP can be done in one - stage procedure for correction of vestibule fistula in the neonatal period with safety. The most important keys are direct rectovaginal dissection, and good perineal reconstruction .Our experience suggests this technique safe, easy, cosmetically and functionally good and feasible in all cases of neonatal rectovestibular fistulas. On the other hand, we think that to establish this conclusion, further studies with high number of patients and long term follow-up still required.

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ANOVESTIBULAR FISTULA IN NEONATES : OUTCOME OF SINGLE STAGE REPAIR

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TECHNICAL VIEWS IN SONDGRASS URETHEROPLASTY: WHICH IS BETTER?

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Abstract

Purpose/Background: *Snodgrass introudced his technique for repair of distal hypospadius and its use is extended to proximal types. There are various technical views in performing the technique as regarding the uretheral plate creation, types of covering layer and uretheral stenting. We aim to evaluate these various aspects and assess different complications concluding which of these views are better.*

Patiens/ Methods: *Between March 2007 and April 2012, 320 cases of hypospadius operated by Snodgrass uretheroplasty. These cases were classified into two groups based on two technical views in Snodgrass uretheroplasy. Group A; composed of the first 115 patients done. In this group of patients, the urethral plate defining incisions and the sagittal deep longitudinal incision were extended to the tip of glans and the neourethera was reconstructed over a 6-10 Fr silicone stent based on child age and covered by a dorsal perpetual flap. On the other hand, Group B included the lately 205 patients done.The urethral incisions and the deep longitudinal incision extended to midglans level and the neourethera was reconstructed over size 6- 10F catheter and covered by lateral dartos flap .In both group of patients, the catheter was left for 3-4 days postoperatively.*

Results: *Complications occurred in 45 patients with a rate of 14.06%. A uretherocutaneous fistula occurred in 8 patients of total (2.5%), six cases in group A (5.22%) and two cases in group B (0.98%). Reoperation by two layers repair was needed in 6 cases 6 months postoperatively with good outcome. The other 2 cases healed on conservative management after metal stenosis was managed by dilatation. Ten patients (3.13%) developed meatal stenosis and had thin and weak urinary stream 1 month after uretheroplasty. These cases responded to*

regular dilatation for 3 months except one patient for whom meatoplasty was performed.

Penile torsion was recorded postoperatively in 13 patients (total rate of 4.06%), nine cases in group A (7.83%) and 4 cases in group B(1.95%). Eight cases were mild and don't need intervention. The other five cases need degloving and adjustment of penile direction which were done 8 months to 1 year postoperatively. It is clear that there are statistically significant differences between the two groups regarding the occurrence of uretrocutaneous fistula, meatal stenosis and postoperative penile torsion (P-value = 0.027, 0.028, 0.013 : respectively). Contrary there is no significant difference between the two groups regearding the final cosmetic appearance.

Conclusion: *Snodgrass ureheroplasy is an excellent method for management of both distal and prximal types of hypospadius with few complications especially with increasing learning curves. The technical view of making the ends of both the uretheral plate defining incisions and midline releasing incision at a midglanular level, the use of ventral dartos flap ,limited penile degloving and uretheral stenting not more than 3 days yield a good functional and cosmotic results.*

Keywords: *Tubularized incised plate uretheroplasty, Snodgrass, Hypospadias.*

Introduction

In 1994, Snodgrass described the tubularized, incised plate (TIP) uretheroplasty for distal penile hypospadias repair[1-2]. Recently its application was extended to proximal hypospadias with encouraging results[3].

The principle steps in the repair are deep longitudinal incision of the uretheral plate which allows tubularization of urethral plate with ease and supporting the repair with additional layer between the neourethera and the overlying skin to avoid the occurrence of uretherocutaneous fistula[4]. The distal limit of the deep longitudinal incision varies between surgeons, either to the level of midglans or to the tip of glans. The covering flap of the neourethera is usually raised from the perpetual skin, but that may result in penile torsion as well as devascularization of the perpetual skin, which may be used in

reconstruction of the penile skin[5]. Alternatively, a lateral dartos flap has been used to cover the neourethera in order to avoid these complications. However, despite the frequent modifications in hypospadias repair, complications such as fistulae, uretheral strictures, meatal stenosis, penile torsion, persistent chordee, infections and wound dehiscence still have been described by investigators[6].

In this paper we describe our experience with Snodgrass TIP uretheroplasty and comparing two ways of operative repair regarding the postoperative complications and outcome with special emphasis on meatal stenosis, fistulae formation, residual chordee and penile torsion.

Material and Methods

This is a retrospective study in which we reviewed our result of Snodgrass TIP uretheroplasty in hypospadias repair that were done Between March 2007 and April 2012 at pediatric surgery department, Maternity and children hospital, Madinah Munawarah, Saudi Arabia. Cases of proximal hypospadias, hypospadias with severe chordee, and cases underwent previous uretheroplasty were excluded from the study. The procedures were done by the same team. The data collected included; demography of the patients, type of hypospadias, the extent of urethral plate incision, the type of the flap used to cover the uretheroplasty, the surgical outcomes and follow up duration. Comparison of the two groups regarding meatal stenosis, occurrence of fistula, penile torsion and cosmetic appearance were done.

The total cases retrieved were 320 cases. Based on the techniques used we divided our patients into 2 groups. Group A; composed of the first 115 patients done. In this group of patients, the urethral plate defining incisions and the sagittal deep longitudinal incision were extended to the tip of glans and the neourethera was reconstructed over a 6-10 Fr silicone stent based on child age and covered by a dorsal perpetual flap. On the other hand, Group B included the lately 205 patients done. The urethral incisions and the deep longitudinal

incision extended to midglans level and the neourethera was reconstructed over size 6- 10F catheter and covered by lateral dartos flap .In both group of patients, the catheter was left for 3-4 days postoperatively.

Data was collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis, (version 13; Inc., Chicago, IL, USA). Qualitative data was expressed as frequency and percent and compared using Chi-square Test. P-value was considered significant at level of ≤ 0.05.

Surgical technique:

All cases were done while the patient in supine position under general anesthesia and with supplement of caudal analgesia. The caudal analgesia composed of 0.25% bupivacaine in a dose of 1 ml/kg body weight.

A stay suture was placed at the dorsal side of the glans for handling in all cases. The urethral plate was outlined at a width of 6-8 mm. A U-shaped incision is made extending along the edges of the urethral plate and stopped at 2-3 mm from the tip of the glans. A longitudinal incision was made at the midline from the posterior edge of hypospadiac meatus to the distal extent of the urethral plate to widen the neourethera in group A patients and to the midglans level in group B patients. (Fig. A and B). The urethral plate is tubularized over a 6-10 Fr fenestrated silicone catheter with a continuous 6-0 PDS absorbable suture to create the neourethera. A circumferential incision 5mm to 1cm proximal to the coronal sulcus was extended from each edges of the urethral plate. Then, the penile skin is degloved in various degrees from 1cm proximal to hypospadiac meatus as were done in group A or down to the penoscrotal junction as in group B. A well vascularized subcutaneous tissue flap from the dorsal preputium was used to cover the neourethera in patients of group A, while a laterl dartos flap was used in group B (Fig. C and D).

The glanular wings were approximated by 6-0 vicryle absorbable suture and the distal

ends are fixed to underlying neourethera at 5 and 7 o'clock by the same type of suture. After completion of the repair, the urethral stent was fixed to the glans penis with 3/0 silk suture. All patients were admitted to the hospital postoperatively and usually discharged after the catheter was removed between the 3rd and 4th day postoperatively. A slit and vertically oriented oval meatus, conical glanular shape with direct urinary stream are the criteria of good results.

Results

We performed 320 cases with mean age of 1.5 years (range 8m - 8 year). The positions of the urethral meatus in the whole sample and in both groups were demonstrated in table 1. Two-hundred eighty-eight (95%) of the 320 patients eventually got an adequately functioning neourethera with slit-like meatus at the tip of the glans The length of the neourethera created after chordee release was 1.3-5.4cm (mean 3.1cm). Complications occurred in 45 patients with a rate of 14.06%. (Table 2). The distribution of these complications among the two groups was demonstrated in table 3. A uretherocutaneous fistula occurred in 8 patients of total (2.5%), six cases in group A (5.22%) and two cases in group B (0.98%). The location of these fistulae was sub- coronal in two patients and distal penile in seven. Reoperation by two layers repair was needed in 6 cases 6 months postoperatively with good outcome. The other 2 cases healed on conservative management after metal stenosis was managed by dilatation. Ten patients (3.13%) developed meatal stenosis and had thin and weak urinary stream 1 month after uretheroplasty. These cases responded to regular dilatation for 3 months except one patient for whom meatoplasty was performed.

Penile torsion was recorded postoperatively in 13 patients (total rate of 4.06%), nine cases in group A (7.83%) and 4 cases in group B (1.95%). Eight cases were mild and don't need intervention. The other five cases need degloving and adjustment of penile direction which were done 8 months to 1 year postoperatively.

From Table; 2 it is clear that there are statistically significant differences between the two groups regarding the occurrence of uretrocutaneous fistula, meatal stenosis and postoperative penile torsion (P-value = 0.027, 0.028, 0.013 : respectively). Contrary, there is no significant statistical difference between the two group regarding the final cosmetic appearance.

Table 1: Types of hypospadias and number of cases in the studied sample.

Types of hypospadias	Total sample 320 cases.	Group A. 115 patients	Group B. 205 patients
Glanular	33	18	15
Coronal	90	30	60
Distal penile	150	50	100
Midpenile	47	17	30

Table 2: Complication rates among the studied patients (320 patients).

Type of complication	No	Percentage
Uretherocutaneous fistula	8	(2.5)
Meatal stenosis	10	(3.13%)
Postoperative penile rotation	13	(4.06%)
Wound infection	5	(1.56%)
Catheter blockage	9	(2.81%)
Total	45	(14.06%)

Table 3: Comparison of complication rates among the two groups.

Types of complications	GroupA (115cases)	Group B (205cases)	p-value
Uretherocutaneous fistula	6 (5.22%)	2 (0.98%)	0.027
Meatal stenosis	7(6.09%)	3 (1.46%)	0.028
Postoperative penile rotation	9 (7.83%)	4(1.95%)	0.013
Wound infection	2(1.74%)	3(1.46%)	0.059
Catheter blockage	4(3.48%)	5(2.44%)	0.41
Total	28(24.35%)	17(8.29)	-
Total	28(24.35%)	17(8.29%)	

*P-value significant if < 0.05

Table 4 : Some Modifications of supportive tissue covering of TIP uretheroplasty and rates of fistula formation.

Covering tissue of TIP repair	References	Date	No . of patients	No .of fistulas (%)
Lateral dartos flap	Al-Hunayan et al [4]	2003	83	(5%)4
De-epithelializied preputial flap	Jayanthi [10]	2003	110	1(1%)
De-epithelializied preputial flap	Baccala et al [11]	2005	101	2(2%)
Parauretheral dartos flap	Mustafa [12]	2005	15	1(7%)
Double dartos flap	Bakan and Yildiz [13]	2007	45	0
Combined Mathieu and Snodgrass	Elganainny et al [14]	2010	101	8(7.9%)
Modified preputial flap	El-Kassaby et al [15]	2012	764	16(2%)

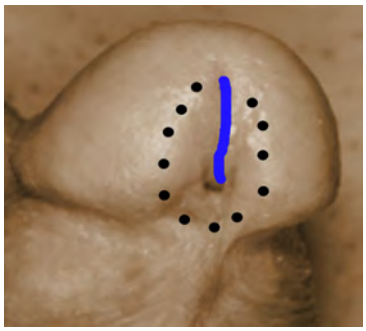

Fig. A : Outline the extent of U-shaped incision and the midline urethral plate incision in group A patients (up to the tip of the glans)

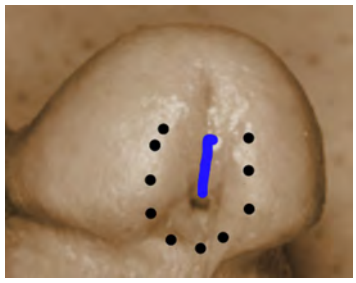

Fig. B : Outline the extent of U-shaped incision and the midline urethral plate incision in group B patients (up to the mid-glans level)

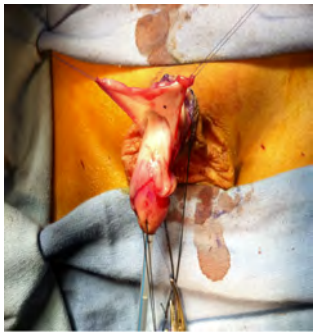

Fig. C : The raised preputial flap in group A patients.

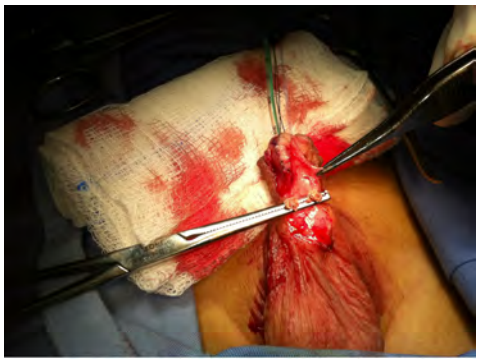

Fig. D : The raised lateral dartos flap in group B patients.

Discussion

TIP uretheroplasty become the popular technique for repair of primary hypospadias since 1994[2]. The surgical goals of hypospadias repair are; full straightening of the penis, formation of a hairless urethra of uniform caliber and adequate size, positioning of the meatus at the tip of the glans, and normal penile appearance with minimum of complications. Up till now whatever the technique used in hypospadias repair complications like fistula, meatal stenosis, urethral flap necrosis and dehiscence still encountered.

The longitudinal split of urethral plate described by Snodgrass is considered a significant progress in urethral plate preserving surgery. It permits tension-free tubularization of the urethral plate to form a neourethera of an adequate size. In our cases; in group A patients, we used to put the first neouretheral tube stitch near to the tip of the glans, distal to the middle glanular level. We found this technique was associated with increased incidence of meatal stenosis (6.09%), in comparison to group B where no more than one stitch beyond middle glanular level was taken during uretheroplasty and were found to have lower rate of meatal stenosis (1.46%). These findings agreed with AI-Hunayan et al and Mehmet et al results[4][7] whom

reported marked decrease in the rate of meatal stenosis with the 2nd method (24% to 15% and 18% to 5%; respectively). Another way we had used was to secure the neourethera to the glans to decrease the risk of retraction of the urethra and hence the development of meatal stenosis. This had not been described by Snodgrass et al in their initial report[1]. However, on their final reviews[6,8], they had mentioned that the meatus should be stitched to the glans at 5 and 7 o'clock to improve cosmetic result as well as decrease meatal stenosis. In the same context, Mehmet et al[7] advised to secure the meatus to the glans at 3-points (4, 6, 8 o'clock). Meatal stenosis in our patients was managed by regular dilatation and meatoplasty was required in one case. Another important step in Snodgrass repair is the interposition of a barrier layer (flap) between the neourethera and the overlying skin aiming to decrease the rate of uretherocutaneous fistula formation. The most popular flap used is the prepuital flap. However, mobilization and ventral transposition of the flap around one side of the penile shaft may cause penile torsion, especially if the flap is of inadequate length and laid-on with tension. Moreover, the dissection of the flap may jeopardize blood supply to the dorsal skin that may be used for resurfacing closure and predispose to skin loss and failure of the repair.

To avoid penile torsion, a modification in the way of mobilization of the preputial flap has been described[9]. A window in the flap at the midline was created, and then pulling the penile shaft through it so as to transfer the dartos flap ventrally over the neourethera .However in the later technique, the size of the flap may be inadequate to cover the repair when the ventral skin is deficient.

Another modification in flap creation that has been described in literature is to raise a lateral dartos ventral flap and cover the neourethera. The last technique was claimed to be associated with a low fistula rate [4]. In our experience we used both types of flaps; the perpetual flap in group A patients and the lateral dartos flap in group B. We found a higher

rate of fistula formation and penile torsion among group A (5.22% and 7.83%; respectively) in comparison to 1.46% and 1.9% in group B. this difference is statistically significant. We found the lateral dartos flap is usually easily raised and mobilized to the midline. In addition to that it is a good option in cases where circumcision has been done prior to uretheroplasty, as the preputial flap is not there in these cases Some of other modifications of supportive tissue covering TIP uretheroplasty and the rate of fistula formation that have been published in literature are summarized in Table 4. [ref; 4,10,11,12, 13,14,15].

Other factor which may affect the penile alignment is the degree of penile skin degloving during hypospadias repair; whether to do complete degloving to the penoscrotal junction or just limited to area around the urethral meatus . Turial et al.[16] recommend limited degloving of the penile skin in order to limit the need of a large covering layer of the neourethera, whereas, Selami S. and Warren S.[17] supported our way in doing complete degloving of the penile skin to provide full erection and prevent postoperative torsion and / or chordee. Other point of controversy in TIP uretheroplasty is the use of urethral stent. Those who advise stenting argue that the stent keeps the dorsal midline incision stretched open and limits the chance that it may heal primarily and thereby lose the benefit of the dorsal incision[10]. On the other hand, others didn't stent their patients and no case of uretherocutaneous fistula, urethral stricture or meatal stenosis had occurred[16,18]. We had stented all our patients in both groups for 3-4 days. This short period of stenting help in easily drainage of urinary bladder without fear of patient's fear of voiding due to surgery. In addition to that it helps haemostasis and stopping of bleeding post -operatively. In the same time this short period avoids the problems of catheter blockage, bladder irritation and long hospital stay.

As this study is retrospective, it has its limitation and further prospective comparative study is recommended. In conclusion, we

believed that Snodgrass TIP uretheroplasty is an excellent method for hypospadias repair.

Conclusion

Snodgrass ureheroplasy is an excellent method for management of both distal and prximal types of hypospadius with few complications especially with increasing learning curves.The technical view of making the ends of both the uretheral plate defining incisions and midline releasing incision at a midglanular level, the use of ventral dartos flap ,limited penile degloving and uretheral stenting not more than 3 days yield a good functional and cosmotic results.

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TECHNICAL VIEWS IN SONDGRASS URETHEROPLASTY: WHICH IS BETTER?

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EFFECT OF PREOPERATIVE PULMONARY ARTERY PRESSURE ON SURGICAL OUTCOME IN CHILDREN BELOW TWO YEARS WITH VENTRICULAR SEPTAL DEFECT (VSD)

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Abstract

Background: *Ventricular septal defect (VSD) is among the most common congenital heart anomalies. Now, primary VSD closure is the surgical treatment of choice for symptomatic patients. Long-standing and neglected cases of ventricular septal defects may develop progressive pulmonary obstructive disease with severe pulmonary hypertension which may affect surgical outcome significantly including mortality, morbidity and lifestyle.*

Methods: *160 patients with VSD below two years old with a mean age of 12.44 ± 5.76 months and a mean weight of 7.77 ± 2.05 kgs, were operated upon and their preoperative, operative and postoperative data were studied. The patients were divided into two groups according to their preoperative mean pulmonary artery pressure. Group I < 55 mmHg (mild and moderate pulmonary hypertension) and Group II > 55 mmHg (severe pulmonary hypertension).*

Results: *Analysis of the data showed, significant correlation between most data with higher morbidity and mortality in Group II.*

Conclusion: *Early intervention gives us the best surgical outcome with the least complications and mortality, and with less hospital stay and better life style.*

Introduction

Ventricular septal defects (VSDs) are among the most common congenital heart anomalies; isolated VSDs represent about 20% to 30% of all congenital

cardiac malformations and have a prevalence of 1 to 2 per 1,000 live births. [1]

Long-standing and neglected cases of ventricular septal defects may develop progressive pulmonary obstructive disease with severe pulmonary hypertension which may affect surgical outcome significantly including mortality, morbidity and lifestyle. [2]

Early definitive repair, before the development of significant histologic changes in the pulmonary arteries and before irreversible pulmonary vascular remodeling occurs, reduce the incidence of postoperative pulmonary hypertension. The incidence of pulmonary hypertension was higher among patients who underwent repair at an older age. [3]

Children with VSD are prone to develop perioperative elevations in PVR and this may complicate the postoperative course. Treatment strategies should focus on the underlying etiology, and should be continually re-evaluated not only from the stand point of the absolute PA pressure, but also the overall circulation and systemic perfusion. Rather than treat a specific or target PA pressure, the relationship of the PA pressure to the systemic artery pressure, along with the function of the right ventricle are critical. [4]

Pulmonary hypertensive crisis is described as an acute increase in pulmonary arterial pressure followed by reduction in cardiac output and a decrease in O_2 saturation. This syndrome occurs most commonly after repair of a congenital cardiac defect associated with a large left-to-right shunt and pulmonary arterial hypertension. Pulmonary hypertensive crisis appears to occur as a result of hypoxemia, hypothermia, hypercarbia, acidosis, or use of α-adrenergic inotropic agent and often occurs after suctioning of the endotracheal tube. Treatment includes hyperventilation with 100% O_2, alkalinization, sedation with intravenous fentanyl or morphine, paralysis with intravenous pancuronium and the use of pulmonary vasodilators. [5]

Several intravenous vasodilators, including the nitric oxide

donors nitroprusside and glycerol trinitrate, the phosphodiesterase inhibitors amrinone and milrinone, the eicosanoids prostaglandins E1 and prostacyclin I2, tolazolineand isoproterenol have been used to treat postoperative patients with elevated PVR. [6]

Endothelial dysfunction during and following cardiopulmonary bypass may have deleterious effects on the pulmonary circulation, which may explain the limited efficacy for this type of PH of endothelium-dependent intravenous vasodilator therapy with nitroglycerine or prostaglandins. Therefore, endothelium-independent pulmonary vasodilators may be indicated to treat persistent PH early after congenital surgery. The agents with the most selectivity for vasodilating the pulmonary vasculature are the gas nitric oxide (NO) and Sildenafil. [7]

Sildenafil produces a strong pulmonary vasodilative response by increasing the intracellular cGMP concentration through inhibition of phosphodiesterase-5 (PDE5), an enzyme that degrades cGMP. Sildenafil is given at a dose of 0.5 mg/kg every 4 to 6 hours. Sildenafil could solve several disadvantages of inhaled NO. Sildenafil doesn't need a special delivery system and rebound of PH doesn't occur after discontinuation of sildenafil as common after discontinuation of NO. [8]

A significant cause of morbidity and mortality is pulmonary vascular disease. Patients with high pulmonary vascular resistance operated on beyond infancy have a poor long-term prognosis because of progression of their pulmonary vascular disease. Reactive or persistent pulmonary hypertension has been one of the most significant causes of morbidity and mortality after operation for congenital heart disease. Preoperative pulmonary hypertension is the most significant risk factor for the development of postoperative pulmonary hypertensive events and deaths related to this complication. [9]

Patients and Methods

In this study we evaluated the effect of preoperative pulmonary artery pressure on surgical outcome after closure of VSD. This study was done on 160 patients

below two years of age. We classified them into two equal groups each of them included 80 patients. This classification was according to the degree of preoperative mean pulmonary artery pressure measured by echo. Group I patients with mPAP below 55mmHg (mild and moderate pulmonary hypertension) and group II patients with mPAP above 55mmHg (severe pulmonary hypertension).

All patients were operated upon under cardiopulmonary bypass. Myocardial protection was achieved by local cooling and coronary perfusion with cold cardioplegic solution.

All patients were subjected to pre, intra and postoperative studies with follow up visit after 6 months. Correlations between the preoperative, operative and postoperative data were presented with concentration on the impact of preoperative pulmonary hypertension on surgical outcome.

The preoperative data included clinical picture, routine laboratory investigations, chest x-ray, echo cardiographic findings specially the size and site of the ventricular septal defect, pressure gradient across the defect, Right ventricular pressure (RVP) and pulmonary artery pressure (PAP) and cardiac catheterization data. Cardiac catheterization was done for 30 patients of group II with very high pulmonary artery pressure or bidirectional shunt by echocardiography.

The operative data included aortic cross clamp time, total bypass time, type and size of the ventricular septal defect, pulmonary artery pressure before and after cardiopulmonary bypass by direct needle measurement, weaning off bypass and complications after bypass.

The postoperative data divided into early postoperative data and late follow up data after 6 months. The early postoperative data included the time of mechanical ventilation, inotropic support, vasodilators or sedation, pulmonary hypertensive crisis, the period of stay in the ICU, the period of stay in the hospital and the patient condition on discharge

clinically, by x-ray and echocardiography. The late follow up data after 6 months included the patient evaluation clinically, by x-ray and echocardiography.

Results

In group I the age ranged between 5 and 24 months with mean age of 12.9 ± 5.69 months. Their weight ranged between 5 and 12 Kilograms with mean weight of 8.56 ± 2.1 kgs.

In group II the age ranged between 5 and 24 months with mean age of 12 ± 5.84 months. Their weight ranged between 4.5 and 12 Kilograms with mean weight of 6.97 ± 1.6 kgs.

Comparative study between the two groups of patients regarding the mean age by using ANOVA test, we found no significant statistical difference between group I and II with P value > 0.05. As regard the mean weight, we found a significant statistical difference between group I and II with P value < 0.05.

The most frequent sign in chest x-ray was increased cardiothoracic ratio in 60% of group I and 100% of group II.

Comparative study between the two groups of patients regarding the mean ischemic time by using ANOVA test, we found no statistically significant difference between group I and II (P value > 0.05). Regarding the total bypass time there was marked significant difference between the two groups with P value < 0.001 as shown in chart (1).

Comparative study between the two groups of patients regarding the mean ICU and hospital stay periods by using ANOVA test, we found marked statistically significant difference between group I and II (P value < 0.001) as shown in chart (3).

Early postoperative complications detected by clinical examination, chest x-ray and echocardiography were as follows.

In group I, Five patients had chest infection with grade I dyspnea, 1 patient had superficial wound infection in contrast to 10 patients had chest infection, 7 pa-

tients with grade I dyspnea and 5 patients with grade II-III dyspnea due to prolonged ventilation. 4 patients had superficial wound infection in group II.

In group I, there was very mild increase in cardiothoracic ratio in 12 patients in contrast to mild increase in cardiothoracic ratio in 34 patients. And 14 cases with dilated pulmonary artery shadow.

In group I, 14 patients had small residual VSD (2-3ml), 9 cases had residual pulmonary hypertension ranged between 25 and 37 mmHg, 12 cases had mild to moderate pericardial effusion, 1 case had severe tricuspid regurge and 7 cases had mild to moderate tricuspid regurge. In group II, 17 patients had small residual VSD (2-3ml), 28 cases had residual pulmonary hypertension ranged between 28 and 65 mmHg. 13 cases had mild pericardial effusion. 18 cases had mild to moderate tricuspid regurge. 4 cases had severe tricuspid regurge.

Late postoperative follow-up (up to 6 months) revealed the following:

In group I, all cases were normal as regard there hemodynamics, chest condition and wound scar. In group II, 12 patients had recurrent chest infection with grade I dyspnea. 2 patients had wound Keloid formation.

In group I, all cases were with normal x-ray and normal cardiothoracic ratio. In group II, there was mild increase in cardiothoracic ratio in 20 patients. 12 cases with dilated pulmonary artery shadow.

In group II, 3 patients had small residual VSD (2 ml). No cases had residual pulmonary hypertension. 4 cases with right ventricular hypertrophy. 1 case had moderate tricuspid regurge. 2 cases had mild tricuspid regurge. In group II, 6 patients had small residual VSD (2 ml). 18 cases had residual pulmonary hypertension. 12 of them ranged between 26 and 35 mmHg and 6 cases with mean pulmonary artery pressure between 36 and 60 mmHg. 24 cases with right ventricular hypertrophy. 6 cases had mild tricuspid regurge.

As regard the postoperative mortality, in group I, two patients died in the ICU one of them due to ARDS and shock lung with failure of weaning from ventilation and the other due to bad chest condition which required recurrent and prolonged ventilation.

In group II, 16 patients died. 15 patients died in the early postoperative period; 8 of them due to attacks of pulmonary hypertensive crisis, 2 patients due to hemodynamic instability, 1 patient died after hemodynamic instability aggravated by an attack of hypertensive crisis, 1 patient died on table in the operating room after failure of weaning from bypass due to persistent severe pulmonary hypertension, 1 patient died due to bad chest condition which required recurrent and prolonged ventilation, 1 patient died after 48 hours of extubation due to sudden hypoxic arrest, 1 patient died due to bad chest condition together with progressive deterioration of hemodynamics and 1 patient died after 3 months of surgery after hospitalization for 3 days because of heart failure.

There is a significant difference in early and late postoperative morbidity and mortality between the two groups as shown in charts 4 and 5.

Table (1): Incidence of symptoms and signs in patients of the two groups.

Symptoms and signs	Group I		Group II	
	No. of cases	%	No. of cases	%
Dyspnea on effort(feeding)	34	42.5	50	62.5
Heavening of cardiac apex	0	0	18	22.5
Accentuated second sound on pulmonary area	50	62.5	72	90
Ejection systolic murmur on pulmonary area	20	25	60	75
Harsh pansystolic murmur along left sterna border	80	100	48	60
A symptomatic	30	37.5	8	10

Table (2): Echo-cardiographic findings of the two groups.

Echo-cardiographic findings		Group I		Group II	
		No of cases	%	No of cases	%
Pressure gradient across VSD	>60 mmHg	24	30	0	0
	30– 60 mmHg	48	60	4	5
	< 30 mmHg	8	10	76	95
Mean pulmonary artery pressure		37.2 ± 9.5		66.4 ± 9.1	
Size of VSD	< 0.5 cm	16	20	2	2.5
	0.5 - 1.5 cm	62	77.5	68	85
	> 1.5 cm	2	2.5	10	12.5
Site of VSD	Perimembranous	52	65	32	40
	Subarterial	16	20	32	40
	Inlet	4	5	6	7.5
	Muscular	8	10	10	12.5
Right ventricular hypertrophy		30	37.5	66	82.5

Table (3): Cardiac catheterization for 30 cases of group II.

Catheter data	30 patients of Group II
Mean PAP mmHg	74.13 ± 5.65
PVR Wood's unite	4.2 ± 1.29
SVR Wood's unite	10.84 ± 2.94
PVR/SVR %	40.05 ± 12.47
PVR/SVR % after O_2	26.83 ± 9.34
Qp L/min	13.87 ± 2.22
Qs L/min	8.48 ± 1.36
Qp/Qs	1.65 ± 0.21
Qp/Qs after O_2	2.12 ± 0.27

Table (4): Operative data for patients of the two groups.

Operative data		Group I		Group II	
		No of cases	%	No of cases	%
Method of closure	Direct closure	8	10	0	0
	Patch closure	72	90	80	100
Mean total bypass time ± SD		48.81 ± 8.51		58.26 ± 16.81	
Mean cross clamp time ± SD		33.08 ± 5.3		35.24 ± 5.68	

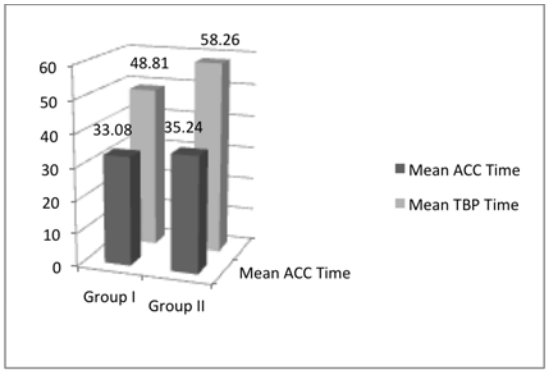

Chart (1): Comparative study between the two groups of patients regarding the mean ischemic time and the total bypass time by minutes.

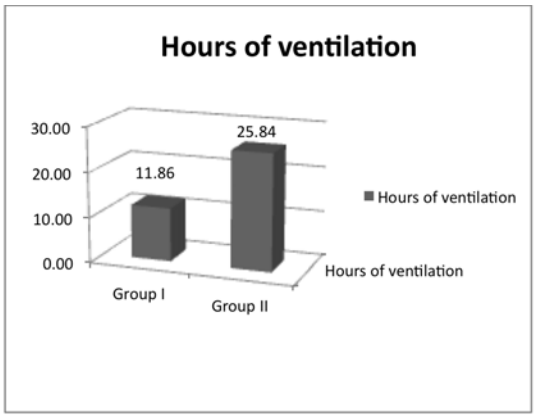

Chart (2): Comparative study between the two groups of patients regarding the mean ventilation time.

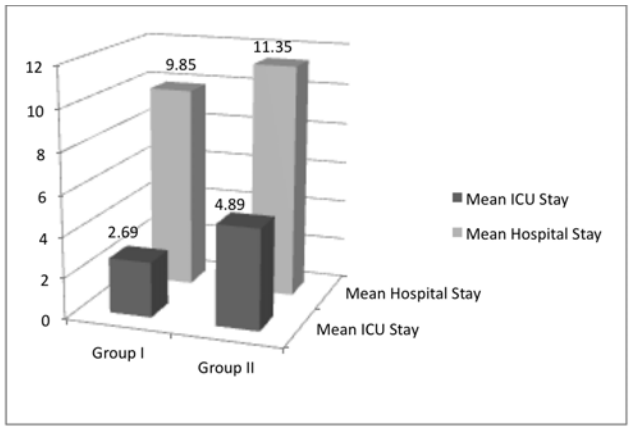

Chart (3): Comparative study between the two groups of patients regarding the mean ICU and hospital stay periods.

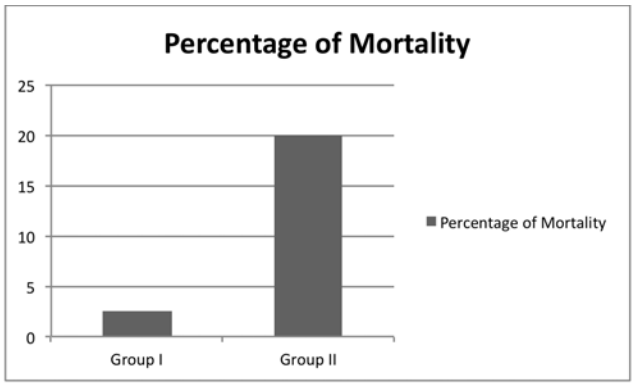

Chart (4): Postoperative mortality.

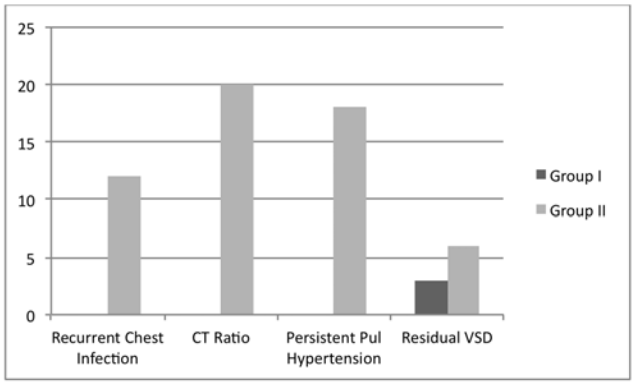

Chart (5): Follow-up visit after 6 months.

Discussion

The age of all patients included in this study ranged between 5 and 24 months with mean age of 12.44 ± 5.76 months. This range of age is different from other series which tends to interfere with VSD either so early before increase pulmonary vascular resistance or to study patients at a wider range of age without studying the impact of age on the outcome as shown in our study. Scully et al., 2010[10] studied 215 patients in similar surgical series underwent surgical repair for isolated ventricular septal defects with age ranged between 20 days and 18 years.

In the present study, cardiac catheterization was done for 30 patients in group two with very high pulmonary artery pressure or bidirectional shunt by echocardiography. Vida VL et al., 2006[11] recommended the same criteria when they studied 100 children and performed cardiac catheterization in 32 patients (32%) to gather more information about their pulmonary vascular reactivity to 100% inspired fraction of oxygen (FiO_2). Selected for cardiac catheterization were patients older than 2 years of age or who had bidirectional shunts across the VSD, or both.

We didn't sedate nor paralyze patients routinely unless indicated. Schulze-Neick et al., 2001[12] in similar series routinely sedate, mechanically ventilate, and paralyze all patients (with vecuronium, midazolam, and morphine) throughout the study. With mean time of ventilation of 42 hours for their patients in comparison to 11.86 hours for group I in our study and 25.84 hours for group II. In spite of sedating patients on ventilator by Schulze-Neick et al., 2001[12], three patients (20%) developed pulmonary hypertensive crisis and required more sedation and more time of ventilation as reported., Regarding the period of ventilation, the mean time of ventilation in group I with mild to moderate pulmonary hypertension was 11.86 ± 12.07 hours in contrast to 25.84 ± 27.49 hours in group II. 16 patients in our study required prolonged ventilation for more than 48 hours. This means that only 10% of all patients required prolonged ventilation for more than 48 hours with a mean

of 76.4 hours in contrast to the study by Schulze-Neick, 2001[12] who reported prolonged ventilation for 40% of the patients with a mean of 160.8 hours.

The pulmonary hypertensive crisis is a major risk factor in postoperative mortality after surgical closure of VSD with pulmonary artery hypertension.[3] In our series, among patients of group II with severe pulmonary artery hypertension, 16 (10% of all patients) patients developed pulmonary hypertensive crisis and 9 patients of the 16 who developed crisis died. In many series the incidence of pulmonary hypertensive crisis in patients with pulmonary artery hypertension is high for example: 20% of cases in the study of Schulze-Neick, et al. 2001[12], 9.5% of cases in the study of Farah Peiravian, et al. 2007[13] Dividing patients according to the degree of their preoperative pulmonary artery hypertension gives our study significant correlations as regard the relation between the preoperative pulmonary artery pressure and the surgical outcome. In other studies the patients with normal pulmonary artery pressure weren't excluded and even those with elevated pulmonary artery pressure weren't quantified.

The total operative mortality in the present series was closely related to the degree of the preoperative pulmonary artery pressure and there was a big difference in the operative mortality between the two groups. Group I with preoperative mild to moderate pulmonary hypertension showed operative mortality of only 2 cases which presents 2.5% of the group while the Group II with preoperative severe pulmonary hypertension showed operative mortality of only 16 cases which presents 20% of the group.

The results in our series were matched with other series that had the same major risk factor for mortality which is the severe pulmonary hypertension. Roos-Hesselink, et al. 2004[9] studied 176 patients underwent VSD closure reported high mortality of 10.8% and attributed this to preoperative high pulmonary artery pressure with additional 2.3% (4 cases) late sudden death due to

persistence of pulmonary hypertension after VSD closure.

Conclusion

It is not safe to postpone surgical closure for large VSD or VSD with increased pulmonary flow after 1 year depending on patient's clinical condition as the resulting increased pulmonary vascular resistance may reduces the amount of left-to-right shunt and partly ameliorate signs of heart failure. Early detection and surgical correction of patients with VSD and increased pulmonary flow is the best safe guard against progression or persistence of pulmonary hypertension and pulmonary vascular changes and consequently, early intervention gives us the best surgical outcome with the least complications and mortality, and with less hospital stay and better life style.

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EPIDERMAL GROWTH FACTOR GENETIC POLYMORPHISM PREDICTS RISK OF HEPATOCELLULAR CARCINOMA IN EGYPTIAN PATIENTS WITH HCV-RELATED CIRRHOSIS

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Abstract

Background: *Epidermal growth factor (EGF) and its receptor play critical roles in cell proliferation and tumerogenesis. EGF is one of the candidate gene for HCC. The EGF gene is mapped to chromosome 4 (4q25) and it consists of 24 exons. A functional polymorphism in the EGF gene has been linked to increased cancer susceptibility. In Egypt, the growing incidence of HCC, which is nearly doubled over the last decade is parallel with that Egypt is plugged with highest prevalence of HCV in the world, ranging from 6 to 28%.*

Aim of the Study : *This study aimed to investigate the assosciation between the EGF +61A/G polymorphism and the risk for hepatocellular carcinoma (HCC) in HCV- related cirrhotic Egyptian patients.*

Subject and Methods: *we analyzed 133 (HCC) (group I) & 105 HCV-related cirrhotic patients without any focal lesion (group II). All patients were subjected to thorough clinical examination, routine liver function tests, abdominal ultrasound (US), Triphasic computed tomography (CT), α-feto protein (AFP) & PCR HCV. The diagnosis of HCC was verified histologically, or based on the finding of typical radiological features in two image examinations (US &CT) or by a single positive imaging technique associated with AFP>400ng/ml. Following DNA isolation from peripheral blood, EGF +61A/G polymorphism was assessed by polymerase chain reaction-restriction fragment length polymorphism. Hardy-Weinberg equilibrium (HWE) was assessed using χ^2 tests. Univariate and multivariate logistic regression analyses were used to calculate odds ratio (OR) and 95% confidence intervals (95% CI).*

Results: *The EGF +61 genotypes frequencies in HCC were AA (24.8%), AG (32.3%), and GG (42.9%) and in cirrhotic control group, AA (57.1%), AG (34.3%), and GG (8.6%). The carriage for allele G of EGF +61A/G SNP was significantly associated with development of HCC compared with long term cirrhotic group (OR=4.0404, 95% CI 2.3275. 7.014, p= 0.0001).*

Conclusion: *Our data suggest an increased risk to develop HCC in Egyptian patients with HCV carrying the G allele of EGF +61A/G SNP.*

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, and the third most common cause of cancer mortality[1,2]. This tumour, which arises from hepatocytes, is often associated with liver cirrhosis resulting from chronic liver diseases. Among the environmental risk factors, the prevalence of chronic hepatitis B (HBV) and C (HCV) virus infections is directly linked to the incidence of HCC [3].

In Egypt, the incidence of HCC has been nearly doubled over the last decade[4], and Egypt has simultaneously been plagued with the highest prevalence of HCV in the world, ranging from 6% to 28%[5]. The prevalence of serological markers of HCV infection in patients with HCC is nearly 80%[6]. The incidence and mortality rates for HCC are virtually identical, reflecting the overall poor survival of patients with this tumor.

The definite molecular mechanism of hepatocarcinogenesis are far from clear. The pathogenesis of HCC is quite diverse and influenced by a variety of environmental and genetic factors of the host. Functional polymorphisms that influence an individual's susceptibility to liver cancer include gene products involved in activation of cell proliferation. Gene polymorphisms of these candidate gene (s) play key roles in individual susceptibility to liver cancer. Thus genetic polymorphisms may explain why individuals with shared environmental exposures do not always share cancer morbid [7,8].

Despite recent improvements in surveillance programs and diagnostic tools, only 30-40% of HCC

patients are eligible for liver resection or transplantation, the only curative treatment options to date[9]. For this reason, identification of molecular markers associated with increased risk of HCC would better define high-risk populations of HCC, helping to improve prevention and treatment strategies.

Epidermal growth factor (EGF) gene is mapped to long arm of chromosome 4 (chromosome 4q25). It is encoded by a 4.8-kilobase (kb) mRNA transcripted from a 110-kb gene containing 24 exons [10]. EGF and EGF receptor (EGFR) interaction plays a pivotal role in cell proliferation, differentiation, and tumorigenesis of epithelial tissues. In recent years, numerous studies have associated a single-nucleotide polymorphism involving an A-to-G mutation at position 61 of the 5' untranslated region of the EGF gene (61 A/G, rs4444903) with the risk of tumorigenesis in multiple human cancers[11,12,13]. EGF is one of the candidate gene for HCC.

The aim of the current study was to evaluate genetic factors related to individual susceptibility to HCC on top of HCV. Specifically, individual genetic differences in EGF +61G/A (rs4444903) polymorphism was investigated in HCC cases and long term HCV-related cirrhotic patients.

Study Population:

This case-control study enrolled 274 patients, all of which had DNA available, 238 (133 patients with HCC and 105 patients with HCV-related liver cirrhosis) had successful DNA genotyping. Patients were recruited at Tropical Medicine, Mansoura University Hospital between 2008 and 2011. All patients were subjected to thorough clinical examination, routine liver function tests, abdominal ultrasound (US), Triphasic computed tomography (CT), α-feto protein (AFP) & PCR for HCV. The diagnosis of HCC was verified histologically, or based on the finding of typical radiological features in two different liver imaging studies showed a mass lesion with characteristics of HCC (vascular enhancement, wash out), or by a single positive imaging technique associated with AFP>400ng/ml. The exclusion criteria were as

follows:, autoimmune liver disease, alcohol abuse, hepatitis B virus or HIV coinfection, hemochromatosis , diabetes mellitus.

All participants provided informed written consent. The research protocol was reviewed and approved by Medical Mansoura University Ethics Committee for Human Subject Research. Approval for this study was granted by the research boards at the Mansoura university.

EGF ELISA.

Aliquot of stored plasma (at -80°C) of our study group was used for assay of plasma EGF using RayBio^{R} Human EGF ELISA Kit (RayBiotech, Inc.). The procedures were done according to manufacturers instructions as follow: Each sample was diluted 10 fold with provided diluent. Each sample and standard were analyzed in duplicate. 100 µl of each standard and sample into appropriate wells were incubated over night at 4°C followed by 4 times washing and addition of 100 µL of biotinylated antibody for 1 h and 100 µL of TMB substrate solution for 30 min. Finally, 50 µL 50 µl of Stop Solution was added to each well. The plate was read immediately in a plate reader (Sunrise TECHAN) with an excitation wavelength of 450 nm. The standard curve was generated for every set of analysis. Samples assayed using the standards provided in the kit and the results were expressed in ng/ml.

Dna Extraction And Genotyping:

DNA genotyping Laboratory Testing

Blood specimens were processed immediately after collection and DNA were stored at -70°C until subsequent testing. Genotyping analysis was done on genomic DNA isolated from lymphocytes using QIAamp DNA Blood Mini Kits (Qiagen, Germany).

The EGF rs4444903 A>G gene polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay as previously described by Guoyang., et al., 2009 [14].

PCR primers were designed to amplify products of exactly 248 pb using Primer3 (v. 0.4.0) (http://

frodo.wi.mit.edu/) software to generate more appropriate allele-discriminating DNA fragments. A DNA fragment of 248 bp containing the A/G polymorphism of EGF rs4444903 gene polymorphisms was amplified using specific primers: forward primer 5'-AGCAAAGCTGAGTCATTCCACc-3', and reverse primer 5'-TGTTTCTTTGGAAGCCAGTAAGA-3'. PCR was conducted on the ABI 2400 (Applied Biosystems, Foster City, CA, USA) in a system with total volume of 25 µl containing 1 ul genomic DNA, 2.5 µl 10xPCR Buffer, 2.5 mM MgCl2, 0.3 µM each primer, 0.4 mM dNTP, AND 0.40 µl Taq DNA polymerase (Qiagen Germany). The cycling parameters were: initial preheating 94°C for 10 min; 35 cycles at 94°C for 1 min , 56°C for 1 min s, 72°C for 1 min s; and a final extension step at 72°C for 10 min. For each PCR product, 5 µl the PCR reaction was then electrophorsed using 1.5 agrose gel stained with ethidium bromide to check a PCR reaction. Ten microliter of remaining PCR products were digested using 10 x NE buffer and 2.0 U of appropriate restriction enzymes (AluI). 10 % of our sample were selected randomly to re-genotype at different set as quality control group. The results of the genotyping showed 100% concordance of our study group and control group.

LIVER BIOPSY:

Liver biopsies were obtained with an 18-gauge or larger needle with a minimum of five portal tracts and were routinely stained with hematoxylin-eosin stain. Biopsies were interpreted according to the scoring schema developed by the METAVIR group. Needle liver biopsy specimens were examined by a pathologist unaware of the laboratory results. METAVIR score was used to stage fibrosis (F0-F4). Fibrosis was scored on a 5-point scale: F0, no fibrosis; F1, portal fibrosis alone; F2, portal fibrosis with rare septae; F3, portal fibrosis with many septae; F4, cirrhosis [15].

Statistical Analysis:

Statistical methods. Hardy-Weinberg equilibrium (HWE) was assessed in cases and controls separately using χ^2 tests. Unconditional logistic regression was used to analyze the case-control

study. Although cases and controls were frequency matched on age and gender, we adjusted for age, adult BMI, and pack-years of smoking using continuous variables, and gender and smoking status (current, ex-smoker, or never smoker) Results using indicator variables. Crude odds ratios (OR) and adjusted ORs (AOR) with 95% confidence intervals (95% CI) were calculated for homozygous (G/G) and heterozygous (A/G) genotypes using the wild-type genotype (A/A) as the reference. Survival curves for OS and FFS were constructed using the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards models for both OS and FFS were used to adjust for clinical covariates identified as being significant in univariate analyses (e. g., stage, performance status, age, gender, and smoking history).

For the serum analysis, we first assessed assay variability by comparing the percent differences in absolute serum levels between each of the triplicate samples and the serum level corresponding with the arithmetic mean of the raw data obtained from the triplicate serum analysis (termed the average serum level). The average of these percent differences (assay variability) was compared with the percent differences in absolute serum levels across different individuals (interindividual variability). The average serum level was used for all subsequent analyses. EGF serum levels were first analyzed using the Wilcoxon rank sum test. Then, we compared G/G with A/G + A/A using t tests. Because serum EGF levels were not normally distributed, we log transformed the serum levels first before parametric testing. The statistical packages Statistical Analysis System 9.1 9 (SAS) and R were used for all analyses.

Results

Our study was included 133 HCC cases and 105 patients with long term HCV-related liver cirrhosis without any radiological finding for focal lesion. All patients were recruited from the Tropical Medicine Department, Mansoura University Hospital. The clinical characteristics of our study cohort are summarized in Table 1.

Table (2) shows the distribution of the different EGF genotypes among patients with HCC and cirrhotic HCV patients. EGF polymorphyism was as follow in patients with HCC 42.9% G/G, 32.3% G/A, and 24.8% A/A and in cirrhotic control group was 8.6% G/G, 34.3% G/A, and 57.1% A/A. Also our finding showed thal subjects carriage allele G of EGF +61A/G SNP was significantly associated with development of HCC compared with long term cirrhotic group (OR=4.0404,95% CI 2.3275. 7.014, p=0.0001) (Table 3).

Table (1) : Clinical Characteristics of patients with HCC and liver cirrhosis.

	HCC (133) N(%)	**Liver cirrhosis (105) N(%)**	**Significance**
Sex: M	118(89.4)	90(85.7)	χ^2=0.7, P=0.4
F	14(10.6)	15(14.3)	
	Mean ±SD (Median)	**Mean ±SD (median)**	
Age	56.1±6.2(57.0)	56.5±6.2(57.0)	t=0.4,P=0.7
Alb	2.9±0.5(2.9)	3.4±0.5(3.4)	t=8.1, P≤0.001
Bil.T	8.3±4.0(7.2)	3.3±0.9(3.3)	t =12.4, P≤0.001
Bil.D	3.0±0.8(2.5)	0.8±0.3(0.8)	t =9.4, P≤0.001
GPT	50.6±22.8(48)	44.1±8.2(38)	t =2.4, P=0.018
GOT	44.6±12.7(43.0)	43.2±12.4(41)	t =0.8, P=0.4
AP	10.9±4.6(10.0)	25.5±1.9(8)	t =6.6, P≤0.001
AFP	1172.5±1746.1(592)	25.5±51.8(9)	Z*=12.3, P≤0.001
EGF.serum	527.4±130.6(523.0)	278.2±85.5(295.0)	t =16.9, P≤0.001

*Z of Mann-Whitney test

Table (2) : Distributions of the EGF +61A/G polymorphism between the two grpups.

	HCC (133) N (%)	**Liver cirrhosis (105) N (%)**	**Significance**
EGF genotype			
A/A	33 (24.8)	60 (57.1)	χ^2=30.3
A/G	43 (32.3)	36 (34.3)	P≤0.001
G/G	57 (42.9)	9 (8.6)	
Allele frequencies			
A	123 (46.6)	155 (73.8)	χ^2=35.7,
G	141 (53.4)	55(26.2)	P≤0.001

Table (3) : Association between EGF +61 A/G SNP and development of HCC.

	OR	95 % CI lower	high	Chi-square	*P*
Overall G	4.0404	2.3275	7.014	24.04	<.0001
G (%)	2.8082	1.535	5.1374	10.57	0.001149

OR= Odds ratio

Table (4) : Logistic regression of independent predictors of HCC.

	B	P	OR(95CI)
Alb (continuous)	-3.6	0.019	37.3(1.8-69.8)
EGF(continuous)	0.1	0.018	1.05(1.01-1.1)
Bil.T(continuous)	2.4	0.013	10.6(1.6-68.9)
Constant	-53.5		
Model χ^2	312.7, ≤0.001		
% correctly predicted	99.2		

Discussion

HCC is a complex, heterogeneous malignancy, the pathogenesis of which involves multiple genetic and epigenetic alterations and modulation of molecular signaling pathways implicated in malignant transformation of hepatocytes and tumor progression[16].

Cirrhosis associated with HBV and/or HCV infection and alcohol is the most well established environmental risk factor for HCC around the world. In fact, cirrhosis is considered a precancerous stage to some extent, although only a fraction of cirrhosis patients and HCV-infected individuals develop HCC later in life[17]. Moreover, some patients without known risk factors eventually develop HCC[18]. Therefore, genetic predisposition may contribute to the process of hepatocarcinogenesis.

Dysregulation of the EGF/ EGFR signaling pathway, is thought to be important in early hepatocarcinogenesis[19]. A functional polymorphism in the 5' untranslated region of the EGF gene (61*G) that modulates tissue-specific EGF gene expression has been associated with multiple

human malignancies including HCC[12,20]. This study aimed to evaluate the assosciation between the individual genetic differences in EGF +61G/A (rs4444903) polymorphism and the risk for HCC development in HCV patients.

Previous studies evaluated the association of the EGF +61A/G polymorphism and the development of HCC have yielded conflict results. Our findings seem to suggest that the role of genetic factor in predisposition and/or pathogenesis of HCC. The result confirmed the possible key role for EGF in the mechanism of progression of HCV-cirrhotic liver into HCC stage. Our study found around a 4-fold increased HCC risk with the G allele of + 61 SNP of EGF. As for +61A/G polymorphism of EGF, our result reveled that EGF +61 A allele could be a protective allele against HCC on the top of HCV in Egyptians after adjusting for confounders and other clearly defined HCC risk factors.

The result of this study was in agreement with some studies. Tanabe et al. (2008), found a significant association of G allele of EGF +61A/G SNP and development of HCC with odd ratio around 4.0[21]. Recently Abu Dayyeh et al., (2011) reported a significant association of EGF genotype G/G with increased risk for HCC American Caucasian as well as Afro-Americans[22].

On the other hand, Qi et al. (2009) failed to find a significant association between EGF +61A/G SNP and risk of HCC in Chinese patients with chronic hepatitis B virus infection[23]. This may be due to using healthy individual as control group or may be due to anther loci is linked to HCC on the top of HBV.

EGF promote cell survival, growth, cell proliferation, differentiation and tumorigenesis via the activation of several integrated signaling pathways[24]. Overexpression of EGF is also associated with growth and invasion of some malignant tumors via autocrine and paracrine pathways[25]. Results from clinical trials indicate that therapies directed against EGFR are promising in the treatment of

a variety of cancers, including esophageal cancer [26].

One mechanism by which the EGF gene polymorphism may lead to increased risk of HCC is by modulating EGF levels. Tanabe et al. (2008) found that EGF secretion was 2.3-fold higher in 61G/G hepatocellular carcinoma cell lines compared to A/A cell lines, and that mRNA transcripts with the G allele showed a longer half-life and increased stability. Tanabe et al. (2008) concluded that the EGF polymorphism rs4444903 is associated with risk for development of hepatocellular carcinoma in liver cirrhosis through modulation of EGF levels [21].

The EGF/EGFR signaling pathway has been shown to be an important mediator of hepatocyte proliferative capacity and liver regeneration in response to chronic injury [27]. Modulation of EGF levels rather than alteration in EGF receptor expression has been suggested to be the mediator of this regenerative liver response[19]. These studies lend biological plausibility to our observation of lower liver fibrosis progression rates among subjects with the 61*G functional polymorphism in the 5' untranslated region of the EGF gene, which predicts increased EGF mRNA expression in hepatocytes and stability in serum. They argue that the observed association between the EGF 61*G functional polymorphism and HCC is not mediated by a more aggressive liver fibrosis course, but perhaps more likely by the inability to downregulate the EGF pathway once cirrhosis has developed, leading to early hepatocarcinogenesis and uncontrolled progression of early HCC.

Conclusion

Our finding suggest increased risk to develop HCC in patients with HCV- related cirrhosis carrying the G allele of EGF +61A/G single nucleotide polymorphism. Our study recommend that the EGF genotype can be used for clinical prediction of patients with chronic hepatitis C and advanced fibrosis/cirrhosis who have relative HCC risk for early detection and management of HCC.

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SERUM AND SYNOVIAL GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) LEVELS AS MARKERS OF DISEASE ACTIVITY AND SEVERITY IN RHEUMATOID ARTHRITIS

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Abstract

Aim of Study: This work aims to determine serum and synovial levels of the granulocyte colony stimulating factor (G-CSF) in order to evaluate their utility as biomarkers of disease activity and/or severity in rheumatoid arthritis (RA) patients. Methods: Thirty patients with established RA as well as a control group of 20 apparently healthy individuals matched for age and sex to our patients were included and thoroughly examined. RA disease activity was assessed using the modified disease activity score of 28 joint count (DAS28) while, disease severity was evaluated using the clinical spread severity Index (SSI). Plain radiography was done for both knees as well as affected joints with evaluation according to Laresn scores. Serum and synovial levels of (G-CSF) were measured by the ELISA technique. Conclusion: Our results reveal that G-CSF has a potential role in driving joint inflammation and also underscore that G-CSF would likely atherapeutic target in RA.

Introduction

Rheumatoid arthritis (RA) is a chronic polyarticular disease manifesting as painful inflammation of the synovial tissue with progressive joints destruction [1].

Once the disease becomes active and chronic, it will likely become progressively worse. Moreover, if active disease has been present for one year in particular joint, cartilage loss is properly irreversible [2].

During active phases of disease, large numbers of activated neutrophils are found in the

synovial fluid of both very early RA and established RA patients. As a source of proinflammatory mediators such as IL-1β, CXCL8 and TNFα, activated neutrophils clearly contribute to the complex cytokine milieu of the inflamed joint [3].

Intermittent neutrophil accumulation within the synovial fluid of RA patients results in the degradation of extracellular matrix proteins that are crucial for the lubricative function of synovial fluid. Activated neutrophils have also been found at the cartilage pannus interface, where they may promote joint erosion more directly [4].

The colony-stimulating factors (CSFs) are a family of four cytokine growth factors originally identified by their ability to support the proliferation and differentiation of hemopoietic progenitor stem cells into mature monocytes/macrophages and granulocytes. The CSF family comprises the lineage-specific members, macrophage CSF (M-CSF or CSF-1) and granulocyte CSF (G-CSF), and the nonlineage-specific members, granulocyte-macrophage CSF (GM-CSF) and multi-CSF [also called interleukin (IL)-3 [5].

G-CSF increases the production and release of neutrophils, mobilizes hematopoietic stem and progenitor cells and modulates the differentiation, lifespan, and effector functions of mature neutrophils. G-CSF may also exert effects on macrophages, including expansion of monocyte/ macrophage numbers, enhancement of phagocytic function and regulation of inflammatory cytokine and chemokine production. G-CSF is currently used to treat neutropenia and to mobilize hematopoietic stem cells for transplantation[6].

Neutrophil trafficking into tissues is a key step during inflammation, and relies on tightly regulated sequential adhesion events mediated by adhesion molecules expressed by both neutrophils and endothelial cells[7].

Therefore, during inflammatory conditions such as RA, G-CSF may promote inflammation via

effects on myeloid cells, endothelial cells, or both[8].

Aim of the work

The aim of this work is to measure both serum and synovial levels of the granulocyte colony-stimulating factor (G-CSF) in rheumatoid arthritis (RA) patients in order to evaluate their correlations and utility as biomarkers of disease activity and /or severity.

Patients and Methods

Thirty patients with established RA attending the outpatient clinic and inpatient unit of Rheumatology, Rehabilitation and Physical Medicine department at Benha university hospital were recruited. All patients fulfilled the 1987 American College of Rheumatology (ACR) criteria for RA [9].

A control group of 20 apparently healthy individuals suffering from traumatic synovitis excluding metabolic and inflammatory disorders matched for age and sex to our patients' group was also included. All of participants gave informed consent.

- All patients were submitted to full history taking and physical examination according to a standard protocol sheet.

- Swollen (SJC 28) and tender joints (TJC 28) counts of the RA patients were recorded. Assessment of disease activity was done using the modified disease activity score of 28 joint count (DAS28)[10]. Its calculated using the formula :

$$DAS\ 28 = 0.56\ \sqrt{(TEN\ 28)} + 0.28\ \sqrt{(SW\ 28)} + 0.70\ Ln\ (ESR) + 0.14\ (\ GH$$

The final score is calculated and graded as:

Low activity = DAS 28 scoring <3.2

Moderate activity = DAS28 scoring 3.2-5.1

High activity = AS scoring >5.1.

At the time of the study, patients were taking different treatments for RA for at least 6 months [Non-steroid ant-inflammatory drugs (NSAIDs) and disease modifying anti-rheumatic drug (DMARDs)]. All patients were taking non-steroid ant-inflammatory drugs (NSAIDs) and disease modifying anti-rheumatic drug (DMARDs) 12 patients were taking

Methotrexate IM 10-15 mg per week + prednisolone 5m-10mg per day, 10 patients were taking Methotrexate IM 10-15 mg per week + hydroxychloroquine 200mg per day, and 8 patients were taking Leflunomide 20mg per day + hydroxychloroquine 200mg per day.

Each patient was assessed for the clinical articular severity of the disease using the spread/ severity index (SS index)[11] : Each set of joints (distal, proximal, and metacarpophalangeal, wrists, elbows, shoulders, jaw, neck, lumbar spine, hips, knees, ankles, and metatarsophalangeal) was scored as 0 = normal, 1 = tender only, 2 = swollen only, 3 = tender and swollen, and 4 = deformed or operated upon. The worse side was the one that was scored. For sets of joints such as the distal interphalangeal joints, the score of the majority was the one that counted. The SS index score was calculated from the sum of the scores of all of the sets of joints (maximum score 52).

- **Radiological study :**

Antroposterior and lateral views of both knee joints were obtained and assessed using Larsen Index scores[12] and were graded as follow : grade 0 = normal; 1 = slight abnormality with one or more minor lesions (periarticular soft tissue swelling, periarticular osteoporosis, and slight joint space narrowing); 2 = definite early abnormality with erosion (not obligatory) and joint space narrowing; 3 = medium destructive abnormality (erosion obligatory); 4 = severe destructive abnormality (bone deformation present); and grade 5 = mutilating abnormality (gross bone deformation).

- **Laboratory studies**

The following laboratory investigations were measured according to standard methods.

- Full blood picture, hemoglobin concentration (gm/ dI).
- Erythrocyte sedimentation rat (mm/h) by Westergren method [13].
- C-reactive protein (CRP).
- Rheumatoid factor (RF) test by latex agglutination.

- Blood samples were obtained from all subjects and allowed to clot for 2 hours at

room temperature before centrifugation. Serum samples were stored at -80°C until use.

- Synovial fluid samples (SF) were obtained from RA patients during therapeutic aspiration of knee joints.

At least 7ml of synovial fluid should be obtained since this was the minimum amount required for routine and cytokines assays.

Subjects who had received intra-articular steroid injections within the previous 3 months were excluded.

Synovial fluid was collected in plain, untreated tubes, centrifuged (400c) for 20 min and stored at -20°C prior to CSF assay. Hyaluronidase (150 IU/ml) was added if necessary to assist solubilisation.

Estimation of G-CSF levels in serum and synovial fluid was carried out by Sandwich Enzyme Linked-Immunosorbent assay (ELISA) (BioSource Interntional, Inc h G-CSF kit).

- Statistical analysis

Data were analyzed by SPSS (Statistical Package for the Social Sciences) Software "Version 11" (SPSS, 2001).

Discrete values were presented as counts and percentages. Continuous values were expressed as mean ± SD. Comparison of discrete data between two independent groups was done using Chi-square test.

Comparison of continuous data between independent groups was done using student t-test. Linear correlation was measured using Pearsons correlation coefficient. A probability level of P-value <0.05 was considered significant and the level <0.01 was considered highly significant.

Results

Thirty patients suffering from RA were included into this study. Their ages ranged between 28-75 years with a mean of 47.1 years. ± SD 10.92 years. They were 25 females (83.3%) and 5 males (16.7%).

A control group of 20 apparent-

ly healthy individuals, age and sex matching our patients : 25 females (83.3%) and 5 males (16.7%), whose ages ranged between 30-69 years with a mean of 47.1 years ± SD 10.9 years were also included. These subjects suffered from traumatic synovitis excluding metabolic and inflammatory disorders.

Table (1): Demographic features of the studied groups.

Group	Age Mean ± SD years	Males		Females		Total (100%)
		No	%	No	%	
Patients (n=30)	47.17 ± 10.92	5	16.7	25	83.3	30
Control (n=20)	47.10 ± 10.9	4	20.0	16	80.0	20
t	0.1284	Incsignificant				
p	> 0.05					

P >0.05 =Insignificant difference

Table (2): Clinical features of RA patients.

Parameter / *Value*	*Mean ± S.D.*
Duration of disease (years)	7.57 ± 5.03
Morning stiffness (minutes)	57.4 ±35.0
visual scale analogue(mm)	5.36 ±1.63
Spread/severity index score	26.27 ±13.61
DAS index	Low activity N=9 Moderate activity N=11 High activity N=10
The Larsen Grading Scale	Grade I N=8 Grade II N=7 Grade III N=6 Grade IV N=9

DAS= Disease activity score.

Table (3): Comparison between patients and controls regarding serum and syovial G-CSF levels.

RF / G-CSF Mean ± SD (Pg/ml)	*Patients*	*Controls*	*T*	*P*
Synovial	1.36 ± 0.69	0.47±0.07	1.89	< 0.05*
Serum	0.875±0.531	0.47±0.04	1.51	< 0.05*

p<0.05* =Significant difference

Table (4): Comparison between serum and synovial levels of G-CSF as regards to RF.

RF / G-CSF Mean ± SD (Pg/ml)	*Positive (n = 24)*	*Negative (n=6)*	*T*	*P*
Synovial	1.48 ±0.69	0.88±0.46	2.02	< 0.05*
Serum	0.95 ± 0.55	0.54±0.21	1.99	< 0.05*

p<0.05* =Significant difference

Table (5): Comparison between serum and synovial levels of G-CSF in the according to presence of subcutaneous nodules.

Sc Nodules / G-CSF Mean ± S.D. (Pg/ml)	*Present (n = 12)*	*Absent (n =18)*	*T*	*P*
Synovial	1.97 ±0.43	0.959±0.51	5.67	< 0.01**
Serum	1.32 ± 0.5	0.579±0.3	5.13	< 0.01**

p< 0.01** = High significant difference

Table (6): *Comparisons of serum and syovial G-CSF levels among RA patients according to different grades of radiological knee joint changes*

X ray Grade / G-CSF Mean ± S.D.(pg/ml)	I (N=8)	II (N=7)	III (N=6)	IV (N=9)	F	p
Synovial	1.16±0.83	0.94±0.62	1.76±0.7	1.76±0.7	2.48	> 0.05
Serum	0.76±0.5	0.55±0.42	1.16±0.7	1.04±0.42	2.1	> 0.05

p >0.05 = Insignificant difference

Table (7): Comparisons of serum and syovial G-CSF levels among patients with different grades of disease activity.

Grade of activity / G-CSF Mean ± S.D.(pg/ml)	Low activity (N=9)	Moderate activity (N=11)	High activity (N=10)	F	p
Synovial	0.77 ± 0.45	1.38 ± 0.72	1.88 ± 0.37	9.60	< 0.01**
Serum	0.41 ± 0.21	1.00 ± 0.56	1.15 ± 0.45	7.02	< 0.01**

p<0.01** = High significant difference

Table (8): Comparison among patients with different medication regarding serum and syovial G-CSF levels.

Medications / Parameter	MTX+ prednisolone (n=12)	MTX + prednisolone (n=10)	Leflunomide + HchQ (n=8)	F	p
	Mean± S.D.	Mean± S.D.	Mean± S.D.		
Synovial G-CSF	0.81±0.42	1.77±0.39	0.70±0.40	.08	>0.05
Serum G-CSF	1.4±0.68	1.2±0.59	1.1±0.61	1.1	> 0.05

P >0.05 = Insignificant difference MTX = Methtrexate
HchQ = Hydroxychloroquine

Table (9): Correlations between synovial G-CSF levels with clinical and laboratory parameters of RA.

Clinical & laboratory parameters	*Synovial G-CSF*	
	R	*P*
Duration of disease (years)	+ 0.055	> 0.05
Morning stiffness (minutes)	+ 0.741	< 0.01**
Visual analogue scale (mm)	+0.477	< 0.05*
spread/severity index score	+0.671	< 0.01*
Larsen index scores of knee joints	+0.335	> 0.05
Activity index score	+0.643	< 0.01**
ESR mm/ 1st hour	+0.844	< 0.01**
Hemoglobin (gm/dl)	-0.624	< 0.01**

P >0.05 = Insignificant difference P<0.05* = Significant differ
P <0.01** = High significant difference

Table (10): Correlations between serum G-CSF levels with clinical and laboratory parameters of RA.

Clinical & laboratory Parameters	*Serum G-CSF*	
	R	*P*
Duration of disease (years)	+ 0.004	> 0.05
Morning stiffness (minutes)	+ 0.730	< 0.01**
Visual analogue scale (mm)	+0.487	< 0.05*
Spread/severity index score	+0.623	< 0.01**
Larsen index scores of knee joint	+0.261	> 0.05
Activity index score	+0.548	< 0.01**
ESR mm/1st hour	+0.793	< 0.01**
Hemoglobin (gm/dl)	-0.559	< 0.01**
Synovial fluid G-CSF	+ 0.897	< 0.01**

P >0.05 = Insignificant difference P<0.05* =Significant differe
P<0.01** = High significant difference

Discussion

The granulocyte colony-stimulating factor (G-CSF) is a well known regulator of granulopoiesis, but the role of endogenous G-CSF in inflammatory joint disease has not been extensively explored[14].

G-CSF has been shown to activate endothelial cells, modulate the expression of leukocyte adhesion molecules , and enhance angiogenesis, and may induce chemokine production [15].

G-CSF also enhances neutrophil and macrophage phagocytosis and prolongs neutrophil survival [16].

Local tissue production of G-CSF might therefore mediate adhesion and trafficking of myeloid cells through the endothelium, as well as promote local cellular activation, function, and survival within inflamed tissues [17].

Our results demonstrated that serum and synovial G-CSF levels of RA patients were higher than their levels in the controls with a highly significant difference between both groups ($p<0.01$)

The serum and synovial G-CSF levels were also higher in patients with a positive rheumatoid factor (p value <0.05) and those with subcutaneous nodules (p value <0.01) compared to other RA patients and the difference were statistically significant.

Our data supported those of Nakamura et al. (2000)[18]., who studied serum and synovial G-CSF levels via solid-phase radio-immunoassay (RIA) in 61 patients with RA versus 20 healthy controls and found that these levels were higher in RA patients

Investigating the correlations of serum and synovial G-CSF levels with different clinical and laboratory parameters of RA showed that both levels of G-CSF significantly and positively correlated with duration of morning stiffness ($p<0.01$), VAS ($p< 0.05$), spread severity index score ($p<0.01$), DAS score ($p<0.01$), ESR values ($p< 0.01$), while they correlated inversely with HB concentrations ($p<0.01$).

In agreement with our results, Nakamura et al. (2000)[18] found significant correlations of both serum and synovial G-CSF levels with other clinical parameters such as morning stiffness ($p<0.05$), visual analogue scale ($p<0.05$), Ritchie articular index ($P<0.05$) functional capacity, ($P<0.05$) and DAS 28 ($p<0.05$) as well as laboratory parameters such as ESR ($p< 0.05$), HB ($p<0.05$) and rheumatoid factor ($p<0.05$), but they found no significant correlation of both with disease duration.

Bell et al. (1995)[19], found that CSF activity was more prevalent in inflammatory fluids, with particularly significant differences noted between RA and osteoarthritis. Their results revealed no significant differences between seropostive and seronegative RA groups although there was a trend towards a higher prevalence of G-CSF in rheumatoid factor - positive group but this did not reach statistical significance. This coincided with Nakamura et al. (2000)[18], who reported that serum G-CSF in the active RA was significantly higher than in the inactive group ($p<0.05$).

Regarding the absence of a significant differences of either serum or synovial fluid G-CSF levels among our patients on DMARDs and those on steroids, these results were agreement with Nakamura et al. (2000)[18], who stated that there is no relationship between G-CSF level and therapy with any particular agent while, Bell et al. (1995)[19] found that samples from subjects not currently taking non-steroidal antinflammatory drugs (NSAIDs) showed significantly lower levels of G-CSF than NSAIDs treated subjects and attributed this to the effects of unsuppressed prostaglandins since cyclo-oxygenase inhibitors, can potentiate the production of cytokine-induced G-CSF from cultured synovial fibroblasts.

Collectively, these data are consistent with many other human and animal studies[14,18,20,21,22], which suggested that G-CSF has an important role during inflammatory arthritis, as was reported by Eyles et al. (2008)[8], who found that serum G-CSF is elevat-

ed during collagen induced arthritis (CIA), which is the major murine model of RA correlating with the onset of clinical features while G-CSF–deficient (G-CSF-/-) mice as well as,[14], G-CSFR-/- mice were markedly protected from CIA. They also proved that G-CSF could directly regulate neutrophil trafficking in vivo, that explained to them why G-CSF blockade produced a rapid effect even after the onset of CIA.

Administration of neutralizing anti-G-CSF antibodies to WT mice after the onset of CIA prevents disease progression. Protection of G-CSF-/- mice in CIA was associated with reduced neutrophil production, but these studies did not address whether G-CSF itself may have other proinflammatory effects[8].

G-CSF exerted contrasting effects in humoral and cellular autoimmune diseases. In humoral diseases such as systemic lupus erythematosus and RA, polarized T cell phenotypes and inflammation often display a complex relationship to the pathogenesis(23). In inflammatory arthritis, neutralization of endogenous G-CSF markedly reduced the progression of disease to the same extent as anti-TNF treatment[24].

Infiltration of target tissues by neutrophils is characteristic of many inflammatory conditions, and neutrophils are a major leukocyte population found within inflamed joints in RA and CIA[25]. Circumstantial evidence suggests that some of the beneficial effects of RA therapies such as methotrexate[26] and TNF inhibitors[27] may relate to reduced neutrophil recruitment or function. Despite this, there are surprisingly few studies on the role of neutrophils in the pathogenesis of inflammatory diseases such as RA.

G-CSF is used clinically to treat chemotherapy-associated neutropenia and to mobilize hematopoietic stem cells for transplantation. G-CSF therapy is generally well tolerated by patients and donors in these clinical settings, however numerous reports link G-CSF administration with vascular complications and exacerbation of underlying inflammatory conditions, including Felty's syndrome

and rheumatoid arthritis (RA) (28,29,30,31).

Conclusion

Collectively Our results reveal that G-CSF has a potential role in driving joint inflammation and also underscore that G-CSF would likely atherapeutic target in RA. Further studies are needed to suggest the dose should be taken if G-CSF is needed to restore neutrophil counts in inflammatory arthritis .

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SERUM AND SYNOVIAL GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) LEVELS AS MARKERS OF DISEASE ACTIVITY AND SEVERITY IN RHEUMATOID ARTHRITIS

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EVALUATION AND EVOLUTION OF FETAL ECHO IN PREDICTION OF CARDIAC CONGENITAL ANOMALIES

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Abstract

Objective: *To evaluate the efficacy of pre-natal fetal echo in prediction and accurate diagnosis of congenital heart disease compared with postnatal infant echo diagnosis.*

Patients & Methods: *300 pregnant women of high risk to congenital heart disease at the second trimester (22-28 weeks of gestation) fetal echo was done and followed up until delivery then postnatal echo was done and comparison between the two diagnosis of pre and post natal echo in congenital heart disease.*

Results: *There were (52) cases of heart anomalies, detected prenatally and proved the diagnosis by postnatal echo and there were 4 cases didn't diagnose prenatally and diagnosed only postnatally.*

Conclusion: *Evaluation of the fetal heart at routine fetal anomaly scan is effective method in detection of congenital heart disease and it has high specificity and sensitivity when compared with postnatal diagnosis.*

Keywords: *Fetal echo, Congenital cardiac anomalies, Prenatal heart defect.*

Introduction

Congenital heart disease are the most common type of congenital anomalies and the major cause of death in newborn. It affects between 1-8 per 1,000 live births and 27 per 1000 stillbirths[1].

The risk of congenital heart anomalies increases if one parent has congenital heart disease or if there was a previous affected child with congenital heart disease. The risk of recurrence when a previous sibling had congenital heart defects about 2% and with 2 affected siblings the risk is 16%[2].

Etiology of the cardiac anomalis depends on interplay of multiple genetic and environmental factors, including maternal diabetes mellitus or collagen disease, exposure to drugs e.g lithium and viral infection such as Rubella and chromosomal abnormalities account for less than 5% of parents, heart disease are found in >90% of fetuses with triosomy 18 or 13 and 50% of trisomy 21% and 40% of those with turner syndrome[3].

Risks are increased with most of genetic syndromes (either dominant or recessive). Approximately 6% of infants born with cardiac anomalies had positive family history of congenital heart disease, whereas approximately 12% has abnormalities in the chromosomes[4].

In the past the cardiac lesion couldn't be recognized antenatally[5].

But now the prenatal detection of the majority of cardiac anomalies especially the major one as well as some of the minor forms, become easy by fetal echo in the second trimester of pregnancy[6].

Cardiac abnormalities detected during routine obstetric evaluation of ultrasound examination and fetal anomalies scan showed some variation between countries and between centeres in the same country[7].

The suspicious cases and high risk pregnancies referred for specialist in fetal echo cardiography to achieve high pre-natal dection rate for congenital heart diseases[8].

The concept of prenatal screening for congenital heart disease was introduced in the United Kingdom in 1986[9].

Fetal echo cardiography is a prenatal non-invasive diagnostic procedure for cardiovascular anomalies which is not diagnosed by routine U.S screening examinations[10].

The anatomy of normal heart in mid-trimester fetus can be identified using 2D sonar, the absence of air-filled lungs makes the fetal heart more accessible to ultrasonic examination it makes it easier to trace the course of the great vessels to and from the heart[11].

Cardiac anomalies detected prenatally tend to be more severe than those missed on prenatal ultrasound. Thus the survival rate of only 17% for cardiac defects detected prenatally is much worse than the survival rate of approximately 80% for anomalies undetected before birth[12].

Patients and Methods

This study included 300 pregnant ladies whom visited feto-maternal unit in Obstetrics Department in Zagazig University Hospitals for routine antenatal care and anomaly scan from May 2011 to May 2012.

For every lady: detailed history was taken, both general and abdominal examinations, and ultrasound examination were performed. The pregnant ladies were chosen according to the following criteria:

- All pregnant at second trimester (22-28) weeks gestation.
- High risk pregnancies either due to maternal factors or fetal factors such as:
- Age ≥ 35 y.
- Diabetic
- Collagen disease
- Viral infection
- Family history of congenital heart disease.
- Exposure to teratogens in early pregnancy.
- Previous history of delivered cardiac baby.
- The father of the fetus has congenital heart disease.
- The pregnant female suffer from cardiac lesions.
- Increased nuchal translucency at 10-14 weeks of gestation.
- The detection of an extra cardiac fetal anomaly on ultrasound scan.
- Fetal hydrops.

An ultrasound scan performed in the second trimester (22-28) weeks of gestation to confirm:

- Fetal viability.
- Number of the feti.
- Placental localization.
- Amniotic fluid (amount & turbidity).
- Anomalies scan.
- Fetal echo: the following data will be fulfield.
- Cardiac axis
- Cardiac size in relation to chest size.
- 4-chamber view.
- Chamber symmetry.
- Atrioventricualr septum.

- Inter-atrial septum and foramen ovale.
- Inter-ventricular septum.
- Outflow tract (the right and the left).
- Rate and rhythm of fetal heart.
- Doppler.

We used high resolution 2-dimensional ultrasound equipment with a cineloop facility (volusion 730) with 4.5 MHZ curved array transducer.

The routine fetal heart examination took 2-3 minutes whenever an abnormalities was detected, the detailed cardiac assessment spent 10 minutes. The picture recorded on the ultrasound machine.

Technique:

First, the fetal presentation and lie should first be diagnosed, then determine the fetal right side and left side. The usual scanning sequence began with the long axis of the thoracic spine and assessment of the disposition of the abdomen and thoracic organs as an abnormal disposition was associated with complex cardiac anomalies. The heart occupies nearly 1/3 of the thorax and shifted to the left and its axis is about 45° to the left of the anteroposition axis of the fetus.

The probe was rotated right angle till 4 chamber view of the heart was seen, it was best obtained from an anterior or left lateral approach to avoid the ribs and the spine by tilling the probe cephaled toward the spine with minimal sliding and angling up the best 4 chamber view was obtained. In this view we can examined both atria and both ventricles, atrioventricular valves, interatrial septum and foramen ovale, interventricular septum and pulmonary venous connections, to determine the right ventricles from the left one by presence of moderator band in it. To identify the insertion of the tricuspid valve along the interventricular septum was more apical than mitral valve insertion.

Evaluation of the cardiac outflow tract achieved by slight angulation of the transducer from the 4-chamber view. By turning the transducer while keeping the left ventricle and the aorta in the

same plane we obtained the left heart outflow tract view, while by moving the transducer cranially and tiliting slightly in the direction of the left shoulder we obtained the right heart outflow tract view.

The left heart outflow tract view, we demonstrated the left ventricles and aortic outflow tract and the anterior wall of the aorta was in continuity with interventricualr septum. While in the right heart outflow tract view we demonstrated the right ventricle, the main pulmonary artery originates from the anterior ventricle and bifuricates into a large vessels, the ductus and 2 small vessels, there are 2 arches (aortic and ductal arch) can be achieved in longitudinal view, the aortic arch emanate 3 vessels but the ductal arch has no vessels eminated moving the probe more cranialy, we saw the aorta arising from the left ventricle, angluating the probe cranialy towards the right shoulder we demonstrated the aorta arises in the center of the heart, more cranialy we obtained 3 vessels view (aorta, pulmonary artery and superior vena cava).

Color flow mapping, Doppler and M-mode were done.

Post natal examinations:

All infants born alive with previous fetal echo examination were referred to the pediatric cardiology unit in Zagazig University Hospital to examine their heart in the first day of life with ultrasound machine Evivid with transducer 3 to 7.5 MHz.

The neonatal study included: 2-dimensional pediatric echocardiography, subcostal view, 4-chamber view, five-chamber view, long and short-axial parasternal view and suprasternal view, with examination of the anatomy of the atria, valves, ventricles, septum, right and left out flow tracts and the great vessels. M-mode measurements for evaluation of right and left ventricular dimensions, thickness and motion of the ventricular wall and color Doppler were achieved.

Results

From 300 pregnant ladies of high risk factors for congenital heart diseases, there were 52 cases diagnosed by the fetal echo

that had congenital heart anomalies whom proved by postnatal infant echo in the first day of life and 248 pregnant ladies had normal fetal echo views from them 4 cases diagnosed by postnatal infant echo that had congenital heart anomalies which missed at fetal echo examinations.

The mean age of the pregnant ladies = 33 y as they ranged from 22 to 44 years.

All were pregnant at 22 to 28 weeks of gestation with the mean = 25 w.

Abnormal cardiac findings at fetal echo = 52 cases (17.3%) and 248 cases looks normal (82.7%).

The evidence of CHDs was higher in presence of exposure to teratogens or positive family history of fetal hydrops. The lowest incidence was related to the maternal age. There is no relations between CHDs and the cardiac father.

The most common findings of congenital heart disease during the fetal echo examination is discovered at 4-chamber view (44 cases out of 52 i.e 85% rater than others views as shown in Fig. (1).

The most common congenital heart disease diagnosed prenatally was atrioventricular defects as well as VSD.

The moving real time images more accurate in interpretation than the static pictures.

All cases which predicted antenatally had congenital heart disease confirmed after delivery by echo in the first day of life had a sensitivity = 92.8% and positive predictive value = 100%.

And there were 4 cases diagnosed postnatally not predicted antenatally, they were small muscular ventricular septal defect. So, fetal echo diagnosed 52 cases of 300 and the infant echo diagnosed 56 cases of 300 so the positive predictive value = 100% and the negative predictive value = 98.3% with sensitivity = 92.8% and specificity = 100% and the accuracy = 98.6%. The positive predictive value is = 100% as no fetus diagnosed having cardiac malformation was later identified as normal postnatally.

Table (1): Shows the indications of fetal echo.

Indications	No. of cases	%
Maternal age > 35 y.	50	16.6%
Previous cardiac sibling	15	5.0%
Maternal diabetes	40	13.3%
Non cardiac sonographic anomalies	30	10.0%
Polyhydraminos	25	8.3%
+ve family history	22	7.3%
Cardiac pregnant patient	10	3.3%
Collagen disorders	15	5.0%
Fetal hydropes	18	6.0%
Nuchal transluceny (at 10-14)	11	3.7%
Exposure to teratogens	16	5.3%
Father's fetus has cardiac anomalies	5	1.6%
Viral infections during pregnancy	43	14.3%
Total	300	100%

Table (2): Shows the results of abnormal findings fetal echo.

Cardiac defects	No	%
Fallot tetralogy	5	9.6%
Pericardial effusion	2	3.8%
Coronary fistula	1	1.9%
Hypoplastic left heart syndrome	6	11.5%
Atrioventricular septal defect	10	19.2%
VSD	10	19.2%
ASD	8	15.3%
Aortic stenosis	1	1.9%
Mitral atresia	2	3.8%
TGA	3	5.8%
Cardiomegally	4	7.7%
Total	52	100%

TGA : Transposition of great arteries. ASD: Atrial septal defect
VSD: Ventricular septal defect.

Table (3): Correlation between the fetal echo indication and percentage of cardiac anomalies.

Indications	No. of cases	%
Maternal age > 35 y.	3/50	6.0%
Previous cardiac sibling	2/15	13.3%
Maternal diabetes	3/40	7.5%
Non cardiac sonographic anomalies	4/30	13.3%
Polyhydraminos	5/25	20.0%
+ve family history	6/22	27.3%
Cardiac pregnant patient	1/10	10.0%
Collagen disorders	3/15	20.0%
Fetal hydropes	11/18	61.1%
Nuchal transluceny (at 10-14)	1/11	9.1%
Exposure to teratogens	9/16	56.3%
Father's fetus has cardiac anomalies	1/5	20.0%
Viral infections during pregnancy	3/43	6.99%
Total	52	100%

Table (4): Indication of termination.

Indication of termination	No	%
Hydrops fetalis	2	28.6%
Neural tube defect	1	14.3%
Fetal distress	4	57.1%
Total	7	100%

CHDs : Congenital heart diseases.

Table (5): The outcome of pregnancy in diagnosed congenital heart disease.

Outcome	**No**	**%**
Term	32	61.5%
Preterm	8	15.4%
IUGR	3	5.8%
Termination	7	13.5%
Neonatal death	2	3.8%
Total	52	100%

IUGR: Intrauterine growth restriction.

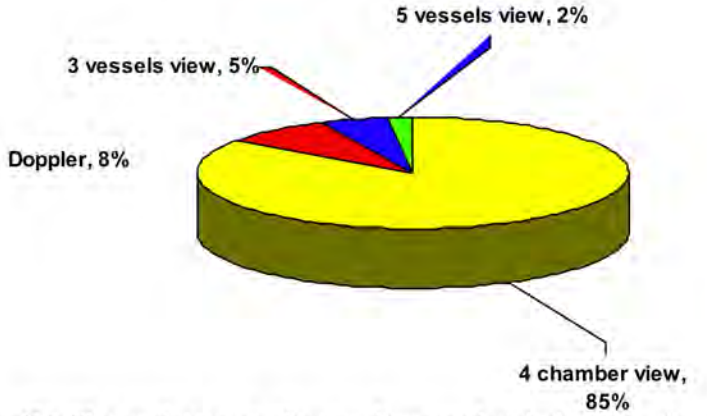

Fig. (1): Shows that most of cases diagnosed at 4-chamber view.

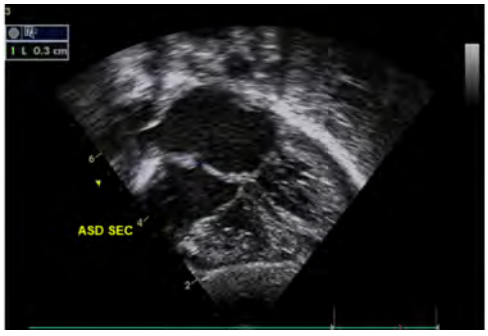

Fig. (2): Atrial septal defect.

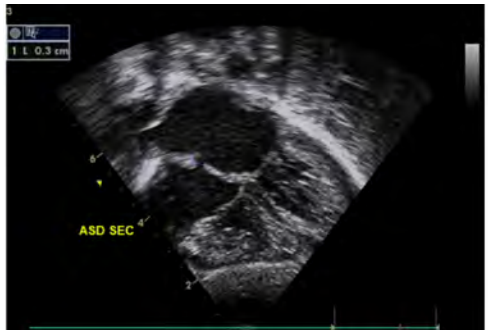

Fig. (3): Atrio-ventricular canal.

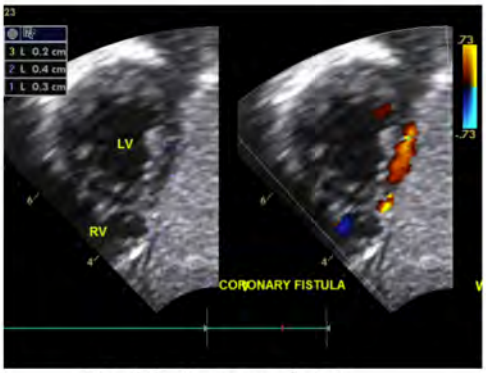

Fig. (4): Coronary fistula .

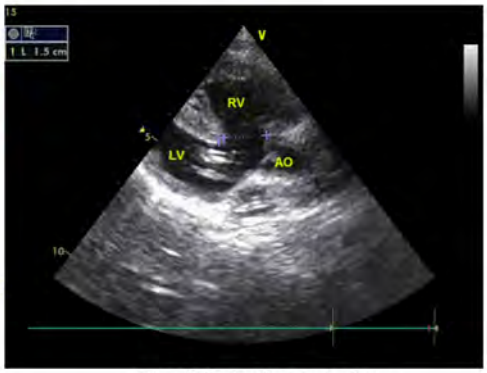

Fig. (5): Fallot tetralogy.

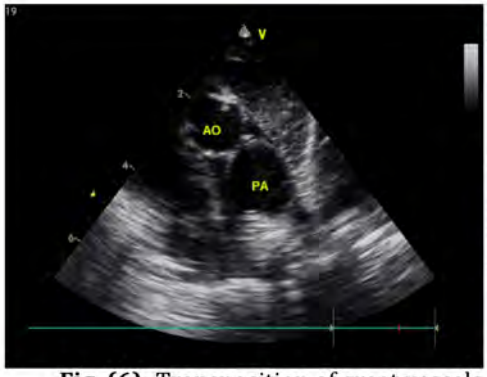

Fig. (6): Transposition of great vessels.

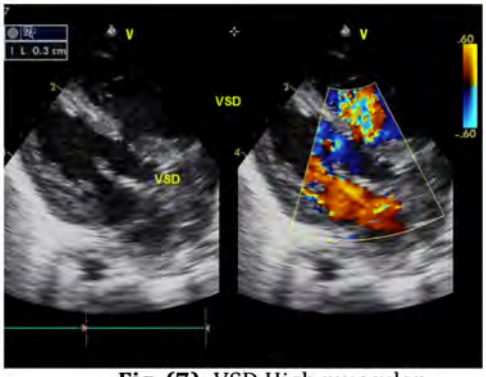

Fig. (7): VSD High muscular.

Discussion

Fetal echo is an important tool in predicting the CHDs. Development of the heart occurs in the first 6-7 weeks of gestation. So any congenital heart diseases develop during this period some of them pass unnoticed during the fetal life and others result in fetal heart failure as in fetal hydrops and some cardiac anomalies apparent only later in life[13].

This study reviewed 300 fetal cardiac examination and the indications of the fetal echo showed in table 1 and the type of cardiac anomalies which detected prenatally is proved by echo postnatal. We obtained correct and complete diagnosis in 92.8% of cases. All examinations were done transabdominally by a single operator to avoid personal variations either in prenatal or postnatal echo. Our data showed that congenital heart disease was predicted in 17.3% of the studied cases, this rate is higher than the rate reported by Reuven et al.[14] who found cardiac anomalies in 5.8% of their studied cases and this variations referred to that our study operated at high risk groups of CHDs. But Reuven et al., had the primary indications for performing fetal echo were suspected fetal anomalies or fetal arrhythmia detected by routine pre natal ultrasound or performing fetal echo due to maternal request.

This study agree with most of other studies which have shown more than 60% confirmation of abnormality for high risk groups

as nuchal transluceny and exposure to teratogenes and diabetic mother as in Ozkutulu et al.[15] study.

According to our data the high rate of cardiac anomalies was found among fetuses referred for fetal echo due to exposure to teratogens or positive family history and the lowest incidence was related to the maternal age and this disagree with Lynch et al.[16], study which showed the highest rate of CHDs in their population was seen in fetuses referred because of an abnormal cardiac examination or routine screening ultrasound (55.6%) following the detection of other fetal anomalies (8.2%).

And also there is a difference between our data and the Jscarvalho et al.[17] data which had the highest reported rate with routine ultrasound screening 75% and their data were completed on postnatal echo, but the absolute number of CHDs encountered is not large, just 40 cases of major CHDs, 30 cases diagnosed antenatally and the others 10 cases which misdiagnosed at fetal echo, diagnosed at postnatal cardiac examinations. SO the prediction rate of fetal echo is 75% and in our study the prediction rate is 92.8%.

Universal screening for congenital heart disease at routine prenatal ultrasound examination were the most effective method to improve the prenatal detection rate of serious cardiac anomalies. Bader[18], study showed 35% of fetal echo performed because of abnormal cardiac findings on routine prenatal sonography.

The American college of obstetrics and gynecologist recommend that 4-chamber view be evaluated during the routine prenatal sonographic examinations at second trimester[19].

It is easier of sonographer to recognize complex cardiac defects when associated with extracardiac anomalies and detection of a minor cardiac anomalies is more difficult[20].

There are many reports have established the ability of fetal 2D and Doppler echo to distinguish normal from abnormal fetal

hearts. There is further evidence establishing the accuracy of fetal echo for specific and accurate diagnosis of congenital cardiac anomalies particularly with an experienced sonographer[20].

Gottliebson et al.[21] study demonstrated that fetal echo can describe the segmental cardiac anatomy with high accuracy and give the detailed analysis of congenital heart anomalies with accuracy >90% and our study proved this as the accuracy of fetal echo in prediction of congenital heart anomalies = 98.4%. Nomiyama et al.[22], showed that even in late gestation the fetal aortic isthmus could be visualized >85% of the time.

We also agree that fetal cardiac examination should be a routine part of the pre-natal echo in order to achieve this in our country a nation training program similar to that carried out by Hunter et al.(8) in northern England is needed for obstetric ultrasonographers.

We believe that the fetal echo must be a part of routine fetal sonographic examination in our developing country as there is a higher incidence of pollutions and exposures to teratogenes and the prenatal consanguinity is high and the birth rate is very high.

Our data show similar detection rates of CHDs following these common referral indications or differ with other studies that were performed without any medical indications or performed due to maternal request.

The 4 cases whose diagnosis was missed by fetal echo and diagnosed by postnatal echo were had a small muscular ventricular septal defect and none was diagnosed as having a significant cardiac anomalies.

Conclusion

Evaluation of the fetal heart is an essential part of a routine prenatal ultrasound examination a 4-chamber view is the best to detect most of cardiac anomalies. Fetal echo is highly sensitive and specific in detecting CHDs prenatally proved by postnatal echo so establishing it as a routine obstetric ultrasonographic examination will increase the prediction rate of cardiac anomalies prenatally.

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EVALUATION AND EVOLUTION OF FETAL ECHO IN PREDICTION OF CARDIAC CONGENITAL ANOMALIES

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MAGNESIUM SULPHATE VERSUS PHENTOLAMINE AS A TECHNIQUE OF DELIBERATE HYPOTENSION FOR ENDOSCOPIC SINUS SURGERY

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Abstract

Background: *This prospective randomized study was designed to compare the efficacy of intravenously administered of either magnesium sulphate or phentolamine as a technique of hypotensive anesthesia during functional endoscopic sinus surgery (FESS).*

Patients and methods: *one hundred and fourteen patients undergoing functional endoscopic sinus surgery (FESS), the magnesium sulphate group (57 patients) received magnesium sulphate 40 mg /kg intravenously as a bolus before induction of anesthesia and 15mg /kg/ h by continuous intravenously infusion during the operation. phentolamine group (57 patients)received phentolamine 5 mg intravenously as a bolus dose before induction, then intraoperative infusion 2mg/h. Intraoperative, heamdynamics, field visibility, blood loss, duration of surgery as well as postoperative recovery were evaluated .*

Results: *In the magnesium sulphate group, there was a significant reduction of blood loss 155(17)ml versus 214(15) ml in phentolamine group, more better surgical filed conditions and reduction in surgical time 106±3.4min in magnesium sulphate group versus 111±2.2min in phentolamine group, however, the extubation time was longer in magnesium sulphate group 8.40(2.49) versus. 6.44(2.40) in phentolamine group. Mean blood pressure was decreased in both groups but without significant differences between both groups. With respect to heart rate, phentolamine group showed significant increase in comparison to mag-*

nesium sulphate group. The intraoperative anesthetic requirements (fentanyl and isoflurane), were significantly reduced in the magnesium sulphate group.

Conclusions: *We conclude that both magnesium sulphate and phentolamine induced hypotension caused by continuous infusion during general anesthesia in functional endoscopic sinus surgery (FESS), however, there was significant reduction of intraoperative blood loss and improvement of operative field visibility, as well as reduction of duration of surgery and anesthetic requirements but there was significant delay in extubation time in magnesium sulphate group compared to phentolamine group.*

Introduction

Bleeding is a major problem in Endoscopic Sinus Surgery (ESS), which may impede surgical progress and associated with complications [1]. Anesthetic management during ESS is aimed to minimize bleeding and to establish a perfect surgical field, which improve the surgical conditions [2]. Deliberate hypotension is a technique which has been used to decrease intraoperative bleeding and improve the quality of the surgical field for better visualization during endoscopic sinus surgeries[3].

Different pharmacologic agents have been used to produce deliberate hypotension such as direct vasodilators, alpha-2 drenergic agonists, beta adrenergic antagonists, alpha and beta adrenergic antagonists as μ-receptors agonist and N-methyl Daspartate antagonist as magnesium sulphate[4]. Phentolamine is a competitive nonselective ∝1- and ∝2-adrenergic receptor antagonist, it produces hypotension by direct vasodilatory effect on vascular smooth muscle and subsequent baroreceptor mediated reflex tachycardia [5].

Magnesium sulphate was used to induce deliberate hypotension by limiting the outflow of calcium from the sarcoplasmic reticulum and produces a vasodilating effect by increasing the synthesis of prostacyclin and inhibiting angiotensin converting enzyme activity [4,6,7].

The main objective of this prospective randomized blind study was to compare the efficacy of using magnesium sulphate or phentolamine in endoscopic sinus surgery (ESS) as regards their efficacy in providing better quality of the surgical field by inducing deliberate hypotension.

Patients and Methods

In the present prospective randomized blind study, 114 adults (aged from 20-40 years) ASA physical status I and II patients undergoing endoscopic sinus surgery were included. A written informed consent was obtained from all patients after approval by the Local Ethics Committee. Patients with hepatic, renal, cardiovascular, neuromuscular or haematological disorders were excluded. Those with history of using sedatives, narcotics or anticoagulants were also excluded. Patients were randomly assigned to receive either magnesium sulphate (g M) or phentolamine (g ph).

Anesthesia was induced by propofol 2 mg/kg intravenously and fentanyl 1 μg/kg , atracurium 0.5 mg/ kg was injected intravenously to facilitate tracheal intubation. Patients in the magnesium group received an intravenous bolus of magnesium sulphate 40 mg/ kg in a total of 100 ml saline over 10 min before induction followed by continuous infusion of 15 mg/kg/ h until the end of operation.The phentolamine group received phentolamine 5 mg over 10 min before induction followed by infusion 5mg /h until the end of surgical procedure.

Anesthesia was maintained with 1-2 MAC isoflurane and lungs were ventilated with oxygen and air at a ratio of 1:1 and atracurium increments of 0.1-0.2 mg/ kg. Ringer's solution was in fused continuously at a rate of 5 ml/kg/h. Mean arterial pressure (MAP), heart rate (HR) and oxygen saturation were recorded before induction of anesthesia (baseline), 1 min after induction of anesthesia, 1 min after intubation then every 15 min intraoperatively, 1 min before extubation and 5 min after extubation, intraoperative end tidal CO2 was recorded. Blood samples for serum magnesium were obtained before and after surgery (3 ml of venous blood was

withdrawn into clean and dry tube and lefted to form clot then the serum was separated by centrifugation and oliquoted and stored at -20Cº until assay using (EBRAMANNHEIM SPECTROPHOTOMETER C SN, 7356) with IVD. Biotecnica instrument S.P.A. via licenza 18.00156 Rome (italy) kits the normal reference range (1.9-2.5mg/dl)[8]. The target MAP during operation was between 60 and 70 mmHg. If hypertension or tachycardia more than 20% of the preoperative value occurred, fentanyl 1 µg/kg was given intravenously. Isoflurane concentration was manipulated according to targeted MAP. The level of anesthesia was monitored using bispectral index (BIS), the BIS monitor strip electrodes (BIS Sensor-Aspect Medical Systems Inc., ms 14796, ms 15115 for adult) were placed on the forehead, the target value was (40-60). Intraoperative fentanyl consumption was also recorded.

If hypotension or bradycardia more than 20% of the preoperative value occurred, 5 mg ephedrine for hypotension or 0.5 mg atropine for bradycardia was given intravenously. Intraoperative blood loss was estimated by measuring the contents of the suction bottle after subtraction of the amount of saline used for irrigation.

Bleeding in the operative field was subjectively evaluated by the same surgeon every 20 min , the surgeon who was not aware of the selected hypotensive agent was asked to assess the quality of the surgical field according to the quality scale proposed by Fromme and colleagues [9].

0 = no bleeding.
1 = slight bleeding - blood evacuation not necessary.
2 = slight bleeding - sometimes blood has to be evacuated.
3= low bleeding - blood has to be often evacuated. Operative field is visible for some seconds after evacuation.
4= average bleeding - blood has to be often evacuated. Operative field is visible only right after evacuation.
5= high bleeding - constant blood evacuation is needed.

At the end of surgery, study drugs were discontinued and

isoflurane was discontinued on the time of nasal packing. Combination of atropine (0.02mg/kg) and prostigmine (0.05mg/kg) was administered to reverse the neuromuscular block. Duration of surgery, extubation time(min), response to verbal command (min) and orientation time (min) were recorded. all data were collected by a blinded observer who was unaware of the patients' assignment group.

Patients were transferred to the postanaesthesia care unit (PACU). In the PACU oxygen saturaion, MAP and HR and were recorded on admission and then 15 and 30 min later. Pain was evaluated using visual analogue scale (VAS) on arrival to PACU, then after 15 min and after 30 min starting from 0 for no pain to 100 for worst pain. If VAS was >40, ketorlac 30 mg intravenously was administered.

Any adverse effects and signs of neurological toxicity of magnesium were recorded. Nurses of the PACU were blinded to patients' group allocation.

Sample size was calculated by using t test for mean in G *power 3.1.5 program. According to pilot study (10 patients in each group) we calculated that 57 patients per group were sufficient to give p value 0.05 significant with confidence interval of 95% when mean value± SD of field score index in group(m) was 2.3±.8 and group (ph) was 1.8±.8.

Statistical Analysis

The statistical analysis of data done by using excel program for figures and SPSS (SPSS, Inc, Chicago, IL).program statistical package for social science version 17. The description of the data done in form of mean (+/-) SD for quantitative data, and Frequency & proportion for Qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative date student t-test was used to compared between two groups. Chi square test was used for qualitative data N.B: P is significant if ≤ 0.05 at confidence interval 95%.

Results

One hundred and fourteen patients ASA physical status I and II of both sex undergoing functional

endoscopic sinuses surgery were included (magnesium group n=57, phentolamine group n=57). The two groups were similar in terms of type of surgery and all patients underwent the same surgery by the same surgeon.

With respect to age, sex and weight, there was no statistical significant differences in both groups (table 1). There was a significant reduction in surgical time ($P<0.001$) of 106±3.4in magnesium group vs 111±2.2min in phentolamine group (table 1). The mean blood loss in the magnesium group was reduced [155 $^{(17)}$ ml vs 214 $^{(15)}$ ml in phentolamine group] (table 1). The operative field conditions were significantly better in the magnesium group up to the end of surgery (Fig. 1).

With respect to intraoperative haemodynamics parameters, the blood pressure was significant decreased in both groups in comparison to the basal value however, the preoperative, intraoperative and postoperative values of MAP were not statistically significantly differed between both groups. With respect to heart rate, there was a significant increase in the intraoperative heart rate in the phentolamine group in comparison to the magnesium group, there was no episodes of hypotension (MAP<50 mm Hg) or arrhythmia in both group (fig. 2, 3) also, the use of ephedrine was not statisticaly significant differed in both groups, however 5 cases in magnesium group needed atropine injection in comparison to no cases in the phentolamine group.

BIS showed no significant difference between both groups, perioperative O2 saturation, intraoperative end- tidal CO2 showed no significant differences in both groups. Postoperative VAS (visual analogue scale) and analgesic request showed no significant difference between both groups (table 2).

There was a significant reduction in the MAC of isoflurane (fig 4) and intraoperative fentayl consumption was significantly decreased in the magnesium group, the three parameters of recovery (extubation time (min), response to verbal command (min), orienta-

tion time (min) were significantly prolonged in magnesium group in comparison to phentolamine group (table 3), postoperative magnesium levels were significantly higher in the magnesium group than in phentolamine group as shown in (table 2).

Table (1) patient's demographic data,duration of anesthesia (min), duration of surgery (min) , amount of blood loss(ml)

Variable	G M (no= 57)	Gph(no=57)	p- value
Age (years)	30±6.7	31±6.5	0.42
Gender (male –femal)	30/27	32/25	0.7
Weight (kg)	77(±5)	78(±4)	0.24
duration of surgery (min)	106±3.4	111±2.2	<0.001**
Amount of blood loss(ml)	155(17)	214(15)	<0.001***

**Significant reduction in duration of surgery in gM
***Significant reduction in blood loss in g M

Table (2): VAS (visual analogue scale) , Analgesic request, Preoperative magnesium (mmol/l), Postoperative magnesium (mmol/l)

Variable	G M (no= 57)	Gph (no= 57)	p- value
VAS 0	33.5 (6.9)	35.5 (6.6)	0.11
VAS 1	33.6 (6.1)	34.8 (5.4)	0.26
VAS2	28 (6.2)	28 (7.4)	0.99
Analgesic request	12	14	0.65
Preoperative magnesium (mmol/l)	1.8±0.3	1.7±0.32	0.08
Postoperative magnesium(mmol/l)	2.2±0.62	1.8±0.46	<0.001***

***Significant increase in the postoperative magnesium level in gM

Table (3): Intraoperative fentanyl consumption (μ/kg), extubation time(min), response to verbal command (min) , orientation time (min)

Variable	G M (no= 57)	Gph (no= 57)	p- value
Intraoperative fentanyl consumption (μ/kg)	1.2(0.23)	2.2(0.34)	<0.001*
Extubation time(min)	8.40(2.49)	6.44(2.40)	<0.001*
Response to verbal command (min)	9.34(1.22)	7.23(1.45)	<0.001*
Orientation time (min)	10.69(1.5)	8.53(1.43)	<0.001*

*Significant prolongation in extubation time, Response to verbal command , Orientation time with significant decrease in theIntraoperative fentanyl consumptionin g M .

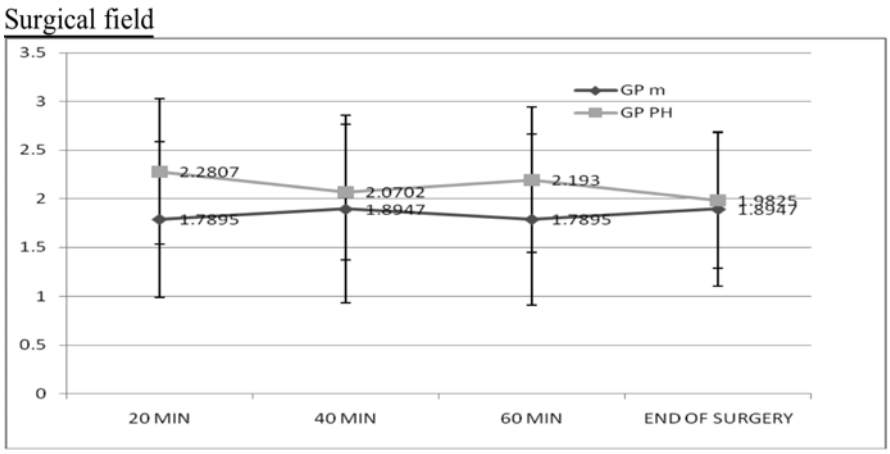


Fig (1) : Better surgical field in g M

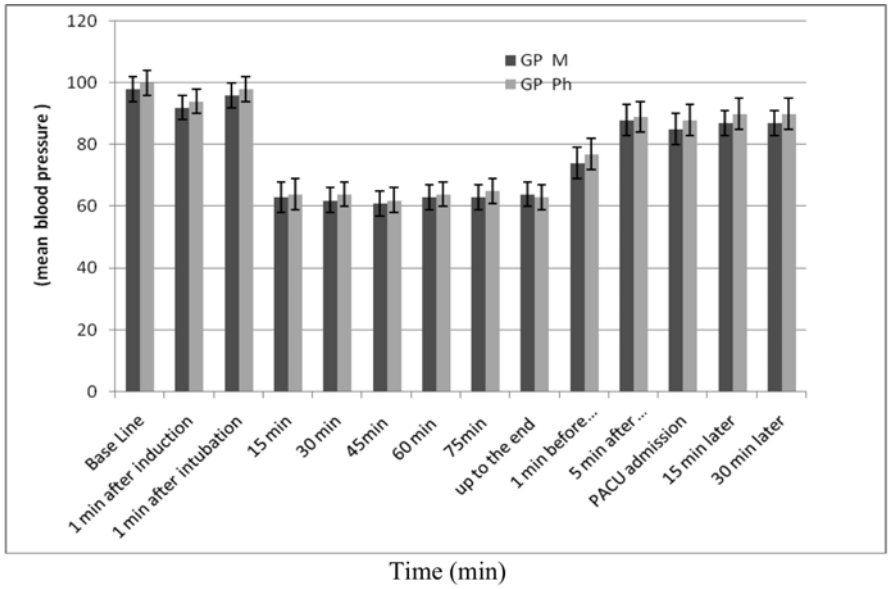
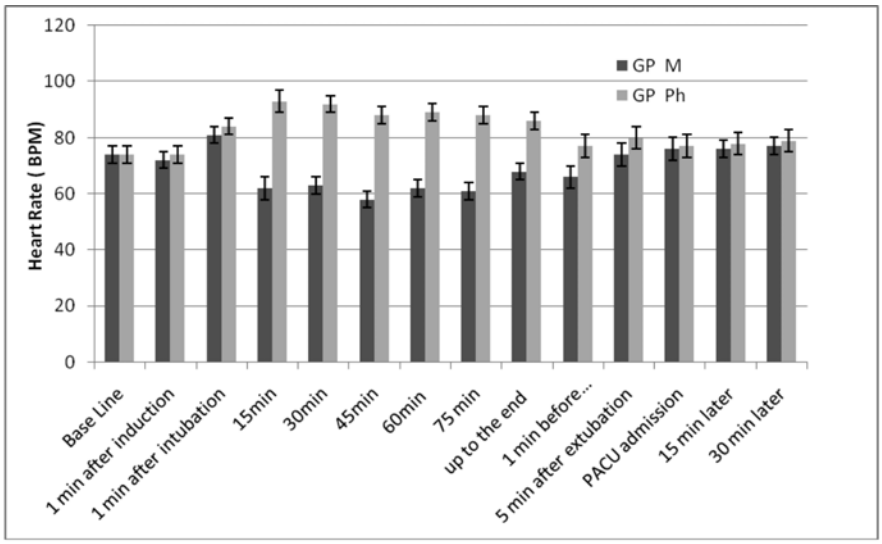
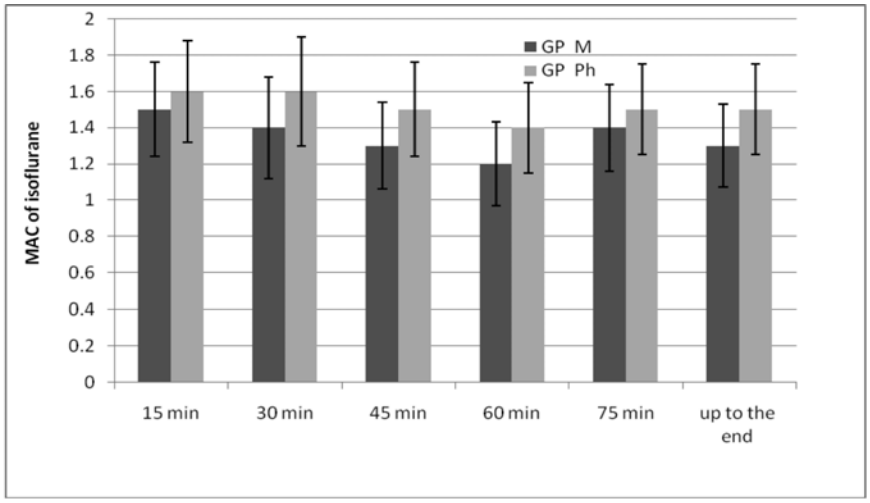


Fig (2): No significant differences between both groups in mean blood pressure .



Time (min)

Fig (3) : Significant reduction in the intraoperative heart rate (beat per minute BPM) in gM



Time (min)

Fig (4) : Significant reduction in MAC of isoflurane in g M

Discussion

Endoscopic sinuses surgery is a technique which carries great potential benefits for the treatment of many nasal conditions[10]. Good visualization is important because small anatomical structure and vascularity of the nose limit access of endoscope and even minor hemorrhage may diminish completing the surgery[11].

General anesthesia is often preferred because of the discomfort and incomplete block that may accompany topical anesthesia as well as providing hypotensive anesthesia[4]. Maintaining moderate controlled hypotension (mean blood pressure 60-70 mmHg) which is important in improving visibility in the operative field which result in faster surgery and reduced risk of major complications such as massive hemorrhage, skull base defect, orbital hemorrhage and blindness[12].

In the present study, both magnesium sulphate and phentolamine group achieved the targeted hypotension however, better surgical field and condition in the magnesium sulphate and subsequent reduction in the amount of blood loss compared to phentolamine group. This result may attributed to the mechanism of action of magnesium sulphate for deliberate hypotension through activation of membrane Ca ATPase and Na–K ATPase involved in transmembrane ion exchanges during depolarization and repolarization phases, and thus stabilize cell membrane and intracytoplasmic organelles[13]. Hypotension induced by magnesium during surgery is also explained by its powerful analgesic effect[14]. The analgesic action of magnesium is explained by its antagonistic effect at N-methyl D-aspartate receptors [15].

In parallel with the present study Crozier et al found that intravenous magnesium sulphate provided a dry field and reduced intraoperative bleeding without reflex tachycardia or rebound hypertension [16].

Moreover Aboushanab et al. concluded that both magnesium sulphate and dexmedetomidine

successfully induced deliberate hypotension and good surgical field visibility but magnesium sulphate was associated with shorter recovery time and earlier discharge from the PACU [17]. The possible mechanisms for reduction of the anesthetic requirements include; antagonism of NMDA receptors in the CNS by magnesium and reduction of catecholamine release by sympathetic stimulation, thus decreasing peripheral nociceptor sensitization or the stress response to surgery [15].

Ryu et al in their study that comparing between remifentanil and magnesium sulphate during middle ear surgery in terms of postoperative pain and other complications, they reported that there was no difference between the groups in terms of the quality of the surgical field or the degree of hypotension. However, rebound hypertension was seen in the remifentanil group, but not in the magnesium group, while the incidence of nausea, shivering and the need for rescue medication for both nausea and pain were significantly greater in the remifentanil group [18].

In the present study, there was significant decrease in the main blood pressure in both magnesium group and phentolamine group, but heart rate in the phentolamine group showed significant increase due to reflex tachycardia in comparison to magnesium group., this parallel with the result of Elsharnouby and Elsharnouby, they reported that the magnesium group infusion during the operation resulted in reduction in mean blood pressure and reduced heart rate, with no episodes of severe hypotension and no patient had rebound hypertension when the magnesium sulphate infusion was stopped [4].

Decrease in heart rate during magnesium sulphate induced hypotension was noticed in the present study and other studies such as the study of Yosry and Othman which compared both magnesium sulphate and sodium nitoprussid for induction of controlled hypotension in choroidal melanoma resection surgery and revealed that magnesium sulphate was associated with significant

decrease of heart rate from baseline compared to sodium nitoprussid that was associated with significant increase of heart rate [6].

Ray et al reported that, perioperative use of both clonidine and magnesium sulphate significantly reduced the consumption of propofol and fentanyl, also magnesium sulphate caused delayed recovery, these results were in parallel with the present study in which the anaesthetic requirements were reduced in the magnesium group but there was a longer emergence time [19].

The mechanism of phentolamine induced deliberate hypotension through competitive nonselective ∝1- and ∝2- adrenergic receptor antagonist with an elimination half-life of 19 minutes after intravenous injection. Phentolamine also promotes histamine release from mast cells, antagonizes serotonergic receptors, and blocks potassium channels [20].

Several studies were reported for the perioperative use of phentolamine as Kobal et al who reported that ∝-adrenergic blockade with phentolamine by continuous infusion was used to blunt large cyclical swings in the patient's blood pressure and heart rate in pheochromocytom, patient received 5 mg of phentolamine mesylate by i.v. bolus injection followed by a continuous i.v. infusion of the drug [21]. However Matthews et al. reported the use of a continuous i.v. infusion of phentolamine for 14 days to manage a trauma patient who was found to have an incidental pheochromocytoma [22].

Moreover Gabrielson et al. described three patients with pheochromocytoma in which the short durations of action of esmolol and phentolamine allowed their safe and effective use for preoperative medical management. All three patients received continuous i.v. infusions of phentolamine and esmolol [23]. Also Mihm et al. described a case in which a continuous i.v. infusion of phentolamine was used as bridge therapy until oral phenoxybenzamine could be started. The phentolamine was infused at a rate of 0.5-3 mg/hr for two days [24].

Another study by McMillian et al. who reported that a continuous infusion of phentolamine was used in a patient with pheochromocytoma to control perioperative hypertensive episodes during surgical adrenalectomy [25]. Up to the auther's recent knowledge, No studies have been published to compare magnesium sulphate and phentolamine regarding their hypotensive effects in patients undergoing endoscopic sinus surgery.

In the present study we conclude that both magnesium sulphate and phentolamine induce hypotension caused by continuous infusion during general anesthesia in functional endoscopic sinus surgery (FESS), however there was a significant reduction of intraoperative bleeding and improvement of operative field visibility, as well as reduction of duration of surgery and anesthetic requirements but with delay in emergence time in magnesium sulphate group compared to phentolamine group.

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MAGNESIUM SULPHATE VERSUS PHENTOLAMINE AS A TECHNIQUE OF DELIBERATE HYPOTENSION FOR ENDOSCOPIC SINUS SURGERY

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TRANSORAL VIDEOENDOSCOPIC ADENOIDECTOMY WITH MICRODEBRIDER VERSUS CURETTAGE ADENOIDECTOMY

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Abstract

Background : *Adenoidectomy is one of the most common procedures performed in children today, either alone or in conjunction with tonsillectomy or insertion of ventilating tubes. The main disadvantage of curettage is that it is a relatively "blind" technique that may lacerate the choanae and torus tubarius, gauge the nasopharyngeal mucosa, or skim the adenoid bulk, leaving behind obstructing tissue, particularly at the Eustachian tube orifices, high in the nasopharynx, and at intranasal protrusions.*

The powered-shaver method with microdebrider has been applied in a number of ways. It may be the primary technique, used as an adjunct to curettage, or coupled with other methods.

Objectives : *The aim of this work was to compare the advantage and disadvantage among transoral videoendoscopic adenoidectomy with microdebrider and traditional Transoral Curette Adenoidectomy.*

Patients and Methods : *Two-hundred patients of both sexes were included in this study all had adenoid (with or without tonsils) hypertrophy. All patients were distributed in two groups. Group (A) ninety (90) patients were underwent transoral video endoscopic adenoidectomy with microdebrider. Group (B) One hundred and ten (110) patients were underwent traditional Transoral Curette Adenoidectomy.*

Direct comparisons between the two groups were being made with regards to operative time, amount of blood loss , presence of possible residual lymphoid tissue, absence of bleeding .

Results : *In this study, we found the mean operative time for transoral video endoscopic adenoidectomy with microdebrider group (17,389*

minutes) is longer than that of traditional Transoral Curette Adenoidectomy group (9.046 minutes), the mean amount of blood loss for traditional Transoral Curette Adenoidectomy group (52.27 ml.) is larger than that of transoral video endoscopic adenoidectomy with microdebrider group (28.33 ml.), the presence of possible residual tissue in traditional Transoral Curette Adenoidectomy group (38,2%) is more than that of transoral video endoscopic adenoidectomy with microdebrider group (0%) and no post-operative bleeding for both groups detected .

Conclusion : *Transoral video endoscopic adenoidectomy with microdebrider is a safe, precise and quick procedure with minimal blood loss, optimal vision, clear view, excellent haemostasis and minimal complication.*

Key words: *Adenoidectomy - Endoscopic - Microdebrider.*

Introduction

Present from early gestation, adenoid growth continues until about 6 years of age, after which atrophy occurs, adenoidal hypertrophy during childhood may both fill the nasopharynx and extend through the posterior choanae into the nose, resulting in nasal airway stenosis, impeding airflow. There is a significant relationship between the endoscopically determined size of obstructive adenoid tissue and symptomatic nasal obstruction in children[1].

Adenoidectomy is one of the most common procedures performed in children today, either alone or in conjunction with tonsillectomy or insertion of ventilating tubes. The widely used conventional curette adenoidectomy was first described in 1885[2] .

The main disadvantage of curettage is that it is a relatively "blind" technique. It may lacerate the choanae and torus tubarius, gauge the nasopharyngeal mucosa or skim the adenoid bulk leaving behind obstructing tissue particularly at the Eustachian tube orifices, high in the nasopharynx or at intranasal protrusions[3] .

A number of Authors have described visualization of the operating field, during surgery, with a laryngeal mirror, trans-nasal or

trans-oral endoscope, these Authors employed curette, suction-coagulator, forceps and trans-nasal or trans-oral microdebrider as surgical tools for the removal of the adenoids[4,5].

The aim of this work was to compare the advantage and disadvantage of transoral video endoscopic adenoidectomy with microdebrider and traditional Transoral Curette Adenoidectomy.

Patients and methods

Two-hundred patients attended outpatient clinic of Benha university hospital &Benha teaching hospital during the period from May, 2011 to November, 2011 were randomly selected with symptoms and signs suggestive adenoid hypertrophy obstructing the nasopharynx as long lasting nocturnal snoring, sleep apnea and open mouth breathing.

Adenoid is the only cause of nasal obstruction. Other causes of nasal obstruction were excluded such as presence of chronic disease, acute rhinitis, allergic rhinitis, septal deviation, anatomical deformities, past history of cleft palate repair, cases with submucous cleft palate were excluded, also cases with bleeding or coagulation defects were excluded.

The study was approved by the medical ethics committee of Benha Faculty of Medicine and informed consents were obtained from the parents of the patients

I- Pre-operative assessment:

1- History taking and clinical examination:

(A) History was taken from the parents and they were asked about:

- Number of recurrent acute attacks of adenotonsillitis in the last year.
- Snoring.
- Night sleep disturbance and weather the child exerts an effort to breathe or not.
- History of chest and heart problems.

(B) Clinical examination:

- Otolaryngology examination: otolaryngology examination was carried out for all patients with stress on:

The nose: it was examined to exclude causes of obstruction oth-

er than adenoid such as deviated septum, allergy or polypi.

2- Radiological examination:

Lateral plain x-ray of the nasopharynx: lateral soft tissue plain x-ray of the post-nasal space and upper airway with slightly extended neck were performed for all cases.

3- Routine investigations:

Pre-operative investigations carried out for all patients were:

i- C.B.C (Complete Blood Picture).

ii- E.S.R. (Erythrocytes Sedimentation Rate).

iii- P.T, P.P.T & I.N.R (Prothmbin, Partial thromboplastin times & International normalized ratio).

iv- F.B.S (Fasting Blood Sugar).

All patients were distributed into two groups.

Group (A): Ninety (90) patients their ages ranged between four (4) to sixteen (16) years underwent transoral video endoscopic adenoidectomy with microdebrider.

Group (B): One hundred ten (110) patients their ages ranged between four (4) to fourteen (14) years underwent traditional Transoral Curette Adenoidectomy.

4- Operative Technique:

The patient was anesthetized under general anesthesia with an oral endotracheal tube. A Boyle-Davis mouth gag was used to open the mouth. The patient was placed supine with a small pillow under the shoulders to allow slight neck extension and was covered with sterile drapes. The palate and uvula were inspected and palpated to exclude a soft palate cleft.

Group (A):

Two soft catheters are introduced through the nasal passages to apply light upward traction to the soft palate, thereby increasing the anteroposterior diameter of the exposed area. The slight stretching of the palate achieved with this maneuver also aids in the recognition of a possible soft palate cleft.

A 70° endoscope with a video attachment was introduced through the mouth to visualize the

nasopharynx and the adenoid mass was identified. A microdebrider with a 40° curved blade, the cutting window of which is on the convex side, was also introduced through the mouth. The instrument was connected to an aspirator and was programmed to alternate rotations, with a rotational speed of 3000 rpm.

Removal of the adenoid tissue starts from the choanal vegetations and proceeds backwards along the vault towards the posterior wall of the nasopharynx.

Generally, the adenoid vegetations appear as a series of parallel cords of tissue with a longitudinal course, more voluminous along the median line and thinner toward the periphery. The most lateral cord is generally in close contact with the torus of the tuba. The smooth point of the microdebrider can be introduced into the furrow between the side vegetations and the structures of the tubaric ostium and the tissue can be completely removed without damaging the mucosa covering the torus tubarius.

At the end of the resection, a pack is placed in the cavity where it is maintained for some minutes. The pack is then removed and the cavity is checked for the presence of possible residual tissue and for the absence of bleeding.

When tonsillectomy is also scheduled, we prefer to perform the adenoidectomy first, but in the case of large tonsillar masses, tonsillectomy may be performed first in order to achieve better vision.

Group (B):

An appropriate-sized unguarded adenoid curette was then used to remove adenoid tissue by routine method.

5- Parameters of working:

Direct comparisons between the two groups were being made with regards to:

A- Intra operative finding:

- Operative time starting from the ending of process of anesthesia to the time of recovery including the time using for removal of the adenoid, the time of haemostasis and the time to ensure cessation of bleeding.

- Amount of blood loss determined by the amount of blood in suction container after removal of the amount of saline & fluids we used intraoprative and the dressing weight by weighting it before and after using with determines the difference in its weight.

B- Immediate postoperative finding:

The cavity was endoscopically checked for:

- Residual lymphoid tissue which may be present in some cases.
- Assessment of bleeding.

6- Statistical analysis:

The results were analyzed by suitable statistical method.

Results

Two-hundred patients of both sexes were included in this study all had adenoid (with or without tonsils) hypertrophy. The patients were distributed in two groups.

Group (A):

Ninety (90) patients their ages ranged between four (4) and sixteen (16) years with a mean age of (9.11) years were underwent transoral video endoscopic adenoidectomy with microdebrider. Ten (10) of them underwent tonsillectomy with adenoidectomy.

Group (B):

One hundred ten (110) patients their ages ranged between four (4) and fourteen (14) years with a mean age of (7.6) years were underwent traditional TransoralCurette Adenoidectomy. Forty fife (45) of them underwent tonsillectomy with adenoidectomy.

Comparisons between the two groups were being made with regards to :

I- Intra operative finding:

1- Operative time:

- Table (2): shows operative time distribution of the 2 groups of the study. The mean operative time for group (B) is significantly shorter (9.046 minutes) than that-of group (A) (17,389 minutes).

- The mean operative timefor group (A) (17,389 minutes) is longer than that of group (B) (9.046 minutes) and the difference was highly significant (P value 0.001).

2- Amount of blood loss:

Table (3): shows distribution of blood loss between the 2 groups of the study group. The mean amount of blood loss for group (A) is significantly smaller (28.33 ml.) than that of group (B) (52.27 ml.).

The mean amount of blood loss for group (B) (52.27 ml.) is larger than that of group (A) (28.33 ml.) and the difference was highly significant (P value 0.001).

II- Immediate postoperative finding:

1- Presence of possible residual lymphoid tissue:

Table (4): shows distribution of presence of residual tissue between the 2 groups of the study group. The presence of possible residual tissue in group (A) is significantly less (0%) than that of group (B) (38.2%).

The presence of possible residual tissue in group (B) (38,2%) is more than that of group (A) (0%) and the difference was highly significant (P value 0.001).

1-Absence of bleeding:

None of our cases of both groups developed postoperativeun controlled bleeding.

Table (1): **Age distribution of the study groups.**

Variable	Groups	Mean	SD	Student t test	P value
Age	A	9.11	3.596	1.407	0.168 NS
	B	7.5	3.61		

Table (2): Show distribution of operative time between the 2 groups of the study.

Variable	Groups	Mean operative time (minutes)	Standard deviation	Student t test	P value
Time	A	17.389	5.72	6.524	0.001 HS
	B	9.046	1.676		

Table (3): Show distribution of blood loss between the 2 groups of the study.

Variable	Groups	Mean amount of blood loss (ml)	Standard deviation	Student t test	P value
Blood loss	A	28.33	5.42	-8.162	0.001 HS
	B	52.27	11.42		

Table (4): Show distribution of Presence of residual tissue between the 2 groups of the study.

Group / Residual tissue	A		B		Total		Z test	P value
	No	%	No	%	No	%		
Present	0	0.0	42	100.0	42	100.0	-	-
Absent	90	57.0	68	43.0	158	100.0	1.767	0.039 S
Total	90	45.0	110	55.0	200	100.0	1.42	0.078 NS
X2 test = 79.56								

Table (5): Show distribution of absence of bleeding in the 2 groups of the study.

Group / Bleeding	A		B		Total		Test	P value
	No	%	No	%	No	%		
Present	0	0.0	0	0.0	0	0.0	-	-
Absent	90	45.0	110	55.0	200	100.0	1.42	0.078 NS
Total	90	45.0	110	55.0	200	100.0	1.42	0.078 NS

Discussion

Adenoidectomy is a common procedure for treating pediatric ear, nose, and throat patients. Nowadays, it is used to treat enlarged adenoids that have not responded to conservative treatments, which are indicated in obstructive sleep apnea, recurrent serous otitis media, and resistant rhino sinusitis[6].

The objective of adenoidectomy is to remove the hypertrophic adenoid tissue that causes nasal airway stenosis leading to pathological restriction of nasal airflow[7].

Adenoidectomy is one of the most frequently performed operations in children. Though not considered a risky surgery, some complications do occur, the most important being bleeding[8].

In this study, which was a comparative prospective one, we found that mean operative time fortransoral video endoscopic adenoidectomy with microdebrider group (17,389 minutes) is longer than that of traditional Transoral Curette Adenoidectomy group (9.046 minutes) and the difference was highly significant (P value 0.001) and The mean amount of blood loss for traditional Transoral Curette Adenoidectomy group (52.27 ml.) is larger than that of transoral video endoscopic adenoidectomy with microdebrider group (28.33 ml.) and the difference was highly significant (P value 0.001). The surgeon found that the oscillating cutting action of the shaver blade minimizes bleeding and continuous suction maintains a clear view thus enhancing safety. Due to the suction and shaving action of the microdebrider drawing the loose tissue into the window, it can remove the tissue down to a less vascular fascial plane, as opposed to the pushing and cutting action of the curette which may leave bleeding tissue behind. Irrigation while using the microdebrider also aids in quicker homeostasis.

Our results was in consistence with Costantini et al., 2008[2] how had described video endoscopic adenoidectomy with microdebrider with mean operating was 12.5 minutes and mean blood loss was 28 ml.

Koltai et al., 1997[3]. review of complete adenoidectomy using microdebrider versus curettes showed that power assisted adenoidectomy was 58% faster (11 min vs. 19 min) but the blood loss (22 ml vs. 32 ml). Stanislaw et al., 2000(5) showed again that operative time was much less with microdebrider (10 min 13 s vs. 12 min 14 s) and also that blood loss was less with microdebrider (17.5 ml vs. 24.0 ml, 27% less). Our results were not in consistence with them according to operative time but were in consistence with them according to amount of blood loss.

Somani et al., 2010[9]. described endoscopic power assisted adenoidectomy with microdebrider. The mean operative time was 12 min 30 s (range, 8-16 min). The average blood loss was 30 ml (range, 24-42 ml). Our results were in consistence with them.

The duration of the procedure is slightly longer than that of adenoidectomy with the adenotome. The organization and preparation times are also longer, while ablation and homeostasis times are substantially the same. However, the slightly longer duration is more than compensated by the greater precision and confidence gained by the surgeon as decided by Costantini et al., 2008[2]. We are agree with them as we found that the oscillating cutting action of the shaver blade minimizes bleeding and continuous suction maintains a clear view thus enhancing safety.

Our experience with the microdebrider was supported by these studies, with respect to completeness of adenoid tissue removal to adequate depth and causing no damage to surrounding structures. The surgeon satisfaction, though a subjective parameter, was definitely high. The postoperative recovery was satisfactory.

In this study we found that presence of possible residual tissue in traditional Transoral Curette Adenoidectomy group (38,2%) is more than that oftransoral video endoscopic adenoidectomy with microdebrider group (0%) and the difference was highly significant (P value 0.001).

Havas and Lowinger, 2002[10].

have drawn attention to the high percentage of residual tissue remaining after traditional adenoidectomy with an adenotome or a curette, especially in the choanal and tubaric regions.

Cannon et al., 1999[4]. used the 4-mm 0 telescope transnasally at the end of the routine transoral adenoidectomy and they found residual adenoid tissue in all cases of their 236 except for 12 cases (5.1%); therefore they stressed that direct visualization is very important during the procedure. Our results were disagreeing with them as we found residual tissue in 38.2% only from all cases of traditional Transoral Curette Adenoidectomy group.

A prospective study involving endoscopic evaluation of cases operated by curette and microdebrider has shown that, following traditional curette adenoidectomy 39% of patients had residual obstructive tissue which was completely cleared by powered shaver adenoidectomy later [10].

Our results were agreeing with them as we found residual tissue in 38.2% only from all cases of traditional Transoral Curette Adenoidectomy group and not found residual tissue in all cases transoral video endoscopic adenoidectomy with microdebrider group.

Stanislaw et al., 2000[5]. found thatthe tissue dissection was more complete and to the appropriate depth with a microdebrider, as opposed to being too shallow or too deep with a curette. The surgeon satisfaction was also greater.

Havas and Lowinger, 2002[10] had demonstrated that in up to 39% of children with clinically significant adenoid hypertrophy, curette adenoidectomy does not achieve adequate removal of obstructive adenoid tissue, especially when there is intranasal extension of adenoid or a bulky mass of adenoid high in the nasopharynx. In such cases the use of powered-shaver technique enables better clearance of obstructive adenoid and our results were agreeing with them.

None of our cases of both groups developed postoperative un

controlled bleeding, developed postoperative un acceptable morbidity or developed postoperative un acceptable complications.

Pagella et al., 2008[11] reported that no significant intra-operative bleeding, post-operative hemorrhage or other complications have occurred. Over the years, there have been advances in the techniques of adenoidectomy, from the conventional trans-oral to endoscopic trans-nasal/trans-oral adenoidectomy. Also Jong and Gendeh, 2008[12] emphasized the advantages of this procedure with respect to that of the conventional technique.

Conclusion

Traditional Transoral Curettage Adenoidectomy is still the most common method of doing adenoidectomy in our Practice. It has short duration and easy operative technique but it also has highly amount of blood loss and highly incidence of presence of residual obstructing lymphoid adenoid tissue.

Transoral video endoscopic adenoidectomy with microdebrider is a safe, precise and quick procedure with minimal blood loss, optimal vision, clear view, excellent homeostasis and minimal complication. It gives a complete clearance of obstructive adenoids. However, the slightly longer duration than curettage is more than compensated by the greater precision and confidence gained by the surgeon. Moreover, it is quite easy to teach using video images. It is highly recommended in cases of recurrent distributed adenoid, partial adenoidectomy, recurrent Eustachian tube obstruction and very selective removal of the adenoid tissue.

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EFFECT OF SELENIUM ON CARBIMAZOLE INDUCED ALTERATIONS IN TESTICULAR FUNCTION IN ALBINO RATS

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Abstract

Carbimazole is an antithyroid drug used in treatment of hyperthyroidism. The use of Carbimazole was associated with various adverse effects in male reproductive system.

Spermatogenesis is an extremely active process with high rate of cell division that leads to high rates of mitochondrial oxygen consumption by the germinal epithelium. However, oxygen tensions in this tissue are low so both spermatogenesis and Leydig cell steroidogenesis are vulnerable to oxidative stress.

Testes and epididymes contain high concentrations of Selenium indicating its vital role during spermatogenesis to improve semen quality. This study was designed to evaluate the effect of Carbimazole on testicular activity in albino rats and the ameliorative role of selenium.

The rats in this study were divided into 4 groups. Group (1) that were served as normal control, Group (2) were orally given Carbimazole Group (3) were orally given sodium selenite and Group (4) that were orally administered Carbimazole and sodium selenite for 8 weeks. Testicular reduced glutathione concentration (GSH), Testicular malondialdehyde (MDA) concentrations, Epididymal sperm count, Sperm motility, Sperm abnormalities, serum testosterone, LH, FSH levels and testicular histopathology were examined. we can conclude that, treating animals with selenium causes improvement in normal testicular function of rats. In addition combination of selenium with Carbimazole showed improvement in testicular alterations induced by Carbimazole in rats.

Introduction

Carbimazole is an antithyroid agent that decreases the uptake and concentration of inorganic iodine by thyroid; it also reduces the formation of diiodotyrosine and thyroxin [1]. Serum Carbimazole is a common oral treatment for hyperthyroidism. On the other hand, the use of Carbimazole was associated with various adverse effects [2]. Carbimazole produced mild necrosis of renal tubules in rats. Carbimazole was capable of inducing acute pancreatitis, pulmonary hemorrhage and necrotizing glomerulonephritis and cholestatic hepatitis in 33-year old female [3].

Reactive oxygen species (ROS) are molecules that have at least one unpaired electron, rendering them highly unstable and highly reactive in the presence of lipids, amino acids and nucleic acids. At physiologic levels, ROS are essential for normal reproductive function, acting as metabolic intermediates in gene regulation, and in facilitated sperm capacitation and acrosome reaction[4]. However, at higher concentrations, they exert negative effects. The main source of ROS production in seminal plasma is leukocytes and immature spermatozoa. Spermatids and mature spermatozoa are deemed highly sensitive to ROS because their membranes are particularly rich in polyunsaturated lipids. By altering membrane integrity, ROS may impair sperm motility and morphology and can lead to sperm cell death [5].

Spermatogenesis is an extremely active replicative process capable of generating approximately 1,000 sperm a second. The high rates of cell division inherent in this process lead to high rates of mitochondrial oxygen consumption by the germinal epithelium. However, the poor vascularization of the testes means that oxygen tensions in this tissue are low and that competition for this vital element within the testes is extremely intense [6].

The testes contain antioxidant defense systems that are of major importance because peroxidative damage is currently regarded as the single most important cause of impaired testicular function[7].

Selenium (Se) is an essential element for normal testicular development, spermatogenesis, and spermatozoa motility and function. Se may protect against oxidative DNA damage in human sperm cells. However, the exact mechanism by which Se eliminates oxidative stress to improve male fertility and semen quality in humans is still controversial [8]. There are at least 25 selenoproteins in the human body, and they help maintain sperm structure integrity[9].

The best-characterized spermatozoal effects of Se deficiency are: important loss of motility, breakage at the midpiece level and increased incidence of sperm-shape abnormalities, mostly of the sperm head. [10].

Selenium is an essential element important in many biochemical and physiological processes including regulation of ion fluxes across membranes, maintenance of the integrity of keratins, stimulation of antibody synthesis, and activation of glutathione peroxidase[11]. Selenium ameliorated the testicular damage and oxidative stress induced by Carbimazole in albino rats[12].

The present work aims to investigate the effect of selenium on histology of testis, serum testosterone level, LH, FSH, Testicular GSH, Testicular MDA and semen analysis alterations induced by Carbimazole in male albino rats.

Materials and Methods

Animals

Thirty two male adult albino rats of locally breaded strain weighing between 150+ 5 g at the beginning of the study were used. They have acclimatized for one week in groups (8/cage) in fully ventilated room at ordinary room temperature. Rats were allowed to water and balanced diet.

At the beginning of the experiment they were divided into 4 groups each contained 8 rats:

Group I : Control normal rats. They received no drugs

Group II: Animals of this group were orally given Carbimazole (1.35 mg/Kg b.w) dissolved in water, daily for 8 weeks [13].

Group III: animals of this group were orally given sodium

selenite (10 µg/Kg b.w) dissolved in water, daily for 8 weeks [14].

Group 4: animals of this group were orally administered Carbimazole (1.35mg/Kg b.w) and sodium selenite (10µg/Kg b.w) for 8 weeks. The treated animals were sacrificed by cervical decapitation after 8 weeks of treatment.

Drugs:

- Selenium (sodium selenite): gray powder 5gm in bottle from (Sigma chemicals Co., U.S.A.).
- Carbimazole: 10 mg Tablet (Ambica Intl Trading).
- Hematoxylin and eosin: (E. Merk, Darmastadt.,) [U.S.A.].
- Formalin (neutral 10% formalin): El-Gomhoria Pharmaceutical Chemi.Ocal Co, ARE. Diagnostics Limited lot. No. 18933.

The procedures used:

Each animal of all groups was killed after the end of the experiments by decapitation and the following were done.

- Blood was collected in a test tube and left to clot then centrifuged for serum separation and serum was collected and stored at - 20°C for hormonal assays.

- Both testes of each animal were separated from the surrounding tissues.The testis tissue washed by ice water, apart was weighed and homogenized in 9 volumes saline 0.9% in homogenizer apparatus. The homogenate was collected and kept at -70°C. GSH activity and MDA were measured [15].

- **Semen collection**

One epididymis was separated rapidly, the tail of the epididymis was scraped longitudinally by scalpel and the semen was collected by stripping of the epididymis then 0.1 ml of saline was added and mixed with the semen for liquefaction [16].

- **Sperm counting**

The semen obtained was diluted with saline at a ratio of 1: 20 and by the hemocytometer slide the sperm count could be calculated using a light microscope [17].

- **Sperm motility**

The right epididymis along with

part of the vas deferens was clamped with a hemostat at approximately the corpus cauda junction. The cauda was then gently dissected with a scalpel blade to allow sperm to emerge from the engorged cauda epididymis, which was then dipped into a plastic Petri dish (35 mm) containing pre-warmed incubation medium (saline). Sperm released into the medium were incubated for approximately 3 min with intermittent gentle swirling to disperse them throughout the suspension. Following the 3-min incubation, an aliquot of the sperm suspension was taken using capillary tubes and loaded into the analyzer. A drop of the collected semen was taken using 100µl capillary tube and loaded into a slide then examined by high power for the number of forward motile sperm per 100 sperms [18].

- **Stained slides :**

One drop of the collected diluted semen was taken into the middle of a clean slide, one drop of the eosin stain was added near to the semen drop then gently mixed and by the edge of another slide the stained semen was spread into the slide and examined by light microscope at lens 40 for the sperm abnormalities and acrosomal deformity [19].

- Hormonal assays: Serum LH, FSH and testosterone levels were measured.

After functional studies were completed, one testis from each rat was removed and was put into a buffered 4% formalin fixation solution and processed with paraffin wax for histopathological examination. Sections 5um were stained with Mayer'shematoxylin and eosin [20].

All groups were subjected to the following investigation:

- Serum testosterone, LH, FSH levels
- Testicular GSH, Testicular MDA
- Epididymal sperm count
- Sperm motility
- Sperm abnormalities and acrosomal deformity
- Testicular histopathological examination

Statistical Analysis:

All data were expressed as

mean ± S.D; data were evaluated by the one way analysis of variance. Difference between groups were compared by Student's t-test with $P < 0.05$ selected as the level of statistical significance.

Results

Administration of Carbimazole (1.35 mg/Kg b.w) dissolved in water, daily for 8 weeks resulted in significant ($P<0.001$) decrease of testosterone, LH & FSH from means of (1.8875±0.280), (2.788±0.203), (2.054±0.123) in control group to means of (1.075±0.205), (2.1625±0.220) & (1.715±0.173). Testicular GSH was significantly reduced ($P < 0.001$) from mean of (10.158±1.355) in control group to mean of (5.929±1.23). Testicular MDA was significantly increased ($P<0.001$) from mean of (4.36±1.193) in control group to mean of (10.88±1.267) (table 1).

The results showed also that administration of Carbimazole resulted in significant ($P<0.001$) decrease of sperm count, sperm motility from means of (15.866±0.276) & (59.1675±0.391) in control group to means of (11.45±0.127) & (34.474±0.248). sperm abnormalities was significantly increased ($P < 0.001$) from mean of (14.695±0.410) in control to mean of (24.165±0.191) in group received Carbimazole (table 2).

Histopathological examination of testicular tissues of rats received Carbimazole only showed degenerative changes of the majority of the seminiferous tubules. These changes were characterized by shrunken, disorganized seminiferous tubules with irregular, buckled basement membrane and incomplete spermatogenesis. Moreover, the seminiferous tubules were almost devoid of spematids and spermatozoa. Vacuolar degeneration of spermatogonia and Sertoli cells was evident. Degenerated germinal epithelial cells were sloughed in the lumina of most seminiferous tubules. Regarding to the interstitium, there were congestion of the interstitial blood vessels and edema that was represented by faint eosinophilic material (Fig. 3) in comparison to the normal testicular tissues of the control group (Fig. 2).

Administration of sodium selenite (10 µg/Kg b.w) dissolved in water, daily for 8 weeks resulted in significant ($P < 0.05$) increase of testosterone from mean of (1.8875±0.280) in control group to means of (2.2625±0.267) & significant ($P < 0.001$) increase of FSH from mean of (2.054±0.123) in control group to mean of (2.51±0.207). Testicular GSH was significantly increased ($P < 0.002$) from mean of (10.158±1.355) in control group to mean of (12.931±1.597). Testicular MDA was significantly reduced ($P < 0.05$) from mean of (4.36±1.193) in control group to mean of (3.231±0.86) (table 3).

The results showed also that administration of sodium selenite resulted in significant ($P < 0.001$) increase of sperm count, sperm motility from means of (15.866±0.276) & (59.1675±0.391) in control group to means of (17.939±0.147) & (60.22±0.224). Sperm abnormalities was significantly decreased ($P < 0.001$) from mean of (14.695±0.410) in control to mean of (11.529±0.333) in group received sodium selenite (table 4).

Histopathological examination of testicular tissues of rats received sodium selenite alone showed normal testicular structure (Fig. 4).

Administration of Carbimazole (1.35mg/Kg b.w) and sodium selenite (10µg/Kg b.w) for 8 weeks resulted in significant ($P < 0.001$) increase of testosterone, LH & FSH from means of (1.075±0.205), (2.1625±0.220) & (1.715±0.173) In group received Carbimazole alone to means of (1.9625±0.262), (3.016±0.228) & (2.5262±0.188) in groups received Carbimazole and sodium selenite. Testicular GSH was significantly increased ($P < 0.001$) from mean of (5.929±1.23) in group received Carbimazole alone to mean of (8.218±0.853) in groups received Carbimazole and sodium selenite. Testicular MDA was significantly decreased ($P < 0.001$) from mean of (10.88±1.267) in group received Carbimazole alone to mean of (6.253±1.814) in group received Carbimazole & sodium selenite (table 5), (Fig. 1-a).

The results showed also that administration of Carbimazole and sodium selenite resulted in significant ($P < 0.001$) increase of sperm

count, sperm motility from means of (11.45±0.127) & (34.474 ±0.248) In group received Carbimazole alone to means of (14.541± 0.327) & (51.614±0.430) in group received Carbimazole and sodium selenite. Sperm abnormalities was significantly decreased (P < 0.001) from mean of (24.165±0.191) in group received Carbimazole alone to mean of (11.919±0.293) in group received Carbimazole and sodium selenite(table 6)&(Fig.1-b).

Histopathological examination of testicular tissues of rats received Carbimazole and sodium selenite showed few seminiferous tubules contained sloughed germinal epithelium (arrows), marked improvement of spermatogenesis (Fig. 5).

Table (1): Effects of Carbimazole on serum levels of testosterone, LH &FSH; testicular GSH and testicular MDA in male rats. **(Mean± SD)** (n = 8).

parameters / Group	Testosterone ng/ml	LH U lu/ml	FSH U lu/ml	GSH (umol/g tissue)	MDA (nmol /g tissue)
Control	1.8875±0.280	2.788±0.203	2.054±0.123	10.158±1.355	4.36±1.193
Carbimazole group	1.075±0.205***	2.1625±0.220***	1.715±0.173***	5.929±1.23***	10.88±1.267***

*** Significant P < 0.001 compared with control normal rats.

Table (2): Effect of Carbimazole on sperm count, sperm motility and sperm abnormalities in male albino rats

parameters / Group	Sperm count $(10)^6$	Sperm motility %	Sperm abnormalities %
Control	15.866±0.276	59.1675±0.391	14.695±0.410
Carbimazole group	11.45±0.127***	34.474±0.248***	24.165±0.191***

*** Significant P < 0.001 compared with control normal rats.

Table (3): Effects of selenium on serum levels of testosterone, LH & FSH, testicular GSH and testicular MDA in male rats. **(Mean± SD)** (n = 8).

parameters / Group	Testosterone ng/ml	LH U lu/ml	FSH U lu/ml	GSH (umol/g tissue)	MDA (nmol /g tissue)
Control	1.8875±0.280	2.788±0.203	2.054±0.123	10.158±1.355	4.36±1.193
Selenium group	2.2625±0.267**	2.9875±0.173	2.51±0.207***	12.931±1.597***	3.231±0.86**

** Significant P < 0.01 compared with control normal rats.

***Significant P < 0.001 compared with control normal rats.

Table (4): Effect of selenium on sperm count, sperm motility and sperm abnormalities in male albino rats

parameters / Group	Sperm count $(10)^6$	Sperm motility %	Sperm abnormalities %
Control	15.866±0.276	59.1675±0.391	14.695±0.410
Selenium group	17.939±0.147***	60.22±0.224***	11.529±0.333***

*** Significant P < 0.001 compared with control normal rats.

Table (5): Effects of combination of selenium and Carbimazole on serum levels of testosterone, LH &FSH, testicular GSH and testicular MDA in male rats. **(Mean± SD)** (n = 8).

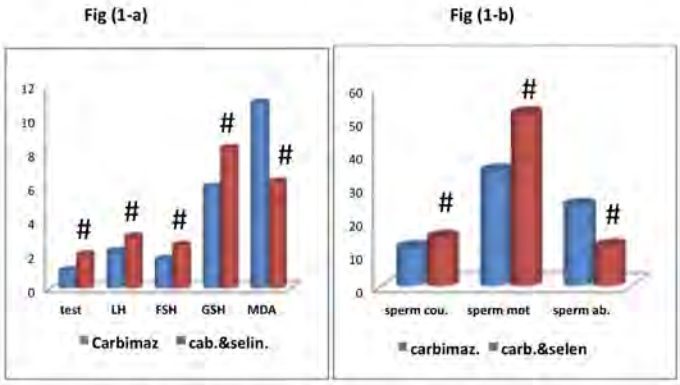
parameters / Group	Testosterone ng/ml	LH U lu/ml	FSH U lu/ml	GSH (umol/g tissue)	MDA (nmol /g tissue)
Carbimazole group	1.075±0.205	2.1625±0.220	1.715±0.173	5.929±1.23	10.88±1.267
Group received carbimazole and Selenium	1.9625±0.262***	3.016±0.228***	2.5262±0.188***	8.218±0.853***	6.253±1.814***

*** Significant P < 0.001 compared with Carbimazole treated rats.

Table (6): Effect of combination of Carbimazole and selenium on sperm count, sperm motility and sperm abnormalities in male albino rats

parameters / Group	Sperm count $(10)^6$	Sperm motility %	Sperm abnormalities %
Carbimazole group	11.45±0.127	34.474±0.248	24.165±0.191
Group received carbimazole and Selenium	14.541±0.327***	51.614±0.430***	11.919±0.293***

*** Significant P < 0.001 compared with Carbimazole treated rats.



Significant (P < 0.001) compared with Carbimazole treated rats.

Fig. (1-a) & Fig. (1-b) Effects of combination of selenium and Carbimazole on serum levels of testosterone, LH &FSH, testicular GSH, testicular MDA, sperm count, sperm motility and sperm abnormalities in male albino rats

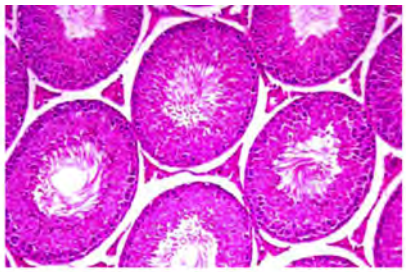


Fig. (2): Cut section of testicular tissues of control normal rats showing no remarkable pathologic changes (H&E×100)

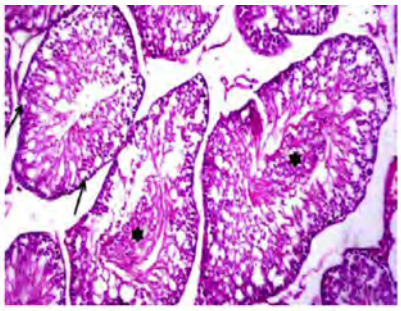


Fig. (3): Cut section of testicular tissues of rats received Carbimazole (H&E×100)

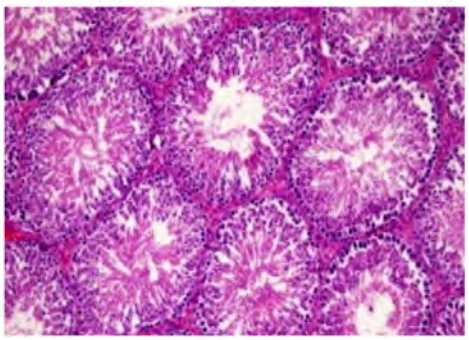


Fig. (4): Cut section of testicular tissues of rats received selenium (H&E×100)

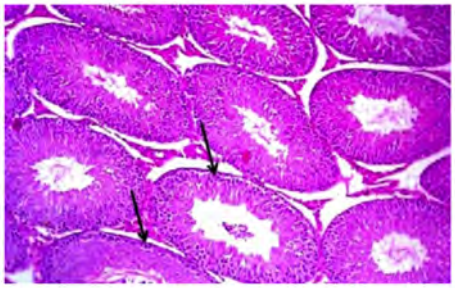


Fig. (5): Cut section of testicular tissue of rats received both carbimazole and selenium (H&E×100)

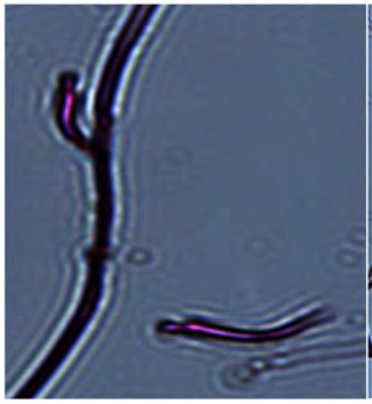


Fig. (6): detached head

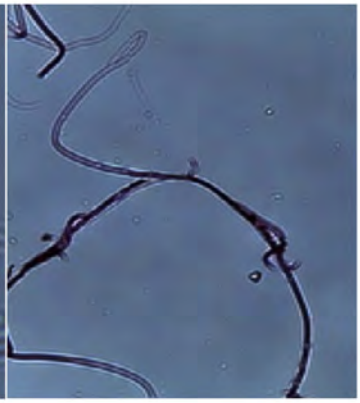


Fig. (7): double head and bifid tail

Fig. (8): damaged acrosome .

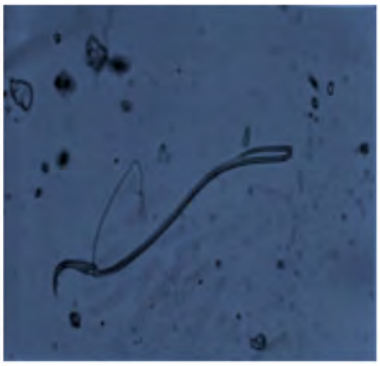


Fig. (9): bended tail

Discussion

Our study revealed that Carbimazole caused significant decrease in testicular reduced glutathione (GSH) activity, serum testosterone; LH & FSH levels with significant increase in testicular MDA level in the testis. As well as it caused significant increase in sperm abnormalities with significant decrease in sperm motility and sperm count.

Histopathological examination of testicular tissue of rats received Carbimazole showed degenerative changes of the majority of the seminiferous tubules. These changes were characterized by shrunken, disorganized seminiferous tubules with irregular, buckled basement membrane and incomplete spermatogenesis. Moreover, the seminiferous tubules were almost devoid of spematids and spermatozoa. Degenerated germinal epithelial cells were sloughed in the lumina of most seminiferous tubules. Regarding to the interstitium, there were congestion of the interstitial blood vessels and edema that was represented by faint eosinophilic material.

These results were in agreement with[13] as they revealed that treating rats with Carbimazole daily for 8 weeks caused distinct histological alterations in prostate gland compared with control group.

Also[21] indicated that superoxide dismutase, catalase and glutathione peroxidase was reduced in erythrocytes of rats treated with Carbimazole compared with control animals. Carbimazole administration caused a significant decrease testicular weight and DNA content in rat pups [22].

High lipid peroxidation with a concomitant decrease in the enzymatic antioxidant status, superoxide dismutase and catalase were recorded in testis of rats treated with Carbimazole [23]. Thus, it is suggested that Carbimazole induced oxidative stress which resulted in the alterations observed in the testes.

Testosterone is required for the attachment of different generations of germ cells in seminiferous tubules. Therefore, low level of intratesticular testosterone may

lead to detachment of germ cells from seminiferous epithelium and may initiate germ cell apoptosis and subsequent male infertility[24]. This finding was parallel to the reduction in epididymal sperm count. Sloughing of germ cells was observed in the lumen of some epididymal ducts indicating testicular dysfunction[25].

Our study revealed that the effect of selenium on normal control rats was significant improvement in testicular function. These results were in agreement with[26] as they concluded that the administration of antioxidants such as selenium or ascorbate to normal animals, not suffering from induced oxidative stress, also appears to improve testicular function, In light of such results, antioxidants have frequently been administered to infertile men in the hope of improving the quality of the semen profile.

Increased generation of ROS in semen affects sperm function, especially fusion events associated with fertilization, and leads to infertility. ROS are known to be generated from spermatozoa and leucocytes and the resultant peroxidative damage causes impaired sperm function. Elevated ROS levels correlate negatively with sperm concentration and sperm motility [27].

Concerning the effect of selenium on rats suffering from induced oxidative stress, the present study indicated that combination of selenium with Carbimazole improved the oxidative status as selenium caused significant increase in testicular reduced glutathione (GSH) activity and significant decrease in testicular MDA content. Also selenium caused significant increase in testosterone, LH, FSH levels and significant increase in sperm count, sperm motility and significant decrease in sperm abnormalities.

These results were in agreement with[28] as they revealed that selenium ameliorated the reduction in the reproductive organs weights, sperm characteristics, Deltamethrin induced oxidative damage of testes and the histopathological alterations of testes, epididymes and accessory sex glands.

Also [29] found that after co-administration of vitamin E and Selenium, serum testosterone level significantly increased parallel to the reduction in LPO concentration. This could be attributed to a relationship between steroidogenesis and ROS.

Our results are also in consistence with[30] who concluded that 0.1 mg/kg selenium (Na2 SeO3) inhibited oxidative stress, apoptosis and cell cycle changes induced by excess fluoride in kidney of rats. Selenium protected rat testes against cadmium-induced oxidative damage[31].

Sodium selenite supplementation significantly protected against exercise-induced testicular gametogenic and spermatogonia disorders, prevented testicular oxidative stress and increased antioxidant status[32].

The effectiveness of combined treatment with selenium and vitamin E in treatment of oligoasthenoteratozoospermia has been studied since Vitamin E is well known to work in synergy with selenium as an antiperoxidant. A prospective, uncontrolled study reported that this drug combination led to statistically significant increases in motility and mean seminal plasma glutathione peroxidase activity. Although no improvements in sperm concentration were documented, and no pregnancies were achieved, the better sperm motion characteristics may be explained by the amplified antioxidant enzyme activity[33]. These results were further confirmed by a more recent randomized controlled trial in which vitamin E and selenium improved sperm motility and lipid peroxidation markers[34].

Selenium reduced the cadmium induced histopathological changes oxidative stress, endocrine disorder and apoptosis in testes of rat,[35]. Co- treatment of vitamin E and selenium revealed a significant reduction in LPO and as a consequence improvement in GSH level[36]. Additionally, Se has a positive effect on ejaculate volume and semen quality in goats[37]. It was reported that the mechanism of chemoprotection of selenium may be related to its antioxidant properties as well as its

ability to interfere with DNA repair pathways [38].

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nanogram (s)	ng	cubic millimeter	cmm
micrometer	um	millilitre (s)	ml
millicurie(s)	mCi	milliequivalent	mEq
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