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RADIOTHERAPY VERSUS STENTING IN MANAGEMENT OF MALIGNANT DYSPHAGIA

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Abstract

Background: *The majority of patients with cancer esophagus present with locally advanced disease and suffering from dysphagia. Stenting and radiotherapy (RT) provide survival benefits along with improvement of dysphagia. Patients and methods: A prospective data of 91 patients with locally advanced or metastatic esophageal cancer who has been treated with either palliative RT, stent or both. Group I had RT only, Group II had stent only and group III had both RT and stent. Results: The median overall survival (OAS) was 169 days in GI, 119 days in GII and 237 days in GIII. The difference between GI & III was statistically significant (p=0.01). Conclusion: Combinations of stent and RT in patients provide survival benefit in patients with malignant dysphagia.*

Key words: *Malignant dysphagia; esophageal cancer, radiotherapy; stent.*

Introduction

Esophageal cancer is diagnosed in about 400,000 patients each year worldwide, and its incidence is increasing [1], it is the sixth leading cause of death from cancer [2]. In Europe, while incidence of SCC has remained stable or declined during the past few decades, the incidence of esophageal adenocarcinoma has been rising. This increase has been more prominent in Northern Europe, notably in the United Kingdom and Ireland [3,4].

The majority of the patients suffering from a cancer of the esophagus presents with symptoms of dysphagia and weight loss because of an obstructive tumor [5].

Several management options have been developed to palliate malignant dysphagia. These include endoluminal stenting or surgery and external beam radiation, brachytherapy, chemotherapy, chemoradiotherapy, laser treatment, photodynamic therapy or ablation using injection of alcohol or chemotherapeutic agents [6,7,8].

Placement of self-expanding metallic stents (SEMS) made up of an alloy, usually nitinol or stainless steel, and deployed using endoscopic or fluoroscopic techniques, is a newer method for relief of dysphagia in these patients [9].

External beam radiotherapy (EBRT) is known to provide durable and effective relief of dysphagia. However, there is a time lag before symptomatic relief occurs, and up to 6 weeks are required for maximum benefit [8].

Survival advantage of combined stenting and radiotherapy was first suggested by Ogilvie et al.[10], other studies [11,12] have also investigated the effect of combined stenting and radiotherapy on survival of patients with advanced esophageal cancer and reported superior results with regard to both relief of dysphagia and survival for stenting followed by radiotherapy in those patients.

In view of promising results of stenting and radiotherapy, we conducted this study to compare stenting alone and radiotherapy with or without stenting in patients of locally advanced cancer esophagus regarding overall survival.

Materials and Methods

This is a prospective data of ninety-one patients with locally advanced or metastatic esophageal cancer who were treated at Northamptonshire Oncology Centre from 1/1/99 till 1/1/2007. Eligibility criteria included patients with locally advanced or metastatic (T3, T4, any N, any M) previously untreated cancer esophagus with Eastern Cooperative Oncology Group [ECCOG] performance status ≤ 3, age greater than 18 years, adequate bone marrow function, renal and hepatic functions. Patients are not eligible to

any radical treatment. Patients included in this study were divided into three groups, group I (GI) 30 Patients received radiotherapy only, 35 patients underwent stenting only (GII) and 26 patients underwent radiotherapy followed by stenting (GIII).

Pretreatment and follow-up evaluation:

Pretreatment evaluation included a detailed history taking, physical examination, and routine laboratory examinations. All patients underwent a baseline CT of the neck, chest and abdomen examination, barium swallow, endoscopic ultrasound, upper gastrointestinal endoscopy and biopsy. All these studies were repeated after one month of end of treatment.

Treatment:

Radiotherapy

Patients were treated by 2 D conventional EBR, which was delivered with linear accelerator 6MV energy, conventional simulation planning two parallel opposing filed was used. The target volume included the initial lesion with a margin of 3cm to 5cm at both proximal and distal ends of the esophagus. The total radiation dose ranged from 20Gy in 5 fractions over one week to 30 Gy in 10 fractions over two weeks.

Stenting

The stent of proper length was selected, placed and released along the site of stenosis by endoesophageal stent introducer. Stent should extend about 2cm proximal and distal to the tumor. After placement, patients were advised to drink adequate warm water, making the stent expand properly. One week after examination was carried out through upper digestive tract fluoroscopy with barium.

Radiotherapy and stent

EBRT was used first and after progression of their dysphagia patients were offered stent placement, radiotherapy dose ranged from 20Gy in 5 fractions to 30 Gy in 10 fractions. Only two patients received 40Gy in 20 fractions over 4 weeks.

Statistical methods: The study endpoint was overall survival and it was calculated from the day of the start of treatment to the day of death or of lost follow-up. The

data were coded and entered into a computer using Statistical Package for Social Sciences (SPSS) version 15.0. Results were expressed as number, percentiles and medians. Categorical variables were compared using chi-square tests. Survival functions (OAS) were estimated using Kaplan Meier test, comparison between survival curves was achieved by the Log Rank test. P value of <0.05 taken to indicate statistical significance.

Results

Patient's characteristics

The characteristics of the 91 patients of groups I, II and III are shown in Table 1. The three patient groups were balanced; the most common pathological type was squmous cell carcinoma 60% in GI, 48.6% in GII and 53.8% in GIII, followed by adenocarcinoma, all patients had dysphagia, 14.3% had metastasis and the most common sites of metastasis were lung and liver.

There was no significance difference between the median radiation dose given for GI and GIII, it was 24.66 and 26.29 in G1and GIII respectively, stent migration occurred in 3 patients of GII and in 1patient of GIII. All patients underwent stent had complications included transient chest pain after stent placement and gastroesophageal reflux.

The median overall survival (OAS) was 169 days (95% CI 96.53-241.46) in GI, 119 days (95% CI 106.48-131.51) in GII and 237 days (95% CI 107.07-366.92) in GIII. The difference between GI &II was statistically insignificant (p=0.86) while the difference between GI &III was significant (p=0.01) (fig 1&2).

Table1. patient's characteristics

	Group I NO. (%)	**Group II** NO. (%)	**Group III** NO. (%)	**p**
Age				
Mean±S.D	69.48±8.93	72.60±6.69	69.96±8.18	0.99
Sex				
Male	18 (60)	22(62.9)	17(65.4)	0.92
Female	12(40)	13(37.1)	9(34.6)	
Pathology				
Adenocarcinoma	7(23.3)	12(34.3)	9(34.6)	
Squmous cell	18(60)	17(48.6)	14(53.8)	0.77
Undiff.carcinoma	4(13.3)	5(14.3)	3(11.5)	
Small cell	1(3.3)	0(0)	0(0)	
Lymphoma	0(0)	1(2.9)	0(0)	
Metastasis				
No	26(36.7)	30(85.7)	23(88.5)	0.95
Yes	4(13.3)	5(14.3)	3(11.5)	
Laser Dilatation				
No	24(80)	28(80)	17(65.4)	0.34
Yes	6(20)	7(20)	9(9.9)	

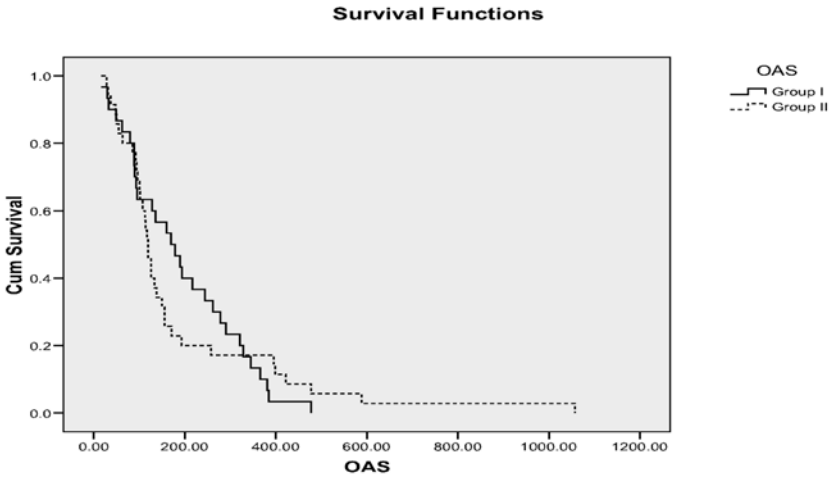

Fig 1. OAS among radiotherapy group and stenting group

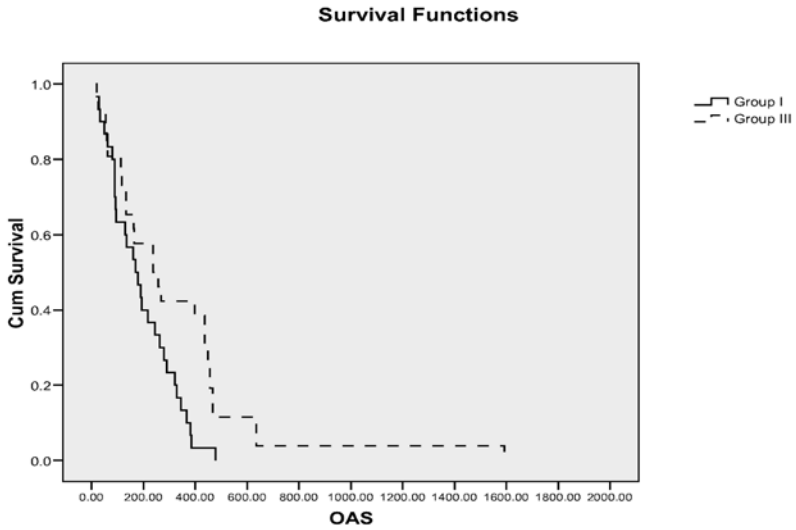

Fig 2. OAS among radiotherapy group and radiotherapy + stenting group

Discussion

Esophageal cancer is increasing in last few years, unfortunately the majority of patients will present with locally advanced or metastatic disease which is difficult to control. Considering this fact, it is important to offer treatment providing adequate and rapid palliation of symptoms especially the obstructive symptoms which reflect on the quality of life. Radiotherapy for esophageal cancer is a relatively effective treatment and provides survival benefits.

In the present study 91 patients enrolled 30 patients received radiotherapy, 35 patients underwent stent and 26 patients underwent stent and received radiotherapy, relief of dysphagia occurs rapidly in stent groups than in radiotherapy alone group [8] and was more standing in stent plus radiotherapy group, recurrence of dysphagia occurs in 8.5% in GII and 3.8% in GIII this is due to tumor over growth on the stent in GII. The median overall survival time was 169 days for patients receiving radiotherapy and this comparable to reported by Slabber et al.[13], who reported 144 days median over all survival, the median radiation dose was 24.66 ± 5.07 in GI and 26.29 ± 6.17 GIII, doses more than 40Gy increase toxicity. Median overall survival for stent only patients was 119 days and this results similar to that reported by Homs et al.[14], and Conio et al.[15], however, Han et al.[12] reported 312 days median overall survival in stent alone patients. The present study shows survival benefits for addition of radiotherapy to stent patients of locally advanced disease, its median overall survival time was 237d days, Han et al.[12] reported 499 days, while Song et al.[16] reported 161 days, this advantage may be due to tumor local control by radiotherapy. Yu et al. in a their trail of offering radiotherapy 4-7 days after stent replacement has reported mean survival of 510 days but this was a very small series[17]. In the future, it can be expected that removable stents will be used as a bridge to surgery or radiotherapy to maintain luminal patency during neoadjuvant treatment. However it is difficult to assess the survival benefit in these approach-

es for each treatment modalities as some patients underwent surgery or radical chemoradiotherapy thereafter [18,19].

Conclusion

Combination of stent and radiotherapy in patients with locally advanced esophageal cancer provide survival benefits along with improvement of obstructive symptoms with acceptable complications.

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OCULAR VESTIBULAR EVOKED MYOGENIC POTENTIALS IN PATIENTS WITH PERIPHERAL VESTIBULAR DISORDERS

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Abstract

One hundred and fifty one subjects classified into five different groups was included in this study. Twenty volunteer was served in the control group, thirty patients with vestibular neuritis, thirty patients with typical BPPV, thirty two patients with Meniere's disease with and thirty nine patients with undiagnosed dizziness. All subjects was subjected to history taking, otological examination, basic audiological evaluation and vestibular testing in the form of bedside examination, VNG, cVEMP and oVEMP recordings. oVEMP and cVEMP parameters in the control and the other four groups was analyzed. Correlation between cVEMP and oVEMP results between different study group reflecting variable degrees of significance suggesting that cVEMP and oVEMP responses may produce characteristic profile in each of theses groups aiding in the diagnosis and differential diagnosis of different peripheral vestibular diseases.

Introduction

Evoked potentials are a well established method of investigating neural projections to the cerebral cortex and can be applied to any situation in which it is possible to evoke a synchronous volley of afferent activity. This is difficult, however, for vestibular afferents using their natural stimulus - rotational and linear movement - because synchronous activation is hard to achieve using imposed movements and the results are prone to stimulus artifact. Recently, Todd et al. demonstrated that vestibular evoked potentials could be produced by an acoustic stimu-

lus capable of activating the vestibular apparatus [1]. An alternate method for synchronously activating the vestibular system is by means of acoustic stimulation, in conjunction with the use of suitable auditory controls. This technique exploits a conserved vestibular sensitivity to sound and has been developed and validated using vestibular evoked myogenic potentials (VEMPs).

Since Colebatch et al. reported surface potentials from the sternocleidomastoid muscles in response to loud clicks i.e., cervical VEMP (cVEMPs), the cVEMP test evoked by air-conducted clicks, galvanic stimulation, and bone-conducted stimuli has become a well-established clinical test of vestibular function[2]. Recently, cVEMP is widely used to assess the sacculocollic pathway in various diseases such as Meniere's disease, vestibular neuritis, vestibular schwannoma, and superior canal dehiscence syndrome[3,4]. Another vestibular evoked responses from the eye muscles produced by bone-conducted sound were also reported[5] namely, ocular vestibular evoked myogenic potentials (oVEMPs).

oVEMPs are short latency mainly negative evoked potentials thought to represent consequently, excitation and inhibition of extraocular muscles. Recently, Rosengren et al reported oVEMPs produced by bone-conducted sound stimulation[5]. The surface potentials recorded in his study group were not consistent with electro-retinal potentials generated by ocular movement. On the basis of circumstantial evidence, they concluded that oVEMPs are likely produced by synchronous activity in the extraocular muscles (i.e. myogenic potentials). The oVEMPs, and the associated eye movements, are distinct morphologically for air-conducted (AC) and bone-conducted (BC) sound, consistent with differing profiles of vestibular afferents being recruited by these stimuli. Todd et al. proposed that AC-evoked oVEMPs were most likely a manifestation of pathways originating from the sacculus, whereas BC-evoked oVEMPs were likely to be due to the excitation of both saccular and utricular afferents[6]. This study was conducted to determine

the usefulness of oVEMPs in dizzy patients.

Methodology:

I- Subjects:

This study was conducted on 151 subjects classified in to 5 different groups:

1) Control group: twenty normal subjects (age ranged between 29 to 42 years) with normal hearing and vestibular functions.

2) Vestibular neuritis group: thirty patients with age range between 34 and 50 years. The criteria for selecting the patients with vestibular neuritis were: (1) typical history of vestibular neuritis, (2) nausea and vomiting, (3) absent or markedly reduced horizontal canal function and (4) positive bedside examination.

3) BPPV group: thirty patients with age range between 30 and 45 years with positive Dix-Hallpike testing for posterior canal BPPV.

4) Meniere's disease group: thirty-two patients with age range between 32 and 43 years. They were selected according to the classification of Committee on Hearing and Equilibrium, American Academy of Otolaryngology-Head and Neck surgery criteria for definite Meniere's disease (1995).

5) Undiagnosed group: thirty-nine patients with age range between 30 and 49 years. To exclude vascular arteriosclerotic changes and systemic aging, patients older than 50 years were not included in the study. Undiagnosed dizziness was based on the following criteria: patients suffering from vertigo or disequilibrium with normal audiometric, bedside vestibular examination and videonystagmography. All of the study subjects had no history of neurological disorders, external, middle ear problems, noise exposure or ototoxicity.

II- Methods:

All participants were submitted to the following:

1- History taking. 2- Otological examination. 3- Basic audiological evaluation in the form of pure tone and speech audiometry using a Madsen two channed pure tone and speech audiometer model Orbiter 922. Tympanometry and acoustic reflex threshold measure-

ments using a GSI immittancemeter TympStar, Version 2 Middle Ear Analyzer. 4- VNG: Computerized 4 channel VNG recording system from Micromedical Inc. Visual eyes using spectrum software version 6.1. 5- Vestibular evoked potentials testing: using a Biologic two channel evoked potential recording system version 4.5.

A) cVEMP testing: EMG activity was ipsilaterally recorded from the upper half of the sterncleimastoid (SCM) muscle using surface electrode, with a reference electrode on the upper edge of the sternum and a ground electrode on the forehead. During each recording session, the subject was instructed to rotate the head towards the contralateral side of the tested ear to keep the SCM muscle under tension. Subjects were instructed to tense the SCM muscle during acoustic stimulation and relax it in between the recording sessions. Tone bursts of 500 Hz with a two-cycle rise/fall time and plateau were used. They were presented at a rate of 3 cycles per seconds (through TDH 39 headphones) at 116 dBnHL and down to threshold. The EMG signal was amplified (10000 times), bandpass filtered (30 to 1500 Hz) and was averaged after 300 sweeps. The analysis window was 50 ms wide. Measurements made on the VEMP response were the threshold (threshold was defined as the lowest value of the stimulus intensity capable of eliciting a visually detectable response), latency of the first positive peak (P1), latency of the next negative peak (N1), and the amplitude difference (N1P1) between the P1 and the N1 amplitudes. The left-right difference in the P1-N1 amplitude was evaluated using the P1-N1 amplitude ratio defined as [(AR-AL)/(AR+ AL)]100, where AR is the P1-N1 amplitude on the right side and AL is the P1-N1 amplitude on the left side.

B) oVEMP testing: EMG activity was contralaterally recorded by inferior electrode below the eye glob using surface electrode, with a reference electrode 2 cm below the primary electrode and a ground electrode on the forehead. During each recording session, the subject was instructed to gaze upward superomedially on a target placed on the roof of the sound

treated room in order to contract the inferior oblique muscle. In the control group, in addition to the previous electrode montage, the EMG was recorded also ipsilaterally, using superior electrode placed on the upper eye lid and the patient was instructed to gaze contralateral. This was done to characterize the best montage for the oVEMP. Tone bursts of 500 Hz with a two cycle rise/fall time and plateau were used. They were presented at a rate of 5 cycles per seconds (through a TDH 39 headphones) at 116 dBnHL and down to threshold. The EMG signal was amplified (10000 times), bandpass filtered (10 to 1500 Hz) and was averaged after 300 sweeps. The analysis window was 50 ms wide. Measurements made on the oVEMP response were the threshold (threshold was defined as the lowest value of the stimulus intensity capable of eliciting a visually detectable response), latency of the first negative peak (n10), and the n10 base line to peak amplitude. The left-right difference in the n10 amplitude was evaluated using the n10 amplitude ratio defined as [(AR-AL)/(AR+ AL)]100, where AR is the n10 amplitude on the right side and AL is the n10 amplitude on the left side.

Results

151 subjects classified into five different groups was included in this study. 20 volunteer was served in the control group with a mean age of 37 years, 30 patients with vestibular neuritis with a mean age of 43 years, 30 patients with typical BPPV with a mean age of 43 years, 32 patients with Meniere's disease with a mean age of 43 years and 39 patients with undiagnosed dizziness and a mean age of 39 years. The study groups with age and sex matched.

Table (1): Mean, standard deviation and p-value of oVEMP amplitude, threshold and latency in the right (R) and left (L) ears in control group.

	Mean	SD	T	p-value
R threshold	*99.25*	*2.45*	*4.46*	*.00*
L threshold	*97.75*	*2.45*		
R amplitude	*1.67*	*.61*	*7.27*	*.00*
L amplitude	*1.68*	*0.88*		
R n10 latency	*9.43*	*0.40*	*0.16*	*.33*
L n10 latency	*9.17*	*0.32*		

Table (1) showed significant difference in oVEMP threshold and amplitude between the right and the left ears with no significant difference in n10 latency in the control group.

Table (2): Mean, standard deviation and p-value of cVEMP and oVEMP amplitude ratios in control group.

	Mean	SD	T	p-value
cVEMP ratio	*7.42*	*20.60*	*4.96*	*.00*
oVEMP ratio	*6.58*	*16.79*		

Table (2) showed significant difference between cVEMP and oVEMP amplitude ratios in the control group.

Table (3): Mean, standard deviation and p-value of oVEMP in affected (A) and un affected (U) ears n10 amplitude, threshold and latency in vestibular neuritis group.

	Mean	SD	T	p-value
A threshold	*108.00*	*8.07*	*1.02*	*.35*
U threshold	*103.50*	*2.74*		
A amplitude	*2.10*	*.99*	*1.34*	*.24*
U amplitude	*2.40*	*.44*		
A n10 latency	*10.60*	*.00*	*.61*	*.57*
U n10 latency	*9.90*	*1.20*		

Table (3) showed no significant difference in oVEMP threshold, amplitude or latency between the affected and unaffected ears

Table (4): Mean, standard deviation and p value of cVEMP and oVEMP amplitude ratios in vestibular neuritis group.

	Mean	SD	T	p-value
cVEMP ratio	*40.00*	*2.50*	*3.12*	*.01*
oVEMP ratio	*32.45*	*9.30*		

Table (4) showed significant difference between cVEMP and oVEMP amplitude ratios.

Table (5): Mean, standard deviation and p-value of oVEMP in affected (A) and unaffected (U) ears n10 amplitude, threshold and latency in BPPV group.

	Mean	SD	T	p-value
A threshold	*110.20*	*4.45*	*4.46*	*.00*
U threshold	*105.50*	*2.74*		
A amplitude	*2.41*	*.36*	*7.27*	*.00*
U amplitude	*3.54*	*.76*		
A n10 latency	*10.20*	*.81*	*8.163*	*.00*
U n10 latency	*10.00*	*.00*		

Table (5) showed significant difference in oVEMP threshold, amplitude or latency between the affected and unaffected ears

Table (6): Mean, standard deviation and significance of cVEMP and oVEMP amplitude ratios in BPPV group.

	Mean	SD	T	p-value
cVEMP ratio	*4.2136*	*.50642*	*4.955*	*.000*
oVEMP ratio	*17.793*	*15.10579*		

Table (6) showed significant difference between cVEMP and oVEMP amplitude ratios.

N.B. In Meniere's disease group, non of the patients produced oVEMP response when the affected ears was stimulated, there oVEMP parameters was not calculated.

Table (7): Mean, standard deviation and p-value of oVEMP right (R) and left (L) n10 amplitude, threshold and latency in undiagnosed group.

	Mean	SD	t	p-value
R threshold	*100.62*	*8.03*	*.32*	*.75*
L threshold	*100.23*	*6.07*		
R amplitude	*2.78*	*1.09*	*1.44*	*.17*
L amplitude	*2.37*	*1.58*		
R n10 latency	*9.50*	*1.29*	*2.21*	*.05*
L n10 latency	*10.03*	*1.68*		

Table (7) showed no significant difference in oVEMP threshold, amplitude or latency.

Table (8): Mean, standard deviation and significance of cVEMP and oVEMP amplitude ratios in undiagnosed group.

	Mean	SD	t	p-value
cVEMP ratio	*15.41*	*21.70*	*3.12*	*.01*
oVEMP ratio	*17.54*	*31.17*		

Table (8) showed significant difference between cVEMP and oVEMP amplitude ratios.

Table (9): F value and significance of cVEMP and oVEMP amplitude ratio between study groups.

	F	Sig.
cVEMP amplitude ratio	*1.37*	*.26*
oVEMP amplitude ratio	*12.93*	*.00*

Table (9) showed significant difference in oVEMP amplitude ratio between study groups.

Discussion

The oVEMP test generated no sex-based difference in this study - n10 latency, threshold and amplitude did not differ significantly between male and female adults and this may agree with sung et al.[7]. There is no significant difference regarding the age between control and the other study groups.

oVEMP responses in the control group:

Incidence of oVEMP responses:

In all subjects in the control group, there was clear cVEMP and oVEMP responses in the 40 ears using AC 500 Hz tone burst at maximum acoustic stimulation (116 dBnHL) with 1 ms rise and fall times and 2 ms plateau down to threshold. Recently, it has been reported that normal cVEMP responses show frequency tuning, with the lowest threshold for 500-Hz tone-burst stimuli[8]. Using the maximal acoustic stimulation, oVEMP were elicited in all ears at 500 Hz, however, using other frequencies, not in all ears produced oVEMP response (80% at 250 Hz, 95% at 1000 Hz, 83% at 2000 Hz). cVEMP were elicited in 100% at 250, 500, and 1000 Hz and 98% at 2000Hz. It is important to get consistently positive responses from all normal subjects for a test to be used as a screening test. In that sense, cVEMP is a more reliable measure than oVEMP and oVEMP responses at 500 Hz are more reliable than those at other frequencies (9).

Thresholds of oVEMP:

The cVEMP and oVEMP responses can be obtained at similar threshold and oVEMP can even be obtained at slightly higher threshold possibly this may be related to the structural differences in the muscles used to record these different responses and to the amplitude of the response, cVEMP response has larger amplitude and can be easily detected at threshold lower than the oVEMP response. Park et al. reported that oVEMP thresholds were higher by about 10 dB than cVEMP thresholds throughout the frequencies[9]. A tone-burst stimulation of 500 Hz also had lower thresholds compared to those at other frequencies in oVEMP and seems to be clinically most appropriate[10].

Amplitudes and latencies of oVEMP:

The amplitudes and latencies of oVEMP were similar to those previously reported and it was reported that the amplitude of cVEMP responses scaled in proportion to the level of tonic activation of the muscles[2,11,12]. In this study, cVEMP P13-N23 peak to peak amplitudes were higher by about 15 times than oVEMP N10 amplitudes at 500 Hz. In this study, the first peak (n1) latency of oVEMP was shorter than the first peak (P1) latency of cVEMP. The difference of the first peak latencies between oVEMP and cVEMP may be due to the different length of neural pathways or different number of synapses between the excitatory and the inhibitory connections [13].

oVEMP in vestibular neuritis group:

In vestibular neuritis group, 13 out of 30 patients (43%) did not give cVEMP response and 6 patients in addition to the 13 that did not give cVEMP[19] out of 30 patients (63%) did not give oVEMP response. The group of patient that produced responses, both cVEMP and oVEMP response amplitude ratios were significantly higher than that of the control group.

AC cVEMP, which are likely due to excitation of saccular afferents in the inferior vestibular nerve, are abolished in only about 20-30% of acute vestibular neuritis ears[5]. Kim et al. reported that abnormal cVEMP results were observed in 25 out of 51 patient (49%) in his study in the form of decreased amplitude or absent cVEMP on the ipsi-lesional side were found in 14 and 11 patients, respectively[6].

oVEMP in BPPV group:

In BPPV group, all patient produced a response, this confirms the hypothesis of the pathological basis of BPPV disease. BPPV arises from damage of the utricular macula and release of the otoconia in the SSCs and thus mainly affects the superior vestibular nerve function and spared the inferior vestibular nerve function. In the present study, there was a significant threshold and latency difference in both cVEMP and oVEMP and significant dif-

ference in the amplitude ratio of oVEMP between BPPV and control group. This can be explained on the basis of not only BPPV arises from damage of the utricle but may be associated by saccular damage in addition there may be significant difference in the recovery of cVEMP pathway before oVEMP pathway as the later is assumed to be multi-synaptic pathway and may be delayed in recovery. Benign paroxysmal positional vertigo is thought to arise from the detachment of otolith from the macula of the utricle; this degenerative process may also affect the macula of the saccule which can be detected by cVEMP and oVEMP testing [7,13].

oVEMP in Meniere's disease group:

In Meniere's disease group, 23 out of 32 patient (71%) produced no cVEMP responses. None of the patient in the study group produced oVEMP response when the affected ear is stimulated.

In Meniere's disease, de Waele et al. stated that AC cVEMP is absent in 55% of affected ears and its absence correlates with low-frequency hearing loss[14]. In the present study, the higher overall prevalence of AC-cVEMP abnormalities in definite MD is likely to reflect saccular involvement. It was expected that AC cVEMP, a predominantly saccular test to yield a significantly larger number of abnormalities than the AC oVEMP, (which is likely to have additional utricular representation). Yet, it was found a higher prevalence of oVEMP than cVEMP abnormalities in our study, with a significantly higher proportion of absent reflexes for AC cVEMP. AC stimulus was nearer threshold for cVEMP thus more likely to yield an absent reflex. oVEMP when compared with the cVEMP is a low-amplitude reflex and thus more likely to fall below the limits of detection. An additional point may be related to the disease duration, in advanced Meniere's disease, there may be more than saccular involvement (responsible for cVEMP generation), the utricle may be also involved (sharing in oVEMP generation) and thus increasing the proportion of oVEMP reflex abnormalities.

oVEMP in undiagnosed group:

In undiagnosed group, 14 out of 39 patients did not give cVEMP response and 6 patients in addition to the previous 14[20] out of 39 patients did not give oVEMP response. The remainder of patient showed responses that do not differ significantly between both ears except in oVEMP latency. The absence of cVEMP and oVEMP responses can be explained on the basis that this group of patient may have saccular dysfunction responsible for the generation of cVEMP and share considerably in the generation of oVEMP response[15]. Some investigators have reported that the symptoms of unilateral otolith dysfunction are compensatory, like those of semicircular canal dysfunction[16]. Thus, patients with complete compensation for a previous saccular disorder may exhibit abnormal cVEMP.

oVEMP provides a new method for examining otolith-ocular pathways. It is a manifestation of the short-latency, phasic VOR. In contrast, oVEMP may remain abnormal, making them a useful new test of otolith function. It has been shown oVEMP can be effective in detecting lesions of central vestibular pathways. Patients with MS who have clinical lesions of internuclear pathways have a high rate of oVEMP abnormalities, with relatively spared cVEMPs. Complementing other evoked potentials used for detecting abnormalities in MS, oVEMP may be valuable for detecting and confirming lesions of otolith–ocular pathways.

Correlation between cVEMP and oVEMP results between different study group reflecting variable degrees of significance suggesting that cVEMP and oVEMP responses may produce characteristic profile in each of theses groups and aid in the diagnosis and differential diagnosis of many vestibular disorders.

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OCULAR VESTIBULAR EVOKED MYOGENIC POTENTIALS IN PATIENTS WITH PERIPHERAL VESTIBULAR DISORDERS

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CORONARY ANGIOGRAPHIC PATTERNS OF PATIENTS WITH METABOLIC SYNDROME

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Abstract

Objectives : *We sought to study the relation between the metabolic syndrome (MS) and the coronary artery disease (CAD) to identify the extension, severity and morphologic patterns of coronary lesions associated with MS.*

Background : *Evidences from cross-sectional and prospective studies demonstrate that MS is associated with an increased risk for coronary atherosclerosis. However the relation between the MS and the morphologic pattern of coronary lesions has not been studied extensively.*

Methods : *The study group consisted of 155 patients, in whom there were indications for coronary angiography for clinically suspected CAD. Coronary angiographic findings including the extent, severity and morphologic patterns of the lesions were compared between both MS group and non MS (NMS) group.*

Results : *Significant increase in the number of diseased vessels, number of diseased segments and Gensini score in patients with MS when compared to those without MS (P=.000, .000, .001; respectively). Significant increase in the number of lesions in LAD (left anterior descending), LCX (left circumflex) and RCA (right coronary artery) in patients with MS when compared to those without MS (.026, .000, .027; respectively). Significant increase in the LAD, LCX and RCA scores in patients with MS when compared to those without MS (P= .028, .000, .006; respectively). Significant increase in the number of complex and simple lesions in patients with MS when compared to those without MS (P.011, .001; respectively).*

Conclusions : *MS is associated with more extensive, severe and complex coronary lesions.*

Key words: *Metabolic syndrome, coronary artery disease, morphologic patterns.*

Introduction

In the last years, MS has caused an authentic revolution in vascular risk stratification and has become one of the most widely used concepts within the health sciences. It describes the accumulation of several risk factors for cardiovascular disease and diabetes within an individual patient [1].

The two underlying mechanisms of MS are abdominal adiposity and insulin resistance. It is exacerbated by age, genetic conditioning, and an inappropriate lifestyle which includes physical inactivity and the consumption of high calorie, high-fat foods, concentrated carbohydrates, and salt. These metabolic alterations occur simultaneously more frequently than would be expected by chance and the concurrence of several factors increases cardiovascular risk over and above the risk associated with the individual factors alone [1].

Several previous studies proved that patients with MS are at increased risk of cardiovascular atherosclerosis. Nathan et al. stated that individuals with MS and DM have a greater incidence and absolute progression of coronary artery calcium compared with individuals without these conditions, with progression also predicting coronary heart disease events in those with MS and DM [16]. However little was studied about the relation between the MS and the character of the coronary lesions. The present study aimed at, first to identify the relation between the MS and the CAD. Second to identify the angiographic and morphological pattern, extension and severity of coronary lesions associated with MS.

Methods

Study population:

We examined 155 patients (83 males, 72 females), admitted to cardiology department at Mansoura Specialized Hospital with suspected CAD for elective coronary angiography. Patients with congenital and valvular heart diseases were excluded. Informed consent was obtained from all patients after detailed description of the procedure.

Study design:

Careful and comprehensive medical history was taken. Cardiovascular risk factors such as smoking and hyperlipidemia were defined according to previous publications [2,3]. All patients underwent a clinical examination with special stress on the cardiovascular system, ECG and echocardiography.

Measurements for the waist circumference, hip circumference, height and weight were obtained from each patient. Blood samples were withdrawn from fasting patients for assay of blood sugar, serum creatinine, lipid profile, by standard laboratory methods.

Coronary angiography:

It was performed in the catheterization laboratory at Mansoura Specialised Hospital by means of COROSCOP (Siemens AG, Munich, Germany), equipped with Quantcor QCA version 2.0 quantitative coronary analysis software. All angiographies were performed by the modified Seldinger technique through femoral artery access. Angiographic scoring was performed by observers who were blinded to the MS state of the patients. Coronary angiograms were interpreted qualitatively and were analyzed in two orthogonal views. Patients were classified as having CAD when their coronary angiograms show any diameter lumen stenosis at any of the coronary arteries. The observers identified the coronary atherosclerotic lesions, ectasia and irregularities of the wall of the coronaries denoting atherosclerosis and they estimated the following:

- Lesions distribution.
- Mean % diameter stenosis.
- Extent; determined by the number occluded segments (from 1 to 27) and the number of occluded vessels.
- Morphology of the lesion being simple or complex; with the complex plaque defined as coronary intraluminal filling defect suggestive or intraluminal thrombus and/or coronary asymmetric narrowing with irregular borders and/or multiple irregularities and/or overhanging edges or with abrupt proximal face or a rough or sawtooth component suggestive of ulcers.
- All abnormal angiographies were assessed by the modified

Gensini score [4]; briefly, location and degree of stenosis (severity), were evaluated. Every lesion was given a score; which is the product of its weighted severity by its weighted location. Weighted location: coronary vasculature was divided into 27 segments and each involved segment was weighted by a value from 0.5 (least important) to 5 (critical location) as follow: lesions in the LM (Left main), 5; lesions in the proximal LAD, 2.5; lesions in the mid LAD, 1.5; lesions in the distal LAD, 1; lesions in the proximal LCX, 2.5; lesions in the obtuse marginal, 1; lesions in the RCA, 1; others, 0.5. Weighted severity: the severity (percentage of stenosis) was weighted as follows: <25%, 1; 26-50%, 2; 51-75%, 4; 76-90%, 8; 91-99%, 16; 100%, 32. The sum of all lesions scores is the Gensini score.

Grouping of patients according to MS: Patients were grouped according to the current adult treatment panel III (ATP III) definition of MS: Presence of any 3 of the following 5 traits: Abdominal obesity, defined as waist circumference in men > 10^2 cm and in women > 88 cm. Serum triglycerides ≥ 150 mg/dl or drug treatment for elevated triglycerides. High density lipoprotein cholesterol (HDL-C) < 40 mg/dl in men and <50 mg/dl in women or drug treatment for HDL-C. Blood pressure ≥ 130/85 mm Hg or drug treatment for elevated blood pressure. Fasting blood sugar ≥100mg/dl or drug treatment for elevated blood sugar [5]. According to this definition patients were divided into 2 major groups: Group 1 for patients with MS and Group 2 for patients without MS.

Statistical methods:

Frequency, mean and standard deviation were used to describe data. Chi-square test was used to test for association between different risk factors and abnormal coronary arteries. Student t-test was used to test for significance of difference in quantitative variables between each two groups. Pearson product moment correlation was used to test for linear relationships between different quantitative variables. Discriminant analysis was used to select variables affecting coronary arteries. P value was considered significant if less

than 0.05. These tests were run on an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS) for windows 7.5 (SPSS Inc., Chicago, IL,USA).

Results

Table (1) : Baseline Characteristics of the study population.

		Total		Metabolic State				χ^2	p
				NMS (60)		MS (95)			
		Count	%	Count	%	Count	%		
sex	Male	83	53.5%	33	55.0%	50	52.6%	0.083	0.773
	Female	72	46.5%	27	45.0%	45	47.4%		
HTN	No HTN	51	32.5%	34	55.0%	17	18.1%		
	HTNive	104	67.5%	27	45.0%	77	81.9%	22.76	.000
DM	No DM	98	63.2%	55	91.7%	43	45.3%		
	DM	57	36.8%	5	8.3%	52	54.7%	34.1	.000
smoking	Non smoker	94	60.6%	40	66.7%	54	56.8%		
	Smoker	47	30.3%	17	28.3%	30	31.6%		
	Ex-smoker	14	9.0%	3	5.0%	11	11.6%	3	0.29
Dyslipid-emia.	Normal	117	75.5%	50	83.3%	67	70.5%		
	Dyslipide-mia	38	24.5%	10	16.7%	28	29.5%	3	0.07

Significant increase in the number of patients with HTN and DM among the group with MS compared to the group without MS.

Table (2) : Clinical Characteristic and ECG findings of the patients with MS and patients without MS:

		Total		Metabolic state				χ^2	p
				NMS (60)		MS (95)			
		Count	% within MS	Count	% within MS	Count	% within MS		
IHD history	Atypical pain	26	16.8%	15	25.0%	11	11.6%	5.42	0.143
	Stable angina	9	5.8%	4	6.7%	5	5.3%		
	Unstable angina	63	40.6%	23	38.3%	40	42.1%		
	Infarction	57	36.8%	18	30.0%	39	41.1%		
Prior Interven-tion	NO	146	94.8%	57	95.0%	89	94.7%		
	PCI	6	3.9%	1	1.7%	5	5.3%	4.38	0.11
	CABG	2	1.3%	2	3.3%	0	0.0%		
ECG	Normal	62	41.1%	28	46.7%	34	37.4%		
	ST-T wave	30	19.9%	12	20.0%	18	19.8%		
	Q-wave	59	39.1%	20	33.3%	39	42.9%	1.61	0.45

No significant difference between both groups regarding the clinical cardiac evaluation.

Table (3) : Characteristics of patients with MS and patients without MS regarding their physical measurements.

	NMS		MS		
	Mean	Std Deviation	Mean	Std Deviation	P
Waist	99.43	13.97	109.16	15.68	.000
Hip	107.38	16.86	109.99	18.51	.051
Waist-hip Ratio	.93	.07	1.01	.18	.000
weight	82.38	19.01	92.57	17.14	.002
Height	159.95	15.39	161.69	9.11	.950
BMI	36.07	35.94	35.64	7.28	.003

Significant increase in waist, hip, waist-hip ratio, weight and BMI among patients with MS compared to those without MS (P= .000, .051, .000, .002 and .003 respectively).

Table (4) : Characteristics of patients with MS and patients without MS regarding their Laboratory findings:

	NMS		MS		
	Mean	Std Deviation	Mean	Std Deviation	P
Fasting blood sugar	104.16	39.49	160.64	89.02	.000
Postprandial Blood sugar	144.00	74.90	195.56	89.83	.000
TC	198.04	44.06	218.75	51.63	.011
TG	122.13	78.70	181.70	76.94	.000
HDL	50.53	22.60	42.03	18.67	.039
Sr. creatinine	.93	.25	.94	.25	.728

Significant increase in fasting, postprandial blood sugar, TC, TG and HDL among patients with MS compared to those without MS (P= .000, .000, .011, .000 and .039 respectively).

Table (5) : Comparative analysis of coronary angiographic findings between patients with MS and patients without MS:

	NMS		MS		
	Mean	Std Deviation	Mean	Std Deviation	P
Vessels number	.82	1.17	1.53	1.23	.000
Segments number	1.17	1.81	2.20	2.05	.000
Gensini score	42.72	84.08	68.43	75.24	.001

Significant increase in the number of diseased vessels, number of diseased segments and Gensini score in patients with MS when compared to those without MS *(P=.000, .000, .001; respectively)*.

Table (6) : Comparative analysis of the number of coronary lesions in each of the coronary vessels between patients with MS and patients without MS:

	NMS		MS		
	Mean	Std Deviation	Mean	Std Deviation	P
LM	.13	.39	.14	.38	.872
LAD	.55	.77	.89	.97	.026
LCX	.17	.46	.56	.74	.000
RCA	.35	.78	.57	.79	.027

Significant increase in the number of lesions in LM, LAD, LCX and RCA in patients with MS when compared to those without MS (P=.872, .026, .000, .027; respectively).

Table (7) : Comparative analysis of the scoring of each coronary vessel between patients with MS and patients without MS:

	NMS		MS		
	Mean	Std Deviation	Mean	Std Deviation	P
LM score	6.50	28.15	8.29	26.06	.547
LAD score	23.15	43.88	35.72	49.11	.028
LCX score	7.37	26.69	11.92	26.33	.000
RCA score	5.97	17.73	12.55	22.94	.006

Significant increase in the LAD, LCX and RCA scores in patients with MS when compared to those without MS (P= .028, .000, .006; respectively).

Table (8) : Comparative analysis of coronary lesions morphology between patients with MS and patients without MS.

	NMS	MS			p
	Mean	std	Mean	std	
Complex lesions	0.57	0.98	1.07	1.37	0.011

Significant increase in the number of complex lesions in patients with MS when compared to those without MS (P.011).

Discussion

MS, a common clinical problem with high prevalence, has been associated with adverse cardiovascular risk and mortality, where acute coronary syndrome significantly affects mortality, morbidity and quality of life in these patients [6,7].

Compared to patients without MS, our patients with MS had more extensive coronary disease. The group of patients with MS showed significant increase in the number of affected coronary vessels and segments. Also they had significantly higher Gensini score. These results are similar to those of other previous studies; Lanz et al. studied the gender specific effect on the relation between MS and coronaries, they reported that both severity and extension scores showed significantly higher values in the MS group, compared with the NMS group. These differences were important in both males and

females. In their work they have found higher scores for the extension and severity of lesions in the MS than NMS group. Interestingly, the impact of MS in CAD scores was more pronounced in females than in males [8].

Butler et al. studied the relation of the MS to quantity of coronary atherosclerotic plaque, they found that patients with MS were more likely to have more plaques and a greater extent of plaque distribution compared to patients without MS. They also concluded that MS was significantly associated with the presence and extent of both calcified and noncalcified coronary atherosclerotic plaque detected using multi-detector computed tomography scan (MDCT). Furthermore, they showed that this association was independent of the presence of individual traditional risk factors and of the Framingham risk score. Their data showed that MS was associated with both higher likelihood of having coronary artery disease detected using coronary MDCT and, if present, more extensive disease compared with patients without MS. This association remained positive even after adjusting for age; gender; smoking; body mass index; history of hypertension, hyperlipidemia, coronary disease, and diabetes; and Framingham risk score [10].

On studying each coronary artery separately; we found that while the LM did not show any significant difference of the number of lesions or the vessel score, the other coronary vessels (LAD, LCX and the RCA) showed significant increase in both number of lesions and score among the MS group. Non significant difference between the two groups regarding the LM could be explained by its wider diameter that needs more amplification of risk factors such as older age (mean age of MS group in our patients was 54.27) or longer duration of risk factors to be present. Actually we found significant positive correlation between the duration of diabetes and the LM affection.

Regarding the morphology of the coronary lesions, our study showed that patients with MS have significant increase in the number of complex lesions com-

pared to patients without MS. According to our knowledge the relation between MS and the morphology of coronary angiographic lesion was not studied before. However Amano et al. studied the impact of MS on tissue characteristics of angiographically mild to moderate coronary lesions. They reported that patients with more than three clustering risks had significantly increased lipid-rich plaque rates, which is consistent with a previous report showing the synergistic effects of risk factors on atherosclerosis. In keeping with the building evidence on the role of inflammation in cardiovascular disease, inflammatory factors have been shown as significant predictors of atherosclerotic plaques [13].

Kawasaki et al. have developed an integrated backscatter intravascular ultrasound (IB-IVUS), which allows analyzing tissue components of coronary plaque in vivo and shows that vulnerable plaques caused by acute coronary syndrome are relevant to increase in plaque burden, including greater lipid pool [14,15]. Regarding the association of MS with vessel atherosclerosis, several studies have shown that the individual components of MS are related to measurements of subclinical atherosclerosis such as carotid intima-media thickness [16]. Few studies, however, have examined the relevance of MS as an entity with coronary plaque, especially with its tissue components. To assess the relevant detail of MS and coronary plaque morphology is more important from a prevention perspective [17].

Amano et al. studied the impact of MS on coronary plaque characteristics. In their study, significant differences in the reference diameter and the percentage diameter stenoses measured by QCA were not recognized between patients with and without MS. However, vessel volume and plaque volume in the conventional IVUS analysis showed a tendency to increase in patients with MS compared with patients without MS. These results suggest that vessels in patients with MS might be compensatorily enlarged to prevent building atheroma from encroaching in lumen, thereby concealing the presence of a lesion

when angiography is performed. In the IB-IVUS analysis, vessels in patients with MS significantly increased in the percentage of lipid volume (%LV) and significantly decreased in the percentage of fibrous volume (%FV). This finding suggests the incidence of lipid-rich plaque. Furthermore, on logistic regression, their study showed that the prevalence of MS was independently associated with lipid-rich plaque. It has been reported that greater plaque volume, including lipid-rich components, is relevant to plaque vulnerability, resulting in increasing coronary events. Taken together, these findings might explain the mechanisms of MS contributing to the increasing risk of cardiovascular events [13].

These findings may contribute to our understanding of the increased cardiovascular event risk in patients with MS and may explain that this risk was increased above the sum of all individual risk factors. The interaction between clinical components of MS and its biologic phenotype, for example, insulin resistance and dyslipidemia, is known to contribute to the development of a vascular proinflammatory state, including increased lipoprotein peroxidation, smooth muscle cell proliferation, accumulation of lipid-laden material, extracellular matrix deposition, and activation of platelets and thrombotic pathways; in summary, all the aspects of atherosclerosis development and progression and its culmination into adverse clinical events [10].

Our results may also have therapeutic implications. Optimally, information about the presence and extent of coronary artery disease may identify patients at low and high cardiovascular event risk in patients with MS. Considering the high prevalence of MS and the obesity epidemic, only targeted therapies to high-risk patients may be safe and cost-effective.

Conclusion

Patients with MS have more extensive, severe and complex coronary lesions compared to those without MS.

Study limitation :

In this study we used coronary angiography to assess the coro-

nary lesions. Coronary angiography is a lumen gram that may not detect early lesions. Other tools like IVUS would be better to demonstrate earlier lesions and would give a better idea about the components and characters of these lesions.

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CORONARY ANGIOGRAPHIC PATTERNS OF PATIENTS WITH METABOLIC SYNDROME

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ROLE OF COLLAGEN CROSS-LINKING USING RIBOFLAVIN/ULTRAVIOLET-A IN KERATOCONUS

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Abstract

Aim : *The aim of this study is to evaluate the safety and effectiveness of riboflavin-ultraviolet type A (UV-A) light rays induced cross-linking of corneal collagen in improving visual acuity and in stabilizing the progression of keratoconic eyes.*

Subjects and Methods : *This is a prospective study of patients attending the outpatient clinic of Mansoura Ophthalmic Center and diagnosed as keratoconus during the period from March 2010 to February 2011 with maximum keratometric (K) reading less than 54 D and minimal corneal thickness greater than 400 microns. The corneal epithelium was removed manually within the central 8 mm diameter area and the cornea was soaked with riboflavin eye drops (0.1% in 20% dextran) for 30 minutes followed by exposure to UVA radiation (370 nm, 3 mW/cm^2) for 30 minutes. During the follow-up period, uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA), slit lamp examination, refraction, and topographic changes, pachymetry, and endothelial cell count were recorded at 3, 6, 12 months.*

Results : *Fifty one eyes of 31 patients with mild to moderate keratoconus were included in the study. All patients completed one year follow up. Fourteen (45%) patients were males and seventeen (55%) were females. The mean age was 24.4 years (range: 17-30 years). Keratoconus was bilateral in 20 patients (65%) and unilateral in 11 patients (35%). There was statistically significant improvement of UCVA from a preoperative mean 0.7 ± 0.07 to 0.48 ± 0.03 at one year ($P < 0.05$). None of the*

eyes lost lines of preoperative UCVA or BCVA. The cylinder values at 1-year examination were statistically significantly (mean 3.7 ± 0 .3 D) less than the preoperative values (mean 4.02 ± 0 .3 D). There was a statistically significant decrease in the mean K value at the apex from the preoperative values and one year values (P < 0.05).The preoperative mean apex K value was (48.27 ± 0.45 D) and changed to (47.65 ± 0.46 D) at one year. Pachymetric reading was reduced from 467.98 ± 5.1 µm preoperatively to 455.27 ± 4.8 µm at 3 months. One-year evaluation showed the pachymetric values increased gradually to 455.68 ± 4.68 µm. Collagen cross-linking did not induce endothelial damage during follow-up. All eyes developed minimal faint stromal haze that cleared in 50 eyes within 1 month. In only 1 eye, this haze resolved within 6 weeks. Other sight threatening complications were not encountered in this series.

Conclusion : *The results show a stabilization and improvement in keratoconus after collagen cross-linking. This suggests that it is an effective and promising method for keratoconus treatment.*

Introduction

Keratoconus is characterized by the development of a non-inflammatory ectasia of the axial or peri-axial region of the cornea and is usually bilateral. Its incidence in the general population is reported to be about one in 2000.[1] Incidences of one in 600 to one in 420 seem more in keeping with the current diagnostic capacity.[2] The thinning and the protrusion in keratoconus induces irregular astigmatism, myopia and scarring resulting in visual loss and mild to marked impairment in the quality of vision. Among the risk factors of this condition is genetics, usually inherited in an autosomal dominant fashion.[3]

As the disease advances, high corneal astigmatism and stromal opacities develop to the point where contact lenses can no longer provide useful vision and penetrating keratoplasty (PKP) becomes necessary to restore visual function. Penetrating keratoplasty is the most commonly performed surgical procedure for keratoconus, but is associated with com-

plications including graft rejection.[4] It is estimated that eventually 21% of the keratoconus patients require surgical intervention (PKP) to restore corneal anatomy and eyesight.[5]

Changes in corneal collagen structure,[6] and extracellular matrix, apoptosis and necrosis of keratocytes, exclusively involving the central anterior stroma, and Bowman's membrane are documented in the structurally weakened corneal tissue in keratoconus. [7]

A new treatment, Ultraviolet corneal collagen cross-linking (CXL), has been recently introduced to reduce the progression of noninflammatory corneal degeneration such as keratoconus, pellucid marginal degeneration, and ectatic corneal disorders after corneal refractive procedures. [8]

Since the early 1990s, basic laboratory studies have demonstrated that CXL caused a long-term increase in corneal biomechanical rigidity by stiffening the human cornea by more than 300%,[9] increasing the collagen fiber diameter by 12.2%,[10] and inducing the formation of high-molecular-weight collagen polymers, with a remarkable chemical stability. [11]

The technique of corneal collagen cross-linking consists of photopolymerization of stromal fibers by the combined action of a photosensitizing substance (riboflavin or vitamin B2) and ultraviolet A rays (UVA) from a solid-state UVA source.[8] Photopolymerization increases the rigidity of corneal collagen and its resistance to keratectasia.[9] The cross-linking effect is not distributed homogenously over the corneal depth. The stiffening effect is concentrated in the anterior 200 to 300 microns of the cornea due to the high absorption of UV light in this area.[9]

Riboflavin has a dual function of acting as a photosensitizer for the production of oxygen free radicals, which induce physical cross linking of collagen, and it gives a “shielding effect” by absorbing the UV-A irradiation (90%), thereby preventing damage to deeper ocular structures. UV-A light of 370 nm wavelength at 3 mW/cm^2 allows approximately 95% of the UV light to be absorbed into the

cornea; thus there is no risk for damage to the lens or retina. Collagen cross-linking is the only treatment that deals with not only the refractive effects of the condition but the underlying pathology. [12]

The aim of this study is to evaluate the safety and effectiveness of riboflavin UV-A light-induced cross-linking of corneal collagen in improving visual acuity and in stabilizing the progression of keratoconic eyes.

Subjects and Methods

This is a prospective study of patients attending the outpatient clinic of Mansoura Ophthalmic Center and diagnosed as keratoconus during the period from March 2010 to February 2011.

Inclusion criteria:

- Completely clear cornea with maximum keratometric (K) reading less than 54 D and minimal corneal thickness more than 400 micron with the absence of any other ocular or systemic disease.

Preoperative examination :

- Slit lamp examination, uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA) measurement, corneal topography (Wavelight -ALLEGRO Topolyzer 1075, Germany), corneal thickness measurement and endothelial cell count using a noncontact specular microscope (Tomy EM-3000) and dilated fundus examination using non- contact Volk 90 lens, and indirect ophthalmoscopy.

Surgical technique :

The procedure was conducted under sterile conditions in the operating room of Al-Nokhba ophthalmic center at Mansoura.After topical anesthetic eye drops (benoxinate hydrochloride 0.4%) every 5 minuet for 30 minutes, the epithelium was mechanically removed within the central 8 mm diameter area using a Beaver blade. Next, riboflavin (0.1% solution, 10 mg riboflavin-5-phosphate in 10 ml dextran 20% solution) (Ricrolin, Sooft Italia, Montegiorgio, Italy) was applied every 3 minutes for 30 minutes until the stroma was completely saturated and aqueous stained yellow. Ultraviolet, A irradiation, was accomplished using a commercially available UVA

system (UV EMITTER Mod. VEGA C.S.O. srl Via degli Stagnacci, 12/E 50010 SCANDICCI (FIRENZE) - ITALY). Before treatment, the intended 3 mW/cm^2 surface irradiance (5.4 J/cm^2 surface dosage after 30 minutes) was calibrated using a UVA meter (LaserMate-Q, LASER 2000, Wessling, Germany). During treatment, riboflavin solution was applied every 5 minutes to ensure saturation and balanced salt solution (BSS®) was applied every 5 minutes to moisten the cornea.

A drop of moxifloxacin hydrochloride eye drops and a bandage contact lens were applied at the end of the surgery.

Postoperative care :

Patients received moxifloxacin hydrochloride eye drops 5 times daily for 1 week. A bandage soft contact lens was applied until re-epithelialization was complete. Then, patient received topical steroid-antibiotic drops (Tobramycin and Dexamethasone) 4 times daily for a week and then tapered over the next 3 weeks; and, Carboxymethylcellulose Sodium (CMC) (0.5%) eye Lubricant 6 times daily for 1 month.

Follow-up was first done after one day, 3 days for contact lens removal, then after one week for prescription of steroid-antibiotic drops (Tobramycin and Dexamethasone) and one month for assessment of corneal haze. Uncorrected visual acuity, BCVA, refractive, topographic changes, pachymetery, and endothelial count were recorded at 3,6,12 months.

• Informed consent was obtained from each patient after the nature of the procedure and its known risks were explained.

Data analysis

The statistical analysis was carried out using the SPSS (Statistical Package for Social Science) program, version 15, utilizing paired's t test for paired data was used to compare preoperative and postoperative data.

Results

Fifty one keratoconic eyes of 31 patients were included in the study. All patients completed one year follow up. Fourteen (45%) patients were males and seventeen

(55%) were females. The mean age was 24.4 years (range : 17-30 years). Keratoconus was bilateral in 20 patients (65%) and unilateral in 11 patients (35%).

•Uncorrected visual acuity :

Visual acuity was measured using the Snellen Metric chart and transformed into logarithm of the Minimum Angle of Resolution (log-MAR) for further statistical analysis as recommended by Holladay [13]. There was a statistically significant improvement from the preoperative values ($P < 0.05$). The preoperative mean UCVA was 0.7 ± 0.07 which changed to 0.6 ± 0.06 at 3 month, 0.49 ± 0.03 at 6 month and to 0.48 ± 0.03 at one year. Eight eyes (15.5%) maintained the preoperative UCVA; twenty seven eyes (53%) gained one line, ten eyes (19.5%) gained two lines and six (12%) eyes gained three to four lines. None of the eyes lost lines of the preoperative UCVA.

•Best corrected visual acuity:

There was a statistically significant ($P < 0.05$) improvement in BCVA between the preoperative and 1-year evaluations. Of the 51 eyes evaluated at 1 year, 43 eyes (84%) experienced at least a gained of 1-4 lines of BCVA. Eight of the 51 eyes (16%) experienced no change in BCVA. There was no statistically significant change from the 6 months examination values and one year values ($P > 0.05$). The preoperative mean BCVA was 0.49 ± 0.03 and changed to 0.42 ± 0.04 at 3 month, 0.27 ± 0.03 at 6 months and 0.26 ± 0.02 at one year.

•Cylindrical refractive error:

The cylinder values at 1-year examination were statistically significant (mean 3.7 ±0 .3 D) less than the preoperative values (mean 4.02 ±0 .3 D). While, there was no statistically significant change from the preoperative values and 3 month values ($P > 0.05$).

•Keratometry :

There was a statistically significant decrease in the mean K value at the apex from the preoperative values and one year values ($P < 0.05$). The preoperative mean apex K value was (48.27 ± 0.45 D) and changed to (47.65 ± 0.46 D) at one year. The K value at the

apex decreased by a mean of 0.62 D from preoperative values. Eighteen eyes (35.3%) maintained the preoperative apex K values, and thirty three eyes (64.7%) showed decrease in K value with range (0.4-2.3 D).Reduction in K value was less than one diopter (< 1D) in eighteen eyes (35.3%) and up to 2.3D (1-2.3D) in fifteen eyes (29.4%). In the one year, there were no statistically significant changes in K readings, compared to six months records, denoting the short term stability of the topographic changes. Progression of corneal steepening from the preoperative value was not observed in any of the treated eyes.

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Visual acuity was measured using the Snellen Metric chart and transformed into logarithm of the Minimum Angle of Resolution (logMAR) for further statistical analysis as recommended by Holladay[13]. There was a statistically significant improvement from the preoperative values ($P < 0.05$). The preoperative mean UCVA was 0.7 ± 0.07 which changed to 0.6 ± 0.06 at 3 month, 0.49 ± 0.03 at 6 month and to 0.48 ± 0.03 at one year. Eight eyes (15.5%) maintained the preoperative UCVA; twenty seven eyes (53%) gained one line, ten eyes (19.5%) gained two lines and six (12%) eyes gained three to four lines. None of the eyes lost lines of the preoperative UCVA.

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- **Pachymetry**

At 3-months post-operative examination, there was a significant reduction in pachymetry (P < 0.05). Pachymetry reduced from 467.98 ± 5.1 µm pre-operatively to 455.27 ± 4.8 µm at 3 months. One-year evaluation showed the pachymetry to increase gradually to 455.68±4.68 µm but still there was a significant reduction in pachymetry than preoperative values (P < 0.05).

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3018104/figure/F0004/

- **Endothelial cell count:**

The mean baseline endothelial cell count was 2624.02 ± 37.3/ mm2. Three months after the procedure, it was 2463.78 ± 32.91/ mm^2; 6 months after, 2587.74 ± 33.89/ mm^2; 12 months after, 2620.27 ± 37.58/ mm2. The difference between the baseline and one year after the procedure was not statistically significant (P > 0.05)http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3018104/figure/F0004/, indicating that CXL did not induce endothelial damage during follow-up. One-year evalu-

ation showed the endothelial cell count to increase gradually to nearly preoperative values Figure (2).

- **Complications**

All eyes developed faint diffuse stromal haze (stromal edema) that cleared in 50 eyes (98 %) within 1 month Figure (3). In only one eye (2 %) the haze cleared within 6 weeks. No other complications were not recorded .

Table (1) : Shows the preoperative and postoperative means of the studied parameters of the eyes included in the study (UCVA, BCVA, cylindrical errors, keratometric readings, pachymetry and endothelial cell count) and the statistical significance of the changes (*P* values).

	Mean preoperative	***Mean postoperative one year***	**P value**
UCVA	*0.7 ± 0.04*	*0.48 ± 0.03*	< 0.05
BCVA	*0.49 ± 0.03*	*0.26 ± 0.02*	< 0.05
Cylinder (D)	*4.02 ± .3*	*3.7 ± 0.3*	< 0.05
Apex K (D)	*48.27 ± 0.45*	*47.65 ± 0.46*	< 0.05
Pachymetry (µm)	*467.98 ± 5.1*	*455.68 ± 4.68*	< 0.05
Endothelial count (/ mm^2)	2624.02 ± 37.3	2620.27 ± 37.58	> 0.05

Figure (1) : shows the topographic changes with reduction of K readings and regularization of corneal shape

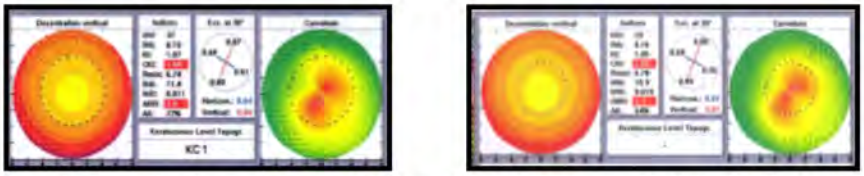

Figure (1): Topographic changes: Preoperative (left), postoperative (right) one year after CLX with reduction of keratometric indices,reduction of the cone size and regularization of corneal shape with improvement keratoconus level.

Figure (2): Endothelial cell count changes: Preoperative (left), postoperative (right) one year after CLX with increase gradually to nearly preoperative values.

Figure (3): Stromal haze (left) which resolved in one month (right).

Discussion

The idea of using corneal collagen cross-linking for corneal stiffening was conceived in Germany in the 1990s. The impressive clinical results initially achieved in Germany have prompted worldwide use of CXL. Currently, there are over 300 centers performing corneal collagen cross-linking in Europe, and the technique has also been used in Canada since 2008. The United States Food and Drug Administration (FDA) recently approved the start of three clinical trials in the United States.[14]

Collagen cross-linking (CXL) is a relatively new method to increase stability of the cornea by inducing additional cross-links between or within collagen fibers using UVA light and riboflavin as photomediators.[15] The efficacy and safety of this new, minimally invasive method for correcting corneal ectasia has been the focus of recent studies. Most work has focused around keratoconus patients, while more recent reports have been published on iatrogenic corneal ectasia as following laser-assisted in situ keratomileusis (LASIK). [16]

In the present study there was statistically significant improvement of UCVA. None of the eyes lost lines of the preoperative UCVA. Eight eyes (15.5%) maintained the preoperative UCVA; (84.5%) gained one to four lines, comparable to that of Arbelaez et al.[12] Also there was significant improvement in BCVA, (84%) experienced at least a gained of 1-4 lines of BCVA and (16%) showed no change in BCVA. There was no statistically significant change from the 6 months examination

values and one year values.

In contrast, El-Raggal[17] found (6.67%) lost 1 line of preoperative value and Hersh et al. [18] found (1.4%) lost 2 or more Snellen lines of BCVA. The cause of UCVA loss in these patients is unclear and did not appear directly related to refractive error or change in corneal topography.

Mean cylinder refraction in this study showed a statistically significant decrease with a significant improvement in uncorrected and best corrected visual acuity 12 months postoperatively. Several studies supported this significant reduction in mean cylindrical power.[8],[19],[12]and[20] In a study done by El-Raggal[17] there was no statistically significant change in mean cylindrical refractive values from the preoperative values (P>0.05). However there was a significant improvement in UCVA and BCVA.

A prospective study published in 2003 reported the earliest clinical experiences in a series of 23 eyes with moderate or advanced progressive keratoconus. During follow-up, which lasted for between 3 months and 4 years, not only disease progression was at least halted, but, in 70% cases, there was also a statistically significant improvement in BCVA, correlating with a reduction of the maximal keratometry readings by 2 diopters. [8]

In the present study, there was a statistically significant decrease in the mean apex K reading between preoperative value (48.27 ± 0.45 D) and 3 months value (48.01 ± 0.46 D) and 3months (48.01 ± 0.46 D) and 6 months (47.67 ± 0.46 D). There was no significant change between 6 months and one year (P > 0.05). This result is comparable to several studies [12],[17]and[18], and contrasts with the findings of others [21] and [22] who reported a continued decrease in maximum K values after the one year follow up.

In the present study, there was a significant reduction in pachymetry at 3 months than the preoperative values (P < 0.05). It reduced from 467.98 ± 5.1 µm preoperatively to 455.27 ± 4.8 µm at

3 months. One-year evaluation showed the pachymetry to increase gradually (455.68±4.68 μm) but still there was a significant reduction in pachymetry than preoperative values. This corresponds to the findings of other studies [12] and [23].

This reduction in corneal thickness could correspond to the apoptosis that occurs after the treatment (2 to 3 months) and the repopulation that occurs thereafter. Based on this finding, the authors strongly suggest that when the cross-linking treatment is combined with an additional treatment, a healing interval of approximately 2 to 3 months should be respected to avoid complications caused by the additional damage of the added procedure.[12]

Regarding endothelial cell count, there were not statistically significant changes at the end of one year indicating that CXL did not induce endothelial damage. These findings were supported by several previous studies[19],[23] and[20].

The lack of evidence of endothelial cell loss is an important safety consideration in assessing this new procedure. Follow-up in this patient was not long enough to assess the long-term effectiveness of Collagen cross-linking. However, the results appear promising. [23]

Sight threatening complications were not found in this study denoting the safety of this procedure. The main complication was faint diffuse stromal haze which cleared in 50 eyes within 4 weeks and within 6 weeks in one eye. El-Raggal,[17] reported results similar to the current study.

In conclusion, riboflavin-UVA corneal cross-linking is a safe measure to increase stability of the cornea and may arrest or even reverse the progression of keratoconus at least in the short term as demonstrated by this study. It is technically simpler, cheaper and less invasive than other treatment modalities for keratoconus. It could be performed in many patients with non advanced keratoconus (K reading less than 54 D).

In addition, surgeons must be

aware of potential complications of the procedure reported above. It would be preferable to treat earlier stages of progressive keratoconus in an effort to better preserve visual acuity.

Some limitations of this study include the limited follow-up and a relatively small number of patients. Further studies with more patients and longer follow-up to evaluate the duration of the stiffening effect are recommended. No analysis of the fellow eye was done in this study; such analysis is also indicated in the future.

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CLINICO-PATHOLOGICAL CORRELLATION BETWEEN EXPRESSION OF CONNEXIN 26 AND BREAST CANCER

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Abstract

Hypothesis: *The role played by CX26 in breast cancer is controversial. Some studies had described CX26 as a tumor suppressor conversely, others as a tumor promoting protein thus, the exact role of CX26 is yet to be precisely resolved, as it may embrace prognostic, predictive and therapeutic significance.*

Methods: *Our study examined 144 patients, 2 patients with bilateral synchronous breast cancers, so we had 146 tumor specimens. We had 70 patients (with72 tumors) presented a Cx26 positive status, while, the remaining 74 patients (with 74 tumors) presented a Cx26 negative status. We compare between the results of the two groups.*

Results: *Cx26 positivity was associated with large sized tumors, clinically palpable axillary lymph nodes, infiltration of more axillary lymph nodes after their pathological examination, advanced tumor stage, high tumor grade and positive lymho-vascular invasion.*

Conclusions: *These results suggest that Cx26 positivity was associated with poor prognosis of patients of breast cancer.*

Introduction

Breast cancer is the most common cancer and the second leading cause of cancer deaths in women in North America[1]. Breast cancer is not a single entity; it is a multifactorial disorder[2-4]. Prognostic factors for breast cancer are in part derived from the tumor and in part from the host .Host factors include age at diagnosis, race, genetic and familial factors. Tumor factors include tumor size, histological

grade, histological type, lymphatic spread, vascular spread, nodal involvement status and other factors[5].

Connexins are transmembrane proteins form intercellular communicating channels termed gap junctions (GJ), ensuring electrical and chemical coupling between cells playing a major role in development, growth and differentiation of cells [6-10].

The function of gap junctions affects the development of tumors according to the stage of tumorigenesis. Gap junctions act as tumor suppressors, while , the re-expression of connexins in migrating tumor cells may help these cells to invade surrounding tissue, and intra- and extravasate [7].

The role played by CX26 in breast cancer is controversial. Many studies described CX26 as a tumor suppressor, others as a tumor promoting protein. Thus, the exact role of CX26 is not yet precisely determined, as it may embrace prognostic, predictive as well as therapeutic significance [11-14].

Patients & Methods

(A) Patients :-

This study included 144 female patients with breast cancer admitted at Mansoura University Hospital and Oncology Center Mansoura University over 48 months between January 2008 and January 2012. Their age ranged from 34-80 years with the mean age of 50 years.

Evaluation of the patients:

Preoperative assessment: All patients were subjected to the following: Detailed history taking, Clinical examination, laboratory investigation, radiology including: Sonomammography, Abdominal sonography and bone scan.

Surgical technique: Conservative Breast Surgery (CBS): for patients had primary tumor less than 5cm (T2), not multicentric, axillary lymph node (N1) without distant metastasis (M0). Patients did not fulfill these criteria underwent Modified radical mastectomy.

Postoperative management: Post operative care: wound care, follow up and discharge was on the second postoperative day,

patients advised to follow up in our OPD, sutures and drains were removed 14 days postoperative unless there was any wound complication.

Adjuvant therapy: 129 patients (88.6%) received adjuvant radiotherapy, 141 patients (97.9%) received adjuvant chemotherapy in the form of 6 cycles of 5-flurouracil, Adriamycin, cyclophosphamid (FAC) and 117 patients (81.2%) were treated with hormonal therapy in the form of Tamoxifen 20mg/day.

Postoperative follow up included: clinical examination every 3 months, sonomamography for the patients who were treated with conservative breast surgery every 3 months, chest X-ray, pelviabdomoinal sonography and bone scan every 6 months. The mean follow-up period of these patients was 31 months, ranging from 14 to 40 months.

Pathological assessment of the tumor specimen: Each tumor specimen was assessed as regard: tumor size, lymph node status, histological type, grade, lympho-vascular invasion, ER, PR and Cx26 status (negative or positive and the degree of positivity).

(B)- Immunohistochemistry : Estimation of Cx26 in breast tissue by immunohistochemical method (13): Breast tissue samples were fixed in10% neutral buffered formalin, then processed to paraffin blocks which were cut into 3 µm thick tissue sections. Prepared sections were cut on charged slides. Each prepared slide was subjected to the following steps: deparaffiniztion, rehydration in alcohol, washing in Phosphate Buffer Saline PBS (Ph 7.2), blocking of endogenous peroxidase then, it was washed in (PBS) buffer solution, antigen retrieval was performed, cooling of the slides then washing with PBS buffer, each slide was covered by protein blocking solution. Connexins were investigated using polyclonal goat Cx26 antibody (1:500 dilution; Santa Cruz Biotechnology, Santa Cruz, USA), and then washed in PBS three times; we applied the streptavidin biotin peroxidase complex technique to visualize the complexes of connexins and their specific antibodies

(LSAB kit; Dako, Glostrup, Denmark), and then washed in PBS, covered by a film of avidin enzyme conjugate then washed in PBS, Diaminobenzidine (DAB) chromogen was applied to each slide then washed in PBS, Haematoxylin was then added as counter stain and then slides were washed in water until the blue color appeared, Dehydration of the slides, followed by addition of xylene, Finally, each slide was covered by the mounting media to preserve them, in negative controls (slides which did not stained by the antibody to be used as a control), the primary specific antibody was omitted for the immunohistochemical staining, expression of Cx26 was analyzed in 10 different tumor fields and its presence was assessed according to a 3 point scale (0 = <10% positive cells; 1+ = 10-50% positive cells; 2+ = >50% positive cells) this assessment was a quantitative one. The specimens were then divided into connexin positive (levels 1+ or 2+) and connexin negative (level 0) tumors based on this grading, also, the intensity of staining was assessed as mild, moderate and severe, this assessment was a qualitative one.

(C)-Statistical Analysis: Data was analyzed using SPSS (Statistical Package for Social Sciences) version 14. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Quantitative data were tested for normality by Kolmogrov-Smirnov test. Normally distributed data were presented as mean ± SD. Student t-test was used to compare between two groups. $P < 0.05$ was considered to be statistically significant. The Kaplan-Meier method was used to perform survival analysis. Statistical significance between survival curves was assessed by the log-rank test.

Results

Table 1 shows that : The relationship between Cx26 expression and

- Clinical size of the tumor (T), showed that there was a statistically significant difference (p-value=0.006), in the meaning of, Cx26 expression was positive in T3 (55.6%, 40/72), while, Cx26 was negative in T2 and T1{(56.8%, 42/74) and (8.1%, 6/74) respectively}. Also in T3 tumors most of

these tumors 40 of 66 (60.6%) were Cx26 positive.

- Clinical lymph node status (N), showed that there was a statistically significant difference (p-value=0.001), in the meaning of, Cx26 expression was positive in N1 (86.1%, 62/72), while, Cx26 was negative in N0 (37.8%, 28/74). Also in tumors with N1 status most of these tumors 62 of 108 (57.4%) were Cx26 positive.

- Clinical stage, showed that there was a highly significant statistical difference (p-value=0.000), in the meaning of, Cx26 expression was positive in stage IIIA (36.1%, 26/72), while, Cx26 was negative in stage IIA and stage I {(27%, 20/74) and (10.8%, 8/74) respectively} as shown in figure 1. Also in patients with stage IIIA most of their tumors 26 of 34 (76.5%) were Cx26 positive.

- Overall recurrence and metastasis, showed that overall recurrence and metastasis were higher in patients with Cx26 positive tumors (28.6%, 20/70) than in patients with Cx26 negative tumors (27%, 20/74) though not statistically significant (p-value= 0.836).

- Mortality due to disease related causes, showed that the occurrence of mortality due to disease related causes was higher in patients with Cx26 positive tumors (8.6%, 6/70) than in patients with Cx26 negative tumors (5.4%, 4/74) though not statistically significant (p-value=0.080). Also in patients died due to disease related causes most of their tumors 6 of 10 (60%) were Cx26 positive. Survival curves of breast cancer patients according to the expression levels of Cx26 are shown in figure 2.

Figure 2 shows that: 6 patients with Cx26 positive tumors (red line curve) died due to disease related causes, the first patient died after 14 months of follow up and the last patient died after 33 months of follow up. 4 patients with Cx26 negative tumors (blue line curve) died due to disease related causes, the first patient died after 14 months of follow up and the last patient died after 31 months of follow up.

Table 2 shows that: The relationship between Cx26 expression and:

- Histological grade of the tumor, showed that there was a statistically significant difference (p-value=0.018), in the meaning of, tumors with grade II represented mainly a negative Cx26 status (73%, 54/74), while, tumors with grade III represented mainly a Cx26 positive status (22.2%, 16/72) .Also in tumors with histological grade III, most of these tumors 16 of 26 (61.5%) were Cx26 positive.

- Lympho-vascular invasion, showed that there was a highly significant statistical difference (p-value=0.000), in the meaning of, Cx26 expression was positive in case of positive lympho-vasculr invasion (88.9%, 64/72), while, Cx26 was negative in case of negative lympho-vascular invasion (75.7%, 56/74) as shown in figure 3.Also in tumors with positive lymph vascular invasion, most of these tumors 64 of 82 (78%) were Cx26 positive.

- Pathological LN status, showed that there was a highly significant statistical difference (p-value=0.000), in the meaning of, Cx26 expression was positive in case of PN2 and PN3 {(41.7%, 30/72) and (27.8%, 20/72) respectively}, while, Cx26 was negative in case of PN1 and PN0 { (35.1%, 26/74) and (29.7%, 22/74) respectively}.Also in tumors with pathological lymph node status PN3, most of these tumors 20 of 28 (71.4%) were Cx26 positive.

- (ER and PR), showed that there was a significant statistical difference (p-value=0.001) in the meaning of tumors with positive Cx26 presented mainly a (positive both ER and PR) status (83.4%, 60/72), while tumors with negative Cx26 presented mainly a (negative both ER and PR) status (29.7%, 22/74) .Also most of the tumors with positive both ER and PR (60%, 60/100) were Cx26 positive, while tumors with negative both ER and PR (81.5%, 22/27) were Cx26 negative.

Table 1: Relationship between Cx26 expression and Clinical data of the patients.

Patients data	Connexin26 expression		P-value
	Negative	Positive	
Clinical size of the tumor (T):			
T1 (<2cm).	6 (8.1%)	0 (0%)	**0.006**
T2 (2-5cm).	42 (56.8%)	32 (44.4%)	
T3 (>5cm).	26 (35.1%)	40 (55.6%)	
Clinical LN:			
N0	28 (37.8%)	10 (13.9%)	**0.001**
N1	46 (62.2%)	62 (86.1%)	
Clinical stage:			
I	8 (10.8%)	2 (2.8%)	
IIA	20 (27%)	6 (8.3%)	**0.000**
IIB	38 (51.4%)	38 (52.8%)	
IIIA	8 (10.8%)	26 (36.1%)	
Overall recurrence and metastasis:	20 (27%)	20 (28.6%)	0.836
Mortality :			
-Disease related	4 (5.4%)	6 (8.6%)	0.080
-Non disease related (road traffic accident)	0 (0%)	4 (5.7%)	

Table 2: Relationship between Cx26 expression and Pathological data of the tumors.

Pathological data	Connexin 26 expression		P-value
	Negative	Positive	
Histological grade			
I	2 (2.7%)	4 (5.6%)	**0.018**
II	54 (73%)	52(72.2%)	
III	10 (13.5%)	16 (22.2%)	
Without grade	8(10.8%)	0(0%)	
Lympho –vascular invasion:			
Positive	18 (24.3%)	64 (88.9%)	**0.000**
Negative	56 (75.7%)	8 (11.1%)	
Pathological LN status:			
PN0	22 (29.7%)	18 (25%)	**0.000**
PN1	26 (35.1%)	4 (5.6%)	
PN2	18 (24.3%)	30 (41.7%)	
PN3	8 (10.8%)	20 (27.8%)	
ER(+) and PR(+)	40 (54.1%)	60 (83.4%)	**0.001**
ER(-) and PR(-)	22 (29.7%)	5 (6.9%)	
ER(+) and PR(-)	8 (10.8%)	5 (6.9)	
ER(-) and PR(+)	4 (5.4%)	2 (2.8)	

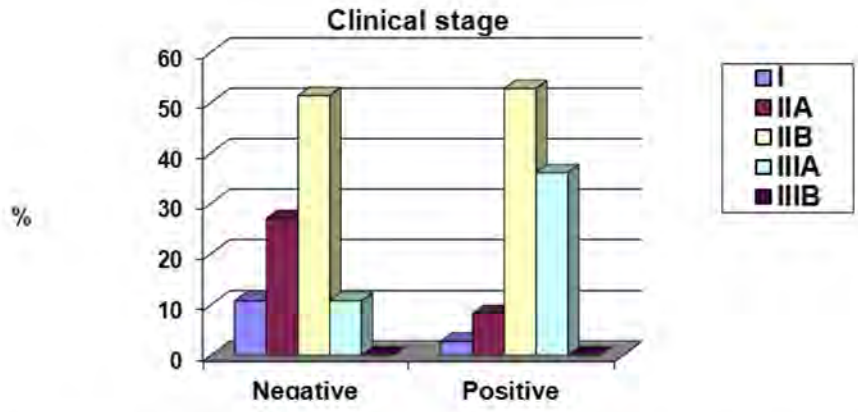

Figure 1. Relationship between Cx26 expression and clinical stage of the tumor.

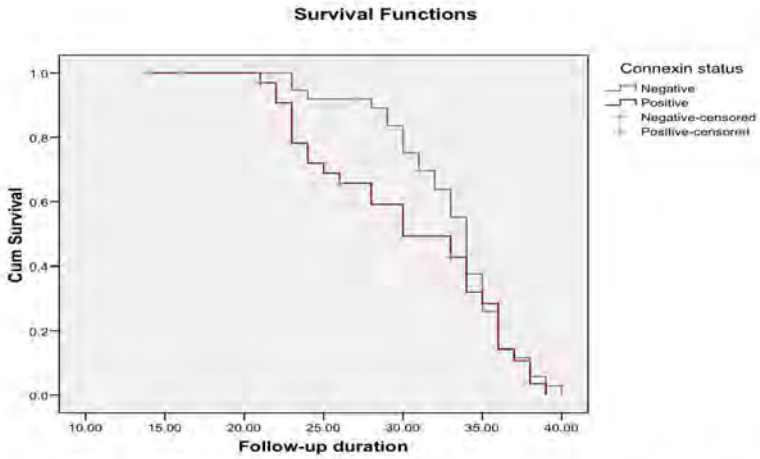

Figure 2. Survival curves of breast cancer patients according to the expression levels of Cx26.

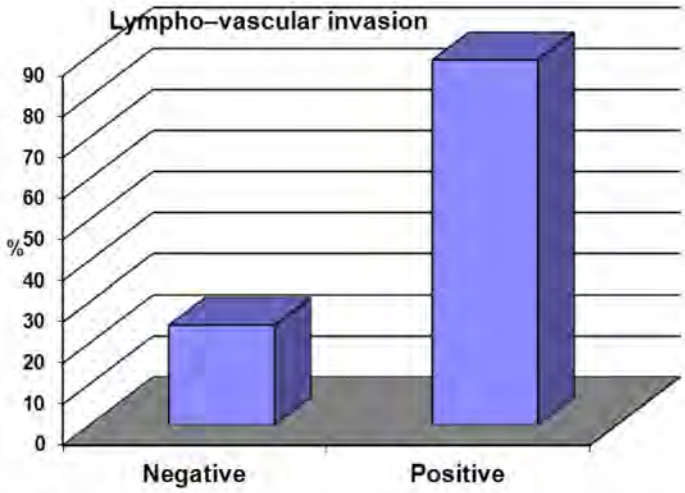

Figure 3 : Relationship between Cx26 expression and lymphovascular invasion.

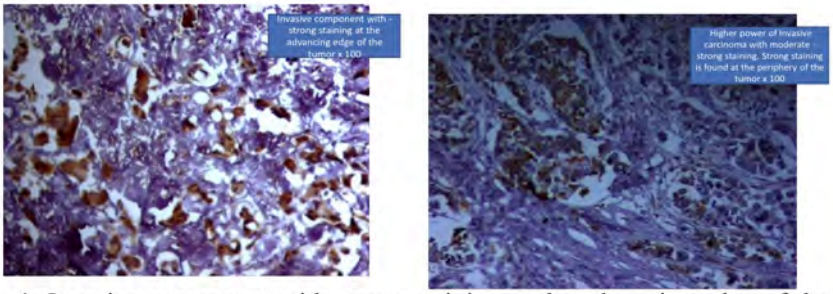

Figure 4: Invasive component with strong staining at the advancing edge of the tumor X100. Higher power of invasive carcinoma with moderate-strong staining .Strong staining was found at the periphery of the tumor X100.

Discussion

Our study demonstrated that Cx26 expression was positive in clinically and pathologically large sized tumors (T3) while, Cx26 was negative in clinically and pathologically small sized tumors (T1 and T2). This means that Cx26 positivity is associated with tumors of large size. The previous results were in agreement with Naoi et al (2007) who found that there was a statistically significant association between Cx26 expression and large tumor size (P = 0.013)[15]. In contrast to our results, Qin et al (2002) found that Cx26 over expression was associated with inhibition of tumor growth, possibly via altered interaction between fibroblastic growth factor and its receptor [16]. This difference may be attributed to the difference between the methodology of our study and theirs. Conklin et al (2007) found that there was no statistically significant value between Cx26 expression and tumor size (p-value = 0.980)[17]. Cx26 expression was positive in clinically palpable axillary lymph node (N1), while, Cx26 was negative in clinically non palpable axillary lymph node (N0).After pathological assessment of the removed axillary lymph node, we found that Cx26 positive tumors were associated with PN3 andPN2, while Cx26 negative tumors were associated with PN1and PN0. This means that Cx26 positivity is associated with clinically palpable axillary lymph nodes and infiltration of more axillary lymph nodes after their pathological examination. Our results are in accordance with those of Kamibayashi et al (1995) who suggested that connexins may play an important role in the extravasation of cancerous cells into lymphoid tissues by formation of gap junctions between tumour cells and endothelial cells in lymph node vessel[18]. In contrast to our results, Naoi et al (2007) found that no statistically significant association between Cx26 expression and lymph node metastasis (p-value= 0.862)[15]. Conklin et al (2007) found that there was no statistically significant value between Cx26 expression and lymph node status (p-value = 0.807)[17]. Overall recurrence and metastasis were higher in patients with Cx26 positive tumors than in patients with Cx26 negative tumors though

not statistically significant. This means that Cx26 positivity may be associated with increase in overall recurrence and metastasis. Such an increased metastatic potential of tumor cells with a high expression of Cx26 may be attributable to the formation of a heterologous (Cx26-Cx43) gap junction between the CX26-expressing tumor cells and Cx43-expressing vascular endothelial cells[19,20] resulting in the increased invasion of tumor cells into the blood vessels [21,22]. Our study showed that, grade III tumors were mainly Cx26 positive tumors, while, grade II tumors were mainly Cx26 negative. This means that Cx26 positivity was associated with tumors with high histological grade. This result was in agreement with Naoi et al (2007) who found that there was statistically significant association between Cx26 and histological grade of the tumor (p-value= 0.043), most of tumors with histological grade III were Cx26 positive (72.2%, 16/22)[15]. Jamieson et al (1998) found that greater portion of grade III tumors were Cx26 positive (73.3%, 11/15) compared to grade II tumors (33.3%, 4/12) (23). In contrast to our results, Conklin et al (2007) found that no statistically significant association between Cx26 expression and histological grade of the tumor (p-value = 0.364)[17], this difference may be due to the difference in sample size, they worked on 346 tumor specimens. Cx26 expression was positive in case of positive lympho-vasculr invasion, while, Cx26 was negative in case of negative lympho-vascular invasion. This means that Cx26 positivity is associated with lympho-vascular invasion.This result was in agreement with Naoi et al (2007) who found that blood vessel invasion was higher in Cx26-positive tumors (5.1%, 4/78) than in Cx26-negative tumors (1.4%, 1/74), though there was no statistical significance (p-value=0.214). Lymphatic vessel invasion was significantly (p-value=0.001) more frequently observed in Cx26-positive tumors (39.7%, 31/78) than in Cx26-negative tumors (14.9%, 11/74)[15]. Over expression of connexins facilitate transendothelial migration through the formation of heterocellular GJIC between breast cancer cells and the endothelium[24]. Cx26-positive tumor cells are mostly

localized in the periphery (invasion front) of a tumor, where blood and lymphatic vessels are also most frequently seen. Thus, it is speculated that Cx26-positive tumor cells localizing in the invasion front, even if the number of such tumor cells is small, seem to have a good chance of invading the adjacently existing lymphatic vessels[15]. The frequency of ER or PR negativity was higher among the Cx26 negative tumors than Cx26 positive tumors and most of ER or PR negative tumors were Cx26 negative though not statistically significant. In contrast to our study, Naoi et al (2007) found that frequency of ER negativity was higher among the Cx26 positive tumors (29.5%, 23/78) than Cx26 negative tumors (27%, 20/74) though not statistically significant (p-value = 0.906). Most of ER negative tumors were Cx26 positive (53.5%, 23/43), while frequency of PR negativity was higher among the Cx26 positive tumors (42.3%, 33/78) than Cx26 negative tumors (24.3%, 18/74) with statistically significant value (p-value = 0.008). Most of PR negative tumors were Cx26 positive (64.7%, 33/51)[15]. This difference may be attributed to difference between our results and theirs regarding Cx26 expression. In their study positive patients were more than negative patients, while, in our study positive patients were less than negative. Also it may be due to ER+ and PR + breast cancer was higher in developed countries[25,26]. Conklin et al (2007) found that there was a statistically significant association between Cx26 expression and ER+ status (p-value= 0.030). The relation between Cx26 expression and PR + status showed that there was no statistically significant association (p-value=0.637)[17]. This difference may be due to the difference in sample size, they worked on 283 tumor specimens in case of ER and 307 tumor specimens in case of PR.

Conclusion

Cx26 positivity was associated with large sized tumors, clinically palpable axillary lymph nodes, infiltration of more axillary lymph nodes after their pathological examination, advanced tumor stage, high tumor grade and positive lymho-vascular invasion. Also Cx26 positivity might be associat-

ed with increase in the overall recurrence and metastasis (bone, brain, liver) except lung metastasis but without statistically significant value. From the previous points we concluded that Cx26 expression might be associated with more aggressive tumors and bad prognosis of patients with breast cancer so it could be used as a useful prognostic factor for breast cancer but this conclusion needs further study. We recommend to do further studies with large sample size in multicentre, with evaluation of Cx26 in primary tumors, axillary lymph nodes and metastasis to get more accurate results.

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CLINICO-PATHOLOGICAL CORRELLATION BETWEEN EXPRESSION OF CONNEXIN 26 AND BREAST CANCER

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EFFECTS OF HEPATITIS C VIRUS VIRAL LOAD IN SERUM AND SEMEN ON COMPUTERIZED SEMEN ANALYSIS PARAMETERS

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Abstract

Background: *It was reported that viral infections contribute to male infertility either by direct toxic effects on cells in the male reproductive tract, and/or indirectly by causing a local inflammatory, or immunological, reaction.* ***Methods:*** *30 patients matching our inclusion and exclusion criteria were evaluated with computerized semen analysis (CASA). 15 of the patients were fertile and 15 were infertile, in the same time 30 fertile healthy controls were also evaluated by CASA.* ***Results:*** *2.35% of the HCV infected patients were infertile. There were non significant differences between CASA parameters of patients with positive and negative HCV in semen. A significant positive correlation was found between the viral loads in blood and teratozoospermic index. A strong significant negative correlation was found between HCV RNA viral load in semen and sperm deformity index. Significant differences were also seen when comparing infertile HCV infected patients and controls regarding straight line velocity, percentage of straightness and normal morphology percentage.* ***Conclusions:*** *HCV affects male fertility mainly through sperm motility and morphology. No significant differences in CASA were found between fertile and infertile HCV infected patients.*

Introduction

Hepatitis C virus is a member of Hepacivirus genus of the Flaviviridae family. To date, six major HCV genotypes with a large number of subtypes within each geno-

type are known[1]. Hepatitis C virus is a major cause of chronic liver disease worldwide. > 200 million people are infected with HCV, with ≥ 70% of acute infections progressing to chronic active hepatitis[2]. The prevalence of HCV infection in Egypt approximates 12%[3]. Though HCV infection is a serious public health problem, some aspects of its biology are still not well understood, such as its transmission through seminal fluid and sexual transmission[4]. It was reported that viral infections contribute to male infertility either by direct toxic effects on cells in the male reproductive tract, and/or indirectly by causing a local inflammatory, or immunological, reaction[5]. The effect, if any, of HCV infection on male fertility is still being debated[6].

Patients & Methods

This study was conducted on 60 males. 30 of them were suffering from HCV infection (with positive HCV RNA Real time PCR in serum) & the other 30 were healthy fertile controls.

The 30 HCV infected patients (proved by HCV RNA RT PCR test in serum) were included in the study after a survey on 637 randomly selected HCV positive (by ELISA test for HCV antibodies in serum) patients in the reproductive age attending the tropical medicine outpatient clinic and inpatient department beside the andrology clinic of the Mansoura University Hospitals during the period between August 2010 and February 2012.

Our 30 patients as well as the 30 controls were in the reproductive age. Patients in both groups A and B were subjected to thorough history taking, general and genital examination, quantitative Real time PCR test for HCV in blood, quantitative Real time PCR test for HCV in semen and computerized semen analysis (CASA) according to WHO criteria in 2010.

Controls Group was subjected to thorough history taking, physical and genital examination and computerized semen analysis (CASA) according to WHO criteria in 2010 through the same machine, soft ware and operator used for the other 2 groups.

Results

We found that 15 of the HCV infected patients (Group B) were infertile among the 637 HCV infected patients we examined and we found that the prevalence of infertility among HCV infected males matching our inclusion and exclusion criteria was 2.35% (15 in 637). HCV RNA was detected in four patients out of thirty HCV infected patients (Groups A and B) and the load ranged from 161 IU /ml to 178000 IU /ml.

Rest of patients [26] gave negative results and the load if present was below the detection limit (<12 IU /ml). So we estimated that the percentage of HCV infected patients (Groups A and B) that found to be able to pass the virus in semen in a detectable level was 13.33%. 3 out of the 4 patients with positive test were fertile (Group A) and only one patient was infertile (Group B). So HCV was detected more in semen of fertile HCV infected patients (75% of these patients are fertile). Median level of HCV RNA viral load in serum showed no significant difference in both groups of infected patients (Groups A and B) as shown in table 1.

Also the median level of HCV RNA viral load in serum showed no significant difference between patients with positive and negative tests for detection of HCV RNA in semen.

Different CASA parameters were studied in patients with positive HCV RNA RT PCR test in semen in comparison to patients with negative HCV RNA RT PCR test in semen where non significant comparison was found (see tables 2 and 3).

On the other hand, when we correlated between HCV RNA viral load in serum of HCV infected patients and CASA parameters a significant positive correlation was found between the viral load in blood and teratozoospermic index.

Non significant positive correlation was demonstrated between viral load in blood and percentage of progressive motility, percentage of non progressive motility, percentage of total motility, mean angular degree of movement, amplitude of

lateral head displacement, percentage of straightness, normal morphology percentage, sperm deformity index and multiple anomalies index. While Non significant negative correlation was demonstrated between viral load in blood and semen volume, semen PH, sperm concentration, sperm Count, percentage of immotile sperms, curvilinear velocity, straight line velocity, average path velocity, beat cross frequency, percentage of linearity and percentage of wobble. Then we repeated this correlation on each group (A and B) and nonsignificant correlation was found. To study the effect of HCV RNA viral load in semen on CASA parameters in patients found to be RT PCR positive for the virus another correlation was done where a strong significant negative correlation was found only between HCV RNA viral load in semen and sperm deformity index. On comparing the 3 groups (A, B and Control) regarding CASA parameters a significant differences between the 3 groups were seen regarding percentage of straightness and normal morphology percentage. We then compared between every 2 of the 3 groups.

Nonsignificant differences regarding the CASA parameters were found on comparing Group A with Group B. But when we compared between Group A and controls we found significant differences between the 2 groups regarding percentage of progressive motility, percentage of total motility and percentage of immotile sperms. Significant differences were also seen when comparing Group B and Controls regarding straight line velocity, percentage of straightness and normal morphology percentage.

Further studies were applied on group B patients (infertile HCV positive patients). Firstly we correlated between HCV RNA viral load in serum and duration of infertility. A significant positive correlation was discovered. Secondly we compared between HCV RNA viral load in sera of patients with primary and secondary infertility. Nonsignificant difference was found between patients. Then we correlated between duration of infertility and semen parameters. Non significant positive correlation was discovered between duration of infertility and percentage of pro-

gressive motility, percentage of total motility, curvilinear velocity, straight line velocity, average path velocity, amplitude of lateral head displacement, percentage of linearity, percentage of wobble, percentage of straightness, teratozoospermic index, sperm deformity index and multiple anomalies index.

While Non significant negative correlation was demonstrated between duration of infertility and semen volume, semen PH, sperm concentration, sperm count, percentage of non progressive motility, percentage of immotile sperms, mean angular degree of movement, beat cross frequency and normal morphology percentage. Finally no significant differences were found comparing semen parameters between patients presented with primary and secondary infertility.

Table (1): Comparison between HCV RNA viral load in sera of patients of Groups A and B.

HCV RNA in serum (IU/ml)	***Group A***	***Group B***	**p**
Median	**134250**	**394184**	**0.633**
Minimum	**20000**	**15000**	
Maximum	**3616082**	**2200000**	

Mann-Whitney test (nonsignificant test, $P > 0.05$).

Table (2): Effects of HCV RNA viral load in semen on different CASA parameters measuring Count and Morphology.

	HCV RNA RT PCR in semen < 12 IU/ml		HCV RNA RT PCR in semen ≥ 12 IU/ml		T	P
	Mean	Standard Deviation	Mean	Standard Deviation		
Conc. (millions)	75.93	+/- 48.63	86.90	+/-76.07	-0.391	0.699
Count (millions)	186.95	+/-127.72	276.52	+/-266.76	-1.120	0.272
N. Morph. (%)	57.14	+/- 10.12	56.43	+/-10.72	0.132	0.896
TZI	2.13	+/- 0.48	2.38	+/-0.19	-1.005	0.323
SDI	0.94	+/- 0.28	1.08	+/-0.23	-0.923	0.364
MAI	2.30	+/- 0.45	2.50	+/-0.36	-0.819	0.420

Significance tested with Student t-test and $P < 0.05$ was considered significant.

Table (3): Effects of HCV RNA viral load in semen on different motility parameters in CASA.

	HCV RNA RT PCR in semen < 12 IU/ml		HCV RNA RT PCR in semen ≥ 12 IU/ml		t	p
	Mean	Standard Deviation	Mean	Standard Deviation		
PR (%)	51.61	+/- 20.70	44.00	+/-22.57	0.678	0.504
NP (%)	13.50	+/- 8.39	9.15	+/-8.15	0.970	0.341
Imm. (%)	34.90	+/- 25.23	46.80	+/-27.44	-0.870	0.392
TM (%)	62.79	+/- 25.66	53.20	+/-27.36	0.691	0.495
VCL (µm/s)	46.68	+/- 10.87	44.09	+/-5.67	0.463	0.647
VSL (µm/s)	31.95	+/- 8.07	33.46	+/-5.63	-0.359	0.722
VAP (µm/s)	35.52	+/- 8.35	36.71	+/-5.59	-.0275	0.785
MAD (')	49.25	+/- 8.50	41.40	+/-7.15	1.747	0.092
ALH (µm)	3.23	+/- 1.07	2.26	+/-0.46	1.772	0.087
BCF (Hz)	5.07	+/- 0.65	5.00	+/-0.89	0.189	0.852
LIN (%)	65.76	+/- 7.85	72.77	+/-8.42	-1.648	0.111
WOB (%)	74.62	+/- 6.18	81.00	+/-6.52	-1.912	0.066
STR (%)	86.20	+/- 4.25	88.28	+/-3.95	-0.918	0.367

Significance tested with Student t-test and $P < 0.05$ was considered significant.

Discussion

Male infertility affects 15% of reproductive age men worldwide [7]. Male factor infertility accounts for up to half of all cases of infertility and affects one man in 20 in the general population [8]. It is estimated that 15% of couples attempting to conceive are not able to do so within 1 year[9]. In our study in order to include 15 infertile HCV infected patients matching our inclusion and exclusion criteria (Group B) we examined 637 HCV infected patients and we found that the prevalence of infertility among HCV infected males matching our inclusion and exclusion criteria was 2.35%.

So the prevalence of infertility among HCV infected males when other factors that may contribute to the problem of infertility are to some degree excluded as in our study was only 2.35%. This is much lower than the prevalence of infertility in general population (15%).

Lower prevalence of infertility detected in our study may be due to the strict inclusion and exclusion criteria applied during selection of our patients. This low prevalence may allow us to suggest that the role of HCV infection as a causative factor of male infertility is present but minimal. Few studies assisting the effect of HCV infection on male fertility were done one study was a retrospective analysis by Chu et al in 2006 [10] that concluded that men co-infected with HCV and HIV-1 does reasonably well undergoing ART and that the transmission risk of viruses to their partners and children is absent. This coincides with our suggestion that the role of HCV as a causative agent of male infertility is minimal. No significant differences in CASA parameters were found in our study when we compared between infertile HCV infected patients and fertile HCV negative controls apart from that regarding straight line velocity, percentage of straightness and normal morphology percentage.

The differences in the percentage of normal sperm morphology as well as in 2 of the parameters measuring sperm motility could suggest that the effect of HCV infection on male fertility may be

through the effect on sperm morphology and/or motility.

Also on comparing the 3 groups (A, B and Control) regarding semen parameters significant difference between the 3 groups was seen regarding the percentage of straightness and normal morphology.

This suggestion can be supported by our findings when we compared CASA parameters between Group A (Fertile HCV infected patients) and fertile HCV negative controls of significant differences between the 2 groups regarding percentage of progressive motility, percentage of total motility and percentage of immotile sperms. When comparing Group A with Group B we found no significant differences between the 2 groups regarding CASA parameters.

Moretti et al in 2008 [11] studied the effect of chronic hepatitis B and hepatitis C on sperm quality. They analyzed sperm quality from selected patients with chronic HBV or HCV infections. Semen samples were examined by light and transmission electron microscopy (TEM). Despite normal sperm concentration, they observed reduced motility. TEM analysis highlighted that 35.7% of patients showed generally good semen quality. However, significantly higher values of apoptosis and necrosis, compared with controls, were observed, demonstrating spermatogenetic alterations. They concluded that the fertility index was significantly lower in HCV- and HBV-infected patients versus controls. This study agrees with our suggestion of an effect of HCV on sperm motility.

In Saudi Arabia Mansoor et al in 2011 [6] tried to determine the prevalence of HBV, HCV and HIV in a group of infertile couples attending a tertiary care unit. They performed a retrospective cross-sectional observational study. All patients were screened for HBV markers, anti-HCV, anti-HIV types I and II using enzyme linked immunoassay technique. They found that the overall HCV prevalence in this group was 0.5%. All females were negative for HCV. This study supports our suggestion that the role of HCV as a causative agent

of male infertility is minimal.

Garrido et al in 2005 [12] evaluated semen characteristics in 136 samples provided from 125 males. They also included a control group of 125 males matched by age and length of sexual abstinence. At the time of semen retrieval, 70 of them were infected with HIV (45 also with HCV), and 55 of them with HCV alone.

Semen analysis, according to the WHO criteria, of HIV- and HCV-affected patients showed no differences from that of non-infected males. Semen analysis performed in this study was routine analysis and same results were found in our study when we assessed count, motility and morphology. But significance differences between cases and controls were seen using CASA parameters.

Safarinejad et al in 2009 [12] evaluated reproductive endocrine profile, sperm chromosomal abnormalities, and semen quality in patients with chronic hepatitis C. In patients with chronic hepatitis C, the mean total sperm count, motility and normal morphology was significantly lower than in controls. In this study unlike ours patients with liver failure were not excluded and CASA was not done. This may explain the difference in results regarding sperm count.

Hofny et al in 2011 [13] studied 57 male patients with chronic hepatitis C virus (HCV) infection and found a statistically significant decrease in semen volume, sperm count, and progressive sperm motility and a statistically significant increase in abnormal sperm morphology compared with healthy controls.

Vicari et al in 2006 [14] evaluated the sperm abnormalities in young infertile patients with hepatitis B or C virus infection beside evaluation of the additional negative influence of varicocele on sperm parameters in these patients. Forty-two infertile patients with HBV (n=23) or HCV (n=19) infection were studied.

Sperm parameters were compared to those of a group of 30 patients with primary infertility due to causes different from liver dis-

eases and/or varicocele. HBV patients showed median sperm parameters (sperm density, total number, viability, forward motility and morphology) significantly worse than those found in patients with HCV. In our study we excluded patients with HBV infection.

Researchers concluded that patients with HBV infection show worse sperm parameters compared with HCV patients. The additional presence of varicocele further impairs sperm output in HBV patients. This may explain the relatively lower prevalence of infertility in our patients reminding that we excluded cases of HBV and varicocele from our study.

In our study in order to avoid the effect of liver failure on male fertility we included only HCV infected patients with compensated liver functions. To our knowledge no other study evaluating effect of HCV on male fertility included only compensated patients.

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EFFECTS OF HEPATITIS C VIRUS VIRAL LOAD IN SERUM AND SEMEN ON COMPUTERIZED SEMEN ANALYSIS PARAMETERS

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EFFECT OF CIGARETTE SMOKING ON SPERM DNA FRAGMENTATION IN MEN WITH NORMAL AND ABNORMAL SEMINOGRAM

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Abstract

Introduction: *Abnormalities in male genom characterized by damaged sperm DNA may be an indicative for male subfertility regardless of routine semen parameters and these parameters don not reveal sperm DNA defects. The etiology of sperm DNA damage is multifactorials; it may be due to intrinsic (eg .protamine deficiency, ROS and apoptosis) or extrinsic factors as- including cigarette smoking,and varicoceles*

Aim of the work (Objective): *This prospective, randomized study was designed to investigate the impact of cigarette smoking on semen parameters and DNA fragmentation of human spermatozoa in an attempt to know the pathophysiology of male infertility.Study the degree of DNA fragmentation in the spermatozoa of smokers and nonsmokers of men with normal and abnormal seminogram, was the second objective.*

Patients & Methods: *100 patients were enrolled for the study. The patients attending the clinic were asked for routine seminal analysis, hormonal assay, and Doppler examination of the inguinoscrotal region. Patients who showed abnormal seminograms were selected for the study.The group with abnormal seminograms was subdivided into three subgroups; 20 non smokers, 20 smoker without varicocele and 20 smokers with varicocele. Forty fertile age matched men were used as control group and were subdivided into 20 smokers and 20 non smokers.*

Results : *The mean values of semen parameters concerning the con-*

centration, motility, morphology and linear velocity were significantly lower in smokers than non smokers in the groups with normal and abnormal seminograms. Moreover, the differences were also significantly lower if the smokers in the two groups with abnormal and normal seminograms were compared. The Comet assay in the groups with normal and abnormal seminograms showed a significantly higher percentage of DNA fragmentation in smokers than non smokers. Moreover, the difference in sperm DNA fragmentation in group with abnormal was significantly higher in smokers with varicocele than smokers without varicocele (p=0,000)

Conclusion: *The conventional semen parameters such as sperm density, motility, morphology and PH in the semen samples are not sufficient for evaluation of reproductive activity. Sperm DNA integrity must be evaluated in addition to routine sperm parameters. This will indicate the quality of the spermatozoa and could have better diagnostic and prognostic values if compared with routine seminal analysis alone. Smokers have statistically significant lower semen volume, sperm count and sperm motility compared with non-smokers.*

Introduction

Over the past two decades, there has been an increasing body of evidence that several environmental toxicants may impair semen quality and thus male fertility in animals as well as in human. Even exposure to episodic seasonal air pollution can have deleterious effects on human sperms[1]. Among different air pollutant, cigarette smoke contains toxic chemicals, mutagenic and carcinogenic compounds, which can adversely affect male fertility too [2] Cigarette smoke contains more than 4000 different chemical compounds, such as nicotine, nitrosamine, polycyclic aromatic hydrocarbons, cadmium and carbon monoxide. Some of these substances were found in seminal plasma at concentrations proportional to those in the serum, suggesting their crossing of the blood - testis barrier[3]. Thus, the seminal plasma of smokers can be considered as a toxic environment for spermatozoa.

Several studies showed a correlation between the consumption of tobacco and an alternation of semen quality[4] and nuclear integrity[5]. Cigarette smoking is linked to significantly increase levels of seminal reactive oxygen species (ROS) which is responsible for oxidative stress[6]. This is why smoking may induce alterations of sperm plasma membrane and high degree of DNA fragmentation[7].

Abnormalities in male genom characterized by damaged sperm DNA may be indicative for male subfertility regardless of routine semen parameters[8]. and these parameters don not reveal sperm DNA defects.. There appears to be threshold of sperm DNA damage beyond which embryo development and subsequent pregnancy outcome are impaired. Clinical evidences suggest that damage to human sperm DNA might adversely affect reproductive outcomes and that spermatozoa of infertile men possess substantially more sperm DNA damage than do spermatozoa of fertile men[9]. The present work was therefore carried out to study the impact of smoking on the nuclear DNA damage in human spermatozoa.

Patients and Methods

The study was carried out upon adult males attending the outpatient clinic of andrology department in Mansoura University Hospital during the period from December 2008 to January 2010.

All patients were subjected to:

1. Thorough history taking:

Personal history: including Name, age, job, and address and special habits mainly cigarette Smoking (duration of smoking, number of cigarettes /day). The smokers included in the study were those who smoked cigarettes on a regular basis for at least 1 year. The men who never smoke or stopped smoking one year prior to examination were considered nonsmokers[10].

Smoking index (SI) is calculated (number of cigarettes/day x years of smoking) and classified as mild < 2000 moderate 200-600 and heavy > 600[11].

Fertility history: Infertile de-

fined as failure to conceive within one year of regular unprotected intercourse. The duration, type, previous investigation and treatment of infertility were reported. Then the female partner was evaluated (age and history of infertility in previous marriage).

Medical history of diseases with possible adverse effect on fertility (systemic diseases, fever, drug abuse,.....etc).

Sexual history: Libido: (present- diminished-absent). Erection: Adequate or inadequate. Ejaculation: (normal-premature-painful-absent).Timing and frequency.

2. Thorough clinical examination of the patient:

Physical examination: as regard height, weight, and spans, signs of hypogonadism, galactorrhea, as well as signs of kartagner or Kallman syndrome. Signs of systemic or endocrinal disease were taken in consideration.

Genital examination:

- **Penis:** position of urethral meatus, penile curvature, scars, plaque, and urethral discharge

- **Scrotal Content:** testes (site, volume and consistency).Scrotal swelling. Epididymis: head, body and tail, thickened, tender or cystic.

- **Examination of spermatic cord:** Vas deferens (palpable or non-palpable, beaded or thickened). Examination for varicocele in both erect and supine postures, during both quiet respiration and Valsalva's maneuver to detect abnormal visible or palpable veins within the spermatic cord and around the tests with comparing both sides.

- Per rectal (P/R) examination of the "prostate" and "seminal vesicle.

3. Investigations:

A. Semen analysis.
B. Hormonal assay: (testosteron, FSH, LH, prolactin, estradiol).
C. Doppler examination of the inguinoscrotal region.

Our patients were divided in to two main groups:

Group I: Sixty infertile men

with abnormal seminogram and normal sex hormone level were taken and categorized into three subgroups.

a) Smokers without varicocele (n=20).
b) Smokers with varicocele (n=20).
c) Non smokers without varicocele (n=20).

Group II: Forty fertile men with normal seminogram and normal sex hormone level were used as control groups which categorized into two subgroups:

a) Smokers without varicocele (n=20)
b) Non smokers without varicocele (n=20).

Fertile men were those who had no history of fertility problem and those whose partners become pregnant spontaneously within one year of regular unprotected intercourse.

Exclusion criteria :

Patient with history of diseases or factors with possible adverse effect on fertility such:

- Systemic diseases: D.M., T.B., chronic respiratory tract diseases, others.
- Fever in past 6 months.
- Surgery: orchiectomy, vasectomy.
- Urinary tract infection.
- Sexually transmitted diseases.
- Epididymitis.
- Pathology possibly causing testicular damage (orchitis; mumps or others, and testicular injury or torsion).
- Testicular maldescent and its treatment; either surgical or medical and age of treatment.
- Environmental and/or occupational factors.
- Excessive consumption of alcohol.
- Drug abuse.
- Patients with azoospermi.

Both group with abnormal seminogram and control group were subjected to the Sperm nuclear DNA fragmentation test using COMET Assay (single cell gel electrophoresis assay).

Semen analysis:

The samples were collected after a minimum of 48 hours and not longer than 7 days of sexual abstinence.

The semen samples were analyzed according to World Health Organizatio [12] using computer-assisted method of semen analysis.

Doppler examination of the inguinoscrotal region :

A portable ultrasonic blood flow detector was used with built in printer, 10 Mhz frequency probe and speaker for detection of changes in the blood flow sonic waves. (Hadeco, Smartdap 20Ex, Hayashi Denki Co., LTD.).

Sperm nuclear DNA fragmentation test using COMET Assay (single cell gel electrophoresis assay).

Procedure:

Sperm cells were prepared and washed with HEPES-bufferd EBSS (Earles balanced salt solution) rapidly at room temperature, sparet into miniature agarose gels on microscope slides and lysed in situ to remove DNA associated proteins and allow the compacted DNA in the sperm to relax. Lysis buffer (Tris 10mmol/l, 0.5mol/l EDTA and 2.5 mol/l NaCI, pH 10) contained 1% Triton X-100, 40 mmol/l dithiothreitol and proteinase K, 100 μg/ml). Microgels were then electrophoresed (20 min at 25V). The comet assay conducted in alkaline (pH>12) buffer (Tris 10 mmol/l containing 0.08 mol/l boric acid and 0.5 mol/l EDTA)[13]. The slides stained with a DNA-specific fluorescent stain (SYBR Green I) and examined using a microscope attached to a digital camera. This is followed by visual analysis of the staining DNA and calculating fluorescence by manual scoring to determine the extent of DNA damage [14].

Comet image analysis: The head region of a comet represents unfragmented DNA that does not migrate outside the region of the nucleus while tail represents DNA which migrates due to fragmentation and loss of structure.

Statistical Analysis :

The statistical analysis of data was done by using excel program and SPSS program statistical package for social science version 10. The description of the data was done in form of mean (+/-) standard deviation (SD) for quantitative data and frequency & proportion for qualitative data. The

analysis of the data was done to test statistical significant difference between groups[15] Munor et al., 2002).

Results

Tables (1, 2) showed Semen parameters of groups with abnormal seminogram and control groups. In groups with abnormal seminogram the mean values of semen parameters concerning concentration, motility, the percentage of normal morphology and liner velocity of smokers were significantly lower than those of non smokers. Moreover, the semen parameters were significantly lower regarding the mean of concentration, motility, velocity, and normal morphology in smoker with varicocele compared with smokers without varicocele (table 1). In control groups the mean values of semen parameters concerning motility, normal morphology and liner velocity of smokers were significantly less than those of nonsmokers (Table 2).

Table (3) showed comparison between smokers without varicocele in the studied groups regarding Semen parameters. The mean values of most of semen parameters concerning concentration, motility, normal morphology and velocity in smokers with abnormal seminogram were significantly poor compared to those of smokers with normal seminogram.

Table (4, 5) showed Comet assay in groups with abnormal seminogram and control groups. The mean value of DNA fragmentation in the spermatozoa of groups with abnormal seminogram was increased significantly in both smokers with varicocele and smokers without varicocele compared with those of nonsmokers. Moreover the difference in sperm DNA fragmentation was significantly higher in smokers with varicocele than smokers without varicocele (table 4). Also, the mean values of DNA fragmentation of smokers with normal seminogram were significantly higher than those of nonsmokers (table 5).

Table (6) showed Effect of smoking index on sperm DNA fragmentation in studied groups. The mean percentage of DNA fragmentation of smokers with abnor-

mal seminogram was significantly higher than those of smokers with normal seminogram for all grade of smoking index.

Table (7) showed the higher percentage of more than 30% of the spermatozoa with DNA fragmentation assessed by comet was detected only in the group with abnormal seminogram in smokers with varicocele .

Table (1): Semen parameters of groups with abnormal seminogram.

Semen parameter	Smokers (no varicocele) n=20	Smokers with Varicocele n=20	Non smokers (no varicocele) n=20	T P1	T P2
	Mean SD	Mean SD	Mean SD		
Volume (ml)	5.07 ± (1.56)	5.46 ± (1.41)	5.06 ± (1.92)	0.020 0.984	0.829 0.412
Concentration (million / ml)	24.52 ± (5.89)	13.39 ± (3.91)	31.48 ± (6.69)	3.494 0.001**	7.045 0.000**
Grade motility (%)	20.09 ± (4.35)	10.94 ± (2.56)	25.57 ± (3.84)	4.227 0.000**	3.120 0.000**
Grade a+b motility (%)	34.45 ± (6.38)	19.34 ± (2.31)	38.40 ± (5.36)	2.119 0.041*	9.959 0.000**
Velocity (µm/sec)	37.60 ± (9.39)	28.50 ± (8.72)	54.65 ± (10.69)	5.357 0.000**	3.175 0.003**
Linear velocity (µm/sec)	23.26 ± (6.30)	15.80 ± (6.03)	36.16 ± (8.25)	5.561 0.000**	3.826 0.000**
Linearity index (µm/sec)	61.82 ± (4.92)	54.78 ± (8.80)	72.30 ± (9.49)	4.388 0.000**	3.124 0.003
Normal morphology (%)	23.11 ± (5.29)	11.4 ± (4.01)	29.40 ± (7.96)	2.970 0.001**	9.980 0.000

P1: Nonsmoker's vs smokers without varicocele; P2: smokers without varicocele vs smokers with varicocele
Significant ($P \leq 0.05$) highly significant ($P \leq 0.01$) Very highly significant ($P \leq 0.001$)

Table (2): Semen parameters of groups groups with normal seminogram.

Semen parameters	Smokers n=20	Non smokers n=20	T	P
	Mean SD	Mean SD		
Volume (ml)	4.95 ± (1.75)	4.62 ± (1.95)	0.548-	0.587
Concentration (million / ml)	61.46 ± (11.27)	67.56 ± (14.77)	1.469	0.150
Grad a motility (%)	39.41±(6.291)	54.00 ± (5.58)	7.761	0.000**
Grade a+b motility (%)	49.69 ± (6.56)	60.00 ± (4.79)	5.678	0.000**
Velocity (µm/sec)	67.9500 ± (7.96)	78.156 ± (8.97)	3.806	0.001**
Liner velocity (µm/sec)	51.89 ± (8.47)	61.52 ± (8.07)	3.679	0.001**
Linearity index (µm/sec)	76.11 ± (5.44)	78.71± (5.85)	1.454	0.154
Normal morphology (%)	52.35 ± (6.97)	63.10 ± (1.77)	6.693	0.000**

Table (3): Semen parameters of smokers in the studied groups.

Semen parameter	Smokers with abnormal seminogram (no varicocele) n=20	Smokers with normal seminogram (no varicocele) n=20	T	P
	Mean SD	Mean SD		
Volume (ml)	5.07 ± (1.56)	4.95 ± (1.75)	0.227	0.822
Concentration (million / ml)	24.52 ± (5.89)	61.46 ± (11.27)	12.994	0.000**
Grad a motility (%)	20.09 ± (4.35)	39.41 ± (6.291)	11.305	0.000**
Grade a+b (%)	34.45 ± (6.38)	49.6890 ± (6.56)	7.446	0.000**
Velocity (µm/sec)	37.60 ± (9.39)	67.9500 ± (7.96)	11.021	0.000**
Liner velocity (µm/sec)	23.26 ± (6.30)	51.8890 ± (8.47)	12.129	0.000**
Linearity index (µm/sec)	61.82 ± (4.92)	76.1145 ± (5.44)	8.715	0.000**
Normal morphology(%)	23.11 ± (5.29)	52.3500 ± (6.97)	11.586	0.000**

Table (4): Comet assay in groups with abnormal seminogram

Comet assay	Smokers (no varicocele) n=20	Smokers with varicocele n=20	Non smokers (no varicocele) n=20	T P1	T P2
	Mean SD	Mean SD	Mean SD		
% DNA fragmentation	23.70 ± (4.62)	33.85 ± (6.80)	9.80 ± (3.61)	10.600 0.000**	5.520 0.000**

Table (5): Comet assay in control groups with normal seminogram.

Comet assay	Smokers (no varicocele) n=20	Non smokers (no varicocele) n=20	T	P
	Mean SD	Mean SD		
% DNA fragmentation	9.80 ± (3.61)	4.15 ± (1.23)	6.633	0.000**

Table (6): Effect of smoking index on sperm DNA fragmentation in studied groups

Smoking index	Smokers with abnormal seminogram n=20	Smokers with normal seminogram n=20	T	P
	Mean SD	Mean SD		
Low	23.66 ± (4.60)	8.45 ± (3.91)	8.5	0.001**
Moderate	24.6 ± (4.93)	11.4 ± (2.51)	6.7	0.001**
Heavy	26.33 ± (5.77)	- -	-	-

Table (7): Percentage of sperm DNA fragmentation of the studied groups.

			Abnormal seminogram		Normal seminogram			Total
			Smokers (no varicocele)	Smokers with (no varicocele)	Non Smokers varicocele	Smokers (no varicocele)	Non Smokers (no varicocele)	
Comet assay	<15	Count % within group			20 100.0%	20 100.0%	20 100.0%	60 60.0%
	15-		20 100.0%	6 30.0%				26 26.0%
	30-			12 60.0%				12 12.0%
	>=45			2 10.0%				2 2.0%
Total			20 100.0%	20 100.0%	20 100.0%	20 100.0%	20 100.0%	100 100.0%

DISCUSSION

Diagnosis of male infertility has mainly been based on the semen parameters; sperm concentration, motility and morphology. It has become apparent that none of these parameters are reliable markers for evaluation of the fertility potential of a couple[16]. A search for better markers has led to an increased focus on sperm chromatin integrity testing in fertility work-up and assisted reproductive techniques. During the last couple of decades, numerous sperm DNA integrity tests have been developed[17].

The quality of sperm DNA is very important in maintaining the reproductive potential of men. Sperm DNA is being recognized as an independent measure of sperm quality that may have better diagnostic and prognostic capabilities than standard sperm parameters[9].

There are several causes of DNA damage; mainly smoking, varicocele, oxidative stress, histone-protamine ratio, radiation and high temperature [18].

Cigarette smoking is a widely recognized health hazard, yet despite worldwide antismoking campaigns, some people continue to consume cigarettes on a regular basis, and the highest prevalence of smoking is observed in young adult males during their reproduc-

tive period[19]. It has been suggested that cigarette smoking affect every system involved in the reproductive process.[20].

It was observed in the present study that smoker had lower sperm count, motility and morphology in both fertile and infertile groups.

Concerning the sperm count, it was lower in smoker versus the non-smokers. The difference was statistically significant in infertile groups. This is more or less in agreement with findings of Kunzel et al.[21] and Reina Bouvet et al.[22] who reported that smokers had significantly lower sperm count versus nonsmokers in infertile men. Our result was also in agreement with finding that heavy smokers show statistically significant lower sperm concentration compared to mild and non smoker's[19].

Concerning the motility it was reported in the present work that there was very high statistically significant drop in the percentage of motile sperm with in smokers if compared with non-smokers. In agreement with our finding Hassa et al.[23] had reported that cigarette smoking was associated with statistically significant decrease in the total number of motile sperms.

As regard the effect of smoking on sperm morphology, it was observed in the present work that smokers had high statistically significant elevated percentage of sperm with abnormal forms. Our results support the works of Reina Bouvet et al.[22] and Niu et al.[24] who concluded that smokers had statistically significant higher number of abnormal sperm morphology versus nonsmokers. Chi et al.[25] reported that smokers had high percent of head-piece sperm defects versus non smokers. Gaur et al.[26] mentioned that heavy smoking produces teratozoospermia.

Nicotine and its metabolites have been implicated to mediate the harmful effects of smoking on sperm parameters. Also, increased copper and lead loading in smoking subjects' mediat oxidation in seminal plasma have been implicated[27].

Oxidative stress produced by some cigarette smoking components could be one of the mechanisms involved in the change of standard sperm parameters and spermatozoon nuclear quality[28].

The functions of accessory sex glands were reduced significantly in smokers. Detached ciliary tufts (DCTs) were observed in the semen of men associated with a high incidence of smoking. It has been postulated that DCTs originate from the epididymal epithelium, possibly shed as part of epididymal involvement as a result of testicular pathology caused by several agents. In addition, seminal mast cells were detected at higher frequency among smokers[20].

On the other hand our results regarding semen paramerers contradict with those of Oldereid et al.,[29] and Trummer et al.,[30] who reported no statistically significant difference in sperm concentration, morphology and motility between non-smokers, ex-smokers and smokers. This difference may be due to the difference in type, duration, number of cigarette smoking and method used for semen analysis between our study and the above mentioned works.

In the present study the Comet assay in the groups with normal and abnormal seminograms showed that the mean values of sperm DNA fragmentation were significantly higher in smokers than non smokers. Also the mean percentage of DNA fragmentation of smokers with abnormal seminogram was significantly higher than those of smokers with normal seminograms at all degrees of smoking index light, moderate or severe.

Many studies reported that exposure to smoking; environmental industrial toxins, genetics and oxidative stress are known to cause sperm DNA fragmentation and infertility[31-32].

Belcheva et al.[7] reported a significant increase of population of apoptotic spermatozoa in ejaculates of smokers. They also reported some degree of DNA damages with high frequencies of double- and single- stranded breaks in the spermatozoa of smokers compared

with non smokers. However, this difference is not statistically significant. Sperm DNA integrity of healthy smokers remains in the normal range.

Ereinpeiss et al.[33] also reported a significant proportion of men with otherwise normal semen parameters but with high sperm DNA damage.

Moreover, in agreement with our result, Niu et al. [24] who observed that smokers have a significantly higher percentage of spermatozoa with DNA fragmentation than non smokers These findings support the suggestion that smoking could have impact in male reproduction that is not apparent in the usual parameters of semen analysis [31].

On other hand Sergerie et al.,[34] using TUNL technique for detection of DNA fragmentation, they had reported that the percentage of DNA fragmentation was not statistically different in heavy, light and non smokers. Their results concluded no association between smoking and DNA fragmentation of spermatozoa of healthy men. This controversy to our results may be due to different techniques used for detection of DNA fragmentation. In present study, comet assay was used which is sensitive, simple and can detect non uniform responses within a mixed population [35].

It was observed in the present study that the mean values of DNA fragmentation in the spermatozoa of the groups with abnormal seminogram were increased significantly in both smokers with varicocele and smokers without varicocele compared with non smokers. Moreover, the difference in sperm DNA fragmentation was statistically significant higher in smokers with varicocele than smokers without varicocele.

Similar to our results, Smith et al. [36] found sperm DNA fragmentation in patients with varicoceles. They concluded that the presence of varicocele is associated with high levels of DNA fragmentation even in the presence of normal semen profile. Moreover, Blumer et al.[37] reported that men with varicocele show highly significant sperm DNA fragmentation. Also,

significantly decreased percentages of spermatozoa with abnormal chromatin and spermatozoa with DNA fragmentation after varicocelectomy were reported by La vignera et al.[38].

Oxidative stress can occur when there is an imbalance between the production of ROS and the scavenging ability of antioxidants in the seminal plasma [39]. The presence of oxidative stress in infertile men suggests its role in the pathophysiology via several mechanisms acting in synergisms which impair the sperm characteristics and functional capacity[40]. In the present study, a higher percentage of more than 30% of the spermatozoa with DNA fragmentation assessed by Comet was detected only in the infertile groups with abnormal seminogram in smokers with varicocele.

Sperm DNA damage is clearly associated with male infertility and abnormal spermatogenesis, but small percentage of spermatozoa from fertile men also possess a detectable levels of DNA damage[41]. The etiology of sperm DNA damage is multifactorials; it may be due to intrinsic (eg .protamine defficiency ROS and apoptosis) or extrinsic factors which include, cigarette smoking, genital tract inflammation, testicular hyperthermia and varicoceles[42]. About 40% of idiopathic infertility had high levels of sperm DNA fragmentation [43].

Mostafa et al., [44] who reported that recent studies at molecular levels have demonstrated that varicocele can cause testicular DNA damage, apoptosis and raised levels of reactive oxygen species.

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EFFECT OF CIGARETTE SMOKING ON SPERM DNA FRAGMENTATION IN MEN WITH NORMAL AND ABNORMAL SEMINOGRAM

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INTERNAL ILIAC ARTERY LIGATION FOR INTRACTABLE POSTPARTUM HEMORRHAGE.
IS IT A LIFE SAVING PROCEDURE?

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Abstract

Background: *Postpartum hemorrhage (PPH) is a major cause of worldwide maternal mortality ranging from 13% in developed countries to 34% in developing countries. Uterine atony is the commonest cause of life threatening obstetric hemorrhage. In women not responding to medical treatment. The traditional surgical treatment to perform an emergency hysterectomy. Bilateral ligation of internal iliac (hypogastric) arteries (BIL) is an alternative life-saving operation.*

Patients and Methods: *This study was conducted in Mansoura University hospital from 2008 to 2012. Thirty women presented by postpartum Hemorrhage where included in the study during this period. All operative procedures were performed by the same consultant team of obstetrician and surgeon. All patients had bilateral ligation of internal iliac artery, if bleeding does not stopped & continued unabated decision for hysterectomy was taken.*

Results: *Seventeen patients (57%) had vaginal delivery and 13 patients (43%) had caesarean section. All patients had sings of hypovolaemic shock. Twenty patients are multipara and ten patients are primiparous. Their age rage from (19-40 years) Mean 27+4.5 years. Three were three maternal deaths in out study. Hysterectomies were done despite Bilateral IIAL in nine patients. There were liver impairment in nine patients and renal impairment in four patients.*

Conclusion: *Bilateral internal iliac artery ligation is safe and effective procedure in treatment of post partum hemorrhage, prevent hyster-*

ectomy in women with uterine atony and facilitates repair or hysterectomy when indicated in traumatic PPH.

Key words: *internal iliac artery ligation, postpartum hemorrhage, Hysterectomy.*

Introduction

Postpartum hemorrhage (PPH) is a major cause of worldwide maternal mortality ranging from 13% in developed countries to 34% in developing countries[1]. It is reported to be responsible for over 125 000 maternal deaths each year and is associated with morbidity in 20 million women per year[2].

Classically. PPH has been defined as a blood loss greater than 500 ml after a vaginal delivery and greater than 1000 ml. after a cesarean section[3].

A more useful and accepted definition of PPH is defined as blood loss sufficient to cause hypovolemia, a 10% drop in the hematocrit or requiting transfusion of blood products (regardless the route of delivery)[4]. PPH of this nature may occur in 4% of vaginal deliveries and up to 6% of cesarean deliveries in developed countries[5].

Uterine atony accounts for 80% of cases of PPH. Other causes include retained placental fragments, Lower genital tract lacerations and uterine rupture[6].

When PPH continues despite aggressive medical treatment. Early consideration should be given to surgical intervention. The choice of procedure will depend on the parity of the women and her desire for childbearing. The extent of hemorrhage and, most importantly, the experience and judgment of the surgeon [7].

Uterine atony is the commonest cause of life threatening obstetric hemorrhage. In women not responding to medical treatment. the traditional surgical treatment to perform an emergency hysterectomy eliminating any possibility of future fertility. Bilateral ligation of internal iliac (hypogastric) arteries (BIL) is an alternative life-saving operation, which preserves reproductive capacity [8].

Internal iliac artery ligation does not produce pelvic ischemia, it merely converts the high pressure arterial flow in the pelvic arteries into a sluggish venous-like flow, allowing clotting and haemostasis; and successful pregnancies have been reported after the procedure[9].

Following ligation of internal iliac artery, there is a reduction of 85% in pulse pressure and 48% in the blood flow in the arteries distal to the ligation. Thereby the arterial pressure approaches the venous pressure and is rendered more amenable to hemostasis by a simple clot formation[10].

The aim of this study is to describe the effectiveness of internal iliac artery ligation for control of life threatening post partum hemorrhage.

Patients and Methods

This study was conducted in Mansoura University hospital from 2008 to 2012.

Thirty women presented by postpartum Hemorrhage where included in the study during this period.

All operative procedures were performed by the same consultant team of obstetrician and surgeon

Women with atonic PPH at vaginal delivery or caesarean section were initially treated with massage and uterotonics such as oxytocin infusion and carboprost injections 250 mg at appropriate intervals. Failure to restore the uterine tone and arrest the blood loss despite these measured led to the decision to do BIIAL ligation.

All the patients in the study had BIIAL by transpertioneal approach.

The women were placed in the semi-lithotomy position as it permitted assessment of vaginal bleeding. When quick access was required in high-risk situations like rupture Uterus or shock, a midline infraumbilical incision was preferred. Otherwise, a Pfannenstiel incision was used. The uterus was pulled out of the abdominal incision and bowels were packed away. After identifying

the ureter at the pelvic brim, the peritoneum was incised starting from a point just proximal to the bifurcation of the common iliac artery proceeding caudally along the external iliac artery for a length of about 8-cm. Using gentle finger dissection, the ureter was retracted medially exposing the retroperitoneal anatomy. Using a long haemostat, the fascia around the internal iliac artery was dissected completely to free the artery from its adjacent structures and to clarify the anatomical relationship of the internal iliac artery with the iliac veins. A right-angled clamp was passed beneath the internal iliac artery from lateral to medial side about four cm distal to its origin. Using a nonabsorbable No-O silk suture the internal iliac artery was double ligated without division.

The procedure is repeated on the other side. All patients had bilateral ligation of internal iliac artery, if bleeding does not stopped & continued unabated decision for hysterectomy was taken.

Results

Seventeen patients (57%) had vaginal delivery and 13 patients (43%) had caesarean section. All patients had sings of hypovolaemic shock. Twenty patients are multipara and ten patients are primiparous. Their age rage from (19 - 40 years) Mean 27 + 4.5 years.

Hysterectomies were done in our study despite Bilateral IIAL in nine patients. There were liver impai-rment in nine patients and renal impairment in four patients. There were three maternal deaths in out study.

Table 1: Patients Characters.

No	Age	Gravidity	Parity	BP	Pulse	Temp	RR	Delivery
1	22	2	2	Cant be assessed		36.6	30	Vag
2	29	3	3	60/30	140	36.9	26	CS
3	30	4	4	50/20	175	36.7	29	Vag
4	21	2	2	70/30	132	37.2	22	Vag
5	24	2	1	65/20	140	37	23	CS
6	35	3	2	Cant be assessed		36.7	30	Vag
7	26	1	1	55/25	160	37	26	CS
8	19	1	1	70/30	133	36.8	25	CS
9	32	3	3	40/15	160	36.9	27	Vag
10	22	1	1	60/25	125	37	22	Vag
11	20	1	1	Cant be assessed		36.3	9	Vag
22	27	3	3	50/20	168	36.9	27	CS
13	29	2	2	Cant be assessed		36.7	8	Vag
14	36	3	2	60/20	134	37	20	CS
15	38	2	2	70/30	119	37.3	20	CS
16	25	1	1	Cant be assessed		36.5	10	Vag
17	40	8	5	70/40	115	37.4	20	CS
18	19	1	1	40/10	164	36.3	32	Vag
19	22	2	2	Cant be assessed		37	28	Vag
20	24	1	1	70/30	120	37.2	25	CS
21	26	2	2	50/20	143	36.5	27	CS
22	30	3	2	Cant be assessed		36.1	26	Vag
23	28	2	2	50/20	136	36	28	Vag
24	20	1	1	Cant be assessed		36.6	30	CS
25	32	4	3	60/30	140	36.9	26	Vag
26	33	3	3	50/20	175	36.7	29	Vag
27	19	1	1	70/30	132	37.2	22	Vag
28	24	3	3	65/20	140	37	23	CS
29	28	3	2	70/30	123	36.5	22	CS
30	30	2	2	90/40	130	37	23	Vag

(BP. Blood Pressure, RR. Respiratory Rate, Vag. Vaginal, CS.Caeserean Section)

Table 2 : Operative details complications outcome.

Cause	Blood Transfusion	IIAL	Hyster-ectomy	Hospital stay	Fetal outcome	Maternal outcome	Complications
Rup uterus	5L	Yes	Yes	14 days	dead	live	Liver impairment
Cx tear	3L	Yes	No	2 days	live	live	
Atony	7L	Yes	Yes	10 days	live	Dead	Liver and renal failure (dead)
Atony	2.5	Yes	No	3 days	live	live	
Vag, cx tear	4	Yes	No	5 days	live	live	
Rup uterus	14	Yes	No	8 days	live	live	
Uterine incision	12.5	Yes	No	6 days	live	live	Renal impairment
Cx tear	12	Yes	No	5 days	live	live	
Rup uterus	4	Yes	Yes	14 days	dead	live	
Vag, cx tear	13	Yes	No	5 days	live	live	
Rup uterus	6	Yes	Yes	20 days	live	live	Liver and renal impairment
Uterine incision	3.5	Yes	No	8 days	live	live	
Atony	2.5	Yes	No	7 days	live	live	Liver impairment and ARF (CRF)
Vag, cx tear	3	Yes	No	6 days	live	live	
Rup uterus	3.5	Yes	No	2 days	live	live	
Cx tear	3	Yes	No	10 days	live	live	
Atony	4	Yes	Yes	9 days	live	live	
Vag, cx tear	4	Yes	No	12 days	Dead	live	
Atony+vag tear	5	Yes	Yes	16 days	ICH	live	
Vag, cx tear	3	Yes	No	11 days	live	Dead	Liver impairment and ARF (dead)
Atony	3	Yes	No	5 days	live	live	
Cx tear	4	Yes	No	9 days	live	live	Live failure (ischemic hepatitis)
Vag, cx tear	2.5	Yes	Yes	10 days	HIE	live	
Rup uterus	6	Yes	Yes	14 days	dead	live	
Atony	3	Yes	No	2 days	live	live	Viral hepatitis
Rupture uterus	4	Yes	Yes	10 days	live	live	Liver impairment
Vag, cx tear	3	Yes	Yes	3 days	live	live	
Atony	3.5	Yes	No	5 days	live	Dead	Liver and renal failure (dead)
Cx tear	2	Yes	No	4 days	live	live	
Atony	3	Yes	No	3 days	live	live	

(CX. Cervical, Vag. Vaginal)

Table 3: Timing of ligation of IIA

Timing of IIAL	Number of women
At caesarean section	11
Laporatomy after vaginal delivery	17
Re-Laboratory after caesarean section	2

Discussion

There is extensive collateral circulation in the pelvis both between branches of the two-hypogastric trunks, and from the systemic circulation. In the absence of severe local or systemic vascular disease. Serious pelvic ischemia is unlikely to be produced by internal iliac ligation, even with the ligature above the point of division into the two trunks[11].

Kelly[12] first performed ligation of internal iliac artery with a success rate 95% and without any major complication. Mukherjee et al [13] performed 36 cases of internal artery ligation with a success rate of 83.3% in 6 years.

Burchell and Olson[14] showed that within seconds of ligation, blood enters the iliolumbar. Lateral sacral and middle haemorrhoidal arteries in a retrograde fashion. From the lumbar, middle sacral and superior haemorrhoidal vessels. The internal iliac artery immediately below the ligation will still contain blood, but the pressure is markedly diminished owing to the small caliber of the contributing vessels, and clots can form and remain in the bleeding vessel.

When uterus is preserved ligation of these arteries does not hamper future reproductive function [10]. Wagarrachchi and Fernando [15] observed future pregnancy in 50% of the cases following bilateral ligation of internal iliac artery.

There were three maternal deaths in our study one due to Rupture uterus, one due to atony & one due to cervical and vaginal tears all of these patients dies due to delayed interference because they referred late to Mansoura University hospital.

In our study, there were nine hysterectomies despite of BIIAL due to massive and unstopped bleeding

IIAL not only contributes to the prevention of hysterectomy but also in cases where hysterectomy cannot be prevented, it facilitates hysterectomy as in cases of uterine trauma [16]. IIAL Performed at the outset decreases the bleeding, clears the operative field and thus enables the surgeon to avoid blindly clamping and ligating tissues submerged in a pool of blood. This is particularly helpful in reducing the risk of ureteric injury. IIAL also facilitates repair of vaginal lacerations that bleed profusely with each suture through the vaginal wall[17].

In our study, nine patients (30%) had Caesarean hysterectomy after internal iliac artery ligation. This is less than Evans and Mc Shane experience where 8 out of 14(57%) of their patients required a hysterectomy after internal iliac artery ligation[18]. The immediate formation of collateral circulation on the pelvic wall contributes to failure of control of the bleeding and equal to Elsheikh and Elfadul (19)study where they reported 29% hysterectomies after IIAL (4 patients out of 14).

Of the 17 patients we were able to follow up ten patients had pregnancy and seven patients did not desire any more children and using contraception.

Conclusions

Bilateral internal iliac artery ligation is safe and effective procedure in treatment of post partum hemorrhage, prevent hysterectomy in women with uterine atony and facilitates repair or hysterectomy when indicated in traumatic PPH.

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INTERNAL ILIAC ARTERY LIGATION FOR INTRACTABLE POSTPARTUM HEMORRHAGE.
IS IT A LIFE SAVING PROCEDURE?

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ROLE OF SUPPRESSOR GENES AND ANGIOGENIC FACTORS IN HEPATOCELLULAR CARCINOMA IN CIRRHOTIC PATIENTS

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Abstract

Objective: *Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy and the third cause of cancer related deaths in the world. Genetic and epigenetic alterations accumulate under the effect of risk factors (viral infections, aflatoxin exposure.etc). Mutation of p53 gene and overexpression of VEGF are among these important alterations. The aim of this work is to correlate expression of p53 and VEGF in HCC tissue arising in cirrhotic patients with clinicopathological parameters.* ***Materials and Methods:*** *This study was conducted on 70 cases of HCC arising on top of cirrhosis and selected from surgical pathology files of gastroenterology center in Mansoura University during the period from 2008 to 2011. Immunohistochemistry for P53 and VEGF was performed. Nuclear staining for P53 and cytoplasmic staining for VEGF was considered positive.* ***Results:*** *40% of cases were P53 positive. There was statistically positive correlation between age, size and grades of p53 staining (p=0.001). There was significant positive correlation between tumor grade and degree of P53 staining (p=0.000). All cases show negative staining in surrounding cirrhotic tissue. 91.4% of cases were VEGF positive. There was positive correlation between size and VEGF positivity (p=0.355).There was statistical significance between different grades of the tumor and grades of VEGF staining (p=0.003).* ***Conclusions:*** *There was positive correlation between p53 expression and VEGF expression and age of patient, size of the tumor and grade. This should be included in the strategies of target therapy. Absence of p53*

expression in surrounding cirrhotic tissue make it useless as a surveillance marker of high risk cirrhotic patients while high expression of VEGF in surrounding cirrhotic tissue makes it good candidate for surveillance for high risk group patients.

Key Words: *HCC- P53- VEGF- Immunohistochemistry.*

Introduction

Hepatocellular carcinoma is the most common primary hepatic malignancy of adults and the third most common cause of cancer-related deaths[1]. It is a tumor of interest to investigators in many fields, especially to those interested in the pathogenesis of human cancer[2]. There is a wide difference in geographical distribution of HCC with the highest incidence in Asia and sub-Saharan Africa and lowest in USA and Western Europe[3]. The incidence has been doubled in recent years attributed to the rising incidence of hepatitis infection and fatty liver disease related cirrhosis [2].

Hepatocellular tumorigenesis is a DNA disease due to the accumulation of alterations in the genes that control the cell cycle and cell proliferation, as well as, a large number of genetic and epigenetic alterations accumulate during this process. Classically, in the initiation of carcinogenesis, the different risk factors (viral infections, cirrhotic lesions, obesity, aflatoxin exposure, haemochromatosis,.....) contribute to promoting the occurrence of gene alteration in hepatocytes. Then, tumor development process will select the altered hepatocyte with the highest capacity to survive and proliferate. These alterations together with other pathways, such as angiogenesis, are the bases for the carcinogenic process [4].

P53 gene is an important tumor suppressor gene that plays important roles in controlling the cell cycle, cell growth, division and apoptosis by acting as a transcription factor or by forming complexes with other proteins. P53 mutation is frequently detected in HCC. It is associated with lower cellular differentiation and poor prognosis [5]. Abnormalities of p53 are also considered a predisposing factor for hepatocarcinogenesis.

Hence, restoration of a wild-type p53 gene is an attractive approach to the treatment of HCC [6].

Angiogenesis is critical for the growth and progression of various human solid tumors because it enables delivery of oxygen and nutrients [7]. Angiogenesis is regulated by angiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietins, which can be secreted by tumor cells. VEGF has emerged as the central regulator of the angiogenic process in physiological and pathological conditions. VEGF, also known as vascular permeability factor, stimulates the proliferation of endothelial cells [8].

Hepatocellular carcinoma is one of the most vascular solid tumors, in which angiogenesis plays an important role in its development, progression, and metastasis[9]. Significant HCC growth is dependent on angiogenesis, and an increase in tumor dimension beyond 0.5 mm will induce the proliferation of vascular endothelial cells. It has been shown that VEGF expression significantly increases during the course of liver fibrosis through cirrhosis till HCC development[10]. Antiangiogenic strategies that target angiogenic molecules and inhibit activation of various types of cells that participate in the angiogenic processes become potential therapies for HCC [11].

The aim of this work is to study the expression of P53 and VEGF in HCC tissue and surrounding cirrhotic tissue using immunohistochemistry on liver resection specimens and correlation between these molecular markers and clinicopathological parameters.

Materials and Methods

The study was conducted on 70 cases received in the Mansoura Gastroenterology Center pathology lab. 57 cases were retrospective from 2008 to 2009 and the remaining 13 were collected during the period of the study from 2010 to 2011. All the cases were HCC on background of cirrhosis. Cases arising in normal livers, associated with bilharizial fibrosis only with no evident cirrhosis, or received previous adjuvant therapy or chemo-embolization were

excluded. Resection specimens, as well as total hepatectomy specimens from the patients underwent liver transplant surgery, were used in the study. No Tru cut biopsy material was used. 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[12] and included age, gender, liver pathology (cirrhosis), tumor size (<5 cm vs ≥ 5cm), tumor grade, vascular invasion, and multiplicity[10]. Sixty patients were males and ten patients were females with age ranging from 44 to 69 years (mean =55.06 years). All patients were HCV positive confirmed by PCR.

Immunohistochemical staining:

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. Antigen retrieval was performed by using 0.01 M citrate buffer in case of P53 for 10m, and by EDTA in case of VEGF for 10m. The slides were then incubated overnight with monoclonal anti- p53 (1:150 dilution, Invitrogen; code No.18-7251), and for 1 hour for mouse polyclonal anti VEGF antibody (1: 100 dilution, Invitrogen code No. 726127A). This was followed by biotin-conjugated goat anti-mouse immunoglobulin and horseradish peroxidase-conjugated streptavidin (UltraVision Detection system) DAP was used as chromogenic substrate. The brown precipitate was identified as positive staining. Positive staining of p53 with nuclear localization were graded by counting the number of positive nuclei in 8- 10 randomly chosen medium power (20 x magnification) fields; (-) no positive cells, (+) < 30 %, (++) = 31%- 70% and (+++) > 71% (13). For VEGF cytoplasmic staining of more than 10% of the tumor cells were defined as positive, the intensity of immunoreactivity was graded as weak (+) moderate(++) and marked(+++)[8]. The expression of both markers was correlated with positive, (cancer breast for P53 and cancer colon for VEGF), and negative controls. The expression of both markers was correlated with clinicopathological data collected. For this purpose the patients were divided

into two age groups (< 60 years &≥ 60 years), size (< 5cm & ≥ 5cm), and tumors were graded according to Edmonson and Steiner four tiered grading system [14, 2].

Statistical analysis of data was done by using Excel program and SPSS program (statistical package of social science). Descriptive data are in the form of mean ±standard deviation and frequency of cases. Analytic data was done by comparing groups using Chi- square test and Pearson test for correlation. P is significant if <0.05.

Results

Immuno histochemical expression of p53:

There were forty two (60%) P53 negative cases that showed no evidence of staining or staining of cell nuclei less than 10% (Figure: 1) . Twenty eight cases (40%) were positive. Eight cases (8.6%) were (+) (Figure: 2) four (5.7%) were (++) = (Figure: 3) while eighteen cases (18) (25.7%) were (+++) (Figure: 4). There was negative staining of p53 in the surrounding cirrhotic tissue in all seventy cases (Figure: 5).

As shown in table 1; seventy one per cent of patients 60 years or more show positive staining of p53 and 57.1% of them show high degree of the stain (+++), in contrast to 32.1% of patients under 60 years who were positive and only 17.9% of them show high grade of staining (+++). The difference in p53 positivity between two age groups was statistically significant (p=0.007) and there was statistically positive correlation between age and grades of p53 staining (p=0.001). As regard to gender most of positive P53 cases (22.9%) are male. Of these, fourteen (23.3%) are more than 70% positive (+++). There was no difference between male and female positivity for p53 (p=1.00). In relation to size, most positive p53 cases are 5cm or more in size with fourteen cases (63.6%) of them more than 70% (+++) positivity, however in tumors less than 5cm in size there are four cases (4) (18.2%) also show positivity more than 70% (+++). The difference in p53 positivity in two size groups was statistically insignificant (p=0.67). There was non statistically significant positive correlation between tumor size and grade

of p53 staining (p=0.29). Most of positive cases of p53 were GIII tumors. Fourteen (53.8%) of them are (+++) while four (15.4%) are (+) and only two (7.7%) are (++). GIV tumors showed six positive cases, four of them (66.7%) are (+++). Only two cases (5.6%) of GII tumors are positive with positivity from 10 - 30% of tumor cells (+). No cases of GI were positive for p53 staining. There was significant positive correlation between Tumor grade and degree of P53 staining (p=0.000).

Immunhistochemical expression of VEGF:

Immunohistochemical staining of the 70 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. There were six (8.6%) cases negative for VEGF (Figure: 6). Sixty four (91.4%) of cases were positive for VEGF; eight (11.5%) showed weak staining (+) (Figure: 7), thirty two (45.7%) were of moderate staining (++) (Figure: 8), twenty four (34.3%) showed marked staining (+++) (Figure: 9). In twenty two cases the stain was more intense at the periphery of the tumor than in the centre and in fourteen cases the stain was more intense in the surrounding cirrhotic tissue than the tumor itself (Figure:10).

Table 2 shows that, of patients under the age of 60 years, twenty six cases (46.4%) were moderately positive for VEGF (++), twenty case (35.7%) were markedly positive (+++) and four cases (7.1%) were weak (+). While in patients 60 years or more, six cases (42.9%) were moderately positive (++), four cases (28.6%) in both mild (+) and marked (+++) staining category. None statistically significant positive correlation between age and VEGF positivity was found (p=0.206). The difference between two age groups was statistically insignificant (p=0.2). Twenty six (43.3%) of male cases showed moderate intensity of staining (++) while twenty two cases (36.7%) showed marked intensity (+++). Six (60%), out of the ten (14.3%) female cases, showed moderate intensity (++). The difference between male and female

positivity of VEGF was statistically insignificant (p=0.296). As regard the size of the tumor, ten tumors less than 5cm (45.5%) showed moderate staining (++). Twenty two tumors (31.4%) of 5cm or more size showed (++) degree of staining, eighteen (25.7%) cases showed (+++) degree of staining while four (4) (5.7%) cases were weak (+). The VEGF positivity difference between two size groups was statistically insignificant (p=0.916). There was positive correlation between size and VEGF positivity (p=0.355).

Two GI tumors (100%) show marked degree of positivity for VEGF (+++). Twenty two cases (61.1%) of GII tumors, showed moderate degree of staining (++), while in GIII tumors, the largest number of cases (10) (38.5%) showed marked degree of staining (+++). Four cases (4) (66.7%) of GIV tumors showed marked degree of staining (+++). There was statistical significance between different grades of the tumor and grades of VEGF staining (p=0.003). There were increased number of cases of vascular invasion with increased intensity of VEGF expression and twelve (17.1%) cases showed marked degree of staining (+++). There was positive correlation between VEGF degrees of staining and vascular emboli (p=0.000).

Table (1): Correlation between p53 staining and clinicopathological features.

Clinicopathological feature	Expression of p53				Total
	Positive			negative	
	+ N (% of group)	++ N (% of group)	+++ N (% of group)	N (% of group)	
Age:					
<60	6(10.7%)	2(3.6%)	10(17.9%)	38 (67.9%)	56(100%)
≥ 60	0	2 (14.3%)	8 (57.1%)	4 (28.6%)	14(100%)
Gender:					
Male	6 (10%)	4 (6.7%)	14 (23.3%)	36(60%)	60 (100%)
Female	0	0	4 (40%)	6 (60%)	10 (100%)
Size:					
<5cm	4(18.2%)	0	4 (18,2%)	14 (63.6%)	22 (100%)
≥ 5cm	2 (4.2%)	4 (8,3%)	14 (29,2%)	28 (58,3%)	48 (100%)
Grade:					
GI	0	0	0	2(2.9%)	2 (100%)
GII	2(5,6%)	0	0	34 (94.4%)	36 (100%)
GIII	4(15.4%)	2 (7.7%)	14 (53.8%)	6 (23.1%)	26 (100%)
GIV	0	2 (33.3%)	4 (66.7%)	0	6 (100%)
Multiplicity	0	0	0	2(100%)	2 (100%)
Invasion of liver capsule	0	0	1(50%)	1 (50%)	2(2.9%)

Table (2): Correlation between VEGF staining and clinicopathological features.

Clinicopathological feature	Expression of VEGF				Total
	positive			negative	
	+ N (% of group)	++ N (% of group)	+++ N (% of group)	N (% of group)	
Age:					
<60	4(7.1%)	26(46.4%)	20(35.7%)	6 (10.7%)	56 (100%)
≥ 60	4(28.6%)	6 (42.9%)	4 (28.6%)	0	14(100%)
Gender:					
Male	6 (10%)	26(43.3%)	22 (36.7%)	6(10%)	60 (100%)
Female	2 (20%)	6 (60%)	2 (20%)	0	10 (100%)
Size:					
<5cm	4(18.2%)	10(45.5%)	6 (27.3%)	2 (9.1%)	22 (31.4%)
≥ 5cm	4(5.7%)	22 (31.4%)	18 (25.7%)	4 (5.7%)	48 (68.6%)
Grade:					
GI	0	0	2(100%)	0	2 (100%)
GII	0	22 (61.1%)	10 (27.8%)	4 (11.1%)	36 (100%)
GIII	6(23.1%)	8 (30.8%)	10 (38.5%)	2 (7.7%)	26 (100%)
GIV	2(33.3%)	0	4 (66.7%)	0	6 (100%)
Intrahepatic metastasis	0	1(50%)	1 (50%)	0	2 (100%)
Invasion of liver capsule	0	0	1(25%)	1 (25%)	2(100%)
Vascular empoli	2(7.7%)	10 (38.5%)	12 (46.2%)	2(7.7%)	26 (37.1%)

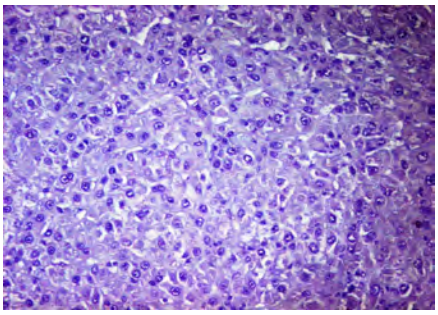

Fig. 1 : Showing negative staining of p53 in the tumor tissue.

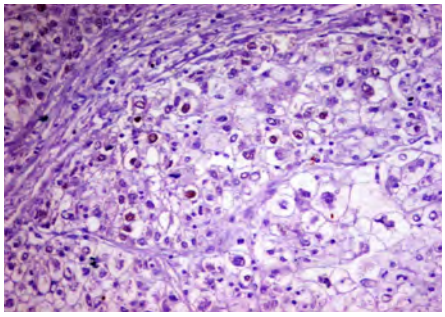

Fig. 2 : Showing mild staining of p53 10 - 30% of nuclei show positive stain.

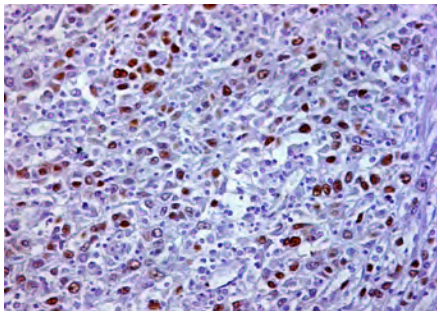

Fig. 3 : The stain of p53 is marked > 70 % 0f cells are positive.

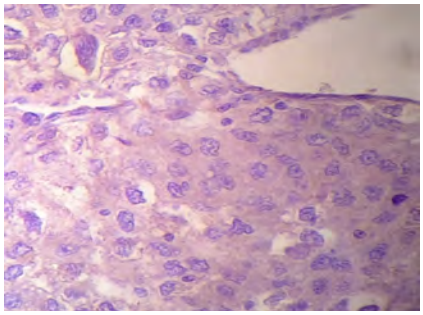

Fig. 5 : Showing negative VEGF in tumor tissue.

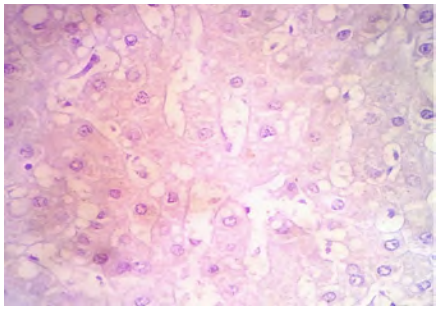

Fig. 4 : Showing negative cirrhotic tissue for p53.

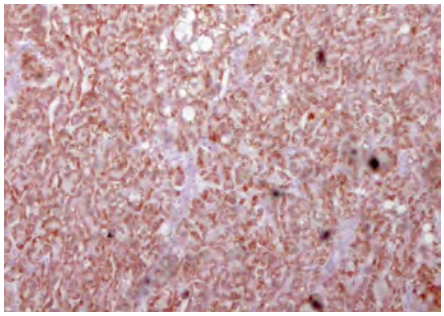

Fig. 6 : show the the intensity of VEGF stain is weak (+).

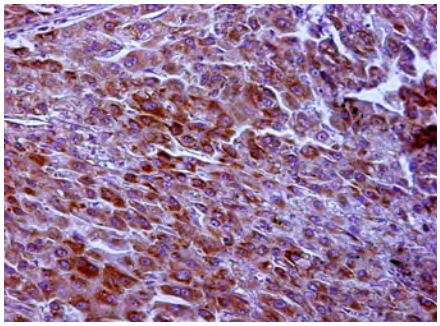

Fig. 7 : The stain of VEGF is moderate (++).

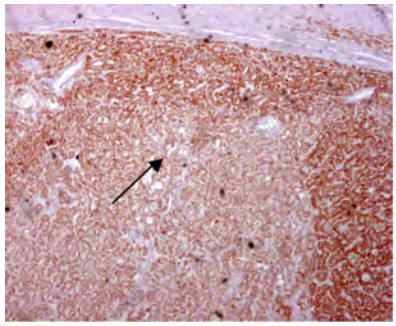

Fig. 9 : Showing increased intensity of theh VEGF stain at the periphery of the tumor more than the center area.

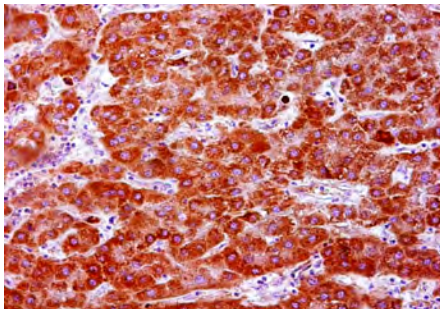

Fig. 8 : The Stain of VEGF is of marked intensity (+++).

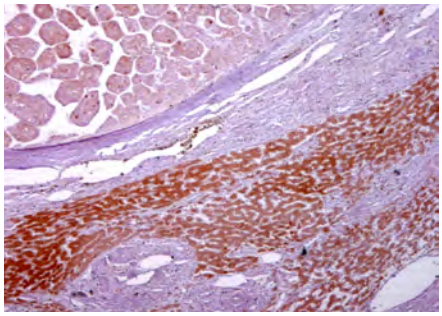

Fig. 10 : Showing intense VEGF stain in the surrounding cirrhotic tissue that HCC tumor tissue.

Discussion

P53 is an important member of apoptosis network; the mutant protein can be detected by immunohistochemical staining. Generally speaking, mutations of p53 were considered relatively late events in HCC development and correlated with tumor recurrence and prognosis [15].

Zhang et al,[16] reported that, several IHC-based studies have shown the proportion of p53-positive HCC cases to vary in the range of 24-69%. In this study, twenty eight cases (40%) were positive. This percentage lies within most of the reported percentages including that of Stroescu et al,[13] and Zhang et al,[16] and others. The cause for the variation in p53 expression may be partially due to the lack of a consistent way of measuring p53 positivity among different studies. Another cause of the discrepancy in the reported percentage of p53-positive tumors is the differences in the p53 mutation and expression according to the relevance of carcinogenic factors and certain unknown molecular mechanisms.

Goodman et al,[2] reported that, there is a direct correlation between Aflatoxin exposure and mutation of P53 especially G to T mutation. In addition, Sanyal et al,[17] mentioned the role of HCV NS2-5 proteins that cause sequestration of P53, inhibition of P21 WAF and induction of oxidative stress. In addition to direct interaction of HCV core protein with P53. In this study all the patients were HCV positive confirmed by PCR as well as there is high incidence of Aflatoxin exposure in the locality which explains the results of P53 positivity which is more toward the higher levels.

Ng et al,[18] reported that the presence of p53 mutations did not show a significant association with tumor size, gender, age, tumor invasiveness, microsatellites and venous permeation, cirrhosis and encapsulation. However, Stroescu et al, [13] mentioned other studies show strong association between p53 overexpresion and large tumors as well as post- resection recurrence.

In our study there was positive correlation between age and p53

positivity grades (p=0.001). On other hand, there was no significant difference between male and female positivity for p53 (p=1.00). There was positive correlation between tumor size and p53 degrees of staining (p=0.29).

Different studies the frequency of mutation of p53 tumor suppressor gene of HCC increased with the increased grading of the tumor[19]. In this study, There was significant positive correlation between the expression of p53 and the grade of the tumor (p=0.000) which is coincident with Tanaka et al, [19].

Different researches were performed to study the expression of biomarkers as p53 in the HCC carcinoma tissue and surrounding liver tissue in order to examine the ability to use these biomarkers for early detection of high risk cases[10]. In correlation with the surrounding cirrhotic tissue, in this study all seventy cases show negative surrounding cirrhotic tissue to p53 stain even with cases of high degree of positivity. This was also reported by Alves et al,[20] while studying expression of p53 in both HCC and surrounding cirrhosis in Brazilian patients which also considered of high incidence area. On contrary, Zhang et al,[16] reported the positivity of P53 in surrounding normal, not cirrhotic, liver tissue in their study but with lesser degree than HCC.

Deli et al,[10] reported that VEGF plays an important role in the angiogenesis of HCC. Studies have demonstrated that increasing expression of VEGF is correlated with aggressive behaviors and a poor prognosis of various human cancers including breast, gastric, esophageal and colorectal cancer. It is reported that expression of VEGF in HCC is correlated with cirrhotic liver.

There is increased level of VEGF in HCV- related HCC as a result of VEGF gene amplification[21,22]. As our cases are all HCV positive, so explain the high expression level of VEGF by 91.4%.

Moon et al,[8] stated that VEGF in HCC is correlated with tumor size. In this study, as regard the

size of the tumor, there was correlation between the size of the tumor and expression of VEGF keeping with Moon et al statement. There was positive correlation between the size and grade of staining with VEGF (P=0.355).

VEGF expression has a significant correlation with vascular invasion, and intrahepatic metastasis. Also positive VEGF expressions in HCC correlate with shortened survival rates [10]. In our study, there was correlation between the VEGF expression and vascular invasion, as there were increased number of cases of vascular invasion with increased intensity of VEGF expression as twelve (46.2%) cases showed marked degree of staining (+++). There was positive correlation between VEGF degree of staining and vascular emboli (0.000).

Yoo et al,[23] reported significant correlation between expression of VEGF and grades of HCC as increased expression in poorly differentiated tumors and decreased in well differentiated ones. There was also positive correlation between grades of the tumor and degree of VEGF expression in this study (p=0.003).

In this study, in twenty two cases (31, 4%), the stain was more intense at the marginal area of the tumor than in the centre. This was also reported by Deli et al, (10). This is 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.

It was reported that, there was significant higher expression of VEGF in the surrounding cirrhotic liver tissue than in HCC (10), a finding detected in our cases. This is as a result of sustained mechanically- reduced blood flow that affects the hepatocytes at the cirrhotic area. This leads to decreased oxygen pressure and strongly up- regulates VEGF transcription and protein synthesis in the cirrhotic area.

Conclusion

There was positive correlation

between p53 expression and VEGF expression and clinicopathological markers such as age of patient, size of the tumor and grade. Absence of p53 in surrounding cirrhotic tissue makes it unavailable for surveillance for high risk patients. VEGF show combined expression in both tumor tissue and surrounding cirrhotic tissue with intense stain in surrounding tissue in some cases. It can be used as marker for surveillance of high risk patients.

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ROLE OF SUPPRESSOR GENES AND ANGIOGENIC FACTORS IN HEPATOCELLULAR CARCINOMA IN CIRRHOTIC PATIENTS

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INTESTINAL PARASITES AMONG SCHOOL CHILDREN IN DAKAHLIA GOVERNORATE

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Abstract

This study was conducted to determine the prevalence of intestinal parasites in children attending schools in Dakahlia governorate along the period from October 2011 to January 2012. A total of 201 samples from children of 4 governmental schools (2 urban and 2 rural) were collected. Prevalence of intestinal protozoa by microscopic examination of stools were in order of frequency: Entamoeba histolytica (26.9%), Giardia (cyst & trophozoite) (17.9%), Giardia cyst (15.4%), E. coli cyst (7.5%), B. homimnis (4.5%), Giardia trophozoite (2.5%), and finally E. histolytica trophozoite (1.5%). There was no statistically significant differences between urban and rural areas except for E. coli cyst (14%, 0.99%) in rural and urban areas respectively ($p<0.001$), and Giardia trophozoite which was absent in urban areas while in rural areas, it was 5% ($p=0.023$). Regarding prevalence of intestinal helminthes in schools. Hymenolepis nana and S. mansoni were the highest (8.5%) each, followed by A. lumbricoides (6.5%), E. vermicularis (4.5%), and finally H. heterophyes (2.5%). There was a statistically significant difference between urban and rural areas regarding prevalence of H. nana being higher in rural children (14% versus 2.97%), also S. mansoni (15%, versus 1.98%) in rural and urban children respectively. While H. heterophyes was (4.95%) in urban children and absent in rural children. A. lumbricoides was 9% in rural and 3.96% in urban children. Impact of negative Giardia infection on children was proven as weight, height, and BMI of infected children with Giardia trophozoite were lower compared to controls. Height residual was significantly lower in patients infected with Giardia trophozoite ($p=0.003$). Also, height residual had a

significant regression in presence of S. mansoni infection. This means that patients with S. mansoni have a lower height for their ages. While weight residual had a significant regression in presence of undigested food, E. coli cyst, S. mansoni, and B. hominis. These results contribute to the growing evidence on the association between intestinal parasitic infection and growth retardation as intestinal parasitic infections contribute significantly to poor growth and malnutrition in children and indicate the need to implement control measures in form of regular deworming, health education and provision of safe water supply.

Introduction

Parasitic intestinal infections continue to be an important cause of morbidity and mortality in the developing world. Intestinal parasites are among the most common infections of school age children in developing countries. As a result of this morbidity, they are at risk of detrimental effects like poor cognitive performance and physical growth [1]. The purpose of this study was to find out the prevalence of intestinal parasitic infection in school children of Dakahlia as well as the impact of such infections on affected children.

Material and Methods

This study was conducted from October 2011 to January 2012 and included 201 schoolchildren attending 4 governmental schools (2 urban and 2 rural schools). A complete clinical sheet was taken from participants including symptoms suggestive of parasitic infection. General and abdominal examination was performed and stool samples were collected in screw capped, labelled plastic containers, which were distributed to patients one day prior to the day of collection. Stool samples were subjected for complete examination - gross and direct microscopic examination (saline and iodine preparation) within 1 to 2 h of its collection. Negative samples were re-examined after formal ether concentration method [5] and by special stains as Trichrome staining technique and modified Ziehl-Neelsen stain. Also Kato-Katz technique [8] was performed and cellophane tape was done for cases with a history of perianal itching.

Statistical Methods

Non-parametric statistical methods were used. Frequency, mean, standard deviation and standard error of mean were used to describe data. Chi-square test was used to test for association between parasitic infections and different risk factors. Regression analysis was used to calculate predicted height, weight and BMI for each child and residual of each variable was calculated (Residual = actual value/predicted value). This residual was compared in children with and without parasitic infections using Mann-Whitney μ test. Also, multiple linear regressions were used to test the contribution of different parasitic infections on these residuals. P value was considered significant if less than 0.05. These tests were run on an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS) for windows Ver. 13 (SPSS Inc., Chicago, IL, USA).

Results

Table (1): Prevalence of intestinal protozoa and helminthes among studied school children

Protozoa	*(%)*	*Helminthes*	*(%)*
***E. histolytica* cyst n=54**	26.9	***H. nana n=17***	8.5
***E. histolytica* trophozoite n=3**	1.5	***H. heterophyes n=5***	2.5
***E. coli* cyst n=15**	7.5	***S. mansoni n=17***	8.5
***Giardia* cyst n=31**	15.4	***E. vermicularis n=9***	4.5
***Giardia* trophozoite n=5**	2.5	***A. lumbricoides n=13***	6.5
***Giardia* (cyst & trophozoite) n=36**	17.9		
Blastocystis hominis n=9	4.5		

Table (2): Prevalence of intestinal protozoa and helminthes among urban and rural school children.

	Urban		Rural		χ^2	P
	n	%	n	%		
***E. histolytica* cyst**	28	27.7	26	26.0	0.076	0.783
***E. histolytica* trophozoite**	2	1.98	1	1.0	0.328	0.567
***E. coli* cyst**	1	0.99	14	14.0	12.316	<0.001
***Giardia* cyst**	18	17.8	13	13.0	0.896	0.344
***Giardia* trophozoite**	0	0.0	5	5.0	5.179	0.023
***Giardia* (cyst & trophozoite)**	18	17.8	18	18.0	0.001	0.974
Blastocystis hominis	4	3.96	5	5.0	0.127	0.722
H. nana	3	2.97	14	14.0	7.895	0.005
H. heterophyes	5	4.95	0	0.0	5.077	0.024
S. mansoni	2	1.98	15	15.0	11.002	0.001
E. vermicularis	4	3.96	5	5.0	0.127	0.722
A. lumbricoides	4	3.96	9	9.0	2.110	0.146

Table (3): Height residual in non-infected versus infected children with *Giardia* trophozoite and *S.mansoni*:

Height residual	*Giardia trophozoite*	*S. mansoni*
-ve (n=196)		*-ve (n=184)*
Mean	**0.047**	**0.123**
Std. Deviation	**1.360**	**1.358**
Std. Error Mean	**0.097**	**0.100**
+ve (n=5)		*+ve (n=17)*
Mean	**-1.844**	**-1.331**
Std. Deviation	**1.5777**	**1.061**
Std. Error Mean	**0.705**	**0.257**
t	**3.058**	**4.293**
p	**0.003**	**<0.001**

Table (5): Results of multiple linear regression of height residual on *S. mansoni* infection.

Variable	*Partial R*	*Standard error of partial R*	*t*	*p*
S. mansoni	-1.454	0.339	-4.293	<0.001
Constant	0.123	0.099	1.429	0.213

R: Regression coefficient.

Table (6): Results of multiple linear regression of weight residual on other variables.\

Variable	*Partial R*	*Standard error of partial R*	*t*	*p*
Undigested food	0.759	0.309	2.456	0.015
E. coli cyst	0.931	0.344	2.703	0.007
S. mansoni	-0.831	0.326	-2.551	0.012
B. hominis	0.939	0.436	2.153	0.033
Constant	-0.113	0.102	1.110	0.268

R: Regression coefficient

Discussion

In Egypt, parasitic diseases constitute a common health problem especially in children that leads to malnourishment and impairment in physical and mental development [16].

Community based studies of parasitic diseases often focus on pediatric population. Previous studies found a consistent correlation between rates of infection in children and its rates in adult population thus; it reflects the burden of such infections on the community[7]. In the present study, 201 children from 4 schools (2 urban and 2 rural schools) were chosen. Eighty eight of them were females and 113 were males. Number of urban students was 101, (48 females and 53 males). Number of rural students was 100 (40 females and 60 males). Regarding symptoms in schools, abdominal pain was the commonest complaint, it was present in 39 cases (19.4%) followed by diarrhea in 37 cases (18.4%), perianal itching in 15 cases (7.5%), pallor in 3 cases (1.5%) and finally vomiting in one case only (0.5%). Symptoms were

found to be most common in stool positive cases according to stool analysis and this is logic as presence of parasitic infection leads to the appearance of symptoms according to the parasite found. Abdominal pain was the commonest in positive cases, followed by diarrhea. In comparison between urban and rural schools, as shown in table (2), *E. histolytica* trophozoite was found in (2%) of urban, and (1%) of rural children with a total of 1.5%. *Entamoeba histolytica* cyst was found in 27.8% of urban and 26.0% of rural children with a total of 26.9% (54 out of 201). *Entamoeba coli* cyst was found in 1% only of urban and 14% of rural students ($p<0.001$) with a total of 7.5% (15 out of 201). This could be explained by the fact that urban students receive exclusively treated water which leads to decrease prevalence of hydro-transmission parasites as *E. coli* in agreement with Giraldi et al. (2001). An explanation for higher rates of intestinal parasites among urban students is that high concentrations of people and wastes in urban areas create more opportunities for exposure to pathogens and a correspondingly greater need for the levels of hygiene that adequate water and sanitation make possible. In general, the overall distribution of protozoa prevalence is consistent with that found in other countries where *E. histolytica* and *G. lamblia* are the most common protozoa. However, the percentages do not coincide. This may be due to a variety of factors such as employment of different diagnosing techniques, geographical differences, morphologic, and socioeconomic patterns of populations studied (age, urban versus rural areas)[11]. In general, among school children in our study, there was a higher prevalence of protozoa than helminthes in agreement with [6].

Regarding helminthes in our results: *Hymenolepis nana* and *S. mansoni* were the highest (8.5%) each, followed by *A. lumbricoides* (6.5%), *E. vermicularis* (4.5%), and finally *H. heterophyes* (2.5%). *Heterophyes heterophyes* was significantly higher in urban schools (4.95%) than rural schools (0.0%) with a total of (2.5%; $p=0.024$). *Schistosoma mansoni* was found in 2 students from urban and 15

students from rural schools with a total of 17 students (8.5%; p=0.001). A comparison between urban and rural areas in similar studies revealed that for example in Chennai, *A. lumbricoides* was the most common helminthic parasite detected in rural areas (52.8%) followed by *T. trichura* (45.6%), *A. duodenale* (37.6%), *S. stercoralis* (3.2%) and *H. nana* (1.6%). Under urban settings, intestinal parasites such as *T. trichura* (2.01%), *H. nana* (1.01%) and *A. lumbricoides* (0.50%) were found to have much lower prevalence in comparison to the rural area tested [4]. Presence of *S. mansoni* in 15 student from rural areas and only 2 urban students could be explained by the habit of playing in ponds or river among them, while urban students have little exposure to infection due to absence of this habit and by feedback from those urban students infected with *S. mansoni,* we found both of them were resident in countryside and just moved to Mansoura city. *Ascaris lumbricoides* was found in 13 students 9% of rural and 3.96% of urban children with a total of (6.5%). In Ethiopia, Legesse and Erko[9] found prevalence of *S. mansoni* among schoolchildren of (21.2%) which was much higher than ours (8.5%). This could be explained by the presence of high number of snail intermediate host making new transmission foci in this country.

Our results of multiple linear regressions showed the relation between parasitic infections and anthropometric measures of children especially weight and height for age. There was only a negative impact on weight of children infected with *S. mansoni* (p=0.012), *E. coli* cyst (p=0.007), *B. hominis* (p=0.033), and on those found with undigested food in their stool (p=0.015) leading to a decrease in their weight for age as shown in table. As regard effect of parasites on height, height residual showed a marked regression on presence of *S. mansoni;* (p<0.001) and Giardia trophozoite; (p=0.003) which means that patients with *S. mansoni* and Giardia trophozoite have a lower height for their ages. Our results were in agreement with Cooper,[2] who stated that all forms of chronic intestinal inflammation lead to growth failure

whether by secondary effects on nutrient balance or by more direct effects on metabolism and also in agreement with Parraga et al.,[13] who reported on the effects of treatment for *S. mansoni* on growth and development in Brazilian schoolchildren and concluded that light or moderate intensity infection with *S. mansoni* contribute to growth deficits as evidenced by anthropometric measurements taken from *S. mansoni*-infected children who exhibited chronic malnutrition, with growth retardation in height and were significantly smaller in height and weight compared to their controls. Similarly, in Sao Paulo, Brazil, a study conducted among school children found a weak but significant association between helminth infection and height. However, no significant relationship between parasitic infection and children's weight was detected [10]. In a village in Cote d'ivoire, a study among schoolchildren found that the heaviest intestinal parasitism especially *A. lumbricoides* could have a negative impact on anthropometric measures, nutritional development, and pubertal growth of children[3]. In Mexico, intestinal parasitic infections are regarded as main risk factors associated with poor nutritional status in Mexican schoolchildren according to a study carried by[14] who found a significantly higher weight and height in non-infected versus infected children. In Colombia, a study to establish the associations between intestinal parasitism and undernourishment was undertaken in a village in the Colombian Amazon region. Anthropometric indices were calculated based on height and weight for age. Indices were inversely related to age and number of parasites [12]. Also, in rural Ecuador, children with intestinal parasitic infections were twice as likely as non-infected children to suffer from growth stunting or chronic malnutrition [16].

Conclusion

Based on results from this study, regular screening of children in schools for parasitic infections is required and anthropometric measures should be taken as indicators of malnutrition and growth suppression Also, the wide adoption of school-based helminth control programs will likely re-

quire the active participation of Education Ministry. Finally, we emphasize the importance of nutrition education as well as health education for children and their parents.

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INTESTINAL PARASITES AMONG SCHOOL CHILDREN IN DAKAHLIA GOVERNORATE

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HISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE RENAL CORTEX OF THE ADULT MALE ALBINO RAT AFTER ADMINISTRATION OF GENTAMICIN WITH OR WITHOUT ADMINISTRATION OF VITAMIN E OR EPOIETIN

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Abstract

Background: *Gentamicin is the most common cause of drug induced renal failure. When the drug accumulates in the proximal convoluted tubules, it cause acute tubular necrosis. These effects impair renal function.*

Aim of the work: *This work was carried out to study the histochemical and ultrastuctural changes that occurred in the renal cortex of adult male albino rats after administration of therapeutic doses of gentamicin and the effect of vitamin E or epoietin or both on these changes.*

Materials & Methods: *95 adult male albino rats were divided into three groups. Group I: a control group. Group II: experimental group were divided into four equal subgroups: Subgroup IIa: were injected with gentamicin for 2 weeks. Subgroup IIb: received gentamicin and epoietin for 2 weeks. Subgroup IIc: received gentamicin and vitamin E for 2 weeks. Subgroup IId: received gentamicin and both epoietin and vitamin E for 2 weeks. The kidneys from each rat used for light and electron microscopic examination to study the renal cortex.*

Result: *After two weeks of gentamicin administration in subgroup IIa, the lesion was mainly in the proximal convoluted tubules. The cell lining showed vacuolated cytoplasm, pyknotic nuclei and there were areas of cellular infiltration. The lumen of affected tubules contained epithelial remnants. There was a decrease in the activity of succinic dehy-*

drogenase enzyme activity. Electron microscopic examination revealed loss of the basal infoldings of the basement membrane of cells of PCT with only few scattered mitochondria in the cytoplasm. Some of the nuclei were heterochromatic with irregular outline. The histological architecture of the renal cortex of the subgroups IIb,c,d were not different than in control group I rats.

Conclusion: *The function and the structure of the renal cortex was affected by gentamicin adminstration for more than one week. Combination of gentamicin with epoietin or vitamin E or both can be used to prevent the occurrence of its nephrotoxic effects.*

Introduction

Gentamicin (GM) is an effective aminoglycoside antibiotic, it is widely used as a bactericidal agent against life threatening gram-negative bacterial infections [1].

However, its clinical use is limited, as 30% of patients treated with gentamicin for more than 7 days show signs of nephrotoxicity [2].

Nephrotoxicity is more likely to be encountered when therapy is continued for more than five days as well as with higher doses or in elderly patients [1]. The recommended dose of gentamicin is 3-5mg\kg\day divided into 3 doses or once daily in a dose of 5-7 mg\kg (for no more than 7 days in both) [3].

The signs of tubular damage including loss of brush borders, tubular debris, cellular vacuolations with inflammatory cellular infiltration (4). With electron microscopy the cytoplasm of the cells of the proximal convoluted tubules revealed various intensity [5].

It was mentioned that, aminoglycosides generate many types of free radicals which play an important role in aminoglycoside induced toxicity. These free radicals or cell oxidizers cause cell destruction through breaking down the cell membrane and pericellular protective barrier [6].

The antioxidant at low concentrations delay or prevent oxidation of the free radicals. These antioxidant are either endogenous antioxidant (naturally) as glutathione

peroxidase and vitamin C or exogenous antioxidant as vitamin E and selenium [7].

A number of studies have demonstrated the ability of vitamin E in the protection against the nephrotoxicity induced by gentamicin [4,8 &9].

Erythropoietin (Epo) is a glycoprotein produced by the peritubular interstitial cells in the middle region of renal cortex [10]. It stimulates erythroid proliferation and differentiation by interlacing with specific erythropoietin receptors on red cell progenitors[11&12].

Epo has an important cytoprotective effects on various cell lines and inhibition of apoptotic death-related pathways has been reported in renal tissue, brain and heart [13].

Erythropoietin is a growth factor, it support the growth not only of red cell progenitors but also other cell types [14].

Materials and Methods

A) Materials :

I- Experimental animals :

95 adult male albino rats (weighing about 150-200gm) were used throughout this study. 15 rats were considered as a control group. The rest of rats (80) received different protocols of medications.

Groups of the animals:

I- Control group (group I):

Animals of this group (15 rats) were divided into 3 subgroups (5 rats each):

a) Subgroup Ia control non medicated rats.
b) Subgroup Ib control rats were injected intraperitoneally (IP) with 1ml normal saline for two week.
c) Subgroup Ic control rats were received 1ml oil via gastric tube for two weeks.

II. Experimental group (group II):

Animals of the 2nd group (80 rats) were divided into (4) subgroups (20 rats each):

i. Subgroup IIa :

This subgroup were injected IP with therapeutic dose (6 mg/Kg bw/day) gentamicin sulfate (3), (Epigent, obtained from the

E.I.P.I.C.O. Company-Egypt) for 2 weeks.

ii. Subgroup IIb:

This subgroup as subgroup IIa, received gentamicin in addition simultaneous IP injection of (100 IU\Kg bw\day) epoietin(14) (Recombinant human erythropoietin) for 2 weeks. Epoietin was obtained from the E.I.P.I.C.O. Company-Egypt.

iii. Subgroup IIc:

This subgroup as subgroup IIa received gentamicin in addition to simultaneous administration of 100mg vitamin E (4), (Pharco Company- Egypt) dissolved in oil, via gastric tube, for 2 weeks.

iv. Subgroup IId :

This subgroup as subgroup IIa received gentamicin in addition to injection of epoietin and administration of vitamin E for 2 weeks.

At the end of the experiment, the rats were sacrificed.

The animals were fed on laboratory food and water ad-libitum. At the time of sacrifice, the rats were anaesthetized by IP injection of 50 mg/kg pentobarbital. In each rat, right and left kidneys were dissected, The right kidney was used for light microscopic examination, while the left kidney was used for electron microscopic examination to study the renal cortex.

II- Staining techniques:

1. Paraffin sections were prepared and stained with Haematoxylin and eosin.
2. Frozen sections were prepared and treated histochemically for the study of succinic dehydrogenase enzyme activity (15).
3. Ultrathin sections were prepared (16) and stained for examination with transmission electron microscopic study.

The sections were examined by the transmission electron microscope in:

• Electron Microscopic Unite in Tanta University using Zeiss EM 100 S transmission electron microscope at 60 KV.

Results

I) Histological Structure of the Renal Cortex in Control group I Rats:

Light microscopic examination:

a) Haematoxylin and eosin-:

The renal cortex of adult control rats group I contains renal corpuscles, proximal convoluted tubules, distal convoluted tubules and collecting tubules. The renal corpuscles were formed from glomerulus surrounded by Bowman's capsule. Each glomerulus was formed of numerous tortuous blood capillary loops (Fig. 1). The proximal convoluted tubules occupied the majority of the cortex. The cells lining of these tubules were cuboidal with indistinct boundaries between the cells. The free borders of these cells had striated brush border. The cytoplasm was acidophilic and granular. The nuclei were rounded, basal and vesicular. (Fig. 1).

b)- Succinic dehydrogenase enzyme activity:

Examination of fresh frozen sections in the control renal cortex treated for the activity of succinic dehydrogenase enzyme revealed strong reaction in the proximal convoluted tubules, and distal convoluted tubules. While the glomeruli exhibited a negative reaction (Fig. 2).

Electron microscope:

Examination of The renal corpuscles under the electron microscope revealed the presence of the glomerular capillaries that were surrounded by podocytes which exhibited major and minor processes. (Fig. 3).

The proximal convoluted tubules with E/ M revealed that the cell lining rest on a basement membrane. The cytoplasm contained lysosomes and mitochondria. The mitochondria were tubular and longitudinally oriented inside basal infoldings of the cell membrane. The nucleus was central, rounded and euchromatic. (Fig 4).

The distal convoluted tubule cells showed numerous, tubular and longitudinally oriented mitochondria inside the basal infoldings. The nuclei were rounded and euchromatic. The upper luminal border exhibited few and short microvilli. Compared with the

proximal convoluted tubules, the lumen of DCT appeared wide (Fig. 5).

II) Histological Structure of the Renal Cortex in Experimental Rats:

Light microscopic picture:

a) Haematoxylin and eosin:

* Subgroup IIa:

After two weeks of gentamicin administration, morphological changes were observed in the renal cortex. The lesion was mainly in some of the proximal convoluted tubules while the renal corpuscles, DCT were nearly similar to those of the control group I rats. The cell lining of affected PCT showed vacuolated cytoplasm and pyknotic nuclei. The lumen of affected tubules contained epithelial remnants. There were focal areas of cellular infiltration in the cortical interstitial tissues (fig. 6).

* Subgroup IIb,c,d:

The histological architecture of the renal cortex of these subgroups was not different than in control group I rats. (Fig 7, 8, 9).

c) Succinic dehydrogenase enzyme activity:

* Subgroup IIa:

Examination of a fresh frozen section of the renal cortex for the activity of succinic dehydrogenase enzyme (SDH) revealed that there was a decrease in the activity of SDH enzyme in this subgroup in some PCT while others still showed a strong reaction (Fig. 10).

*Subgroup IIb, Subgroup IIc, Subgroup IId:

Examination of a fresh frozen section of the renal cortex treated for the activity of SDH enzyme revealed that the staining enzymatic activity was not different in these subgroups, (Fig 11,12, 13 respectively) compared to control group I rats.

Electron microscopic picture:

*Subgroup IIa:

Electron microscopic examination of the renal cortex of rat-receiving gentamicin for two weeks revealed many inflammatory cellular infiltrations in the renal interstitium. The cells of PCT showed loss of the basal infold-

ings of the basement membrane with only few scattered mitochondria in the cytoplasm. Some of the nuclei were heterochromatic with irregular outline (Fig. 14).

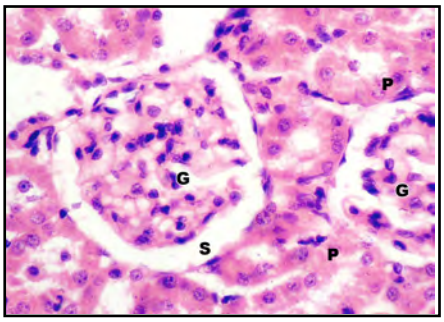

Fig. (1): A photomicrograph of a paraffin section in the renal cortex of control rat of group (I) showing the proximal convoluted tubules (P), it had narrow lumen, acidophilic granular cytoplasm, apical brush border (arrows heads) and rounded vesicular nuclei. The renal corpuscles show glomerulus (G), it's Bowman's capsule had two layers separated by the Bowman's space (S). The Parietal layer lined by simple squamous epithelium (arrows). (Hx&E x 1000)

*Subgroup IIb,IIc and IId:

The ultrastructure of the renal cortex of rats of these subgroups was similar to the control (Fig. 15,16,17).

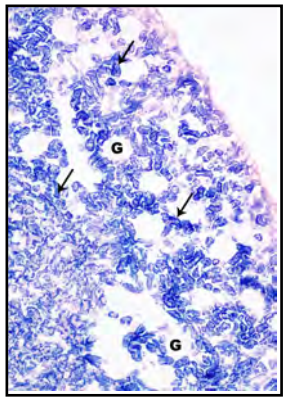

Fig. (2): A photomicrograph of a frozen section in the renal cortex of control rat of group (I) showing a strong reaction in the renal tubules (arrows) and the negative reaction detected in the glomeruli (G).
(Succinic dehydrogenase x 100)

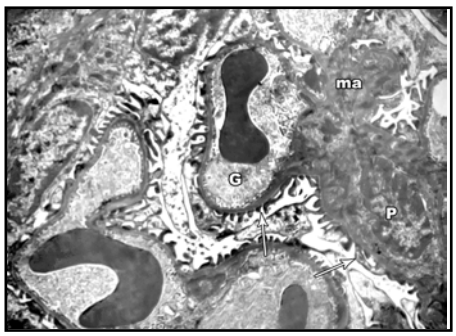

Fig. (3): An electron micrograph of the renal cortex of adult control rat of group (I) showing the renal corpuscle containing the glomerular capillary (G) surrounded by podocyte (P) with major (ma) and minor (arrows) processes. (TEM x 4000)

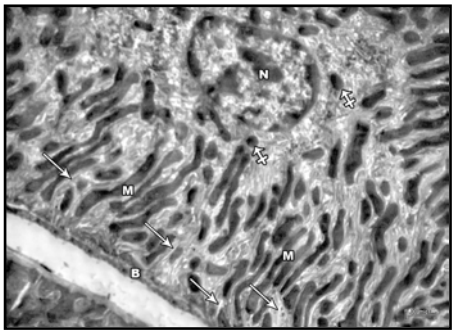

Fig. (4): An electron micrograph of the renal cortex of control rat of group (I) showing the cells of proximal convoluted tubule resting on a basement membrane (B), containing rounded and euchromatic nuclei (N) and lysosomes (crossed arrows). Note the numerous, tubular longitudinally oriented mitochondria (M) inside basal infoldings of the cell membrane (arrows) are detected. (TEM x 6000)

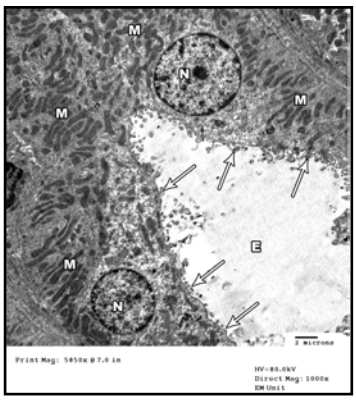

Fig. (5): An electron micrograph of the renal cortex of control rat of group (I) showing the cells of distal convoluted tubule with numerous tubular mitochondria (M) arranged among the basal infoldings of the cell membrane. Note, the rounded euchromatic nuclei (N) and the few short microvilli (arrow) projecting in a wide lumen (E) of the tubule. (TEM x 1000)

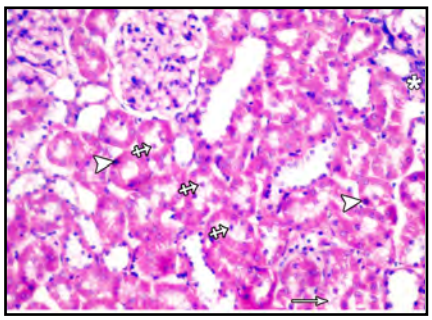

Fig. (6): A photomicrograph of a section in the renal cortex of a subgroup IIa treated rat for 2 weeks showing some of PCT with vacuolated cytoplasm (arrow) and pyknotic nuclei (arrows heads), degenerated desquamated cells in their lumen (crossed arrows). Note the focal cellular infiltration in the interstitial tissue (asterisk). (H&E x 400)

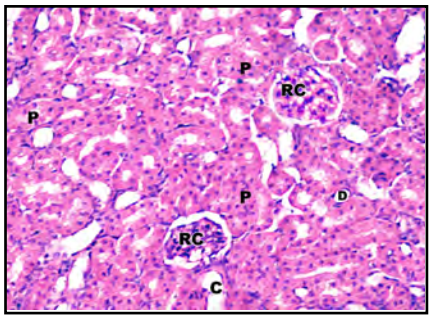

Fig. (7): A photomicrograph of a section in the renal cortex of a subgroup IIb treated rat for 2 weeks show intact histological architecture of renal corpuscle (RC), PCT (P), DCT (D) and collecting tubule (C). (H&E x 400)

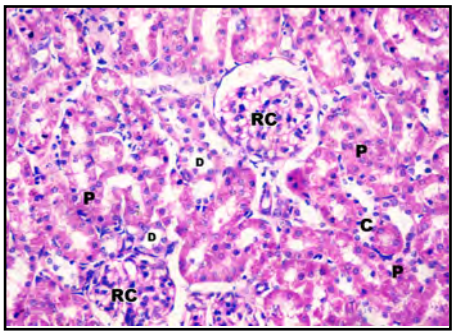

Fig. (8): A photomicrograph of a section in the renal cortex of a subgroup IIc treated rat for 2 weeks showing intact histological architecture of renal corpuscle (RC), PCT (P), DCT (D) and collecting tubule (C).
(H&Ex 400)

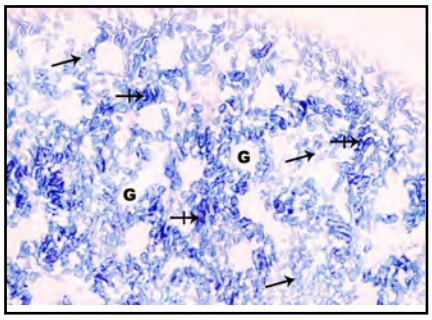

Fig. (10): A photomicrograph of a frozen section of the renal cortex of a subgroup IIa treated rat for two week showing a strong reaction (crossed arrows) in some PCT and a weak reaction in others (arrows). Note the negative staining of the renal glomeruli (G).
(Succinic dehydrogenase x 100)

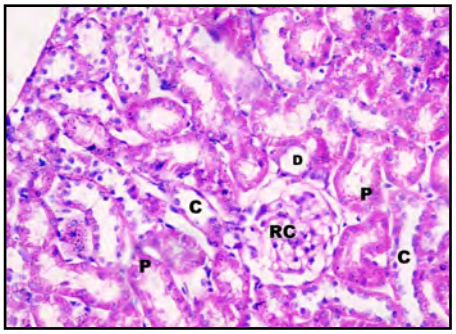

Fig. (9): A photomicrograph of a section in the renal cortex of a subgroup IId treated rat for 2 weeks showing intact renal corpuscle (RC), PCT (P), DCT (D) and collecting tubule (C).
(H&Ex 400)

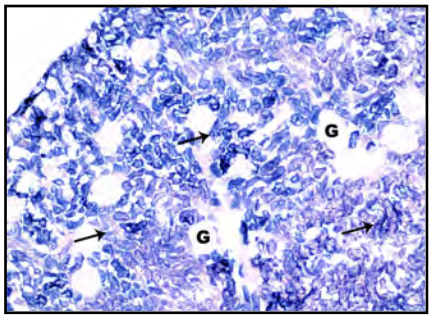

Fig. (11): A photomicrograph of a frozen section of the renal cortex of a subgroup IIb treated rat for two week showing positive staining in the renal tubules (arrows) and negative staining in glomeruli (G).
(Succinic dehydrogenase x 100)

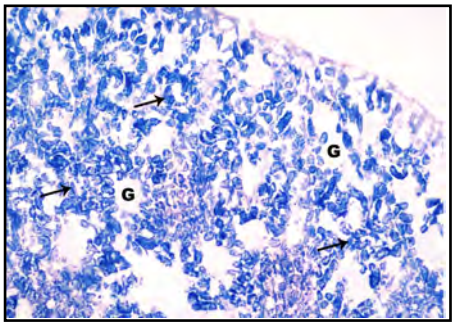

Fig. (12): A photomicrograph of a frozen section of the renal cortex of a subgroup IIc treated rat for two week showing positive staining in the renal tubules (arrows) and negative staining in glomeruli (G).
(Succinic dehydrogenase x 100)

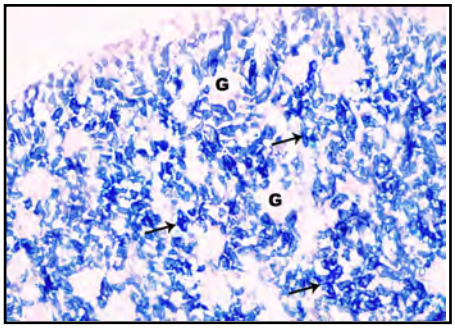

Fig. (13): A photomicrograph of a frozen section of the renal cortex of a subgroup IId treated rat for two week showing positive staining in the renal tubules (arrows) and negative staining in glomeruli (G).
(Succinic dehydrogenase x 100)

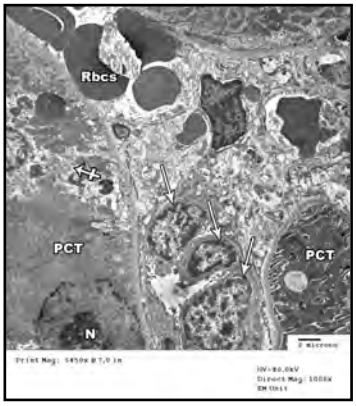

Fig. (14): An electron micrograph of the renal cortex of subgroup IIa treated rats for two week showing cells of proximal convoluted tubule with an area of partial loss of basal infoldings of basement membrane with few scattered mitochondria distributed in the cytoplasm (crossed arrow). Note the nuclear irregularities with condensed nuclear chromatin (N) and the inflammatory cells in the peritubular interstitial tissue (arrows).
(TEM x 1000)

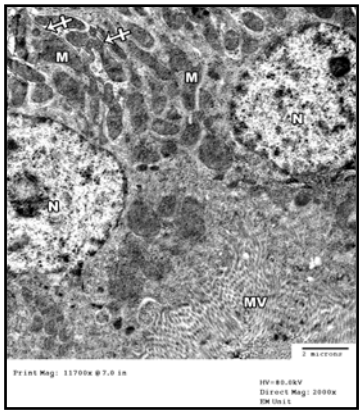

Fig. (15): An electron micrograph of the renal cortex of subgroup IIb treated rats for two week showing two cells of proximal convoluted tubule with the regular basal infoldings of basement membrane (crossed arrows) enclosing the mitochondria (M). Note the presence of euchromatic nuclei (N) with long apical microvilli (MV).
(TEM x 2000)

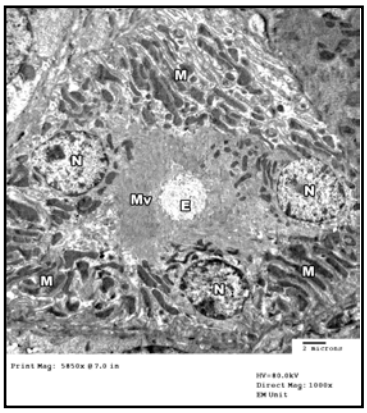

Fig. (16): An electron micrograph of the renal cortex of subgroup IIc with regular basal infoldings of basement membrane (arrows) enclosing the mitochondria (M), euchromatic nuclei (N) and long apical microvilli (MV). (TEM x 1000)

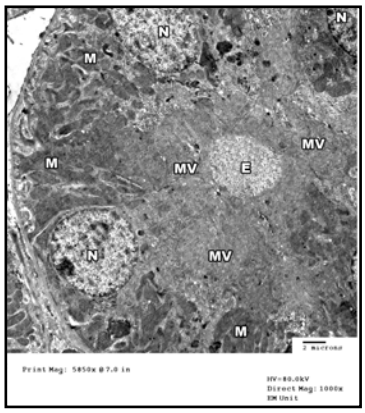

Fig. (17): An electron micrograph of the renal cortex of subgroup IId treated rats for two week showing cells of proximal convoluted tubule with regular basal infoldings of basement membrane (arrows) enclosing mitochondria (M), euchromatic nuclei (N) and long apical microvilli (MV). (TEM x 1000)

Discussion

In the present work, the histological structure of the renal cortex of adult control albino rats were examined with light and electron microscopes, revealed similar structures as those mentioned by many authors[17&18].

Examination of frozen sections of the control renal cortex treated for the demonstration of the activity of SDH enzyme revealed negative reaction in the glomeruli, while the renal tubules (proximal & distal) revealed a strong enzymatic activity specially at their basal parts. The same finding was reported by many researchers[19&20].

After two weeks of gentamicin treatment in the current study, there was a toxic morphological change in the renal cortex detected by haematoxylin and eosin-stained paraffin sections of treated subgroup IIa rats. The lesion was mainly in the proximal convoluted tubules while the renal corpuscles and DCT were nearly similar to those of the control group. The cells lining the affected PCT showed vacuolated cytoplasm and pyknotic nuclei. The lumen of affected tubule contained degenerat-

ed epithelial cells.There were some areas of focal cellular infiltration in the cortical interstitial tissues.

The result of the present study was in agreement with[21&22]. They stated that there was no evidence of any morphological changes in the glomeruli after gentamicin administration in both human and rats. They also noticed some morphological changes in the PCT in the form of vacuolated cytoplasm. Some tubular cells showed rupture of the cytoplasm followed by desquamation of the epithelial residues into the lumina of the PCT and extrusion and karyorrhexis of the nuclei.

5% of filtered aminoglycosides is actively reabsorbed by the proximal tubular cells. Acidic phospholipids were considered the binding site of aminoglycosides in brush-border membrane of proximal tubular cells. Once attached, these agents undergo pinocytosis and enter the cell setting off complex biochemical events that result in tubular destruction[23&24].

As regard the focal mononuclear cellular infiltration that seen in the cortical interstitial tissue (25) explained this finding as kidney injury that caused by gentamicin stimulate an inflammatory response in which chemokines, are secreted to attract leukocytes to damaged areas.

On the contrary,[26] examined the kidney after aminoglycosides administration and noticed that DCT occasionally showed vacuolization and disruption of the basal cell architecture.

The present work showed a decrease in the activity of SDH enzyme at the examination of a fresh frozen section of the renal cortex of rats treated by gentamicin for two weeks. We attributed this decrease in the activity to the effect of gentamicin on the mitochondria as detected by electron microscopic examination of the renal cortex. This finding was agreed with that of[20&27] they found that there was a gradual depletion of SDH enzyme activity in the renal cortex after gentamicin treatment.

Electron microscopic examination of the renal cortex of rat-received therapeutic dose of gentamicin for two weeks in the present work revealed many in-

flammatory cellular infiltrations in the renal interstitium, some cells of PCT showed partial or complete loss of the basal infoldings of the B.M. with only few scattered mitochondria in the cytoplasm. Some of the nuclei were heterochromatic with irregular outline.

The result of this study was consistent with that of[28]. They reported that the cytoplasmic vacuoles that have been described in the PCT resulted from pinocytotic activity at the luminal membrane.

A pinocytotic vacuoles in the cytoplasm of the affected PCT cells as well as swollen and distorted mitochondria which became freely scattered in the cytoplasm as a result of loss of the normal basal infoldings of the basement membrane were detected in the altered PCT[29].

In addition,[30] reported that when tubular cells die, they slough off into the tubule lumen and contribute to cast formation.

Treatment of experimental animals with gentamicin results in apoptosis as well as necrosis of tubular epithelial cells mainly in the proximal tubule in which the drug accumulates[31].

The present work demonstrated that the renal cortex of the treated rats with gentamicin for two weeks with simultaneous administration of Epo didn't develop any histological changes as those observed in the renal cortex of treated rats with gentamicin alone.

The result of this study was similar to those of[32&33]. They mentioned that erythropoietin mediates anti-apoptotic effects in the bone marrow, facilitating maturation and differentiation of erythroid progenitors, also exerts anti-apoptotic effects in the brain, heart and in the kidney, which can limit the degree of organ damage.

Many researches done by [34,35&36], suggest that Epo provides cytoprotection by ameliorating oxidative stress. Epo may exert its antioxidative effects directly by exploiting intracellular antioxidative mechanisms and indirectly by inhibiting iron-dependent oxidative injury.

The present work demonstrated that the renal cortex of subgroup IIc treated rats with gentamicin for two weeks with simultaneous administration of vitamin E didn't develop any histological changes as those observed in the renal cortex of treated rats with gentamicin alone.

The role of vitamin E in ameliorating the toxic changes induced by gentamicin was investigated by[37], stated that vitamin E can prevent the tubular alterations induced by gentamicin. This is possibly due to prevention of lipid peroxidation and the preservation of superoxide dismutase (SOD) and glutathione peroxidase activities.[38] reported that the use of antioxidant like vitamin E improved the renovascular function as well as the histopathological changes caused by aminoglycoside.

The present work demonstrated that the renal cortex of subgroup IId treated rats with gentamicin for two weeks with epoietin and vitamin E, didn't develop any histological changes as those observed in the renal cortex of treated rats with gentamicin alone.

Few studies investigated the effect of both epoietin and vitamin E on the changes that caused by gentamicin administration,[34], found that co-supplementation of epoietin, vitamin E and iron improve the changes caused by gentamicin administration.

Conclusion and recommendation

1. It could be concluded from the present study that, the function and the structure of the renal cortex was affected by gentamicin adminstration.

2. Also, it was proved that epoietin, vitamin E or both markedly reduce these changes. However combination of epoietin and vitamin E were more effective in the prevention of these changes.

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HISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE RENAL CORTEX OF THE ADULT MALE ALBINO RAT AFTER ADMINISTRATION OF GENTAMICIN WITH OR WITHOUT ADMINISTRATION OF VITAMIN E OR EPOIETIN

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ERYTHROPOIETIN MEASUREMENTS IN PATIENTS WITH RENAL CYSTS

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Abstract

Background: *Erythropoietin (EPO) is a sialoglycoprotein hormone secreted primarily by the mature kidneys in response to tissue hypoxia and/or red cell mass decrease. It stimulates erythrocyte production from the blood marrow. Objectives: To estimate hemoglobin and EPO in blood and cystic fluid in patients with renal cysts.*

Methodology: *This case control study included 60 subjects, 30 patients (30 - 60 years of age) with renal cysts, attended urology department at Alwahda Hospital, Derna-Libya, during 2007. In addition, 30 healthy subjects, matched with age and sex were chosen as controls (29-58 years of age). Blood hemoglobin, urea and creatinine were measured. EPO were estimated in serum and cystic fluid by ELISA.*

Results: *Mean values of serum EPO and urea in patients with renal cysts were (29.7±7 mU/ml and 7.2±1.3 mmole/1, respectively), which was significantly higher than the control group (6.2±4.3 mU/ml and 4.4±1.1 mmole/1, respectively, $P < 0.05$). Hemoglobin levels in patients with renal cysts were significantly lower than the control group (11.2±0.6 and 13.2±1.3 gm/dl, respectively, $P < 0.05$). EPO in cystic fluid showed a 15 times higher level than serum EPO.*

Conclusion: *This study suggests that in patients with renal cysts, there is no erythropoietin penetration from cystic fluid into blood and the level of erythropoietin in cystic fluid needs more research.*

Keywords: *Chronic Kidney Diseases; Renal Cyst; erythropoietin; renal anemia; Cystic fluid.*

Introduction

A number of underlying diseases, such as diabetes mellitus and hypertension, can contribute to the incidence of chronic kidney disease (CKD), which in 1996 accounted for 43% and 23% of incident cases of end-stage renal disease (ESRD), respectively [1,2].

Simultaneous with the proactive management of these underlying diseases, these patients should also undergo comprehensive preparatory treatment for entry into ESRD. According to The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative Guidelines (NKF-K/DOQI) recently released for CKD, the evaluation and treatment of patients with CKD requires understanding of separate but related concepts of diagnosis, comorbid conditions, severity of disease, complications of disease, and risks for loss of kidney function and cardiovascular disease. Among CKD patients, the disease stages are defined based on the level of kidney function, thus the rate at which patients approach ESRD varies. Defining the stages of CKD is crucial to the effective management of these patients and requires "categorization" of continuous measures of kidney function [3].

A hypothesis that EPO may be produced in renal cyst (RC) was put forward during an observation of patients with renal cysts coexisting renal failure [4].

The aim of the present study is to estimate hemoglobin and EPO in blood and cystic fluid in patients with renal cysts.

Subjects and Methods

This case control study included 60 subjects, 30 patients (30 - 60 years of age) with renal cysts, attended urology department at Alwahda Hospital, Derna-Libya, during 2007. In addition, 30 healthy subjects, matched with age and sex were chosen as controls (29 - 58 years of age).

Hematological parameters by hematology analyzer H-l Technicon. Blood urea and creatinine were determined by biochemical analyzer RA-XT Technicon, rea-

gents from Technicon [5].

Cyst fluid from patients with renal cysts was obtained by either in vivo aspiration or immediately after nephrectomy. EPO in serum and cystic fluid were determined using Anthos Labtec ht II microtitracic platelets counter and reagents from Boehringer Mannheim (immuno-enzymatic method, ELISA technique [6]. Statistical analysis were done using SPSS software ver. 12. All results were presented as mean ± standard deviation (SD). Student "t" test were used to compare between means. The level of significance was set at P value <0.05 [7].

Results

Table (1) shows that the mean values of serum EPO and urea in patients with renal cysts were (29.7±7 mil/ml and 7.2±1.3 mmole/1, respectively), which was significantly higher than the control group (6.2±4.3 mil/ml and 4.4±1.1 mmole/1, respectively, P < 0.05). Hemoglobin levels in patients with renal cysts were significantly lower than the control group (11.2±0.6 and 13.2±1.3 grn/dl, respectively, P < 0.05).

Table (2) shows that EPO in cystic fluid was 15 times higher level than serum EPO in patients with renal cysts.

Table (1): Mean values of EPO, hemoglobin, urea and creatinine in patients with renal cysts and the control group.

Laboratory Parameters	Patient group(30)	Control group(30)	P-value	Significance
	Mean ± SD	Mean ± SD		
Serum EPO mU/ml	29.7±7	6.2±4.3	< 0.05*	Sig. higher
Hemoglobin level gm/dl	11.2±0.6	13.2±1.3	<0.05*	Sig. lower
Blood urea mmole/1	7.2±1.3	4.4±1.1	< 0.05*	Sig. higher
Serumcreatinine mmole/1	92± 17	88±16	>0.05	Non Sig.

Table (2): Mean values of EPO in serum and cystic fluid among patients with renal cysts.

	Serum EPO mU/ml	Cystic fluid EPO mU/ml	Ratio
Meani SD	29.7±7	433 ±370	14.93
Minimum	3.7	3.1	
Maximum	99.4	935	

Discussion

Perhaps the most common cystic change of all is the appearance of one or more "simple renal cysts" in adults. These cysts may be only a few millimeters in size, or may reach 10 cm or more. They are rarely numerous enough so that intervening normal parenchyma is not recognizable, and they are very unlikely to be the cause for renal failure. These cysts are lined by a flattened cuboidal epithelium and filled with a clear fluid. On occasion, there may be hemorrhage into a larger cyst, and it may appear as a mass lesion that can be difficult to differentiate from a renal cell carcinoma. Endocrine abnormalities.in patients with chronic renal failure are well documented. In some articles acquired cystic kidney disease has been related to improvement of anemia in dialysis patients. It has been suggested that this could be due to erythropoietin production by the cysts [8].

Anemia is a common complication of CKD, mainly due to the inability of the kidneys to secrete enough erythropoietin to stimulate adequate hematopoiesis. Additional factors that may cause or contribute to CKD-related anemia include iron deficiency, severe hyperparathyroidism, acute and chronic inflammatory conditions, aluminum toxicity, folate deficiency, shortened red blood cell survival, hypothyroidism, and hemoglobinopathies such as a-thalassemia [9].

It is recently proposed a nomogram allowing an easy interpretation of serum erythropoietin val-

ues (EPO) by plotting them against hemoglobin using percentiles. They found that EPO correlated inversely with hemoglobin and patients with chronic kidney disease (CKD) of various etiologies preserved the feedback loop although at a lower level, with most patients below the 25th percentile[10].

Although serum EPO concentrations can easily be measured with radioimmunoassay (RIA) or enzyme-linked assay (ELISA), routine measurement of serum EPO concentrations is not practiced widely. K/DOQI guidelines do not recommend EPO measurements to establish the diagnosis of renal anemia in patients with creatinine concentrations >2 mg/dl. The low utilization of measuring serum EPO concentrations can partly be explained by the difficult interpretation since EPO concentrations have to be assessed in relation to the severity of anemia and to the renal function. Relative EPO deficiency may be easily overlooked without correction for these factors since reference values given by the manufacturer are mainly derived from healthy non-anemic subjects. Expression of EPO concentrations in percentiles improves the diagnostic value of measuring EPO concentrations for diagnosing relative EPO deficiency and renal anemia [11,12].

The EPO level is generally increased in patients with acquired cystic kidney disease and renal cancer. This is currently attributed to either production of EPO in the cyst or tumor tissue or to the hypoxic enhancement of EPO production secondary to vascular compression by a renal mass. However, it seemed unlikely that such factors were responsible for the elevated EPO level in our heart failure group, because no cysts or renal tumors were detected by abdominal computed tomography. The erythrokinetic rates in the anemic uremic patients were about one-half the rate in normal hematological stable individuals even though the serum EPO titers were the same or higher in the anemic uremic patients [13].

Serum erythropoietin may be increased in patients on chronic hemodialysis secondary to acquired polycystic disease of the

kidneys. The development of RCC in ADPKD is rare and the association of RCC and ADPKD is controversial. The majority of reported cases have been shown to be an incidental finding either on surgical exploration or at autopsy. The serum EPO level is usually low in long-term hemodialysis patients with anemia. However, increased levels of serum EPO and hemoglobin have been observed in hemodialysis patients because the renal proximal tubule cells produce EPO into the fluid of cysts. Thus, the diagnosis of EPO-producing RCC in such cases is difficult due to overlapping clinical features and marked distortion of architecture [14].

In the present study, the mean values of serum EPO and urea in patients with renal cysts were (29.7±7 mU/ml and 7.2±1.3 mmole/1, respectively), which was significantly higher than the control group (6.2±4.3 mU/ml and 4.4±1.1 mmole/1, respectively, $P < 0.05$). Hemoglobin levels in patients with renal cysts were significantly lower than the control group (11.2±0.6 and 13.2±1.3 gm/dl, respectively, $P < 0.05$).

Hemoglobin and erythropoietin were significantly higher in patients with polycystic kidney disease. Patients without cysts had the lowest levels of hemoglobin and erythropoietin, although no significant difference was found in those with multiple bilateral cysts or in those with 1 - 3 isolated cysts [8].

In another study, serum erythropoietin levels were randomly collected and measured by a sensitive radioimmunoassay in a hemodialysis population. Patients were divided into two groups: those with polycystic kidney disease and those with other kidney diseases. It was demonstrated that polycystic kidney disease patients manifested higher hematocrit, reticulocyte counts, and serum erythropoietin levels when compared to other kidney disease patients. Authors suggested an inappropriately low serum erythropoietin level for the severity of anemia in uremic hemodialysis patients and that greater availability of erythropoietin results in more effective erythropoiesis, even in the uremia environment[15].

In the present study, EPO in cystic fluid showed a 15 times higher level than serum EPO.

It has been demonstrated that cystic fluid and interstitial cells produce erythropoietin independent to the oxygen content, and this has been the main argument for the opinion that PKD patients produce more EPO than others. Although this may be true in early stages of CKD with volume expansion, this effect may be offset in the later stages as a result of uremia. Therefore as PKD progresses, more cysts produce more EPO and may contribute to higher hemoglobin in stages 3 and 4. In stage 5, the inhibitory effect of uremia may block the response of bone marrow to EPO. Our data are limited because the number of patients in the control group is small. Besides this limitation, our results show the utility of the nomogram in a clinical setting and that PKD patients have higher hemoglobin only in early stages [16]. A pronounced enrichment of EPO was observed in cysts with sodium concentrations greater than 100 mmol/liter, suggesting an association with proximal tubular malformations. Findings suggest that single interstitial cells juxtaposed to proximal tubular cysts may produce EPO independent of the oxygen pressure inside the cysts, which ameliorates the anemia during end-stage polycystic kidney disease [17].

The role of EPO in the cystic fluid cannot be unambiguously explained. No evidence of fluid EPO penetration to the serum is provided. A new question arises, whether fluid EPO could be a growth factor for renal cysts, similarly to inflammation mediators or the interleukins which are present in the cystic fluid [18].

Conclusion

This study suggests that in patients with renal cysts, there is no erythropoietin penetration from cystic fluid into blood and the level of erythropoietin in cystic fluid needs more research.

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ACUTE APPENDICITIS : THE RELIABILITY OF DIAGNOSIS AT EL-MERGIB REGION TEACHING HOSPITALS

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Abstract

The aim of the present study was to spotlight different risk and associated factors amenable for incidence of acute appendicitis (A.Ap) and to clarify the validity of procedures done to provisionally diagnosed A.Ap. All cases admitted to surgery department at AL-Khoms teaching hospital and Zeliten teaching hospital, with query appendicitis were taken during period from 1-10-2009 to 31-12-2009. All cases subjected to a well formed questionnaire involved all variables and all cases were followed during period of observation and investigations till open appendectomy were done. Results showed a more predominance of A. Ap. among males than females (ratio of 1.28:1) at all age groups with the commonest age at 10-19 years. Also, it emphasized that the most sensitive and specific symptoms were pain at first (98.9%), then vomiting (67.8%) and anorexia (35.6%), Lavardo score as a diagnostic tool has high sensitively 93.9%, a high positive predictive value (75.6%) if an ultra investigations as C.T. and c-reactive protein were done, because still ultimate diagnosis of A. Ap. in our teaching hospitals depends upon leucocytosis and ultrasonography whose have a low sensitivity (59%, 56.1%) and specificity (58.3%, 75%) respectively, the study recommended that these ultra investigation (C.T and c-reactive protein)Must be done to all patients before appendectomy, furthermore the study opened new challenges in educational career of our students in diagnosis of acute appendicitis.

Keywords: *Risk factors, acute appendicitis, Lavardo score, c-reactive protein.*

Introduction

Appendicitis is the most common acute surgical condition of abdomen; approximately 7% of the population will have appendicitis in their life time, with the peak incidence occurring between the ages of 10 and 30 years. The white blood cell (W.B.C) count is elevated (greater than 10.000 per mm in 80 percent of all cases of a cute appendicitis, unfortunately, the W.B.Cs is elevated in up to 70 percent of patients with other causes of right lower quadrant pain, thus elevated W.B.Cs has a lower predictive value, but serial W.B.Cs measurement (over 4-8 hours) in suspected cases may increase the specificity as the W.B.Cs count often increases in acute appendicitis(ref-l). ultrasonography specificity is 92% but sensitivity does not exceed 85% while C.T. scan sensitivity reaches to 90% to 100% and specificity reaches 95 to 97% [1].

During the period from April 2003 to April 2004, 247 patients underwent appendectomy in Colorado University hospital, the normal appendix in these patients reached 10 cases (4.05%) while actually inflamed cases were (184) with a percent of (75,5%) while those with perforation reached (53) cases with a percent (21.45%) [2].

In U.S.A, the highest incidence of primary positive appendectomy was found in persons aged 10-19 years, males had higher rate of appendicitis than female for all groups (overall rate ratio, 1.4: 1). But the incidence of appendicitis decreased from 1970 to 1984 by about 14.6% the reasons for this decline are unknown [3-4].

Appendicitis remains one of the most common acute surgical disease, its peak incidence occurs more frequently in early adulthood, appendicitis occurs more frequently in males especially at time of puberty. A review of more than (2000) patients with appendicitis demonstrated an overall 1.3: 1 male predominance. A decline in incidence of acute appendicitis from 100 cases per 100.000 population to 52 cases per 100.000 population was demonstrated over a study period (1975-1991) from changes does not seem to be explained by improved diagnosis and the explanation for this

phenomenon remains elusive. Currently 84 percent of all appendectomies are performed for acute pathology, the rate of normal appendectomy average was 16 % of patients, with females comprising 68 % of these patients found to have a normal appendix at exploration [5].

Among British there has been a decline in the incidence of appendicitis over the last 20 years, but the reason for this is unknown. There is an equal incidence in males and females. Appendicitis is uncommon in patients below age of 2 year and above the age of 65 years with a peak incidence between 8 and 14 years in western countries, it is seen more frequently in cities than in rural areas. Only 50% of patients give a typical history. Abdominal pain remains the prime symptom of acute appendicitis. Vomiting occurs in 75 percent of patients, but not prolonged and most patients vomit only once or twice. Most patients give a history of constipation before onset of pain, diarrhea may occur in some patients particularly children, in 95% of patients with acute appendicitis followed with acute appendicitis anorexia is the first symptom followed by abdominal pain which is followed in turn by vomiting, if vomiting precedes pain the diagnosis should be questioned. Temperature is rarely more than 1℃ rise [5-6].

Despite newer imaging techniques, acute appendicitis can be very difficult to diagnose. 1/2 to 2/3 of patients have the classical presentation pain begins in the epigastrium then shifted to RLQ typically occurs, RLQ pain was 81% sensitive and 53% specific for diagnosis, Anorexia is the most common associated symptoms. Vomiting is more variable, occurring in about 1/2 of patients. The white blood cell count (W.B.C) rise is of limited value in diagnosis of acute appendicitis where sensitivity of WBC is 70-90% but specificity is very low. But, a positive predictive value of high W.B.C is 92% and negative predictive value is 50%. Graded compression ultrasonography to diagnose acute appendicitis reported 94.7% sensitivity and 88.9% specificity. But there are limitations of US where retrocecal appendix may not be

visualized perforations may be missed due to return to normal diameter. C,T. (computed tomography) is best choice in diagnosis of acute appendicitis. In one study, CT had greater sensitivity, accuracy, negative predictive value than US (ultrasonography) [7].

More recently suggested laboratory evaluation is the determination of the C-reactive protein level. An elevated C-reactive protein level (greater than 0.8 mg per dl) is common in appendicitis, but some studies disagree on its sensitivity and specificity. An elevated C-reactive protein level in combination with an elevated WBC count and neutrophilia are highly sensitive (97 to 100 percent), if one of these three findings is absent the chance of appendicitis is low [8].

The aim of the study were:

1. To clarify the major risk factors associated with occurrence of acute appendicitis.

2. To spotlight the validity of different procedures done on acute appendicitis patients starting from provisional diagnosis in outpatient department (O.P.D), to observational period in the internal section where lab. Investigations were done till surgery done where the removed appendix may be normal or inflamed.

Subjects and Methods

This study was carried out during the period from October 2009 till end of December 2009 where all provisionally diagnosed acute appendicitis patients in O.P.D at El-Khoms teaching hospital (group I) and at Zeiiten teaching hospital (group II) were taken in the study. A well designed questionnaire involved all risk factors and confounding associated.

With acute appendicitis was applied to study groups. All subject were followed up during observation and investigations done (C.B.Cs, Hb%, and urine analysis) and ultrasonography. Number of study subjected relieved during observation and remaining subjects were followed in operation room to see whether the removed appendix was normal or inflamed.

All data were collected, presented and analyzed using Alvarado score in detection of acute appendicitis [1]. This score gives one point for following [Migratory pain in RLQ, Anorexia, Nausea and vomiting, rebound tenderness, fever, shifting of leucocytosis to left (segmented neutrophils)] and gives two points to [Both RLQ tenderness and leucocytosis]. A score below 5 is strongly against a diagnosis of appendicitis, while a score of 7 or more is strongly predictive of acute appendicitis, in patients with an equivocal score of 5-6, CT scan is used in U.S.A to further reduce the rate of negative appendectomy [1]. Data are analyzed using basic statistical methods according to Armitage 1987.

Results and Discussion

Over all positive predictive value of provisional diagnosis is 62. 9% while highest predictive values 80%, 75% were at age group 50-60, 40-50 years old respectively while, lowest +ve predictive values 53.8%, 58.10%, 58.3% were at age group 30-40, 20-30 and less than 10 years old respectively.

Provisional diagnosis predictive values is statistically higher (PO.01) among males 74.58% than among females 52.22%.

9.1% of inflamed appendix patients show negative lavordo score 33.3% (one third of cases) lavordo score gave and doubt result which needs further investigation especially C.T. to confirm it 57.5% of cases confined by lavando scales.

Acute appendicitis is one of the most important surgical emergencies to which certain percent (reached to 7%) of human population are exposed, but our study is not a community based study, so incidence rate can be deduced, but it resulted that the commonest ages of incidence are 10-19 years (about 35.2%) of studied subjects fall in this age group, and 20-29 years (about 29.5%) of selected subjects occurred within age group, with a male predominance where male to female ratio scored (1.28: 1). This coincided with many studies which reported that primary positive appendicectomy was found in persons aged 10-19 years, male had higher rate of appendicitis than females for all age group (over all rate ratio is

1.4: 1). Other study reported that appendicitis occurs more frequently in male especially at time of puberty (overall rate ratio is 1.3: 1).

Furthermore, the study showed that the provisional diagnosis of acute appendicitis had a more positive predictive value (74.58%) among males than among females (74.83%) with a highly significant difference (PO.001) as shown in tabs (3, 4). Where 61.5% of normal appendix found on open appendectomy were females and only 38.5% were males it was also found that out of 90 appendectomized patients only 54 were inflamed (60%), about 12 (13.3%) were perforated and the normal appendix removed from 24 ones (24.7%) However, these results are nearly consisted with the results of a study carried out at Colorado University hospital (2003-2004) where number of inflamed appendix were 184 (75.5%) and the number of perforated appendix were 23 patients (21.45%), but the normal appendix were 10 cases (4.05%).

Only contradiction to this study were percent of normal which were in our study (26.7%). This may be difference in teaching of diagnosis, large sample size taken in this hospital, but fortunately, other study reported that the percent of normal appendectomy reached to 16 percent with females comprising 68% percent of these patients found to have a normal appendix at exploration and this agrees with our foundations as in table (4) where percent of normality in female reached 61.5% out of the normal.

As regard residual, our study resulted that actually inflamed appendix were statistically higher ($p<0.05$) among Rural patients (63.6%) than among urban ones (36.4%) this was in contrast to findings in western countries, James et al. (2007) reported that acute appendicitis occurs more frequently in cities than is rural areas, our explanation for that controvers is that our region AL-Margib province is considered from demographic point of view, a rural region and urbanization status can't be applied on it.

Lavardo score as a diagnostic tool showed a fairly good results in our study where only (9.1%) of

actually inflamed appendix gave a negative score (less than 5) but already positive score (more than or equal 7) showed in only 57.5% of actually inflamed (sensitivity 57.8% and specificity 66.7%) as shown in tables (7-10), this was due to a high number of doubtful diagnosis (33.3%) cases by Alvarado score which needs further investigations as C,T.,and c-reactive protein if these investigations were applied as in table (10), this will elevate the sensitivity of Alvarado- score to 93.9% these results are consistent with many studies 1, 8, 9.

Classical clinical presentation occurs in 60% of patient underwent appendectomy and this agrees with other studies 5, 6, 7 who reported that 1/2 to 2/3 of patients have the classical presentation, pain was the imminent symptom (98.9%) with 100% sensitivity & very low specificity and with 74.2% positive predictive value (Table 11) after pain, vomiting was more common among those submitted to surgery (67.8%), other studies (5, 6) reported that vomiting occurs in (75%) of patents while another study (7) reported that vomiting occurring in about 1/2 of patients only, vomiting sensitivity in our study was 69.7% and specificity 37.5% was the third common symptom with 40.9% sensitivity and 79.2% specificity with the highest positive predictive value of all symptoms (84.4%) as shown in Table (11B), Table (12) B- showed that leucocytosis > 9000 was evident in 54.4% of appendectomized patients with a sensitivity 59.1% and specifity 58.3% and 79.6% positive predictive value, as regards ultrasonography its sensitivity was 56.1% and specificity 75% and a higher positive predictive value (86.1%) than leucocytosis. This was in agreement as the other studies but our finding is highly lower than these studies, because other studies used computed tomography (C.T.) and c-reactive protein as a more meticulous investigations for diagnosis of acute appendicitis.

It could be concluded that computed tomography (C. T.) and C-reactive protein must be done for all provisionally diagnosed patients as acute appendicitis.

Table (1) : Age and gender distribution of study subjects in the two groups.

Age groups	Group I		Group II		Total	
	No.	%	No.	%	No.	%
<10	4	7.14	8	16.3	12	11.4
10-19	21	37.50	16	32.7	37	35.2
21-29	18	32.14	13	26.5	31	29.5
03-29	6	10.71	7	14.3	13	12.4
40-49	2	3.58	2	4.1	4	3.8
50-59	4	7.14	1	2.0	5	4.8
60+	1	1.79	2	4.1	3	2.9
Total	56	100	49	100	105	100
Mean age (x)	24.8		22.69		23.5	
S.D	±13.6		±13.87		±13.48	
t test = 0.79 P value > 0.05						
Gender	**Group I**		**Group II**		**Total**	
	No.	%	No.	%	No.	%
Mate	31	55.4	28	57.1	59	56.2
Female	25	44.6	21	42.9	46	43.8
total	56	100	49	100	105	100
M / F ratio	1.24: 1		1.33: 1		1.28: 1	

Table (2): the predictive value of provisional diagnosis in age groups and gender in studied subjects.

Age groups	Group I				Group II				Total		
<10	1	0	3	4	0	4	4	8	12	7	5S.3
10-	4	3	14	21	3	2	11	16	37	25	67.6
20-	3	3	12	18	2	5	6	13	31	18	58.1
30-	2	1	3	6	0	3	4	7	13	7	53.8
40-	0	0	2	2	6	1	1	2	4	3	75
50-	0	I	3	4	0	0	1	1	5	4	80
60+	0	0	1	1	0	1	1	2	3	2	66.6
Total	10	8	38	56	5	16	28	49	105	60	62.9

gender	Total No.	Inflamed Apa Apperd	Normal relieved	+ve predictive value
male	59	44	15	74.58%
female	46	22	24	52.22
$x^2 = 7.88$ p values is less than 0.01				

Table (3): Distribution of studied subjects According to residence.

	Group I N=56				Group II N=49				Total N=105			
	Rural		urban		Rural		urban		Rural		urban	
	No.	%	No.	%	No.	%	No.	%	No.	%	%	No.
Provisionally diagnosed subjects	29	518	27	48.2	40	81.6	9	18.4	69	65.7	36	34.3
Ztest	Z=0.27 p>0.05				Z=4.42 p>0.01				Z=0.27 p>0.01			
	Group I N=38				Group II N=28				Total N=66			
	Rural		urban		Rural		urban		Rural		urban	
Actually in Flamed Subject	No.	%	No.	%	No.	%	No.	%	%	No.	%	No.
	19	50	19	50	23	82.1	5	17.9	42	63.6	24	36.4
Ztest	Z=0 p>0.05				Z=3.36 p>0.01				Z= 2.21 p>0.05			

Not significant significant high significant

Table (4): Provisional diagnosis assessment and final diagnosis according to gender.

Gender	Total of provisional diagnosis	Inflamed on appendectomy		Normal & relieved		+v'e predictive value
		No.	%	No.	%	
Male	59	44	66.7	15	38.5	74.58
Female	46	22	33.3	24	61.5	47.83
total	105	66	100	39	100	

Table (5): Final results of appendectomy subjects in the (2) groups.

	Groupl		Group H		Total	
	No.	%	No.	%	No.	%
Inflame appntiy only	34	73.9	20	45.5	54	60
Inflamed & prefrontal	4	8.7	8	18.2	12	13.3
Normal	8	17.4	16	36.4	24	26.7
Total	46	100	44	100	90	100

X^2 - 7.58 p value < 0.05

Table (6): Role of lavardo score in diagnosis of Acute appendicitis patients admitted to surgery of found inflamed.

	Group I		Group II		Total	
	No.	%	No.	%	No.	%
Conclusive - 7	23	60.5	15	53.6	38	57.6
Doubt 8 needs more specific invest 5-6	12	31.6	10	35.7	22	33.3
Absolutely not condic	3	7.9	3	41.7	6	9.1
Total	38	100	2	100	66	100

Table (7): Validity of lavardo score in diagnosis of acute appendicitis patients admitted to surgery.

Lavarado score	Inflamed appendix on appendectomy	Normal appendix an appendectomy	Total
Conclusive Ls=or>7	38	8	46
Not conclusive Ls<7	28	16	44
Total	66	24	90

Sensitivity = 57.8%, Specificities = 66.7%. +ve predicative value = 82.6%, -ve predicative value = 36.4%, Degree of accuracy - 60%.

Table (8): validity of lavardo score in diagnosis of acute A. appendicitis in the two. groups (AL-Khoms & Zleiten).

	Group I			Group II		
Lavarado score	**Inflamed on operation**	**Normal on operation**	**Total**	**Inflamed on operation**	**Normal on operation**	**Total**
Conclusive Ls=or>7	23	5	28	15	3	18
Not conclusive Ls<7	15	3	18	13	13	26
Total	38	8	46	28	16	44

Sensitivity = 60.5%, Specificities = 7.5%, +ve predicative value = 82.1 Sensitivity = 53.6%, Specificities = 1.3%,, +ve predicative value = 83.3%, -ve predicative value = 50%, Degree of accuracy = 63.6% -ve predicative value = 16.7%, Degree of accuracy = 56.5%

Table (9): Validity of lavardo score f further investigations done.

Lavarado score	Inflamed appendix on appendectomy	Normal appendix an appendectomy	Total
Conclusive 7 and do it 5-6	62	20	82
Sorely not conclusive <4	4	4	8
Total	66	24	90

Sensitivity = 93.9%, Specificities = 16.7%, +ve predicative value = 75.6%, -ve predicative value = 50%, Degree of accuracy = 73.3%

Table (10): (A) Distribution of studied subjects according to clinical picture.

	Group I		Group II		Total	
	No	%	No	%	No	%
Classical clinical picture	32	69.6	28	63.6	60	66.7
Pain	46	100	43	97.7	89	98.9
Vomiting	34	73.9	27	61.4	61	67.8
Anorexia	26	56.5	6	13.6	32	35.6
Fever	20	43.5	13	29.5	33	36.7
Dianhoea	2	4.3	14	31.8	16	17.8
Total	46	100	44	100	90	100

(B)- validity of different symptoms of appendectomized subjects

symptoms	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Pain	100	4.2	74.2	100
Vomiting	69.7	37.5	75.4	31.03
Anorexia	40.9	79.2	84.4	32.8
Fever	37.9	66.7	75.8	28.1
diarrhoea	13.6	70.8	56.3	22.9

Table (11) : (A) Distribution of result of investigations done forappendectomized subjects (W.B.Cs count & ultrasongraphy)

	Group I				Group II				Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
T W.B.Cs Leucocytosis	27	71.1	7	87.5	12	42.9	3	18.8	49	54.4
Positive ultras onagrapy	22	57.9	1	12.5	15	53.6	5	31.3	43	47.8
Total	38	100	8	100	28	100	16	100	90	100

(B) Validity of Leucocytosis & ultrasongraphy in diagnosis of acute appendicitis

Investigations done	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
WBCs>9000/c.c	59.1	58.3	79.6	34.2
ultrasonagrapy	56.1	75	86.1	38.3

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ACUTE APPENDICITIS : THE RELIABILITY OF DIAGNOSIS AT EL-MERGIB REGION TEACHING HOSPITALS

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STANDARDIZED COMPREHENSIVE DYNAMIC DIAGNOSTICS AND TREATMENT OF POSTOPERATIVE PERITONITIS

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Abstract

Structural issues of primary interventions, development frequency, mortality and treatment tactics of postoperative peritonitis after emergency and selective abdominal operations were ana-lyzed on huge clinical material. The assessment and opportunity of prognostic integral scale appli-cation, summery of results, which was standardize by protocols of treatment of patients with post-operative peritonitis, adequately estimate risk and selection of optimal tactics of reoperation.

Considering obtained analysis results it is possible to establish a fact that the use of prognostic scales allow not only to adequately assess the severity of main pathology and its complications but also with high degree of accuracy to verify the extent demonstration of polyorgan disfunction and accordingly to identify the group prognosis for mortality risk. Based on above mentioned infor-mation we suggest to use two integral systems to identify the tactics of managing patients with postsurgical peritonitis. To assess the probability of reintervention it is possible to use specifications of PIR and to use the system SOFA (Sepsis-Related Organ Failure Assessment, Vincent J., 1996) for dynamic assessment of the severity of polyorgan disfunction and efficacy of medical activities.

Key Words: *postoperative peritonitis, protocol of treatment, prognostic integral scale.*

Introduction

There is no more dramatical but sometimes even tragical situation among critical conditions of surgical practice than severe early postoperative complications that

require secondary surgical intervention. Early relaparotomy remains to be the main mean of saving patient under development of serious postoperative complications as peritonitis, abdominal bleeding, mechanical obstruction, destructive pancreatitis and others[1,5,7]. Postoperative peritonitis belongs to one of the most complicated and adverse complications[2,3,4,5,8]. Lack of unified standardized tactics of managing of this category of patients is determined by empirical approach to the choice of appropriateness, duration and method of surgical reintervention. **AIM :** the main aim of current analysis is to try the implementation of standardized guidelines of managing of postoperative peritonitis based on different prognostic criteria by adapting them to the individual possibilities of different chains of surgical service of Uzbekistan Health System.

Subjects and Methods

To conduct multicentral analysis we included into research the treatment results of 317 patients with postoperative peritonitis (PP) treated at 17 inpatient facilities that were divided into 3 groups. In group 1 (Republican level) were included 191 patients (79 patients after primary emergency surgery and 112 patients after elective surgical interventions) that were under observation at Republican Specialized Center of Surgery named after academician V.Vakhidov in all abdominal departmetns for the period from 1976 up to 2008 including. In group 2 (city and regional level) were included 96 patients (77 patients after primary emergency surgery and 19 patients after elective surgical interventions) that were under observation at different clinics of city and regional level of surgical service for 2000 - 2008 : Nukus branch of Republican Scientific Center of Emergency Care; Tashkent city hospitals #1 and #7; Tashkent regional multidisciplinary medical center; City clinic of Samarkand; Clinic of Samarkand State Medical Institute. In group 3 (district clinics) were included 30 patients after primary emergency surgery that were observed at district clinics of the Republic of Karakalpakstan for 2000 - 2008.

Female patients - 72 (22.7%), male patients - 245 (77.3%), average age of patients in group 1 was - 47.15±12.09 years, in group 2 - 48.12±13.28 years and group 3 - 35.29±11.47 years. It should be note that in group 3 the average age of patients was ($P<0.05$) less than in groups 1 and 2.

All conducted primary surgeries according to the category of complexity were divided into the following types:

1. General surgical operations: appendectomy, traditional cholecystectomy, palliative treatment of complications of ulcer disease (suture ligation of perforations and discontinue of bleeding), treatment of intestinal obstruction (excision of adhesions, to tunnel a stoma, bypass), rupture of small pyogenic abscesses of abdominal cavity, urgent operation in connection with trauma of abdominal cavity organs without resection technologies, suturing of veins of stomach cardia in order to stop bleeding from varix of oesophagus and stomach under portal hypertension (Patsiora's operation), uncomplicated forms of echinococcosis of abdominal cavity organs, traditional hernioplasty. Put the other way round these are interventions that don't require specific surgical tools, expansion of the capacity of proposed intervention, prolonged reanimation and accordingly experts in specialized fields. This type of operations is conducted at all levels of surgical service and at the same time as a main intervention that is used at district clinics, against of republican and regional clinics.

2. High technological operations: radical treatment of complications of ulcer disease (resection of stomach), calculous cholecystitis complicated by mechanical jaundice laparoscopic cholecystectomy and appendoctomy, collateral choledochoduodenoanatomosis and cholecystoenteroanastomosis, total separation of gastroesophageal collector (method of Nazirov FG), external draining of gall ducts under postcholecystectomic syndrome, operations under acute pancreatitis and hiatal hernia. Performance of these types of surgeries depends on two components; from one side it is a surgical, diagnostical, resuscitation and anestisia equipment of

the clinics and from the other side is the professional skills of a surgeon.

3. Surgeries are mainly being performed in Republican specialized centers are: radical treatment of tumors of gastrointestinal tract (gastrectomy, hemicolectomy, pancreatoduodenal resections), reconstructive-rehabilitative surgeries on gall ducts, reconstructive surgeries on stomach under diseases of operated stomach, radical correction of syndrome portal hypertension (portosystem shunting), peritoneoatrial shunting under diuretic resistant forms of ascites, surgeries on esophagus. This type of surgery is mainly typical for republican chain of surgical service, applies to the category of severe patients and directly depends on particular speciality and professional skills of a surgeon, appropriate equipping of diagnostical, special surgical equipment and instruments, possibilities for dinamycal monitoring by qualiffied specialists of resuscitation and anestesia services.

Certainly we understand the conditional character of this division especially for urgent surgery but for objective analysis of the quality of surgical assistance this gradation is necessary especially due to the fact that the main task for surgical service is a development of tactics-diagnostical and accordingly treatment standards for this severe category of interventions as relaparotomy. Character of primary surgeries is shown in table 1.

Period of developing of postoperational peritonitis is approximately similar in the groups with maximum frequency of 3-5 days - 64.6-70.1% and 6-9 days - 15.2-18.8% of patients.

Prevalence of peritonitis and character of peritoneal exudate have an important role especially in details of treatment and disease prognosis. It was noted that maily by intraoperative way were detected diffusive forms of peritonitis with the most frequency after primary emergency interventions (table 2).

Depending on the specifics of performed surgeries we can note

that in group 1 in 40.5% of cases after emergency operations and in 20.5% after elective interventions, peritonitis was of bilious etiology, in group 2 these indicators were 6.5% and 42.1% respectively. The serous-fibrinosis and purulent character of exudate prevailed in all groups under general postsurgical peritonitis.

The diagnostics of postsurgical peritonitis is a critical problem under unclear clinical picture. Diagnostical monitoring, or putting it more precisely, its quality depends on the level of surgical service.

Considering different applied methods of diagnostics, their comprehension regarding verification of postsurgical peritonitis, depends not only on the level of inpatient facility and respectively diagnostical potential but as well as from the use rate i.e. qualitative dynamic monitoring. Thus based on clinical and laboratory data, postsurgical peritonitis is verified in 63.4% patients of group 1 and only 42.7% in group 2 and 40.0% in group 3 (table 3).

Ultrasound scanning is the simplest method to evaluate the condition of abdominal cavity organs but the comprehension of this method depends on the abilities of the equipment and professional skills of a researcher and at the same time at Republican Specialized Center of Surgery the efficacy of ultrasound scanning was 60.8%, in group 2 - 45.5% and in group 3 only 20.0%. Com-puter tomography is an expensive method and difficult to perform at those conditions however its comprehension is 66.7-67.9%. The most diagnostic value was received by performing laparoscopic diagnostic - 85.7% (in 12 out of 14 patients in group 1).

In table 4 we showed a character of reinterventions in patients with postoperative peritonitis. Depending on ethiology of peritonitis we divided operations according to the following complications: inconsistency of suture after close perforation or applied anastomosis; biliary peritonitis (in-consistency of stump of cystic duct, iatrogenic damage of choledoch, inconsistency of suture of biliodegestive anastomosis of a different ethiology -

cases when peritonitis was developing after pri-mary surgery without verifying provoking factor, was progressing after primary emergency surgery or situations with not verifyied main diagnosis - diverticulitis (Mekkel) with perforation (group 3 - 2 patients that had appendectomy), development of appendicitis with general peritonitis in a patient of group 3 after suturing of perforative ulcer, development duodenal ulcer (group1 - in 2 patients after hemicolonectomy and echinococcectomy) as well as ascetic-peritonitis in a patient with liver cirrhosis after splenectomy that resulted in eventration.

Results

For the last ten years the main direction in development of surgical service for patients with different postsurgery complications became a development and implementaiton of different prognostic system concerning as evaluation of severity of patient's condition so as a possible identication of potential risk of death from this complication. All leading world-wide clinics are working in this direction and by present time the developed prognostic scales were acknowledged by many specialists and their diagnostic value allowed standardizing the evaluation of patients' condition in many countries. Objective evaluation of severity of patient's condition in peritonitis and identifica-tion of the possibility of failure represent certain difficulties. At the moment there are several ap-proaches in solving this task. First, the use of widespread integral scales to evaluate the severity of patients' condition (APACHE, APACHE II, APACHE III, SAPS, SAPS II, SOFA, MODS and some others); secondly, the use of specific scales that had been developed considering particular qualities of peritonitis (the most famous scale - Mangame index of peritonitis, prognostic index re-laparotomy); thirdly, the use of some individual indicators of homeostasis as an independant predic-tor of failure [8]. But this kind of variety of evaluating scales can also be evidence of weakness of the latter. Due to that, after a thorough analysis of literature, we decided on the most optimal options of prognosis estimation and retrospectively identified their diagnostic value for the different level clinics of surgical

service. An analysis on evaluating the probability of reintervention was conducted on identifying prognostic index of relaparotomy suggested as prognostic criteria of surgical reinterventions. The system includes score system according to 8 criteria (performance of first surgery according to emergency indicators; respiratory insufficiency; renal failure; intestinal paresis after 72 hours of postsurgery; abdominal pain after 48 hours of postsurgery; infectious complications at the area of surgery; consciousness disorders; pathological symptoms occuring after 96 hours of postsurgery) at the same time prognostic index of relaparotomy more than 20 points - absolute indicator to relaparotomy. After retrospective analysis it is possible to establish a fact that having the simple use of this index we were able to divide patients according to prognostic index of relaparotomy only in groups 1 and 2. It was not possible to classify medical files of a 3 group as there was no data regarding consciousness dynamic condition or some laboratory indicators were not mentioned. Due to the same reason it was possible to systematize medical files in group 1 only in 164 patients out of 191; in group 2 in 44 patients out of 96 patients. Average value of prognostic index of relaparotomy in analysis groups as well as division of patients according to its indicator is shown in table 5.

Distribution of mortality rate depending on PIR showed that mortality rate was minimum when PIR was less than 20 points and it was 9.7% in group 1 and 14.3% - in group 2. Under PIR 20-22 points the mortality rate had increased sharply and reached - 43.5% and 53.8% respectively; under PIR 23-24 points - 80.9% and 88.9% (figure 1).

Objective integral and quantitative assessment of the severity of patient's condition in a critical condition including abdominal sepsis can not be performed without using objective scale-system of assessing functional disorders (APACHE II, SAPS) and the extent of organ disorders (MODS, SOFA). Between those systems regardless of the fact that they have the same methodological approach there is a principal or strictly speaking

ideological difference. Particularly, the main purpose of objective systems of assessing the severity of patients' condition is prognosis and risk assessment for the lethal outcome. At the same time PACHE II and SAPS are higly specific (90%) regarding success prognosis but less sensitive (50-70%) regarding lethal outcome prognosis. Furthermore these systems are accepted for outcome prognosis and comparative analysis of groups of patients but not for the assessment of individual patients [2]. Exactly for this reason they are not recommended for prognostic assessment of a particular patient and can not be basis for taking a decision in a clinical practice that had been noted at the conference of European society of intensive medicine (1998). Therefore it has to be considered that scales MODS and SOFA are clinically significant instrument for objective assessment of severity of patients' condition and for prognosing outcome of critical condition in abdominal sepsis.

The system of assessment SOFA (Sepsis-Related Organ Failure Assessment, Vincent J., 1996) intended for dynamic assessment of severity of polyorgan disfunction and efficacy of medical activities is the most comfortable and simple for use. Distinctive quality of this scale is its high sensitivity for patients with peritonitis as it is intended especisally for the assessment of septic demonstrations and polyorgan disfunction, on the other hand dynamic indicators reflrect efficacy of current conducting treatment that is also important for managing this difficult category of patients. According to data of distribution, the indicator SOFA of 1-4 points is obtained in 25.3% of patients of group 1 and 26.1% in group 2. Indicator SOFA of 5-8 points in 51.6% and 43.5%, more than 9 points in 23.2% and 30.4% respectively (table 6).

According to distribution as of severity of indicator SOFA the mortality rate in group 1 with SOFA of 1-4 points was - 8.3%, 5-8 points - 40.8% and more than 9 points - 100%, in group 2 - 16.7%, 50.0% and 100% accordingly (figure 2).

Table (1): Frequency of postoperative peritonitis after emergency and elective interventions in diffrent groups

Category of a surgery	Group 1				Group 2				Group 3	
	Emergency n=79		Elective n=112		Emergency n=77		Elective n=19		Emergency n=30	
	No	%	No	%	No	%	No	%	No	%
General surgical category										
Appendectomy	1	1.3%			10	13.0%			12	40.0%
Suture ligation of perforations and discontinue of bleeding under ulcer disease of the stomach and duodenum	7	8.9%			16	20.8%			9	30.0%
Surgeries on small intestine	1	1.3%			14	18.2%			6	20.0%
Surgeries on large intestine	5	6.3%	6	5.4%	15	19.5%	2	10.5%	1	3.3%
Cholecystectomy	2	2.5%	1	0.9%	4	5.2%	6	31.6%	1	3.3%
Echinococcectomy	3	3.8%	3	2.7%	1	1.3%				
Hernioplasty (with resection of intestines)	1	1.3%			4	5.2%				
Patsiora's operation	3	3.8%			1	1.3%				
High technological category										
Stomach resection	13	16.5%	39	34.8%	4	5.2%	2	10.5%	1	3.3%
Choledectomy with draining of choledoch	4	5.1%	2	1.8%	3	3.9%				
External draining of gall ducts	10	12.7%	4	3.6%			1	5.3%		
Collateral choledoectomyanastomosis with enteroenteroanastomosis by Brown	3	3.8%	2	1.8%	2	2.6%	1	5.3%		
Vagotomy with draining operation			6	5.4%			1	5.3%		
Lap .Cholecystectomy			1	0.9%			1	5.3%		
Splenectomy in portal hypertension			1	0.9%						
Separating surgeries under portal hypertension	1	1.3%	4	3.6%						
Category performed mainly at Republican centers										
Reconstructive surgery on stomach	5	6.3%	8	7.1%	1	1.3%				
Gastrectomy	2	2.5%	9	8.0%			1	5.3%		
Hemicolectomy	2	2.5%	4	3.6%	1	1.3%	3	15.8%		
Biliary-enteric anastomosis	11	13.9%	11	9.8%	1	1.3%				
Reconstructive-rehabilitative surgery on hepaticoholec.	5	6.3%								
Pancreatoduodenal resection			8	7.1%			1	5.3%		
Portosystem shunting			3	2.7%						

N.B: percentage is calculated out of a total number of each surgery

Table (2): Prevalence of peritonitis and character of peritoneal exudate

Category of surgery		Group 1				Group 2				Group 3	
		Emergency		Elective		Emergency		Elective		Emergency	
		N=(154)	%	N=(203)	%	N=(148)	%	N=(37)	%	N=(55)	%
Local	purulent	4	5.1%	11	9.8%	6	7.8%	1	5.3%	5	16.7%
Diffusive	serous	2	2.5%	11	9.8%	3	3.9%	1	5.3%	3	10.0%
	purulent	4	5.1%	12	10.7%	6	7.8%	2	10.5%	4	13.3%
	bilious	10	12.7%	8	7.1%	1	1.3%	2	10.5%		
	total	16	20.3%	31	27.7%	10	13.0%	5	26.3%	7	23.3%
General	serous	4	5.1%	12	10.7%	13	16.9%	1	5.3%	5	16.7%
	serous-fibr	9	11.4%	25	22.3%	19	24.7%	2	10.5%	6	20.0%
	purulent	24	30.4%	18	16.1%	25	32.5%	4	21.1%	7	23.3%
	bilious	22	27.8%	15	13.4%	4	5.2%	6	31.6%		
	total	59	74.7%	70	62.5%	61	79.2%	13	68.4%	18	60.0%

Table (3): Comprehension of different diagnostic methods of postoperative peritonitis

Diagostic method	Comprehension of a method					
	Group 1		Group 2		Group 3	
	N=(285)	%	N=(101)	%	N=(20)	%
Clinical and laboratory data	121	63.4%	41	42.7%	12	40.0%
Ultrasound scanning of abdominal cavity	48	60.8%	35	45.5%	6	20.0%
Computerized scanning of abdominal cavity	19	67.9%	2	66.7%		
Survey radiography of abdominal cavity	51	26.7%	15	23.8%	1	14.3%
Contrast radiography	17	32.1%	3	27.3%		
Esophagogastroduodenoscopy	17	39.5%	5	18.5%	1	50.0%
Diagnostic laparoscopy	12	85.7%				

Table (4) : Character of performed reinterventions in patients with postoperative peritonitis.

Character of reintervention	Emergency surgery						Elective surgery					
	Inconsistency of sutures after gastrointestinal tract surgery		Biliary peritonitis		Peritonitis of a different ethiology		Inconsistency of sutures after gastrointestinal tract surgery		Biliary peritonitis		Peritonitis of a different ethiology	
	N=(91)	%	N=(40)	%	N=(50)	%	N=(93)	%	N=(30)	%	N=(8)	%
Group 1												
Applying stoma	4	5.1%					5	4.5%				
Abdominal cavity sanation					7	8.9%	26	23.2%	1	0.9%	3	2.7%
Suturing of inconsistent sutures	28	35.4%	9	11.4%			47	42.0%	9	8.0%		
Intestines resection	1	1.3%										
Hemicolonectomy							1	0.9%				
BDA			1	1.3%					1	0.9%		
Elimination of event. With abdominal cavity sanation					1	1.3%						
External draining of choled.			20	25.3%					10	8.9%		
Rehabilitation of hepaticoholec.			2	2.5%								
Reconstructive surgery on stomach	1	1.3%					2	1.8%				
Suturing of ulcer perforation of duodenum											2	1.8%
Medical laparoscopy (sanation)	1	1.3%			4	5.1%	2	1.8%			3	2.7%
Group 2												
Applying stoma	19	24.7%			3	3.9%	4	21.1%				
Abdominal cavity sanation	1	1.3%	1	1.3%	22	28.6%	1	5.3%				
Suturing of inconsistent sutures	18	23.4%	2	2.6%			5	26.3%	4	21.1%		
Intestines resection	5	6.5%										
External draining of choled.			5	6.5%					5	26.3%		
Hemicolonectomy	1	1.3%										
Group 3												
Applying stoma	11	36.7%										
Abdominal cavity sanation					10	33.3%						
Suturing of inconsistent sutures	6	20.0%										
Intestines resection					2	6.7%						
Appendectomy with abdominal cavity sanation					1	3.3%						

Table 5. Indicators of Prognostic Index of Relaparotomy (PIR) for the moment of surgical reintervention

Group	Average value	PIR < 20		PIR > 20	
		N=(44)	%	N=(164)	%
Group 1 (n=164)	21.4±1.7	30	18.9%	134	81.1%
Group 2 (n=44)	22.7±2.5	14	31.8%	30	68.2%

Table 6 : Distribution of patients with postsurgical peritonitis according to scale indicator SOFA for the moment of surgical reintervention

Distribution of points SOFA	Group 1 (n=95)		Group 2 (n=23)	
	N=(190)	%	N=(46)	%
1-4 points	24	25.3%	6	26.1%
5-8 points	49	51.6%	10	43.5%
More than 9 points	22	23.2%	7	30.4%
Total	95	100.0%	23	100.0%

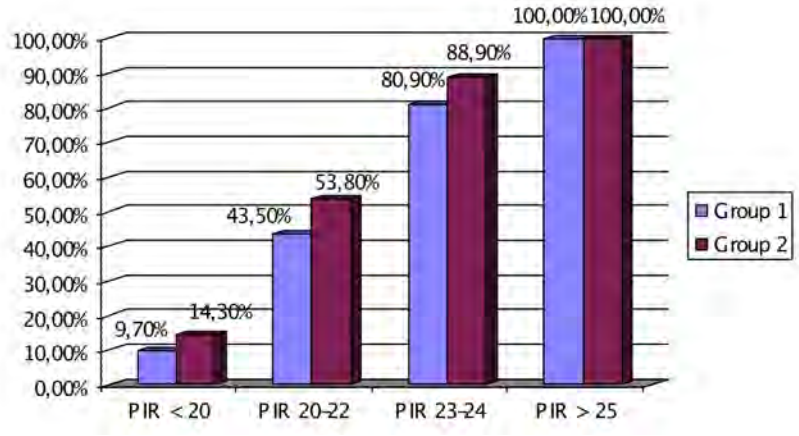

Figure 1: Mortality rate depending PIR indicator.

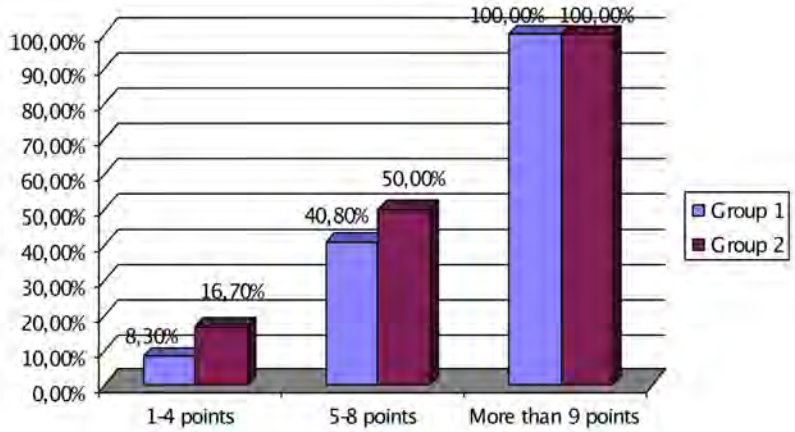

Figure 2: Mortality rate depending on indicator of scale SOFA

Discussion

According to suggested algorithm while suspecting a development of postsurgical peritonitis first of all PIR has to be identified. In case of PIR is less than 20 points it is possible to have a dynamic observation conducting full-scale conservative therapy that includes specific antibacterial therapy, decontamination of gastrointestinal tract, correction of Fluid Electrolite Balance and other disorders. Main value in identifying the efficacy of a therapy is given to a dynamic Ultrasound scanning (dynamic of focal mass, exudate, intestinal peristalsis and etc) as well as for score system of SOFA where regress indicator is an evidence of a positive efficacy of conductig conservative activities. In case of failure of a therapy there will be increase of dynamic indicator of SOFA scale as well as PIR increase. Respectively in case the value of PIR exceeds 20 points than this is an absolute indicator for relaparotomy.

At this stage the following quality should be paid attention. For the last years laparoscopic surgery took a special place and there is progressive increase of the possibilities of this small inva-sive intervention. Despite of small clinical experience of Republican specialized center of surgery in performing reinterventions by laparoscopic method, this method can be reffered to the category of

choice surgery especially because mini-invasive effect in adequately performed operation decreases the risk of developing or progressing of different complications. Of course, the probability of performing laparoscopy depends on many factors but in the view of diagnostic guideline the indications for its use don't not differ from standard indications for this type of intervention in emergency abdominal surgery. Thus we took the possibility of laparoscopic diagnostics into separate direction.

According to a suggested algorithm while having a possibility for performing laparoscopic diagnostics, the reintervetion especially in unclear clinical situations is more advisable to start from this method of surgery. While revising it has to be assessed the extent of peritonitis and its character, and that the source of this complication is verified. In limited or general peritonitis with serous exudate or of enzyme ethiology, lack of inconsistency of sutures or perforation i.e. the factors of causes of peritonitis, the laparoscopic diagnostics can be transferred to a medical laparascopy that considers sanation of abdominal cavity, adequate draining. In laparoscopy verified factor of causes of peritonitis (inconsistency, perforation and etc) or isolated fibrinosis-purulent peritonitis to adequately perform intervention it is advisable to transfer to relaparotomy. But in case of severe Poly-organ insufficiency (PI) with the value of SOFA more than 9 points and extremely high risk of mortality, it is possible to perform medical laparoscopy with a thorough sanation, tamponing the source and adequate draining of abdominal cavity. In relaparotomy the choice of surgical tactics also depends on the severity of PI. If in case of revision serous-fibrinous or enzyme peritonitis were identified and the value of assessment system SOFA is 1-4 points than it is possible to extend interven-tion up to high technological level. For example, complications of undiagnozed ulcer disease (perforation) after conducting intervention concerning different emergency abdominal pathology (cholesistitis, intestinal obstruction, appendectomy and etc) when SOFA is 1-4 points, it is possible to propose performing of stomach resection or vagotomy

with draining operation (picture 3). An exception regarding choice of complexity of the surgery for patients with PI when SOFA is 1-4 points for Republican clinics or regional and city level with availability of specialized departments can be peritonitis of biliary ethiology (under inconsistency of sutures of choledoch or BDA, inconsistency of cystic duct after cholecystectomy of residual multiple choledocholiasis, iatrogenic damages of hepaticocholedoch and etc) when it is possible to perform reconstructive-rehabilitative surgeries using precision technologies.

Conclusions

Postoperative peritonitis is one of the severest and prognostically adverse complications in the structure of those its frequency can reach 31.1-43.8%. Depending on the level of surgical service and accordingly the categories of complexity of performing surgeries, the summary frequency of the mortality after interventions regarding to this complication reaches 51.3% at Republican specialized center of surgery, 63.5% in the inpatient facilities of city and regional level and 36.7% in district clinics. In 81.1 - 90.9% of cases a main reason of mortality outcome is polyorgan insufficiency.

Main predisposing factor for developing or progressing of postsurgical peritonitis is unjustified expansion of operation capacity, not following of compulsory principles of surgical treatment of peritonitis and underestimation of patients' condition.

Available diagnostic potential in patients with postsurgical peritonitis determines their different verified descriptiveness that is only 14.3-50.0% at district clinics, 18.5-66.7% at inpatient facilities of city and regional level and 26.7-90.0% at Republican clinic.

Optimal option of standardizing guidelines on managing this type of patients is the use of prognostic integral scales, combined results of the latter allow to adequately assess the risk of surgical reintervention and accordingly to optimize a choice of treatment tactics at all levels of surgical service of Health system.

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STANDARDIZED COMPREHENSIVE DYNAMIC DIAGNOSTICS AND TREATMENT OF POSTOPERATIVE PERITONITIS

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THE POTENTIAL BENEFICIAL EFFECTS OF PERINDOPRIL OVER CLONIDINE ON DIABETIC NEPHROPATHY IN RATS

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Abstract

Diabetic nephropathy has become a worldwide epidemic, accounting for approximately one third of all cases of end- stage renal disease. About 30-50% of patients with long-standing diabetes will develop diabetic nephropathy.

Improved management of diabetes aimed at improved glycemic control, to avoid initiation of diabetic nephropathy, and antihypertensive treatment blocking the renin-angiotensin system, to avoid its progression and need to be implemented, particularly in high-risk patients.

This study was designed to evaluate and compare the reno protective effects of clonidine and perindopril on rats with diabetic nephropathy.

Diabetic nephropathy was induced in rats by streptozotocin (STZ) 100mg/Kg/single I.P) administration. Diabetic nephropathy rats were randomly divided into one of 3 groups. Diabetic nephropathy group, clonidine (0.03mg/kg/day) treated group and perindopril (1.5mg/kg/day) treated group. Each given orally for 4 weeks starting 4 weeks after STZ injection. Systolic and mean arterial blood pressure (SBP & MBP), were measured by rat tail method. Renal blood flow (RBF) were estimated by flowmeter. Fasting blood glucose (FBG) and 24 hours urine albumin were measured by colorimetric methods. Renal tissue specimens were histopatholgically examined by hematoxylin & eosin staining.

Both drugs significantly reduced SBP, MBP, fasting blood glucose (FBG) and urine albumin (mg/24hours). There was insignificant differ-

ence between hypotensive effects of clonidine and preindopril. Effects of preindopril on RBF, FBG and urine albumin were more significant than that of clonidine. Moreover, histopathological examination of kidney tissues showed improvement of nephropathy in the form of reduction of basement membrane, thickening and infiltration of the inflammatory cells.

From this study we can conclude that, both drugs significantly reduced blood pressure, urine albumin, FBG and RBF with superior effect of perindopril over clonidine.

Introduction

Frequently Hypertension and diabetes mellitus are closely related conditions, their deleterious effects being additive. In type 1 diabetes, hypertension often develops late, whereas in the type 2 diabetes, hypertension diagnosis frequently precedes that of diabetes itself [1].

Overall, 50% to 60% of diabetics over the age of 60 years are hypertensive [2]. Hypertension prevalence reaches 40% in type 1 diabetes and 70% in type 2 diabetics, with a female predominance [3].

Diabetic nephropathy is characterized by persistent proteinuria, decreasing glomerular filtration rate (GFR) and increasing blood pressure [4].

Diabetic nephropathy is an important complication of diabetes for a number of reasons. Firstly, it is a relatively common complication affecting about one of three of patients with IDDM.- secondly, the proteinuria which is the hallmark of widespread damage to small and large blood vessels and a marker for cardiovascular disease which is a common cause of death in these patients. Thirdly, there is increasingly convincing and optimistic evidence that the progression of nephropathy and its associated mortality can be ameliorated by antihypertensive and other treatments if started at an early stage [5].

Beside glycemic control, the systolic blood pressure could be identified as the pre-eminent factor influencing the development of

nephropathy. Hypertension is a crucial determent not only for the loss of kidney function but also for the prospects of survival of diabetics with nephropathy [6].

The lowering diastolic blood pressure below the traditional target blood pressure of 90 mmHg is safe in diabetic patient and should indeed be recommended. This policy results in a reduction of mortality and morbidity superior to that seen with the conventional treatment goal of a reduction below 90 mmHg [7].

The Modification of Diet in Renal Disease (MDRD) study demonstrated that tight blood pressure control to a level of 92 mmHg mean blood pressure (corresponding to a blood pressure of 125/75 mmHg) significantly slowed the deterioration of renal function relative to a less tight blood pressure reduction to 107 mmHg mean arterial pressure (corresponding to 140/90 mmHg) [8].

The renin angiotensin aldosterone system plays a central role in the development of hypertension and progression of end organ damage [9].

Evidence has accumulated during the last few years that ACE inhibitors convey a specific nephroprotective effect in addition to the beneficial effects of blood pressure reduction and therefore are even superior to the beneficial effects of conventional antihypertensives. Comparable studies showed that ACE inhibitors were superior to (β-blockers, diuretics and calcium antagonists in reducing albuminuria [10].

The sympathetic nervous system plays an important role in the pathogenesis and maintenance of essential hypertension. Sympathetic overactivity and excess catecholamines may contribute to the development of other pathological processes associated with elevated blood pressure[11].

Furthermore, sympathetic overactivity can lead to underperfusion of the kidney[12] and several metabolic abnormalities, including changes in glucose metabolism, insulin resistance, a rise in low-density lipoprotein (LDL) and a fall

in high- density lipoprotein (HDL)[13].

Therefore, inhibition of sympathetic nervous system activity may ameliorate not only hypertension but may also of the chronic diseases associated with it [14].

Perindopril erbumine is a once-daily angiotensin-converting enzyme (ACE) inhibitor that effectively lowers systolic and diastolic blood pressure (BP) in patients with mild-to-moderate hypertension [15].

Clonidine (catapress) is a centrally acting antihypertensive agent characterized by its wide therapeutic range. Clonidine is an imidazoline derivative, was originally developed as a nasal decongestant and vasoconstrictor[16].

Clonidine decreases plasma renin activity, presumably as a result of the decrease in sympathetic activity. However, it may directly inhibit the renal release of renin [17]. The inhibition of renin release contributes to the antihypertensive effect of clonidine[18].

The aim of this work is to investigate and compare the potential reno-protective effects of clonidine and prenidopril on (STZ) induced diabetic nephropathy in rats.

Material and Methods

Animals :

Twenty four male adult albino rats of locally breaded strain weighing between 150-250g at the beginning of the study were used. They have acclimatized for one week in groups (6/cage) in fully ventilated room at ordinary room temperature. Rats were allowed to ad libitum, access to water and balanced diet.

At the beginning of the experiment they were divided into 4 groups each contained 6 rats:

Group I: Control normal rats. They receive no drugs; they only receive drug vehicles (methyl cellulose).

Group II: was served to induce nephropathy by administration of a single dose of streptozotocin (STZ) (100mg/kg I.P.), then examined after 1 month.

Group III: was served to study the renoprotective effect of clonidine (0.03 mg/kg/orally) on diabetic nephropathy rat. The drug was administered daily for one month after induction of nephropathy.

Group IV: was served to study the renoprotective effect of perindopril (1.5 mg/kg/orally) on diabetic nephropathy rat. The drug was administered for one month after induction of nephropathy.

Drugs:

- Clonidine hydrochloride (catapress) [Boehringer Ingelheim pharma KG-Germany]. It was supplied as tablet 150µg.
- Perindopril tetra butyllamine (coversyl 10) [Servier Egypt Industries limited]. It was supplied as tablet 10mg.
- Heparin (Novo Industry, Denmark).
- Hematoxylin and eosin: (E. Merk, Darmastadt.,) [U.S.A.].
- Formaline (neutral 10% formaline): El-Gomhoria Pharmaceutical Chemical Co, ARE.
- Streptozotocin (STZ) powder (Sigma chemicals Co., U.S.A).
- Urethane (Ethyl carbamate): (Prolabo, Paris) white crystals 0.6ml/100g 25% freshly prepared solution [19].

All drugs were dissolved in methyle cellulose and all drugs and chemicals were freshly prepared before each experiment.

Clonidine and perindopril were administered orally using a curved gag reaching up to the pharynx for 4 weeks after induction of diabetic nephropathy by (STZ) which was administrated IP.

All groups were subjected to the following investigation:

- Systolic and mean arterial blood pressure (SAP, MAP).
- Renal blood flow (RBF).
- Fasting blood glucose (FBG).
- 24 hour urine albumin.
- Histopathological examination.

- **N.B.** it should be taken in consideration that doses of clonidine and perindopril chosen in this study are based mainly on researches done by other investigators

[20,21], on pilot experiments performed in our study, and also by calculating the therapeutic doses for experimental animals by [22].

- Systolic blood pressure (SBP) was measured by rat tail plethysmography [23].
- Renal blood flow (RBF) was measured by Doppler flow meter [24].

- Fasting blood glucose levels (FBG) : only one drop of blood of the adult rat was obtained by a puncture of the retrobulbar sinus, the capillary end of the glass tube was inserted into the medial canthus of the eye. The sinus was punctured and blood entered the tube by its own pressure forming a free flow of blood. The blood was biochemically investigated for FBG according to [25].

- Urine albumin g/24 h.

- After functional studies were completed, both kidneys were removed and the upper 1/3 of each kidney was put into a buffered 4% formaline fixation solution and processed with paraffin wax for histopathological examination. Sections (5 μm) were stained with hematoxylin and eosin [26].

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 STZ (100 mg/kg/ single I.P) resulted in significant ($P < 0.001$) elevation of both SBP and MBP from means of (122.2 ± 5.3) & (92.3 ± 3.5) in control group to means of (210.6 ± 9.7) & (165 ± 7.2) mmHg with a percentage increase of (↑72.3%) & (↑78.8%) respectively. RBF was significantly reduced ($P<0.001$) from a mean of (10 ± 0.4) cm/s to a mean of (4.7 ± 0.2) cm/s with percentage change (53% ↓). FBG was increased from a mean of (93.4±3.4) to a mean of (240.5 ± 10.6) mg/dl which is significant at

(P < 0.001) compared with the control group with a percentage increase of (↑157.4%). The results showed also that STZ induced diabetic nephropathy produced significant (P < 0.001) increase of urine albumin from (1.05 ±0.08) to (3.9 ±0.13) mg/dl with a percentage increase of (↑271.4%) compared to control group, Table (1), Fig. (2,6).

Histopathological examination of renal tissues revealed diffuse diabetic glomerular sclerotic lesion which is characterized by diffuse thickening of glomerular capillary wall and generalized increase in mesengial matrix in all mesengial regions of all golmeruli fig. (10).

Administration of clonidine (0.03 mg/kg/orally) resulted in significant (P < 0.01) reduction of SBP and MBP with a percentage reduction of (↓38.2%) and (↓37.9%) from a mean of (210.6 ± 9.7) and (165±7.2) mmHg in diabetic nephropathy non-treated group to (130 ± 4.6) and (102.4 ± 4.2) mmHg respectively in clonidine treated group. There was significant (P < 0.01) increase of RBF from a mean of (4.7±0.2) to a mean of (6.1 ± 0.24) with percentage increase (↑29.8%). Regarding FBG in clonidine treated group, results of the present work demonstrated significant (P < 0.05) reduction of FBG from (240.5 ± 10.6) in diabetic nephropathy non treated rats to a mean of (205.5±7.6) mg/dl in clonidine treated group with a percentage reduction of (↓14.5%). Concerning urine albumin the data revealed, significant (P < 0.01) reduction from a mean of (3.9± 0.13) mg/24h in the diabetic nephropathy non treated group to a mean of (2.1±0.12) mg/24h in the clonidine treated group with a percentage reduction of (↓46.1%) (Table 2), Fig. (3,7).

Histopathological examination of renal tissues in clonidine treated rats showed mild thickening of glmoerular capillary wall and mild changes in mesengial matrix Fig. (11).

Administration of perindopril (1.5mg/kg/orally) resulted in significant (P < 0.01) reduction of SBP and MBP from means of (210.6±9.7) & (165±7.2) mmHg in diabetic nephropathy non-treated

group to (125±4.2) & (95.2±3.7) mmHg in perindopril treated group with a percentage reduction of (↓ 40.6%) and (↓ 42.3%) respectively. RBF was significantly (P < 0.001) increased from a mean of (4.7 ± 0.2) cm/s in diabetic nephropathy non treated rats to a mean of (9.8± 0.5) cm/s in perindopril treated group with percentage increase of (↑ 108.5%).

Also there was significant (P < 0.01) reduction of FBG from a mean of (240.5 ± 10.6) mg/dl in diabetic nephropathy non treated rats to (165.7±8.2) mg/dl in perindopril treated group with a percentage decrease of (↓ 31.1%) compared with diabetic non treated group. Also there was with a percentage reduction of (↓ 71.7%) in urine albumin from (3.9±0.13) mg/dl to (1.1±0.05) mg/dl in perindopril treated group which is significant at (P < 0.001). (Table 3), Fig. (4,8).

Histopathological examination of kidney tissues removed from perindopril treated rats revealed minimal changes in glomerular capillary wall and mesengial matrix Fig. (12).

Statistical analysis revealed no significant differences between the effects of clonidine and perindopril on SBP, MBP compared with the diabetic nephropathy group, Table (4).

On the other hand, statistical analysis showed significant (P < 0.05) difference between clonidine and perindopril in improving FBG level of STZ-induced diabetic nephropathy in rats, table (4).

Our study also revealed a significant (P< 0.001) difference between clonidine and perindopril in improving RBF and 24 hour collected urine albumin in experimentally- induced diabetic nephropathy in rats, table (4).

Table (1): Effects of streptozotocin induced diabetic nephropathy in albino rats on SBP, MBP, RBF, FBG & urine albumin **(mean± SE)** (n = 6).

Parameters / Group	SBP (mmHg)	MBP mmHg	RBF cm/s	FBG mg/dl	Urine albumin Mg/24
Control rats	122.2 ± 5.3	92.3 ± 3.5	10 ± 0.4	93.4±3.4	1.05±0.08
Diabetic non treated group	210.6 ± 9.7***	165 ± 7.2***	4.7 ± 0.2***	240.5 ± 10.6***	3.9±0.13***
Percentage change	↑ 72.3%	↑ 78.8%	↓53%	↑157.4%	↑271.4%

*** Significant P < 0.001 compared with control normal rats.

Table (2): Effect of clonidine on SBP, MBP, RBF, FBG and urine albumin **(mean± SE)**, in STZ induced-diabetic nephropathy in rats (n = 6).

Parameters / Group	SBP (mmHg)	MBP mmHg	RBF cm/s	FBG mg/dl	Urine albumin Mg/24
Diabetic non treated group	210.6±9.7	165±7.2	4.7±0.2	240.5±10.6	3.9±0.13
Clonidine treated group	130±4.6**	102.4±4.2**	6.1±0.24**	205.5±7.6*	2.1±0.12**
Percentage change	↓38.2%	↓37.9%	↑29.8%	↓ 14.5%	↓ 46.1

* Significant P < 0.05 compared with the diabetic non-treated rats.
** Significant P < 0.01 compared with the diabetic non-treated rats.

Table (3): Effect of perindopril on SBP, MBP, RBF, FBG and urine albumin **(mean± SE)**, in STZ induced-diabetic nephropathy in rats (n= 6).

Parameters / Group	SBP (mmHg)	MBP mmHg	RBFcm/s	FBG mg/dl	Urine albumin Mg/24
Diabetic non treated group	210.6± **9.7**	165±**7.2**	4.7±**0.2**	240.5±**10.6**	3.9±**0.13**
Perindopril treated group	125±**4.2****	95.2±**3.7****	9.8±**0.5****	165.7±**8.2****	1.1±**0.05****
Percentage change	↓40.6%	↓42.3%	↑108.5%	↓31.1%	↓71.7%

** Significant (P < 0.01) compared with diabetic non-treated group
*** Significant (P < 0.001) compared with diabetic non-treated group

Table (4): Effect of clonidine versus perindopril on SBP, MBP, RBF, FBG and urine albumin **(mean± SE)**, in STZ induced-diabetic nephropathy in rats (n = 6).

Group / Parameters	Diabetic nephropathy (non treated group)	Clonidine treated group	Perindopril treated group
SBP (mmHg)	210.6± 9.7	130±4.6	125±4.6
MBP (mmHg)	165±7.2	102.4±4.2	95.2±3.7
RBF cm/s	4.7±0.2	6.1±2.4	9.8±0.5***
Blood glucose (mg/dl)	240.5±10.6	205.5±7.6	165.7±8.2*
Urine albumin (mg/24h)	3.9±0.13	2.1±0.12	1.1±0.05***

* Significant ($p < 0.05$) compared with clonidine treated group

*** Significant ($p < 0.001$) compared with clonidine treated group

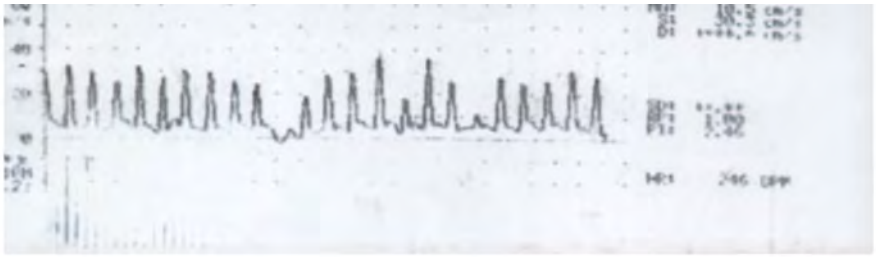

Fig. (1) A trace showing RBF in control normal rat.

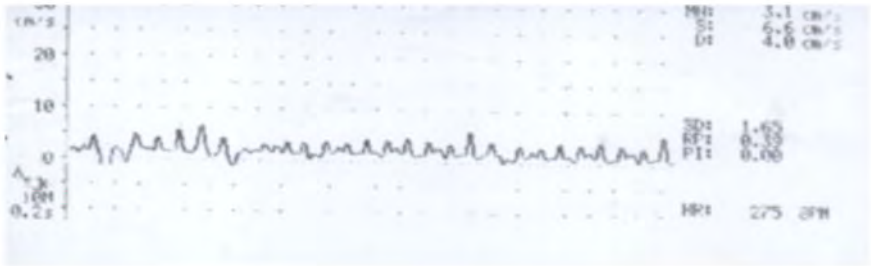

Fig. (2) A trace showing RBF in diabetic nephropathy rats.

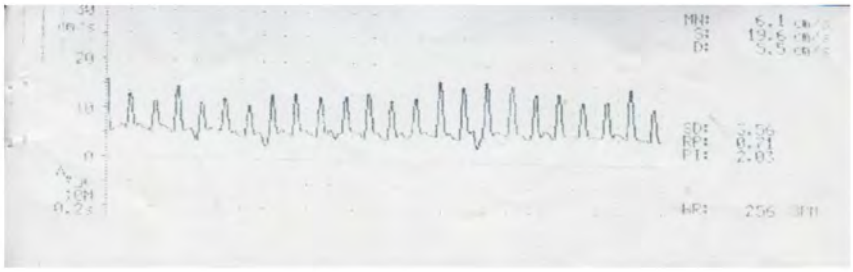

Fig. (3) A trace showing RBF in clonidine treated diabetic nephropathy rats.

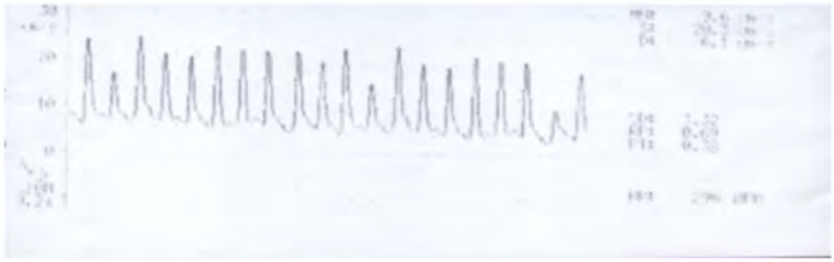

Fig. (4) A trace showing RBF in perindopril treated diabetic nephropathy rats

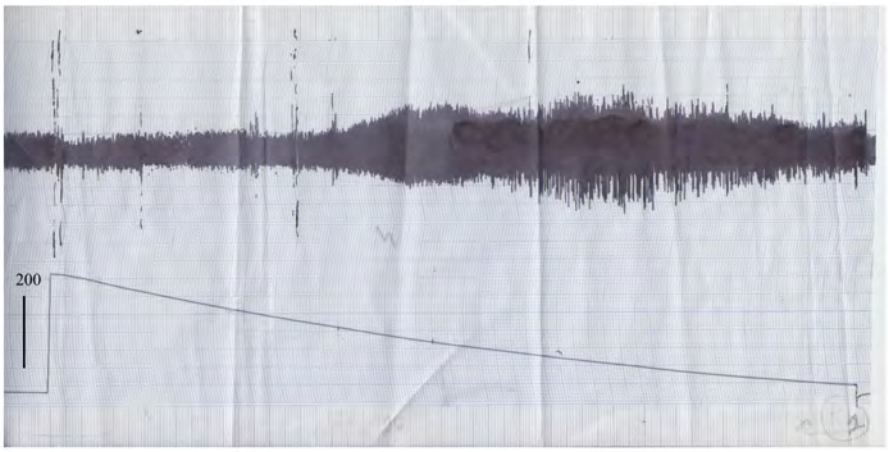

Fig. (5): Blood pressure trace of control normal rat

Fig. (6): Blood pressure trace of diabetic nephropathy rat

Upper trace represents pulse blood flow.
Lower trace represents cuff pressure.

- The systolic blood pressure measured at the start of pulsation and referenced to the pressure curve.
- The mean blood pressure measured at stability of pulsation and referenced to the pressure curve.

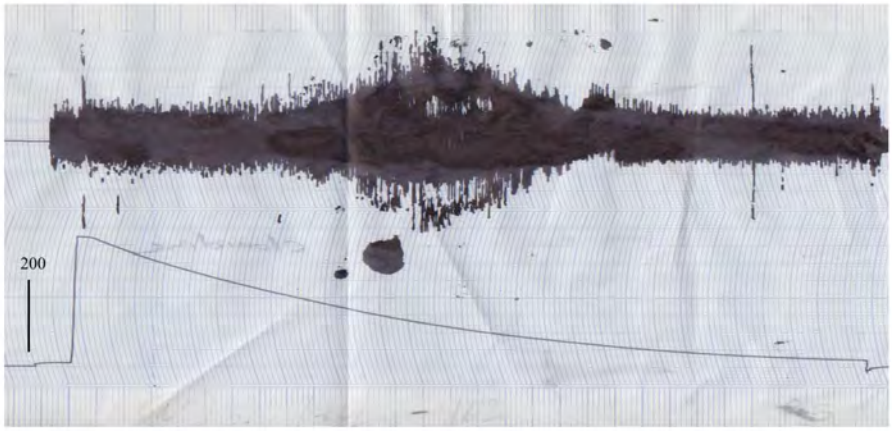

Fig. (7): Blood pressure trace of clonidine treated diabetic nephropathy rat

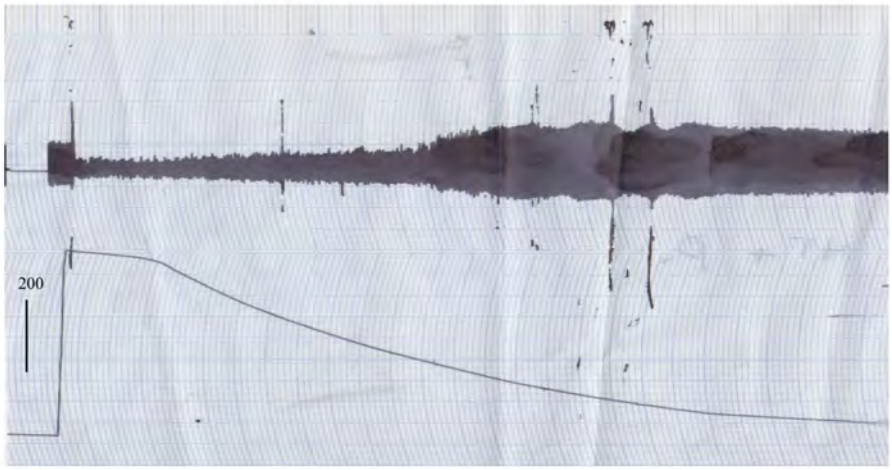

Fig. (8): Blood pressure trace of perindopril treated diabetic nephropathy rat

Upper trace represents pulse blood flow.
Lower trace represents cuff pressure.

- The systolic blood pressure measured at the start of pulsation and referenced to the pressure curve.
- The mean blood pressure measured at stability of pulsation and referenced to the pressure curve.

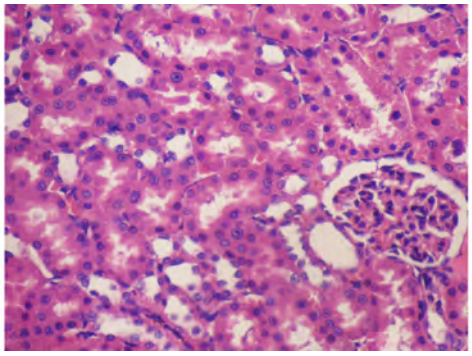

Fig. (9): Cut section of renal tissues of control normal rats showing no remarkable pathologic changes (H&E x 40).

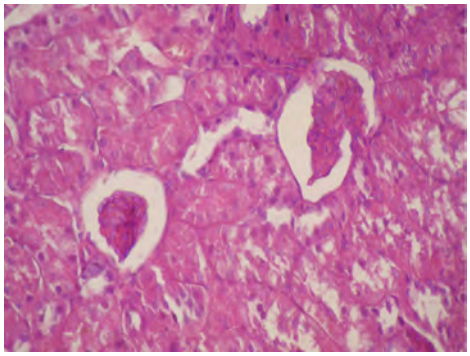

Fig. (10): Cut section of renal tissues of diabetic nephropathy rats (H&E x 40).

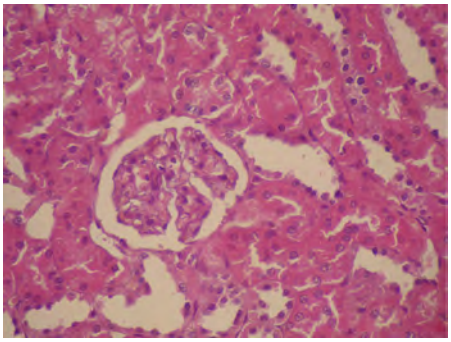

Fig. (11): Cut section of renal tissues of clonidine treated diabetic nephropathy (H&E x 40).

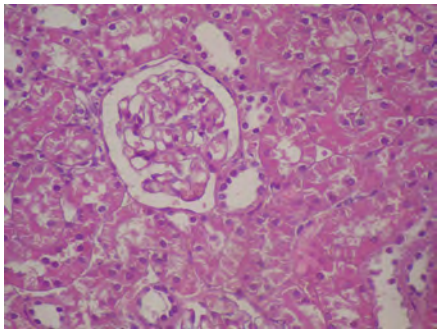

Fig. (12): Cut section of renal tissues of perindopril treated diabetic nephropathy (H&E x 40).

Discussion

Hypertension aggravates both decline in renal function and mortality rates in diabetic patients, and the importance of treating hypertension in diabetics is no longer in question. The United Kingdom Prospective Diabetes Study Group (UKPDS) showed that controlling blood pressure in the type 2 diabetic provides morbidity - mortality benefits at least equal to, possibly greater than, tight diabetic control (1). The need for effective and reliable antihypertensive`agents is therefore underlined.

In the present study, we induced diabetic nephropathy in adult male albino rats by single (I.P.) injection of streptozotocin (STZ) and rats were examined after one month [27].

STZ induced significant increase of SBP, MBP, RBF FBG and 24-hour collected urine albumin in rats. Histopathological examination of renal tissues of diabetic nephropathy rats by light microscop revealed diffuse glomerular sclerotic lesion.

Our study revealed that chronic clonidine administration in diabetic nephropathy rats for 4 weeks produced significant decrease of SBP, MBP, FBG and 24-hour collected albumin with significant increase in RBF in diabetic nephropathy rats. Histopathological examination of renal tissues from clonidine treated diabetic neph-

ropathy rats revealed mild thickening of glomerular capillary wall and mild changes in mesengial matrix.

These results were in agreement with [1]. Acute injection of clonidine (centrally acting sympatholytic drug) in type 1 diabetes (insulin-dependent) with incipient nephropathy reduced arterial blood pressure[28]. The prevention of elevation of blood pressure by clonidine in diabetic nephropathy could be explained by its binding to imidazoline receptors and hence reducing the sympathetic tone and prevention of excess sodium and water reabsorption [1].

Some selective α_2 antagonist appeared to have weak or no blocking effects on the peripheral hypotensive action of clonidine or its related compounds. This suggests the functional dominance of the imidazoline receptors [29].

Chloroethylclonidine caused attenuation of the renal vasoconstrictor responses elicited by the adrenergic stimuli[30].

[31]Studied the effect of prophylactic clonidine administration in STZ-induced diabetes in rats and concluded that clonidine prevented glucose intolerance.

In agreement with these results are that of[32] who had been studied the metabolic effects of rilmendine (Imidazolines structurally-related derivative) in hypertensive patients with metabolic syndrome. They found that both fasting and post-prandial plasma glucose levels were significantly lowered in rilmendine- treated patients.

These data are in agreement with[33] who suggested that central inhibition of sympathetic drive by rilmendine in hypertensive patients could have favourable effects on glucose and lipid metabolism.

Moreover, the results run in consistence with[34] who demonstrated that imidazolines and structurally- related derivatives are able to stimulate insulin release from pancreatic β-cells through inhibition of ATP- sensitive K^+ channels.

Reduction of FBG by clonidine

could be explained by its capacity to increase the concentration of an intracellular factor essential for insulin-mediated activation of glucose disposal, as shown by moxonidine (another selective imidazoline binding drug) and/or reduction of the sympathetic activity with result of improvement of insulin resistance [35].

Our results were disagreed with that of [28] who reported that slow clonidine injection to 12 patients with type 1 (IDDM) with incipient nephropathy did not significantly affect blood glucose concentration.

[36]Reported that increased albumin excretion rate not only indicated renal disease, but also reflected universal vascular damage affecting large and small renal vessels.

The results regarding 24-hour collecting urine are in agreement with[28] who reported that acute clonidine injection in hypertensive type 1 diabetics significantly reduced urinary albumin excretion and functional clearance of albumin in all patients.

Thus the results of the present study may provide an evidence for the beneficial effects of clonidine in the hypertensive diabetic patients by reducing blood pressure, FBC and urine albumin with increasing RBF.

This study also revealed that chronic perindopril administration in STZ- induced diabetic nephropathy rats produced significant decrease of SBP, MBP, FBG and albumin excretion in urine with significant increase of RBF. Our data also demonstrated a renoprotective effect of perindopril in the form of improvement of glomerular and vascular sclerotic lesions in kidneys of diabetic nephropathy rats.

These results run in agreement with that of [37] who studied the effect of renal and metabolic disorders that depend on the renin-angiotensin system in Lyon hypertensive rats associated with diabetes. He reported that perindopril normalized the BP, reduced the plasma lipids, and lowered the proteinuria.

Renal medullary protection is conferred in spontaneous hyper-

tensive rats by ACE inhibition, an effect that, is not obtained with other antihypertensive treatments [21].

Late inhibition of RAS halts the progression of glomerulosclerosis in obese Zucker rat (OZR), reverses mesangiolysis and prevents the decline in glomerular endothelial cell and podocyte numbers [38].

Our results are also in consistence with[39] who suggestes that the intrarenal renin-angiotensin system (RAS) may play an important role in the development of glomerular changes associated with diabetic nephropathy. In their study, they induced diabetic nephropathy in male rats by STZ. They found that, in glomerular extracts of diabetic rats, angiotensinogen and angiotensin II were significantly increased by 2.2 and 1.9 fold respectively. Analysis of glomerular extracts of diabetic rats demonstrated that exogenous angiotensin I was significantly converted into various angiotensin peptides including angiotensin II. Pre-incubation of glomerular extracts with captopril resulted in a 20%-30% decrease in the angiotensin II conversion from exogenous angiotensin I in diabetic and control rats.

Concerning the results about renoprotective effects of perindopril [40] reported that perindopril suppresses the apoptosis induced by endoplasmic reticulum stress in renal tubular in experimental diabetic rats.

Also [41] reported that perindopril attenuates renal tubulointerstitium injury by inhibiting scavenger receptor A (SR-A) over - expression in diabetic rats.

Finally, we can conclude that the use of drugs that inhibit the renin-angiotensin system is an effective way to intervene in the pathogenesis of cardiovascular and renal disorders. Some studies have shown that ACE inhibitors can delay or prevent the deterioration of renal function in chronic renal disease, independently of their antihypertensive effects [42].

The present work revealed a more significant effect of perindopril over clonidine in lowering FBG and 24-hour collected albu-

min with increasing of RBF in STZ-induced diabetic nephropathy rats. On the other hand, we revealed no significant difference between perindopril and clonidine in normalizing SBP & MBP of STZ-induced diabetic nephropathy rats.

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THE POTENTIAL BENEFICIAL EFFECTS OF PERINDOPRIL OVER CLONIDINE ON DIABETIC NEPHROPATHY IN RATS

Hanan T. Emam MD, Ghada A. Abd El Fattah MD and Noha I. Hussien MD

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BONE MARROW INVOLVEMENT BY NON HODGKIN'S LYMPHOMAS IN MANSOURA ONCOLOGY MEDICAL CENTER, A PROSPECTIVE STUDY

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Abstract

Lymphoma is a relatively common malignancy in Egypt, contributing to 12% of all cancer patients, ranking as 4th of all malignant tumors. The presence or absence of bone marrow infiltration by lymphoma has a significant impact on prognosis and treatment options in a variety of NHL. Antrograde 287 bone marrow biopsies were taken for staging patients who were already diagnosed as NHL Lymphomas from nodal or extranodal sites. The trephine biopsies were routinely stained by H&E, Geimsa, reticulin and Masson trichrome stains. Immunohistochemical staining was done using CD20, CD3, CD5, CD23, CD30, CD10 and CD99 monoclones for confirmation and establishing the marrow infiltration.

Positive marrow infiltration was seen in 38.7% of cases by BMB and 8% by BMA. The highest rates of infiltration were noticed in SLL (100%) and HCL (100%) cases, while the lowest rates were in TCL (16,7%) and ALCL (12.5%). The use of IHC staining increased the diagnostic accuracy of bone marrow biopsies in 12.6% (14 cases) of total positive cases. The most commonly seen secondary changes in BMB were myelofibrosis and reduced haematopoiesis.

Key words: *Bone marrow biopsy, NHL, Staging*

Introduction

Lymphoma is a relatively common malignancy in Egypt, contributing to 12% of all cancer patients, ranking as 4th of all malignant tumors after breast, leukemias and bladder cancer [1]. The presence or absence of bone marrow infiltration by lymphoma has a significant impact on prognosis and treatment options in a variety of Non Hodgkin lymphomas (NHL) [2].

Bone marrow involvement by lymphoma is more often apparent on trephine specimens than bone marrow aspirates (BMA), the bone marrow biopsy (BMB) renders important diagnostic information which cannot be obtained from BMA; as architectural features of the infiltrate and its topographic distribution as well as the amount of marrow space occupied by a malignant infiltrate. The negative results on aspirate samples are due to the presence of bone marrow fibrosis which is a frequent finding in lymphomatous infiltrates [3].

Staging bone marrow is generally done using routine bone marrow histology, with ancillary studies as immunophenotyping being performed in some cases to confirm or establish lymphomatous involvement [4].

Aim of work

The aim of this study is to assess the frequency of bone marrow involvement by both BMA and BMB procedures in established cases of lymphomas, and to study the relative frequency, patterns of infiltration in different subtypes and the secondary associated changes.

Material and Method

Antrograde 287 BMB together with their aspirates were taken for patients who were previously diagnosed as NHL lymphoma in Oncology Medical center, Mansoura Univerisity during the period from January 2010 to January 2012. Their H&E, immunohistochemical (IHC) stained slides were retrieved and reviewed. The primary lymphoma cases were 199 diffuse large B cell lymphoma (DLBCL), 30 cases were small lymphocytic lymphoma (SLL), 28 cases were follicular lymphoma (FL), 10 cases were T cell rich B cell lymphoma

(TCRBCL), 8 cases were anaplastic large cell lymphoma (ALCL), 6 cases were T cell lymphoma (TCL), 4 cases were T cell lymphoblastic lymphoma (LL) and two cases were Hairy cell leukemia (HCL), The trephine cores for these patients were fixed in neutral buffered formalin 10% and decalcified in formic acid 8%. Then the trephine cores were processed and stained routinely for H&E, Geimsa, reticulin and Masson trichrome stains. The IHC staining were done using the following monoclones:

1- CD20: monoclonal mouse antibodies, Dako., Catalog No: M 0755, 1ml concentrate.
2- CD 3: monoclonal mouse antibody, Dako. Catalog No: M 7254, 1ml concentrate.
3- CD5: monoclonal mouse antibody, Dako. Catalog No: M7194, 1ml concentrate.
4- CD10: monoclonal mouse antibody, Dako. Catalog No: 110 M -16, 1ml concentrate.
5- CD 23: monoclonal mouse antibody, Lab Vision, Catalog No: 729-s, 1ml concentrate.
6- CD30: monoclonal mouse antibody, Dako. Catalog No: M 7254, 1ml concentrate.
7- CD99: monoclonal mouse antibody, Lab Vision, Catalog No: Ms 294p, 2 ml concentrate.

- Dilution of these monoclones was done as follows; for CD3, CD20, CD5 the dilution was 1/50, for CD23 and CD30 the dilution was 1/25, for CD10 was 1/10 and for CD99 was 1/100

Detection system: Dako LSAB/ HRP kit code No k 0679 and Dako En Vision /HRP kit code No k 4004 and k 4006.

The procedure of immunostain was conducted according to the methods described by Kiernan [5].

Interpretation of immunohistochemical staining:

The results were interpreted in light of the appropriate staining of all positive and negative controls. CD20, CD3, CD5, CD10, CD 99 and CD23: membranous staining CD30: Perinuclear and membranous staining.

Statistical analysis of data was done by using SPSS program (statistical package of social science Version 16). Descriptive data are in the form of mean ±standard deviation and frequency of cases. Analytic data was done by comparing groups using Chi-square test. P is significant if <0.05.

Results

Out of 287 cases of lymphoma for staging, 152 were males and 135 were females with age range from 10 to 77 years and mean age 50.08 years ± 1.42 SD. Positive marrow infiltration was found in 111 cases (38.7%) by BMB (table 1) and in 8% by BMA with significant differences between BMB and BMA (P value <0.001).

Regarding the pattern of infiltration, mixed pattern of infiltration was the commonest of all patterns and in cases of DLBCL. while diffuse pattern was the commonest in SLL, HCL, ALCL, and TCL. The paratrabecular pattern was the commonest in FL cases, while the nodular pattern predominant in T cell LL, There was significant association between the patterns and subtypes of lymphoma (P value = 0.01)(table 2).

Secondary changes in BMB were myelofibrosis in 107 cases, reduced hematopoiesis in 49 cases, reactive T cells in 15 cases, morphologic dysplasia in 7 cases, and ALIP in 4 cases. There was significant association between these secondary changes and subtypes of lymphoma (P value = 0.01) (table 3).

Table (1): IHC results of total cases in bone marrow biopsy.

Primary lymphoma	IHC								
	Total	infiltration BMB	%	CD20	CD3	CD30	CD10	CD99	CD23+ CD5
DLBCL	199	56	28.1%	+	-	ND	ND	ND	ND
SLL	30	30	100 %	+	-	ND	ND	ND	+
FL	28	16	57.1%	+	-	ND	+	ND	ND
TCRBCL	10	2	20%	+	+	ND	ND	ND	ND
ALCL	8	1	12.5%	-	-	+	ND	ND	ND
TCL	6	1	16.7%	-	+	ND	ND	ND	ND
LL	4	3	75%	-	+	ND	-	+	ND
HCL*	2	2	100 %	+	-	ND	ND	ND	ND
Total	287	111	38.7%	111	111	1	20	4	30

ND =not done (+) =positive (-) =negative
DLBCL: Diffuse large B cell lymphoma
SLL: Small lymphocytic lymphoma
TCRBCL: T cell rich B cell lymphoma
ALCL : Anaplastic large cell lymphoma
TCL NOS: T cell lymphoma not otherwise specified
FL: Follicular lymphoma
HCL: Hairy cell leukemia
LL: Lymphoblastic lymphoma

Table (2): Patterns of infiltration in subtypes of primary lymphoma.

Primary lymphoma	Total	Mixed	Nodular	Diffuse	Interstitial	Para-trabecular
DLBCL	56	18	17	5	10	6
SLL	30	12	3	15	0	0
FL	16	4	3	0	2	7
TCRBCL	2	0	0	0	2	0
HCL	2	0	0	2	0	0
LL	3	0	3	0	0	0
ALCL	1	0	0	1	0	0
TCL NOS	1	0	0	1	0	0
Total	**111**	34	26	24	14	13

p value = 0.01

Table (3): Secondary changes in BMB of lymphoma patients.

Secondary BM changes	No	%
Myelofibrosis	107	37.2%
Reduced hematopoiesis	49	17.1%
Morphologic dysplasia	7	2.4%
ALIP	4	1.4%
Reactive T cells	15	5.2%

ALIP: Abnormal localization of immature precursors. P value = 0.01

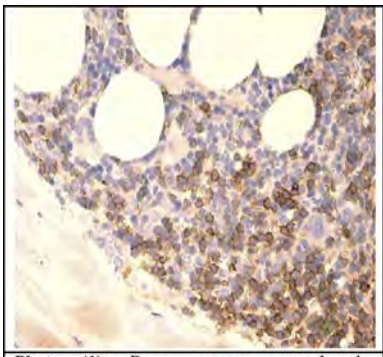

Photo (1): Bone marrow paratrabecular infiltrate, follicular lymphoma CD10x400.

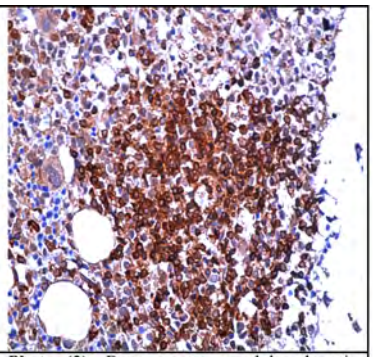

Photo (2): Bone marrow, nodular deposit, follicular lymphoma CD10 x400.

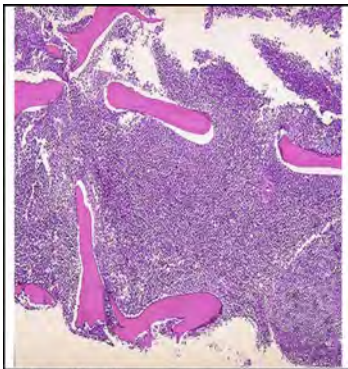

Photo (3): Bone marrow small lymphocytic lymphoma, diffuse infiltrate H&E x200.

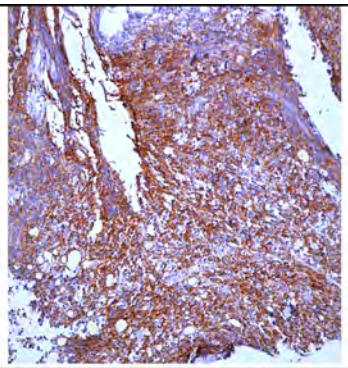

Photo (4): Bone marrow small lymphocytic lymphoma, diffuse infiltrate CD23 x200.

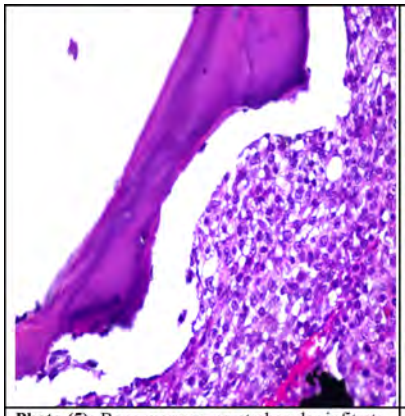

Photo (5): Bone marrow, paratrabecular infitrate, diffuse large cell lymphoma H&E x400

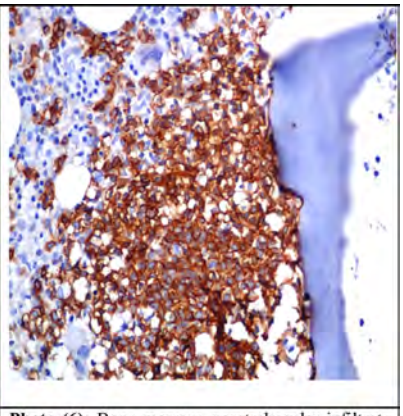

Photo (6): Bone marrow, paratrabecular infiltrate diffuse large cell lymphoma, CD20 x400

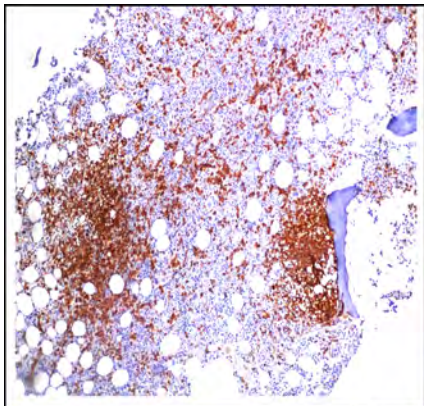

Photo (7): Bone marrow, mixed pattern infiltrate, diffuse large cell lymphoma CD20 x200

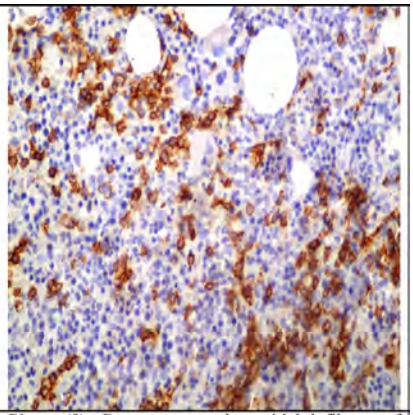

Photo (8): Bone marrow, interstitial infiltrate of diffuse large cell lymphoma CD20x200

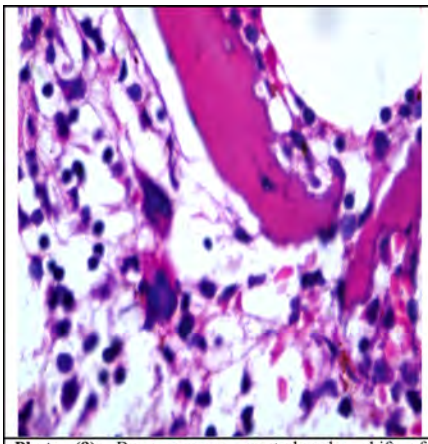

Photo (9): Bone marrow paratrabecular shift of megakaryocytes with abnormal nuclei x1000.

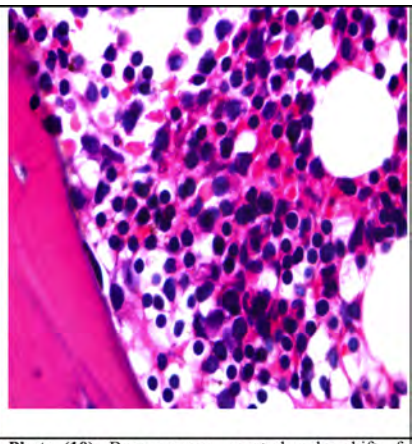

Photo (10): Bone marrow, paratrabecular shift of erythroid precursors H&E x1000.

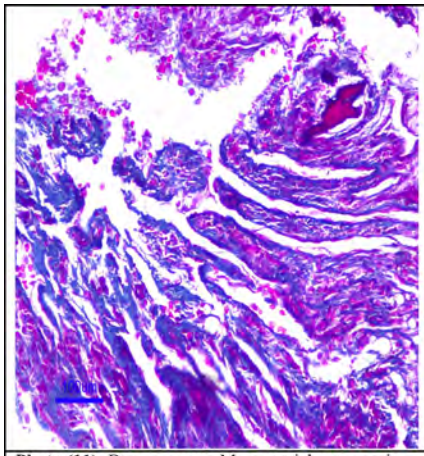

Photo (11): Bone marrow, Masson trichrome stain; fibrosis grade 4 x400

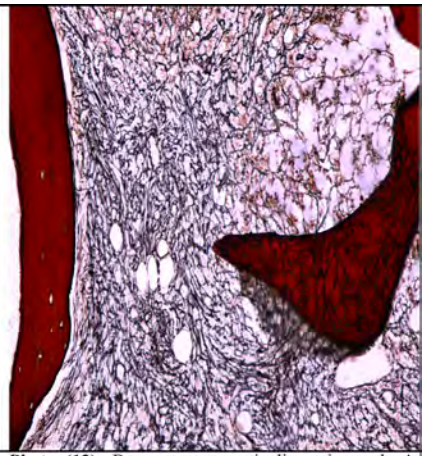

Photo (12): Bone marrow, reticulin stain grade 4 x400

Discussion

The current study included 287 BMB for staging NHL patients, bone marrow was infiltrated in 111 cases (38.7%). Lower frequency of bone marrow infiltration (12.4%-30%) was reported by[6,7], while higher frequency of bone marrow infiltration (44-69%) was detected by others[8,9,10]. The differences between previous studies and the present one could be attributed to inclusion of unequal proportions of patients with early and advanced disease, in addition to different proportions of various histological sub-types of lymphomas which already have variable frequencies of bone marrow infiltration[8].

Bone marrow aspirate in the present study showed positivity in 8% of total cases, especially in B cell lymphomas. Significantly higher rate of positivity (30%) was reported[9]. The lower results of bone marrow aspirate may be related to marrow fibrosis.

In the current study, higher rate of bone marrow infiltration was identified in BCL (39.4%) than TCL (27.8%). Lower rate; 24.4% and 18.9% in BCL and TCL, respectively with predominant BCL was reported[20], while higher rate of infiltration in TCL (16.7%) than BCL (11.4%) was reported[7].

The predominant histological pattern of bone marrow infiltration by lymphoma in this study was the mixed one, which was observed in (28.1%). Similar results were reported in another study[10]. However, this was different from the results of El Bolkainy et al.,who found the predominant pattern of marrow infiltration to be the paratrabecular in 30% of cases[9].

In cases of DLBCL positive bone marrow infiltration was found in 28.1%, other studies showed wide range of marrow infiltration in 8-35%[11,12]. Regarding the pattern of infiltration in DLBCL in the current study mixed and nodular patterns were the most common, while in other studies nodular pattern was the commonest[11,12].

In SLL, 100% of the staged cases showed marrow infiltration, in

other studies similar results; 100% of cases were positive [13,14]. Although the predominant patterns in present study were the diffuse and mixed patterns, in other studies the nodular and interstitial patterns were the commonest[13,14]. This may be related to late stage of SLL and leukemic transformation especially that 13 cases in this work presented with absolute lymphocytosis.

Regarding FL cases, it showed positive marrow infiltration in 57.1 % of cases, and the paratrabecular pattern was predominate. These results were consistant with other studies in which 50–60% of cases were positive and also paratrabecular pattern was the most common [15,16].

Among cases of TCRBCL marrow infiltration was detected in 20% of cases. A higher rate of infiltration was reported in another study which showed marrow infiltration in 33% of cases[17]. The pattern of infiltration in this study was interstitial, while in another study, the pattern was often paratrabecular[17].

Regarding HCL (2 cases), in the current and other studies, bone marrow infiltration was detected in 100% of cases, with diffuse pattern of infiltration [18,9].

In cases of LL, marrow infiltration was found in 75%. In other studies constant marrow infiltration (100%) was seen.[19,9]. The pattern of infiltration in the present study was nodular. In other studies, the pattern of infiltration was ranging from interstitial to diffuse [18,9].

In anaplastic large cell lymphoma, only one case showed marrow infiltration (12.5%) with diffuse pattern of infiltration. Other studies showed marrow involvement in 10 - 30% of cases and the involvement was subtle and undetectable without IHC[19,20].

Regarding T cell lymphoma in this study one case showed BM infiltration (16.7%). Higher rate of bone marrow infiltration (27.8%) was reported in another study[21]. The infiltration pattern was diffuse in this study, while in another study the

infiltration varies from subtle to diffuse and widespread [22].

Importantly in the current study all the positive cases were concordant, similar results were reported by another study [9], however in other studies discordant morphology between the primary and marrow infiltrate was encountered in 29.63% in cases of follicular lymphomas and diffuse large B-cell lymphomas [10,8].

In the present study, myelofibrosis was found in 107 cases (37.2%). The remaining 4 cases were negative for fibrosis, probably may be due to early interstitial infiltrate. This results indicates that immunohistochemistry is more sensitive in detecting marrow infiltration than reticulin and H & E stains, and can aid in increasing the diagnostic accuracy of bone marrow biopsy in detection of occult lymphomas[12]. These results of reticulin stains were different from Kumar et al., who found that reticulin stain helped in detection of scanty focal or interstitial infiltrate, which may not be apparent in routine H and E stains [11].

In this study marrow morphologic dysplasia was found in 7 cases (2.6%), theses reactive changes maintained relative topographic distribution of all series. Inspite of these changes secondary myelodysplasia was not implicated as the blast cells did not exceed 5% of marrow cells. Higher rate of morphologic dysplasia 6.3% was reported by Elbokainy et al [9].

Abnormal localization of immature precursors (ALIP) was found in 4 cases (2.5%) in the current study, and also it was not associated with increase in the marrow blasts. Higher rate of ALIP was found by other authors[23,9]. Reactive T cells were found in 15 cases of FL and DLBCL (5.6%). Similar to Kremer who found a high number of reactive T cells in neoplastic infiltrates of bone marrow in BCL especially FL [24].

Eventually using IHC staining was a valuable tool for staging lymphoma in bone marrow biopsy as it confirmed the diagnosis of positive cases by H&E in 92/106 cases (86.8%) and established the diagnosis of bone marrow infiltration in 14/106 cases (13.2%) with

interstitial pattern of infiltration, which were suspicious by H&E. Similar results were reported by Talaulikar et al., as they found that using IHC techniques increased the diagnostic accuracy of BMB in staging lymphoma in 11% of cases when compared to histology alone [12].

Conclusion

This study highlights the incidence and different patterns of involvement of 287 cases lymphoma in the bone marrow, without discordance between BM morphology and primary site histology.

Bone marrow trephine biopsy showed involvement by lymphoma in 38.7% of cases. The incidence of involvement was higher in B-cell lymphomas when compared with T-cell lymphomas and the predominant pattern of involvement was mixed. The highest rate of infiltration was seen in small lymphocytic lymphoma, and hairy cell leukemia, while T cell lymphoma and T cell rich B cell lymphoma showed the lowest rate of bone marrow infiltration. The use of IHC, increased the diagnostic accuracy by unmasking obscured patterns and morphology.

Bone marrow aspirates had lower diagnostic value in staging NHL than bone marrow biopsy.

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BENHA MEDICAL JOURNAL

BONE MARROW INVOLVEMENT BY NON HODGKIN'S LYMPHOMAS IN MANSOURA ONCOLOGY MEDICAL CENTER, A PROSPECTIVE STUDY

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LEFT VENTRICULAR SYSTOLIC DYSFUNCTION 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 systolic function abnormalities in HCV positive cirrhotics without clinical, evidence of cardiac problem.*

Aim of study: *The study was designed to study systolic function of 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). Moreover, 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.

Conclusion: *HCV infection could be an additional factor to portal hypertension in the pathogenesis of left ventricular systolic dysfunction in cirrhotics.*

Introduction

Hepatitis C virus (HCV) causes not only liver disease but also a broad clinical spectrum of extrahepatic manifestation. Immunological studies demonstrated the presence of HCV in extrahepatic organs including the heart [1,2].

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 [3].

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) [4].

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 [5]. However, fewer studies investigated systolic function abnormalities in cirrhotic patients with HCV

infection without clinical evidence of heart failure.

This study was designed to investigate systolic function 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 or 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 (6) 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 [7].

During echocardiography, a one lead electrocardiogram was recorded continuously internal left ventricular end systolic (LVES), left ventricular end diastolic (LVED), left ventricular interventricular septum during diastole (IVSD) and posterior wall thickness during diastole 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[8]. Each representative value was obtained from the average of the three consecutive measurements.

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 anti-HCV positive, anti-HCV negative and control groups regarding age, body mass index, SBP, DBP, dyspnea and palpitation by one way Anova (Table 1).

Cirrhotics with +ve anti-HCV showed statistically significant higher LVED, LVES diameters as well as significant higher IVST and LVPWT. They also showed statistically significant lower LVEF and LVFS as compared with cirrhotics with -ve anti-HCV. Statistically significant higher MPI of left ventricle is found in cirrhotics with positive HCV infection than other groups, (Table 2).

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 3).

Also, P and SMV and SV maximum flow rates were statistically significant lower in patient groups than the control group (table 3).

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 4).

Table (1): Comparison of clinical features of cirrhotic patients with positive anti-HCV, cirrhotic patients with negative anti-HCV and control group.

Clinical parameters	Patients with positive anti HCV (n = 63)	Patients with negative anti HCV (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 cirrhotic patients with +ve anti-HCV antibodies and cirrhotic patients with –ve anti-HCV antibodies

Table (2): Comparison of some M-mode echocardiographic parameters among the studied groups.

	Cirrhotic patients with positive anti-HCV (n = 63)	Cirrhotic patients with negative anti-HCV (n = 34)	Control (n = 22)	P value	P *
LVEDD (cm)	5.24 ± 0.49	4.74 ± 0.53	4.50 ± 0.39	< 0.001	< 0.001
LVESD (cm)	3.63 ± 0.30	3.08 ± 0.38	2.98 ± 0.25	< 0.001	< 0.001
IVST_d (cm)	1.09 ± 0.17	1.07 ± 0.12	0.94 ± 0.12	< 0.001	0.518
LV PWT_d (cm)	0.96 ± 0.18	1.07 ± 0.08	0.85 ± 0.10	< 0.001	< 0.001
LV EF (%)	65.44 ± 7.25	72.04 ± 6.44	70.61 ± 6.0	< 0.001	< 0.001
LVFS	30.43 ± 4.90	34.98 ± 4.98	33.74 ± 4.42	< 0.001	< 0.001
LV MPI	0.51 ± 0.11	0.47 ± 0.04	0.44 ± 0.05	< 0.001	0.004

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.

P = comparison of all groups

P* = comparison of cirrhotic patients with +ve anti-HCV antibodies and cirrhotic patients with –ve anti-HCV antibodies

Table (3): Comparison of some Doupplex abdominal ultrasound parameters among the studied groups.

	Cirrhotic patients with positive anti-HCV (n = 63)	Cirrhotic patients with negative anti-HCV (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 cirrhotic patients with +ve anti-HCV and cirrhotic patients with –ve anti-HCV

Table (4): 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.

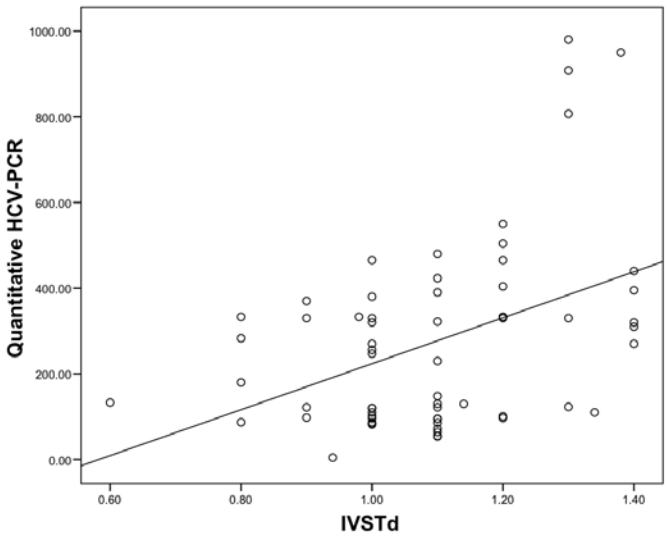

Figure (1): Correlation between quantitative HCV-PCR and IVSTd.

Discussion

Heart in patients with cirrhosis presents with structural and functional abnormalities that have been termed cirrhotic cardiomyopathy [9].

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 [10,11].

Many studies reported diastolic dysfunction that worsen with the presence of ascites and physical stress, less numerous studies reported abnormal systolic response to stress [12]. Some authors reported that their cirrhotic patients, in response to stress were unable to adequately increase their heart rate, stroke volume index and cardiac index. In addition, their LVEF showed a mean negative increase compared to controls. As a consequence, cirrhotic patients presents 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 [13].

Links between hepatitis C virus and dilated cardiomyopathy were previously reported [14,15]. Moreover, findings and new diagnostic tests resulted in increased interest in inflammatory cardiomyopathies and a better understanding of its pathophysiology [16].

Impaired autonomic function is caused by chronic HCV infection (17). 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.

Because HCV infection/ replication itself is not cytopathic, both liver and neurological pathologies in chronic HCV infection are considered by many authors to be immune mediated [18,19].

In this study, it was found that HCV +ve cirrhotics had significantly higher LVED, LVES and IVST than HCV -ve cirrhotics and control. 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 [20- 22].

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.

However, it has been hypothesized previously that portal hypertension itself can be added to the list as one of the 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 [23]. They also reported a significant increase in aldosterone levels among the cirrhotics and they

suggested a role of activated RAAS in the pathogenesis of cardiac contractile dysfunction.

In addition, Torregrosa et al. reported that cardiac alterations in cirrhosis are mild and independent of the etiology of cirrhosis, and consist of increased ventricular wall thickness, a diastolic dysfunction that worsens with the presence of ascites and physical stress, and a basal hyperdynamic systolic function with abnormal systolic response to stress conditioning limited exercise capacity [12].

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 [1,24].

Also, inflammatory cytokines as interleukin-1, -6 or TNF released secondary to HCV infection could account for cardiac involvement in patients infected with HCV.

It has been reported that an association between HCV infection and left ventricle hypertrophy in terms of left ventricle systolic dysfunction could exist [20,21].

Conclusion

HCV infection could be additional factor in the pathogenesis of left ventricular systolic dysfunction in cirrhotics. Cardiac involvement should be considered during follow up of a patient with HCV infection.

Recommendations

- Further studies including large number of cirrhotic patients and controls for accuracy of results.
- Determination of plasma aldosterone levels as well as RAAS and serum levels of cryoglobulins should be added to variables investigated in such studies.
- HLA typing inspite of its higher cost should be done as not all the HCV infected patients exhibited abnormal cardiac findings.
- Autonomic dysfunction should be evaluated in further similar studies.

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LEFT VENTRICULAR SYSTOLIC DYSFUNCTION IN CIRRHOTIC PATIENTS ASSOCIATED WITH HCV INFECTION

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COMBINED THERAPY OF TRANSARTERIAL CHEMOEMBOLIZATION AND THREE DIMENSIONAL CONFORMAL RADIOTHERAPY FOR HEPATOCELLULAR CARCINOMA

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Abstract

Introduction: *Hepatocellular carcinoma is the most severe complication of chronic liver disease. The overall incidence remains alarmingly high in the developing world especially in Egypt. Combined treatment of TACE with 3D-CRT has some advantages. Tumor shrinkage after TACE makes the radiation field smaller, lipiodol uptake of the tumor aids in delineating the target and chemotherapy retained act as a radiosensitizing effect.*

Aim to work *is to evaluate the efficacy and toxicity of combined therapy of three-dimensional conformal radiotherapy (3DCRT) and transarterial Chemoembolization (TACE) for unresectable hepatocellular carcinoma (HCC).*

Methods: *Between August 2009 and August 2011, 15 patients with locally advanced unresectable HCC were enrolled prospectively in this study. TACE was performed first by using mixture of 50mgAdriamycin, 750mg 5-Fluorouracil, 50mg cisplatin, and lipiodol with a median of two courses, followed by 3DCRT (30-50 GY, 1.8GY/day, 5days/week) after 8-10 weeks. Tumor response was evaluated by the European Association for the Study of Liver disease (EASL) recommendation to account tumor necrosis recognized by non enhanced areas on serial computed tomography scans and toxicity was evaluated by the Common Terminology Criteria for Adverse Events Version 4 (CTCAE 4).*

Results: *An objective response was achieved in 5 patients (33.4%), with a complete response in 2 patients (13.3%) and partial response in*

3 patients (20.1%). The overall survival rates were 60%, 20%, at 1 year, and 2 year respectively, with median survival time was 18 months. The progression-free rate at the end of study was 86.7% at the end of study, with median progression free survival (PFS) 18 months. T stage, child-Pugh grade, ECOG performance status, portal vein thrombosis (PVT), number of TACE session and radiotherapy dose were identified as independent predictors for survival by univariate analysis. All are still significant by multivariate analysis except radiotherapy dose. Regarding acute toxicities, most toxicity were mild to moderate, transient and most patients recovered within 1-2week. Radiation induced liver disease (RILD) was developed in13.3%.

Conclusion: *The combined modality is tolerable, effective therapeutic strategy in carefully selected patients and need further trials to confirm the efficacy of this combined therapy.*

Key words: *hepatocellular carcinoma, conformal radiotherapy, trans-arterial chemoembolization.*

Introduction

Hepatocellular carcinoma is the most severe complication of chronic liver disease. The overall incidence remains alarmingly high in the developing world especially in Egypt where it is considered the second most frequent cancer site for males and number one cause of death [1].

Although early diagnosis and curative surgical resection can achieve the best prognosis, the number of patients who could undergo resection is still limited, even for those with small tumors because of the unique characteristics of this tumor, such as multifocality, early vascular invasion, and concurrent liver cirrhosis [2].

Most of the patients with HCC are frequently diagnosed at the intermediate or advanced stages. For these patients, the prognosis has been assumed to be poor, with median survival of less than 1 year. TACE has long been used as a palliative treatment for patients with technically unresectable or medically inoperable HCC with unsatisfactory results [3].

Historically, HCC has been considered a radioresistant tumor; however, this view was likely related to the fact that only low doses

were used originally to treat HCC to avoid toxicity. Three-dimensional conformal radiotherapy (3D-CRT), however, makes it possible to deliver higher doses to the tumor where as minimizing the irradiation of normal liver tissue. Recent studies using 3D-CRT for HCC showed a significant response of 60% to 70% with acceptable toxicities and dose-response relationships[4].

Combined treatment of TACE with 3D-CRT has some advantages. Tumor shrinkage after TACE makes the radiation field smaller, lipiodol uptake of the tumor aids in delineating the target volume, and anticancer drugs retained in the tumor after TACE may have a radiosensitizing effect. Local RT can also eliminate residual tumor cells after TACE. This approach has been applied and several reports have showed that TACE plus RT was more effective than either of these therapies alone[5].

Patients and methods

This study was performed on 15 with inoperable hepatocellular carcinoma, referred to Gastroenterology Center, Internal Medicine hospital, and Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Mansoura University dated from August 2009 to August 2011 inclusive. All the following eligibility criteria were satisfied: HCC confirmed by radiological criteria; Inoperable due to liver cirrhosis or cardiovascular diseases, or technically unresectable because of bulky tumor, partial invasion of the main portal vein or its branches; Single HCC nodules at any site or a few adjacent small nodules closely located within a restricted volume of the liver parenchyma. Single HCC nodules can be divided in large (>5cm) or small size (≤5cm) tumors; Good general condition with ECOG performance status ≤ 2; Liver cirrhosis of Child-Pugh A or B7; Adequate hematological, hepatic and kidney functions.

Pretreatment evaluation :

Pretreatment evaluation included complete history, physical examination, and laboratory studies including viral serology and tumor marker. Pre and post-treatment imaging including multiphasic CT scan of the abdomen.

Treatment:

TACE :

TACE was performed using the Seldinger technique with a mixture of 50 mg of doxorubicin hydrochloride (Adriamycin), 5-Fluorouracil (750 mg), and cisplatin (50 mg) as a total dose were perfused slowly into hepatic arteries, followed by a mixture of iodized oil (10-30 ml; Lipiodol). The total volume of emulsion was judged by the tumor size and achievement of stagnant arterial flow. For the entire group, 1 to 3 courses of TACE were administered with interval range 6–8 weeks.

Radiation therapy :

Patients were treated with external beam irradiation. The prescription dose is the ICRU-50 reference point within the planning target volume, at or near the center of target. With total dose ranged from 30 Gy to 50 GY, 1.8 Gy / fraction once daily and 5 days / week. Gross target volume (GTV) was delineated on CT scan aided by iodine injected by TACE, and the margin of GTV was about 0.5 cm beyond the iodine deposit. CTV = gross tumor + 1cm safety margin in all directions. PTV = CTV + 0.5 cm. 0.5-2.5 cm was added for respiratory motion of the liver. This addition was determined individually using fluoroscopy.

CT scan was given at 5 mm cuts in treatment position with use of intravenous contrast and oral contrast. Cuts were taken from 2 cm above the dome of the diaphragm to the bottom of the kidneys. The target volumes and dose-limiting structures, including the stomach and duodenum, bilateral kidneys, spinal cord, normal liver were delineated. Isodose distribution, multileaf collimators, beam's eye view and dose volume histogram (DVH) were done and treatment plans and DVHs were compared for the plan optimization.

Adjuvant therapy:-

During the treatment, adjuvant therapy consisted of vitamins, liver supportive drugs, and H2-Blockers to relieve side effects and toxicity of treatment.

Toxicity:

Irradiation toxicities were

scored by Radiation Therapy Oncology Group (RTOG) criteria, including acute (within the first 90 days of treatment) and late (more than 90 days post-treatment). Acute toxicity was graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Late toxicity was focused on radiation induced liver disease (RILD) [6].

Patient evaluation :

Response was defined according to the European Association for the Study of Liver disease (EASL)recommendation to account tumor necrosis recognized by non-enhanced areas (EASL Necrosis Criteria). Complete response (CR) was defined as the absence of enhanced tumor areas, reflecting complete tissue necrosis; Partial response (PR) was defined a decrease ≥50% of enhanced areas, reflecting partial tissue necrosis; Progressive disease (PD) was defined as an increase >25% in the size of ≥1 measurable lesion(s) or the appearance of new lesions; and Stable disease (SD) was defined as a tumor response between PR and PD or <50% decrease in enhancing tissue.

Statistical methods

Statistical calculations were performed using SPSS, version 16 for Windows (SPSS Inc. Chicago, IL, U.S.A). Overall survival was estimated using the Kaplan-Meier method. Univariate and multivariate analyses by Cox proportional regression model were performed to identify the prognostic factors for survival. Statistical significance was calculated at the 95% confidence level ($P< 0.05$).

Results

FOLLOW-UP :

Patients were seen on a weekly basis during their RT to monitor symptoms and toxicity. Follow-Up was performed with multiphasic CT. CT scans were obtained within 1 month after 3D-CRT, and then every 3 months. The median follow up period was 18 months (range: 7-24 months). All the patients had been followed up until death or end of the study. The last follow up was performed in August 2011.

At the time of the last follow-up 7 patients were alive with disease, one patient still free from disease and the other 7 patients died with

disease; one patient died from lung metastases, two patient died from RILD, two patient died from liver cell failure, one patient died due to complication of diabetes mellitus, and one patient died from irrelevant psychic disorder.

Tumor Response:

At the last follow up, two patients (13.3%) had complete response (Figure1) and 3 patients (20.1%) had partial response, eight patients (53.3%) have stationary disease and 2 patients (13.3%) had disease progression with response rate 33.4 %.

Survival, local progression-free rate and distant metastasis

The overall survival rates (from the date of first session of TACE) were 60%, and 20% at 1 year, and 2 year respectively, with the median survival time (MST) 18 months (figure 2). The progression-free rate was 86.7%, while median progression free survival (PFS) was 18 months.

Prognostic factors affecting overall survival

By applying univariate analysis to identify the importance of prognostic factors for survival, child-Pugh grade, T stage, PVT, ECOG performance status, number of TACE sessions and tumor radiation dose was statistically significant with P value < 0.05 (P = 0.020, 0.003, 0.020, 0.001, 0.003, 0.048 respectively), in favor of T2 stage, small tumor diameter, ECOG < 2, no thrombosis in portal vein, and Child-Pugh Grade A, large number of TACE and high radiotherapy dose as shown in Figure 3, 4, 5, 6, 7, 8 and table 1.

From Cox regression analysis only T-stage, child-Pugh grade, PVT, ECOG score and number of TACE session remained statistically significant prognostic factors (P=0.016, 0.044, 0.044, 0.015 and 0.016 respectively) but radiotherapy dose became statistically non significant (P=0.127) as shown in table 2.

Tolerance and toxicities:

Four patients were not able to tolerate treatments due to acute hepatic toxicity in one case, and general condition worsened in three cases during irradiation. Acute hepatic toxicity was notable in the form of G1 elevation of total

bilirubin in 20 %, elevation of liver enzymes in 14 patients with 80% CTCAE G1 in 12 patients and G3 in two patients. Elevations of alkaline phosphatase were found in 2 patients (G1) during the early time after 3D-CRT but all recovered eventually. Hematological toxicity not exceeds G2 except in two patients who develop G3 thrombocytopenia. Also, Acute gastrointestinal complication was mild not exceed G2.

Late toxicities were evaluated 3 months after completion of RT. two patients (13.3%) have radiation induced liver disease (RILD). One RILD patients was positive for HBV and the other was positive for HCV. Both were associated with cirrhosis of Child-Pugh B of 7 scoring. Both were treated by supportive hepatic medications to retain their hepatic function, and died of hepatic failure soon after the onset of RILD.

Table 1: Univariate analysis of prognostic predictors for overall survival in 15 HCC patients treated by TACE and 3D-CRT.

Variable		No. of cases	%	MST in months	P value
Age	> 65	5	33.3	20	.667
	≤ 65	10	66.7	15	
ECOG	< 2	10	66.7	21	.001
	=2	5	33.3	11	
T-stage	T2	7	46.7	23	.003
	T3	8	53.3	11	
Child stage	A	12	80	20	.020
	B	3	20	11	
AFP	<400	9	60	20	.927
	≥400	6	40	15	
PVT	No	12	80	20	.020
	Yes	3	20	11	
Etiology	HCV	12	80	11.5	.127
	HBV	2	13.3	22.5	
	Both Negative	1	6.7	20	
GTV	≤125	13	86.7	12	.790
	>125	2	13.3	20	
RT dose	<4500	4	26.7	12	.048
	≥4500	11	73.3	23	
No of TACE	1	4	26.7	10	.003
	2	7	46.7	18	
	3	4	26.7	23	

Table 2: Multivariate analysis of prognostic parameters for overall survival in 15 HCC patients treated by TACE and 3D-CRT.

Parameter	B	SE	Wald	df	Sig	Exp(B)
T-stage	-2.184	0.909	5.769	1	0.016	0.113
Child–Pugh	-1.696	0.844	4.039	1	0.044	0.183
PVT	-1.696	0.844	4.039	1	0.044	0.183
No of TACE	-1.400	0.582	5.788	1	0.016	0.247
ECOG scale	-2.685	1.099	5.972	1	0.015	0.068
RT Dose	-.994	0.652	2.326	1	0.127	0.370

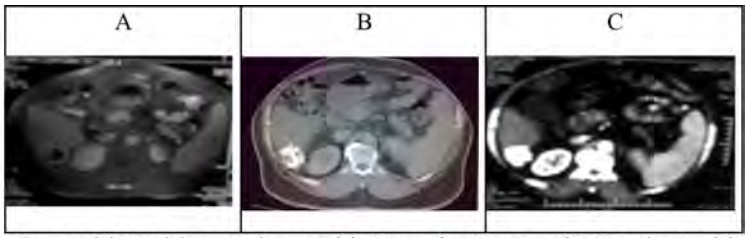

Figure 1 : (A) MRI abdomen at diagnosis. (B) CT scan after 3 sessions of TACE at planning. (C) Follow up CT scan 18 month after Rt showing homogenous uptake of iodized oil and no evidence of viable tissue .

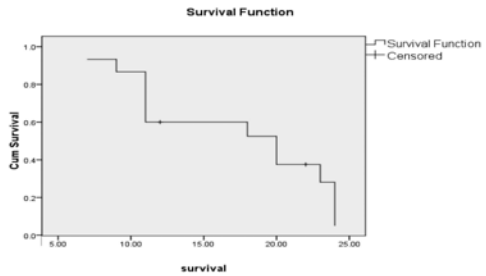

Figure 2: Overall survival of 15 HCC patients treated by TACE and 3D-CRT.

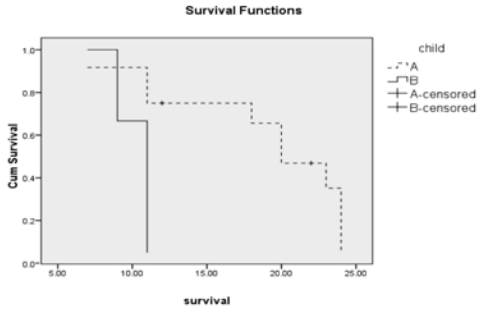

Figure 3: Overall survival and Child–Pugh grades in 15 HCC patients treated by TACE and 3D-CRT.

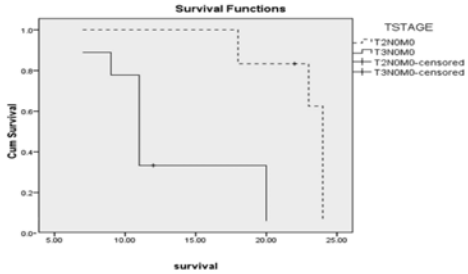

Figure 4: Overall survival and T stage of in 15 HCC patients treated by TACE and 3D-CRT

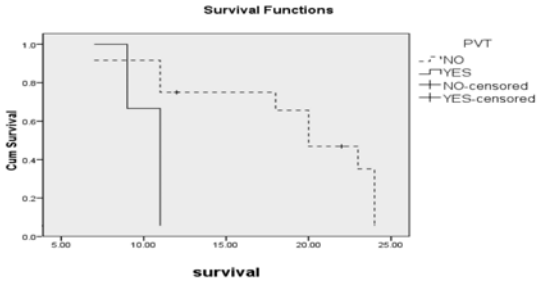

Figure 5: Overall survival and presence of portal vein thrombosis in 15 HCC patients treated by TACE and 3D-CRT.

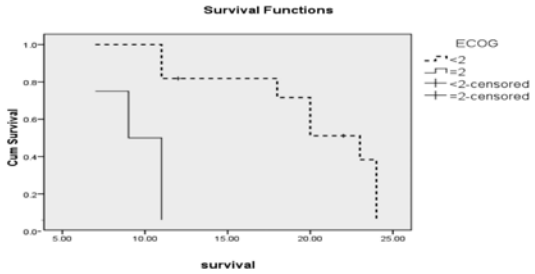

Figure 6: Overall survival and performance status according to ECOG scale in 15 HCC patients treated by TACE and 3D-CRT.

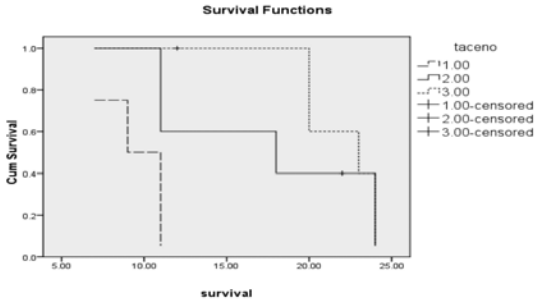

Figure 7: Overall survival and number of TACE sessions in 15 HCC patients treated by TACE and 3D-CRT.

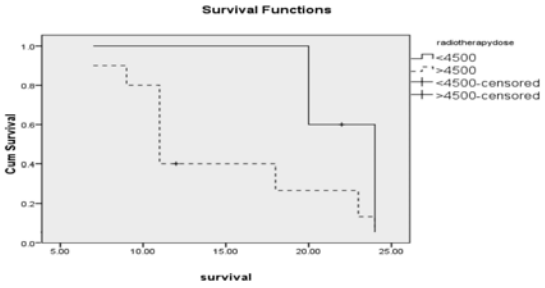

Figure 8: Overall survival and radiotherapy dose in 15 HCC patients treated by TACE and 3D-CRT.

Discussion

Many authors have reported on the results of treatments combining TACE with RT. The methods used for combining TACE and RT are heterogeneous, but 2 strategies can be conceptually described. The first strategy is to use RT as a salvage treatment for unresponsive tumors despite repeated TACE. Seong et al[7] used RT with patients who eventually failed 2 to 10 sessions of TACE. The second strategy is to use RT as a planned consolidation treatment to target residual tumors after TACE. In a study of Li et al[8], RT was planned after 2 cycles of TACE for patients with stage III HCC. Two further cycles of TACE were administered after completion of RT.

The present study is a prospective clinical trial, which is designed to evaluate the efficacy and

toxicity of 3DCRT combined with transcatheter arterial chemoembolization (TACE) in management of unresectable hepatocellular carcinoma. In the present study, our regimen was to deliver RT immediately after 1 - 3 sessions of TACE. This is a planned consolidation treatment to target residual tumors after TACE. This regimen has advantages of reducing liver atrophy resulting from repeated TACE, and so it can be more tolerable to deliver RT. Combined treatment can produce synergistic effects such as the elimination of residual viable tumor cells around the capsule after TACE could be eradicated by RT, especially in large-sized HCC. Also, for some small-sized HCC are resistant to TACE because of low vascularity.

In the present study conventional fractionated RT, i.e. 1.8 Gy per fraction, five fractions a week based on the fact that, liver was considered a late responding normal organ with a low ratio of α/β. The α/β ratio in HCC cells and normal hepatocytes is 11.2Gy and 1-2 Gy, respectively. So, conventional radiotherapy is better than hypofractionated radiotherapy based on calculated biologically effective dose (BED) using the linear-quadratic equation with lower reported morbidity and mortality from RILD in patients receiving 3 DCRT with conventional fractions [9].

In our results, the response rates was 33.4 % (13.3% complete response and 20.1% partial response), 53.3% stationary disease and 13.3% disease progression which is nearly similar to results of Shim et al [10] where, the response rate was 36.8%. In 2005 Zeng et al [11] had reported higher results than ours with objective responses 76%.

The overall survival rates were 60%, and 20% at 1 year, and 2 year respectively which is comparable to previous studies results of Zhou et al.,[12] where the overall survival rates at 1 year, and 2 years were 60%, and 38% respectively.

The median survival time in our study was 18 month which is similar to result of Xu et al.,[13] who reported 18 month median survival but less than results of

Oh et al.,(14) where the median survival were 23.5 month. Also, this results higher than results reported by Seong et al.[15] where median survival (12 month).

Analysis of pattern of treatment failure of studied cases showed 2 failures (13.3%). Intra-hepatic metastasis manifested by appearance of new lesions out of the RT field in one patient (6.7%) and extra-hepatic metastasis (lung metastasis) was found in one patient (6.7%). These results are superior to result of Oh et al.,[14] where 40.0% developed intra-hepatic metastasis or new lesions out of the RT field developed and 32.5% extra-hepatic metastasis. On contrary Zhou et al.,[12] showed lower rates of treatment failure 8%.

In our study from univariate analysis, we found that child-Pugh grade, T stage, PVT, ECOG performance status, number of TACE session, and radiotherapy dose significantly impacted on overall survival in favor of T2 stage, small tumor diameter, no thrombosis in portal vein, Child-Pugh grade A, large number of TACE and higher dose. This result similar to study of Zhou et al.,[12] in that T stage, PVT, irradiation dose and liver cirrhosis significantly impacted on overall survival.

From Cox regression analysis only T-stage, Child-Pugh grade, maximum diameter, PVT, and number of TACE session remained statistically significant prognostic factors which consistent with result of Zhou et al.,[12] in that T stage and hepatic cirrhosis grade (Child-Pugh grade) were demonstrated as independent predictors for overall survival. Also, our result consistent with result of Wu et al.,[16] as tumor size was a significant prognostic factor.

On the other hand in terms of radiotherapy complications as the primary end point in our trial, the most common acute treatment-related toxicities include haematopoietic, hepatic, and gastrointestinal such as leukocyte count decline, total bilirubin, alanine amino transferase level increase, and nausea/ vomiting. Most toxicity was mild to moderate according CTCAE 4. These effects were transient and most patients recov-

ered within 1-2 weeks. No grade 4 toxicities had occurred, except in two patients (13.3%). These patients develop grade 4 thrombocytopenia which was manageable without severe sequelae. These result consistent with result of Oh et al., [14].

As regard late toxicities in our study, the most serious late complication was RILD, which occurred in two patients (13.3%) have radiation-induced liver disease (RILD).which is consistent with result reported by Dawson et al.,[17] where the incidence of RILD has been reported from 4% to 20%. Also, consistent with result of Mornex et al., [18] where more patients develop RILD in Child-Pugh class B.

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REPRINT

BENHA MEDICAL JOURNAL

COMBINED THERAPY OF TRANSARTERIAL CHEMOEMBOLIZATION AND THREE DIMENSIONAL CONFORMAL RADIOTHERAPY FOR HEPATOCELLULAR CARCINOMA

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EVALUATION OF LONG-AXIS MYOCARDIAL FUNCTION WITH 2D STRAIN ECHOCARDIOGRAPHY IN PATIENTS WITH SIGNIFICANT CORONARY ARTERY STENOSIS

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Abstract

Background: *myocardial ischemia or infarction is usually assessed with 2-D echocardiography by detecting the segmental wall motion abnormalities.*

With the introduction of color Doppler myocardial imaging (CDMI), it is currently possible to noninvasively quantify in-plane myocardial deformation (regional strain).

Objective: *To assess and validate 2 Dimensional long axis myocardial strains for detection of myocardial ischemia.*

Patients and methods: *This study included 40 patients categorized into two groups, Group A that included 30 patients with significant coronary artery disease and no visual segmental wall motion abnormalities, who underwent coronary angiography within 6 months before inclusion in the study, their age ranged between 40-60 years, and Group B that included 10 patients with chest pain and normal coronary angiography, age matched with group A as a control group. All of these patients were subjected to full history taking, physical examination, resting 12 leads ECG, coronary angiography and trans-thoracic echocardiography (conventional 2D echo. and 2D myocardial strain).*

Results: *There is significant reduction in 2D long axis myocardial strains in mid anterior septal, mid inferior septal, mid inferior, apical anterior, apical septal and apex in patients have significant LAD disease in compare with control group (Group B)(p value <0.01), while in patients with significant right coronary artery disease, there is significant reduction on 2D myocardial strains at basal anterior, basal inferior septal, basal inferior, mid inferior septal, mid inferior & apical lateral seg-*

ments compared to Group B(p<0.01). In patients with both significant LAD & RCA, there is significant reduction on 2D strains at basal anterior, basal anterior septal, basal inferior septal, basal inferior, basal posterior lateral, mid anterior, mid anterior septal, mid inferior septal, mid inferior, mid anterior lateral, apical anterior, apical septal and apical lateral segments compared to control group.

The sensitivity of 2D myocardial strains for detection of coronary artery disease was 89.6% and specifity 63.6%.

Conclusions: *long axis 2D myocardial strain is a good diagnostic tool for diagnosis of coronary artery disease in compared to usual diagnostic wall motion scoring.*

Introduction

The early identification of acutely ischemic myocardium has both important therapeutic and prognostic implications [(1)].

Myocardial ischemia or infarction is usually assessed with 2-D echocardiography by detecting the segmental wall motion abnormalities [(2)].

With the introduction of color Doppler myocardial imaging (CDMI), it is currently possible to non invasively quantify in-plane myocardial deformation (regional strain). [(3)(4)]

The accuracy of such ultrasonic strain measurements has been recently validated in a comparison with son micrometry in an experimental ischemia model [(5)].

Aim of the work

To assess and validate 2 Dimensional long axis myocardial strain for the detection of myocardial ischemia in patients with significant coronary artery stenosis without visual wall motion abnormalities at rest.

Patients and methods

This study included 40 patients and was conducted at cardiology department, Benha University Hospital.

Exclusion criteria:

1. Patients with previous myocardial infarction.
2. Heart failure.
3. Arrhythmia mainly (AF)
4. Conduction abnormality (LBBB)
5. Significant Valvular lesion.
6. Previous cardiac surgery.

All patients were subjected to:

1- Full history taking:

a- History of coronary artery disease risk factors, including diabetes mellitus, smoking, family history of CAD, hypertension and dyslipidemia.

b- History of angina pectoris, myocardial infarction and dyspnea.

c- History of previous coronary revascularization, PCI, or valve replacement.

2- Full clinical examination:

Thorough physical examination including vital signs (pulse and blood pressure), general examination and local cardiac examination were performed for every patient.

3- Resting 12 leads ECG: for detection of ischemic heart disease.

4- Diagnostic Coronary angiography.

All patients underwent coronary angiography within 6 months before inclusion in this study; the right common femoral artery was punctured by using Seldinger?s technique. Selective coronary angiography was performed using left and right Judkin?s coronary catheters.

5- Trans-thoracic echocardiography examinatio

A- Conventional 2 D echocardiography

It was done using two-dimensional mode from classic windows of apical 4-chamber, apical 2-chamber, apical long axis, left parasternal long and short axes, the following measures were done:

* Left ventricular end diastolic and end systolic diameters were measured by M mode.

* Left ventricular ejection fraction (EF) was measured by M mode.

According to (American Society of Echocardiography's Guidelines) (6)

* Left ventricular wall motions were assessed by 2D mode to exclude patients with resting motion abnormalities from the study.

* Valvular assessment was done by color flow and Doppler studies, to exclude any patients with valvular heart disease.

B-2D longitudinal strain

Echocardiography was performed to all patients within 6 months from coronary angiography, with a commercially available ultrasound equipment and transducer (Vivid 7 dimension, M3S probe, GE Vingmed).

Two-dimensional grayscale images have been acquired in apical four-chamber, apical two-chamber, apical long-axis and three short-axis of the left ventricle at the levels of mitral valve, mid-ventricle and apex using frame rates from 50–70 frames/s. All images have been digitally stored on the hard disk in the machine for off-line analysis. The off-line analysis has been performed independently to the clinical and coronary angiographic results. The left ventricle has been analyzed using a 17 segment model [7].

The endocardial borders have been traced at the end-systolic frame from the three apical views and three short-axis views, and an automated tracking algorithm outlined the myocardium in successive frames throughout the cardiac cycle. After the tracking quality was verified for each segment (with subsequent manual adjustment of the region-of-interest if necessary), myocardial motion was analyzed by speckle-tracking within the region-of-interest bound by endocardial and epicardial borders.

Myocardial longitudinal, strain profiles were obtained and peak systolic strain values were measured. The location of myocardial segments was correlated with coronary artery distribution by using a scheme modified from that proposed by Segar et al. [8].

Anteroseptal, anterior and mid- and apical posteroseptal segments were considered to be a part of the left anterior descending coronary artery (LAD) distribution; lateral and posterior segments were considered to be a part of the left circumflex coronary artery (CX) distribution and the basal-posteroseptal; and inferior segments were considered to be a

part of the right coronary artery (RCA) distribution [8].

The patients according to coronary angiography were divided into two groups:

• **Group (A):** Diseased patients group:

Included thirty patients with significant coronary artery disease and no visual segmental wall motion abnormalities by 2D echocardiography, and underwent for coronary angiography within 6 months before inclusion in these study, and their age ranged between 40-60 years.

All patients had more than 70%coronary artery diameter stenosis in at least one major coronary artery.

• **Group (B): Control group:**

Ten patients with chest pain and normal coronary angiography and their age matched with group A.

STATISTICAL ANALYSIS

The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 12.The results were analyzed by the suitable statistical methods, which included:

• Mean.

• Standard deviation (SD).

• Student's T-test:

• Paired student t-test to analyze data within a group.

• Unpaired student t-test to analyze data in different groups.

Data were considered significant at p value <0.05. [9]

Results

Forty patients were included in this study and divided into 2 groups according to the angiographic findings as follows:

1- Group A: included 30 patients with significant coronary artery lesion (≥ 70% stenosis), 8 of them have LAD lesion, 7 patients with RCA lesion, 13 patients with LAD & RCA lesions, while only 2 patients have both LAD & LCX lesions.

The mean age of this group was (52.8±5), 19 of them (63.3%)were males and 11 (36.7%) were females, 22 patients(73.3%) were hypertensive , 70% (21/30) were diabetics, 46.7% (14/30) were

smokers, 43.3% (13/30) were dyslipidemics and 53.3% (16/30) with positive family history of coronary artery disease.

2- Group B: Included 10 subjects, and age matched with group A with normal coronary angiography, their mean age was (51±4), 40% of them (4/10)were males and 60% (6/10)were females, 60% (6/10) were hypertensive, 50% (5/10) were diabetics, 30% (3/10) were smokers, 40% (4/10) were dyslipidemic and 20% (2/10) have positive family history of coronary artery disease, there is no statistically significant difference between the diseased patients group as compared to the control group in demographic data (P>0.05). Table (1)

Table (2): show, there is statistically significant difference between patients having significant LAD lesion and control group in mean long axis myocardial strain at (mid anterior septal, mid inferior septal, mid inferior, apical anterior, apical septal and apex) that show significant decrease in patients with significant LAD stenosis. (P value<0.01).

Table (3) show there is statistically significant decrease in the mean long axis myocardial strain between patients having significant RCA lesion and control group at (basal anterior, basal inferior septal, basal inferior, mid inferior septal, mid inferior and apical. (P<0.01).

In table (4) there was statistically significant difference between patients having significant 2 vessels disease(LAD& RCA) and control group in mean long axis myocardial strain (P value<0.01) at (basal anterior, basal anterior septal, basal inferior septal, basal inferior, basal posterior lateral, mid anterior, mid anterior septal, mid inferior septal, mid inferior, mid anterior lateral, apical anterior, apical septal and apical lateral) that show significant decrease in patients with significant stenosis in the 2 vessels LAD & RCA.

As regards from table (5) there was statistically significant difference between patients having significant 2 vessels disease (LAD& CX) and control group in mean long axis myocardial strain (P val-

ue<0.05) at (basal anterior, basal inferior septal, basal inferior, mid anterior, mid inferior septal, mid inferior, apical septal and apical lateral) that show significant decrease in patients with significant stenosis in the 2 vessels LAD & CX.

Table (6): show the sensitivity and specificity of 2D echocardiography strain for detection of CAD, the sensitivity was 89.6% and specificity was 63.6%.

Table (1): Demographic data of both groups

		Patients		Controls		Total		Chi-square	
		N	%	N	%	N	%	X^2	P-value
Sex	Female	11	36.67	6	60.00	17	42.50	1.671	>0.05
	Male	19	63.33	4	40.00	23	57.50		
Age	Range	45.0-60.0		46.0-60.0				T=0.973	>0.05
	Mean ± SD	52.867±5.151		51.100±4.358					
HTN	Positive	22	73.33	6	60.00	28	70.00	0.635	>0.05
DM	Positive	21	70.00	5	50.00	26	65.00	1.319	>0.05
DYSLIP.	Positive	13	43.33	4	40.00	17	42.50	0.034	>0.05
Smoker	Positive	14	46.67	3	30.00	17	42.50	0.853	>0.05
FH	Positive	16	53.33	2	20.00	18	45.00	3.367	>0.05

Table (2): comparison between patients with LAD lesion in group A and group B

	Controls	LAD	T-test	
	Mean ± SD	Mean ± SD	t	P-value
basal anterior	-21.100 ± 3.446	-20.250 ± 4.713	-0.442	>0.05
basal anterior septal	-19.700 ± 3.164	-19.500 ± 3.505	-0.127	>0.05
basal inferior septal	-23.800 ± 4.050	-21.250 ± 3.536	-1.402	>0.05
basal inferior	-26.200 ± 5.473	-22.375 ± 7.596	-1.243	>0.05
basal posterior lateral	-19.200 ± 2.394	-17.000 ± 3.423	-1.605	>0.05
basal anterior lateral	-20.400 ± 4.115	-19.500 ± 1.414	-0.588	>0.05
mid anterior	-18.300 ± 4.990	-14.000 ± 4.243	-1.938	>0.05
mid anterior septal	-18.400 ± 2.797	-13.250 ± 2.765	-3.902	<0.01*
mid inferior septal	-20.200 ± 3.425	-13.375 ± 1.408	-5.265	<0.01*
mid inferior	-19.800 ± 3.048	-15.500 ± 4.567	-2.393	<0.05*
mid posterior lateral	-15.300 ± 10.761	-15.000 ± 3.251	-0.076	>0.05
mid anterior lateral	-17.700 ± 2.791	-16.875 ± 3.044	-0.599	>0.05
apical anterior	-16.500 ± 5.126	-8.875 ± 3.482	-3.587	<0.01*
apical septal	-18.500 ± 5.061	-11.500 ± 2.777	-3.500	<0.01*
apical inferior	-13.100 ± 11.338	-0.500 ± 14.412	-2.079	>0.05
apical lateral	-16.600 ± 3.921	-13.375 ± 4.241	-1.673	>0.05
apex	-10.600 ± 3.471	-6.500 ± 1.604	-3.075	<0.01*

Table (3): comparison between patients with RCA lesion in group A and group B

	Controls	RCA	T-test	
	Mean ± SD	Mean ± SD	t	P-value
basal anterior	-21.100 ± 3.446	-15.857 ± 4.413	-2.755	<0.05*
basal anterior septal	-19.700 ± 3.164	-17.571 ± 2.699	-1.446	>0.05
basal inferior septal	-23.800 ± 4.050	-11.286 ± 2.215	-7.392	<0.01*
basal inferior	-26.200 ± 5.473	-13.143 ± 1.464	-6.106	<0.01*
basal posterior lateral	-19.200 ± 2.394	-17.571 ± 3.259	-1.192	>0.05
basal anterior lateral	-20.400 ± 4.115	-17.429 ± 2.699	-1.668	>0.05
mid anterior	-18.300 ± 4.990	-19.571 ± 5.224	0.507	>0.05
mid anterior septal	-18.400 ± 2.797	-17.714 ± 2.138	-0.545	>0.05
mid inferior septal	-20.200 ± 3.425	-14.429 ± 4.541	-2.995	<0.01*
mid inferior	-19.800 ± 3.048	-7.143 ± 3.761	-7.664	<0.01*
mid posterior lateral	-15.300 ± 10.761	-17.571 ± 3.552	0.534	>0.05
mid anterior lateral	-17.700 ± 2.791	-16.000 ± 2.000	-1.377	>0.05
apical anterior	-16.500 ± 5.126	-19.857 ± 3.891	1.458	>0.05
apical septal	-18.500 ± 5.061	-16.000 ± 8.266	-0.776	>0.05
apical inferior	-13.100 ± 11.338	-11.143 ± 8.513	-0.386	>0.05
apical lateral	-16.600 ± 3.921	-10.571 ± 4.860	-2.831	<0.05*
apex	-10.600 ± 3.471	-10.714 ± 7.675	0.042	>0.05

Table (4): comparison between patients with LAD&RCA lesions and control group

	Controls			LAD and RCA			T-test	
	Mean	±	SD	Mean	±	SD	t	P-value
basal anterior	-21.100	±	3.446	-8.077	±	5.171	-6.860	<0.01*
basal anterior septal	-19.700	±	3.164	-13.385	±	4.273	-3.913	<0.01*
basal inferior septal	-23.800	±	4.050	-15.538	±	3.479	-5.260	<0.01*
basal inferior	-26.200	±	5.473	-13.077	±	5.139	-5.904	<0.01*
basal posterior lateral	-19.200	±	2.394	-15.923	±	2.753	-2.990	<0.01*
basal anterior lateral	-20.400	±	4.115	-17.000	±	5.066	-1.726	>0.05
mid anterior	-18.300	±	4.990	-10.615	±	5.221	-3.566	<0.01*
mid anterior septal	-18.400	±	2.797	-14.231	±	2.351	-3.885	<0.01*
mid inferior septal	-20.200	±	3.425	-13.385	±	2.755	-5.295	<0.01*
mid inferior	-19.800	±	3.048	-12.231	±	4.764	-4.371	<0.01*
mid posterior lateral	-15.300	±	10.761	-7.538	±	14.286	-1.431	>0.05
mid anterior lateral	-17.700	±	2.791	-15.000	±	2.799	-2.296	<0.05*
apical anterior	-16.500	±	5.126	-11.000	±	4.865	-2.626	<0.05*
apical septal	-18.500	±	5.061	-10.462	±	4.994	-3.805	<0.01*
apical inferior	-13.100	±	11.338	-7.462	±	3.620	-1.695	>0.05
apical lateral	-16.600	±	3.921	-8.846	±	4.598	-4.266	<0.01*
apex	-10.600	±	3.471	-10.769	±	4.833	0.094	>0.05

Table (5): comparison between patients with LAD&LCX lesions and control group

	Controls	LAD and LCX	T-test	
	Mean ± SD	Mean ± SD	t	P-value
basal anterior	-21.100 ± 3.446	-14.000 ± 5.657	-2.459	<0.05*
basal anterior septal	-19.700 ± 3.164	-16.000 ± 5.657	-1.367	>0.05
basal inferior septal	-23.800 ± 4.050	-16.500 ± 2.121	-2.416	<0.05*
basal inferior	-26.200 ± 5.473	-15.000 ± 7.071	-2.558	<0.05*
basal posterior lateral	-19.200 ± 2.394	-15.500 ± 4.950	-1.732	>0.05
basal anterior lateral	-20.400 ± 4.115	-20.000 ± 2.828	-0.129	>0.05
mid anterior	-18.300 ± 4.990	-9.000 ± 5.657	-2.372	<0.05*
mid anterior septal	-18.400 ± 2.797	-14.000 ± 2.828	-2.029	>0.05
mid inferior septal	-20.200 ± 3.425	-13.000 ± 1.414	-2.834	<0.05*
mid inferior	-19.800 ± 3.048	-10.000 ± 2.828	-4.180	<0.01*
mid posterior lateral	-15.300 ± 10.761	2.500 ± 20.506	-1.900	>0.05
mid anterior lateral	-17.700 ± 2.791	-18.000 ± 0.000	0.146	>0.05
apical anterior	-16.500 ± 5.126	-9.000 ± 1.414	-1.983	>0.05
apical septal	-18.500 ± 5.061	-9.000 ± 1.414	-2.544	<0.05*
apical inferior	-13.100 ± 11.338	-5.000 ± 0.000	-0.972	>0.05
apical lateral	-16.600 ± 3.921	-10.000 ± 0.000	-2.290	<0.05*
apex	-10.600 ± 3.471	-10.500 ± 7.778	-0.031	>0.05

(6): Sensitivity and specificity of the 2D longitudinal strains for detection of CAD

All cases	True cases	False cases
30 patients(Group A)	True +ve=26	False +ve=3
10 patients(Group B)	True –ve=7	False –ve=4

$$Sensitivity = \frac{true+ve}{true+ve+false+ve} = 89.6\%$$

$$Specificity = \frac{true-ve}{true-ve+false-ve} = 63.6\%$$

Discussion

Tissue Doppler imaging (TDI) has been introduced as a new method to quantify regional myocardial function. It provides an analysis of myocardial velocities and regional myocardial displacement, deformation and rate of deformation. [10].

Nevertheless to improve speed, pertinence and efficiency of the diagnosis in many patients, a subjective interpretation of myocardial regional motion and thickness is frequently performed in the intensive care unit (ICU), therefore not necessarily in the best conditions. [11]

The wall motion score assessing regional contractility has been validated. Reproducibility of this method is high for expert observer but it requires dedicated training and remains subjective. [5]

Thus, TDI may offer an alternative quantitative technique that can be used in routine practice. Several parameters have been described in experimental settings. [12]

The aim of this study to assess and validate 2 Dimensional long axis myocardial strain for the detection of myocardial ischemia in patients with significant coronary artery stenosis without visual wall motion abnormalities at rest.

As shown from our results as regards clinical characteristics of patients, there were no statistically significant difference between the diseased patients as regards age ,sex, hypertension , cigarette smoking, diabetes mellitus, dyslipidemia and positive family history of coronary artery disease as compared to the control group ($P>0.05$) as, these results were similar to those reported by Erwan et al who compared tissue Doppler echocardiography and coronary angiography results in coronary artery disease patients. [13]

Coronary angiography of group (A) in this study, revealed that there were (8) patients have single (LAD) significant stenosis, [7] patients have single (RCA) significant lesion, [13] patients have significant 2 vessels (LAD & RCA) lesions and only [2] patients have significant 2vessls (LAD &CX) le-

sions.

Long axis myocardial strain

Myocardial fibers consisted of three different anatomical layers. [14] The innermost sub-endocardial layer of fibers has an oblique clockwise orientation in the longitudinal direction. Recent studies have demonstrated that sub-endocardial layer of fibers mainly contributes to cardiac long-axis function [15]. Zhang et al. (2005) reported that the peak systolic longitudinal velocity and strain rate obtained with tissue Doppler-derived strain rate imaging declined significantly in sub-endocardial infarction segments and acute coronary syndrome when compared with those in controls. [16]

In our study, we found that although there was no visual segmental wall motion abnormalities could be detected in patients with coronary artery stenosis at rest on conventional 2-D echocardiography, the peak systolic longitudinal strain was significantly reduced at (mid anterior septal, apex, apical anterior, apical septal, mid inferior septal and mid inferior) segments in patients having single LAD with >70% Stenosis Group when compared with those in control group as regarded at table [2].

The peak systolic longitudinal strain was significantly reduced at (basal anterior, basal inferior septal, basal inferior, apical inferior, mid inferior, mid inferior septal) segments in patients having single RCA with >70% Stenosis Group when compared with those in control group as regarded at table [3].

The peak longitudinal strain was significantly reduced at (basal anterior, basal anterior septal, basal inferior septal, basal inferior, basal posterior lateral, apical lateral, apical septal, mid anterior, mid anterior septal, mid inferior septal and mid inferior) segments in patients having 2 vessels (LAD&RCA) with >70% Stenosis Group when compared with those in control group as observed at table [4].

It is also found that the peak longitudinal strain was significantly reduced at (basal anterior, basal inferior septal, basal inferior, mid anterior, mid inferior sep-

tal, apical lateral, Apical septal and mid inferior) segments in patients having 2 vessels (LAD&CX) with >70% Stenosis Group when compared with those in control group as regarded at table [5].

Similarly, Zhang et al. (2005) also showed that peak systolic longitudinal strain measured with 2-D strain echocardiography technique reduced significantly in sub-endocardial infarction segments when compared with those in controls. Our study further extended those observations by demonstrating that decrease in myocardial long-axis function exists at rest in the myocardial segments subtended by the coronary arteries with >70% stenosis. [16]

Our findings are in accordance with the previous studies, Yip et al. (2002) reported a decreased longitudinal systolic velocity as measured by myocardial tissue Doppler in patients with left ventricular hypertrophy and normal left ventricular ejection fraction. Similarly, a decreased longitudinal systolic strain as measured by tissue tracking was reported recently in patients with diastolic heart failure. [17]

Liang et al. (2006) revealed a reduction in longitudinal peak systolic strain rate in myocardial segments subtended by >70% coronary stenosis compared with control group. It has been reported that when the coronary artery flow reduced, sub-endocardial myocardium would be the first to suffer and sub-endocardium is more vulnerable to ischemic damage than the mid myocardium or sub-epicardium.(18). When coronary arteries are constricted to reduce total coronary artery flow to approximately 40% of control, endocardial to epicardial flow ratio falls from 1.16 at baseline to 0.37. [19]

Tissue Doppler velocities can detect an ischemic myocardium effectively but strain rate imaging is superior in detection of ischemic myocardium than tissue Doppler velocities alone. [5, 12]

Yao et al. (2008) demonstrated that sub-endocardial flow begins to reduce after 75% stenosis of the left anterior descending coronary

artery by using myocardial contrast echocardiography, whereas the sub-epicardial flow remains unchanged. This pattern of redistribution of flow away from the endocardium may, in part, explain the findings observed in our study. [20], that is sub-endocardial layer of fibers mainly contributes to cardiac long-axis function which was significantly reduced in segments supplied by coronary arteries having >70% Stenosis.

Sensitivity and specificity were 89.6% and 63.6%, respectively for peak systolic longitudinal strain to predict segments subtended by coronary arteries with significant coronary artery stenosis. Table (6)

Tongji Medical College 2010 also reported the same result in study conducted on department of medical ultrasound, Tongji Hospital at 2009, which revealed that Sensitivity and specificity were 74% and 72%, respectively, for peak systolic longitudinal strain to predict segments subtended by coronary arteries with greater than 75% stenosis. [21]

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EVALUATION OF LONG-AXIS MYOCARDIAL FUNCTION WITH 2D STRAIN ECHOCARDIOGRAPHY IN PATIENTS WITH SIGNIFICANT CORONARY ARTERY STENOSIS

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POPULATION BASED EVALUATION OF ANTITHROMBOTIC THERAPY IN PATIENTS WITH ATRIAL FIBRILLATION

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Abstract

Background: *Atrial fibrillation (AF) is the most frequent arrhythmia in clinical practice and is the strongest independent risk factor for stroke. Approximately 50% of all cardiac and systemic emboli occur in individuals with AF.*

Objective: *The aim of this work is to determine the use of oral antithrombotic therapy for treatment of patient with atrial fibrillation in Kalubia government, Egypt.*

Patients and methods: *These present cross sectional analytic study was conducted on 200 AF patients and were eligible for antithrombotic therapy. All patients were subjected to full history taken with emphasis on admission details, thromboembolic and hemorrhagic complications, risk factors, also clinical examinations, investigations including prothrombin time, INR ,resting ECG, and trans- thoracic echocardiography data were collected. The patients were divided into two groups, Group A: patients received oral anticoagulation regularly, and Group B: patients not received oral anticoagulation. Group A subdivided into subgroup A1 {controlled patients with normal INR level (2-3)}, and subgroup A2 (patients with uncontrolled INR).*

Results: *The present study included 200 patients, 83 (41.5%) females and 117 (58.5%) males, their age range from 20 to 87 years old. One hundred and eighty six (93%)of patients with chronic (permanent) AF, 14(7%) with paroxysmal recurrent AF, Forty nine (24.5%) patients presented by rapid AF, and only 4 patients (2.5%) presented by slow AF. The commonest causes of AF in our study was IHD included 117patients (58.5%) ; hypertension found in 80 patients (40%), while rheumatic heart disease presented in 78 (39%) of cases, and finally*

54patients (27%) with heart failure. Seventy six patients (38%) received oral anticoagulant, 36.8% of them with controlled INR, and most of them with prosthetic valves (92 %).Thromboembolic complications were developed in 9 patients(11.7%)all of them in group A2 versus 39 patients(31.4%)in group B. The estimated bleeding complications in patients taking warfarin developed in 10 patients (13.9%),2 patients (3.1%) of them had intracranial hemorrhage.

Conclusion: *Only 38% of patients receive oral anticoagulation regularly, 36.8% of them were properly anticoagulated. Prosthetic valves, rheumatic heart disease, associated with high rate of warfarin use in our study. Intracranial hemorrhages developed in 3.1% with anticoagulated patients, and thromboembolic complications in patients not anticoagulated were 31.4%.*

Introduction

Atrial fibrillation (AF) is the most frequent arrhythmia in clinical practice and is the strongest independent risk factor for stroke. Approximately 50% of all cardiogenic and systemic emboli occur in individuals with AF.(1)

There are three potential therapeutic goals of treatment for patients with AF; these include restoration and maintenance of sinus rhythm, rate control during AF and prevention of thromboembolism(2). In patients with atrial fibrillation, warfarin prevents 62% of strokes .Thus; warfarin has become the recommended treatment for candidates who have atrial fibrillation and at least one additional risk factor for stroke.(3)

Despite clear and consistent recommendations warfarin is suboptimal prescribed. Several factors contribute to suboptimal use of warfarin therapy.(4)

Aim of the work

Aim of this study is to determine the use of antithrombotic therapy for the treatment of patient with atrial fibrillation and to survey the knowledge of antithrombotic therapy for atrial fibrillation in Kalubia government.

Patients and methods

This present cross sectional analytic study was conducted on 200 AF patients in Kalubia government and eligible for antithrombotic therapy during the period from March to October 2009.

Patient with lone AF (no heart diseases or risk factors) were excluded from this study.

The following data was collected:-

1- Full history data was collected with to determined risk factors as DM, HTN, rheumatic heart disease, prosthetic valve, and thromboembolic, hemorrhagic complications.

2- Clinical examinations data.

3- Investigations data was collected included routine laboratory investigations and prothrombin time, prothrombin concentration, and INR.

4- Resting 12 lead surfaces ECG.

5- Trans-Thoracic Echocardiography (TTE) data was collected to determined valvular heart diseases LA & LV size, LV EF.

These patients were divided into two groups:-

(i) Group A: included 76 patients received oral anticoagulation regularly.

They divided into 2 sub groups:

A1: 28 Controlled patients, where INR within the therapeutic range.

A2: 48 uncontrolled patients.

(ii) Group B: included 124 patients not received oral anticoagulation.

Statistical analysis

Data were collected from total patients (group A and B), and expressed as the mean value, standard deviation and percentages.

Kruskal – Wallis test was used to compare different parameters between groups. When inter-group differences were found, Mann-Whitney test was performed to determine which groups were significantly different.

Categorical variables were analyzed with the Chi square test. Paired student's t-test was used to compare different parameters of studied groups.

A value of $P < 0.05$ was considered statistically significant.[5]

Results

The present study included 200 patients, 83 (41.5%) females and 117 (58.5%) males, their age range from 20 to 87 years. One hundred and eighty six (93%) patients have chronic (permanent) AF and 14 (7%) patients have paroxysmal recurrent AF, Forty nine (24.5%) patients presented by rapid AF, 4 (2.5%) presented by slow AF and 146 (73%) patients have controlled AF.

The commonest risk factors of AF in our study were IHD, included 117 patients(58.5%) 27 of them in group A and 90 patients in group B; hypertension found in 80 (40%) of cases 15 of them in group A and 65 patients in group B, while RHD presented in 78 (39%) of cases 59 of them in group A and 19 patients in group B, and finally heart failure found in 54 (27%) of cases, 21 of them in group A and 33 patients in group B. Table (1).

Only 76 (38%) patients received oral anticoagulant (group A), (36.8%) of them (14% from the total study) have controlled INR (group A1), while (62%) of patients not received oral anticoagulant (Group B). Fig. (1).

Married patients were used oral anticoagulant more in group A, 65patients (84.5%) versus 90 patients(71.6%) in group B, and these statistically significant (p value < 0.05), while widow patients were more in group B, 23 patients(18.5%) versus 3 patients (4.8%) in group A with statistical significant (P value < 0.05).

Forty four(57.8%) of patients in group A were living in urban area versus 33 patients (28.2%) in group B, also large number of patients in group B, 91patients (71.8%) were living in rural area versus 32 patients (42.2%) in group A, and these result high statistical significant (p value < 0.001). Table (2).

Table (3) show significant increase in the mean value of both left atrium (LA) and left ventricle (LV) dimensions in patients with group A, it was (5±0.71, 6.5±1.07 respectively) versus (4.4±0.61, 6±1.03) in group B (p value <0.05), also the mean value of LVEF was significantly lower in

group A, it was (48±13%) versus (53.4±14%) in group B (P value<0.05)

Thromboembolic complications in patients who received oral anticoagulation were 9 patients (11.7%) all of them in group A2 versus 39 patients (31.4%) in patients who not received oral anticoagulation (group B), the difference was statistically significant (p value<0.01), while bleeding complications in patients taking warfarin (group A) were 10 patients (13.9%) all of them were in group A1 and 2 patients of them (3.1%) developed intracranial hemorrhage (p value <0.05). Table (4)

Table (1): risk factors of studied groups:-

Group / Risk factors	Group A		Group B		Total	P value
	No.	%	No.	%		
	76	100%	124	100%		
HTN	15	18.8%	65	81.2%	80	<0.05*
DM	15	21.4%	55	78.6%	70	<0.05*
IHD	27	23.1%	90	76.9%	117	<0.05*
RHD	59	75.6%	19	24.4%	78	<0.05*
HF	21	38.9	33	61.1	54	>0.05
Prosthetic valves	23	92	2	8	25	<0.05*

Table (2): distribution of residence and marital stat between studied groups

	Group A N=76	Group B N=124	P value
Residence			
* rural area	32(42.2%)	91(71.8%)	<0.001**
* urban area	44(57.8%)	33(28.2%)	<0.001**
Marital stat			
* single	8(10.7%)	4(4.3%)	>0.05
* married	65(84.5%)	90(71.6%)	<0.05*
* widow	3(4.8%)	23(18.5%)	<0.05*

Table (3): Echocardiography parameters of studied groups

	Mean value & SD		P value
	Group A	Group B	
LA dimension	5±0.71	4.4±0.61	<0.05*
LV dimension	6.5±1.07	6±1.03	<0.05*
LVEF%	48±13%	53.4±14%	<0.05*

Table (4): complications of studied groups

	Group A N=76		Group B N=124	P value
	A1	A2		
Thromboembolic (total)	0	9 (11.7%)	39(31.4%)	<0.01*
* cerebral infarction	0	6(7.7%)	36(29%)	
* lower limb ischemia	0	0(0%)	1(0.8%)	
* transient ischemic attack	0	3(4%)	2(1.6%)	
Hemorrhagic complications	10(13.9%)	0	0	<0.05*
* cerebral hemorrhage	2(3.1%)	0	0	
* ecchymosis	2(3.1%)	0	0	
* other	6(7.7%)	0	0	

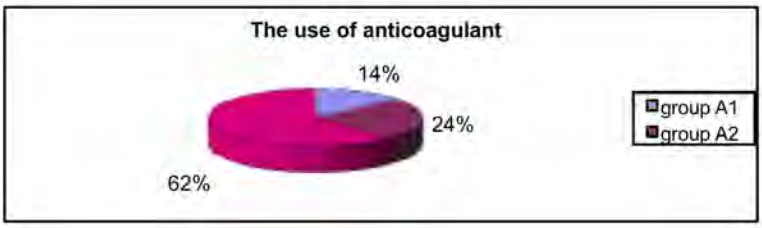

Fig. (1) Oral anticoagulant distribution among the patients

Discussion

Approximately 50% of all cardiogenic and systemic emboli occur in individuals with AF.[1] Trials of anticoagulant and antiplatelet medications to prevent stroke in AF were conducted to interrupt the presumed cardioembolic mechanism of stroke in AF. Despite clear and consistent recommendations, several factors contribute to suboptimal use of warfarin therapy.[4]

The aim of this work is to evaluate the use of antithrombotic therapy for the treatment of patient with atrial fibrillation and to survey the awareness of antithrombotic therapy for atrial fibrillation in Kalubia government, Egypt.

The commonest underlying causes of AF in our study were IHD found in 117 (58.5%) of cases This discordance with Salah , et al., (2007) who, reported that the commonest underlying cause of AF was RHD (70%) while IHD was present in 8.8% of cases.[6] According to current guidelines' recommendations for anticoagulation, all patients of our study were eligible for oral anticoagulation.

Unfortunately, only 76 (38%) patients received oral anticoagulants in our study while, 23 (11.5%) patients received oral anticoagulation and ASA, 91(45.5%) patients received ASA alone in Salah, et al study.[6]

In Taiwan, Lin et al., (2008) reported that only 24.7% of the overall population received appropriate antithrombotic therapy, when patients with risk for bleeding were excluded, the rate of compliance increased to 26.2%, so most of patients with AF in Taiwan did not receive appropriate antithrombotic therapy over the period studied which is in concordance with the current study.[7]

Glazer et al., (2007) found that 59% used warfarin, 28% used aspirin, and 24% neither used warfarin nor aspirin, so many at high risk of stroke did not receive warfarin.[8] In USA Lieva et al., (2009) reported that 79% were being treated with warfarin (to maintain an International Normalized Ratio between 2.0 and 3.0), 17% were being treated with aspirin

325 mg daily and 4%were not being treated with warfarin or aspirin.[9]

We found that only 28 patients with controlled INR, they were 36.8% from patients who received oral anticoagulation (14% from the total study), This is consistent with Salah Atta et al., (2007) reported that only 25 (19%) were properly anticoagulated But Fuster, et al., (2001) reported that only 36.8% of their patients were controlled. [6,10]

In our study patients whose lives in urban areas, married, prosthetic heart valves and rheumatic heart disease were more use of oral anticoagulation than patient's lives in rural areas, widow, patients with hypertension, coronary artery disease, diabetes mellitus, and heart failure.

Lin et al (2008) found that hypertension, coronary artery disease, older age, and bleeding risk factors were associated with low rates of warfarin use.[7]

In our study we found that, high incidence of thromboembolic complications (31.4%) in patients who not received oral anticoagulation versus (11.7%) in patients under anticoagulant therapy and uncontrolled INR.

GO et al., (2003) found that among 11,526 patients, 397 of them developed thromboembolic events (372 ischemic strokes, 25 peripheral embolisms) during follow-up, and warfarin therapy was associated with a 51% lower risk of thromboembolism compared with no warfarin therapy (either no antithrombotic therapy or aspirin).[11]

A nested case-control analysis estimated a 64% reduction of thromboembolism with warfarin compared with no antithrombotic therapy. Warfarin was also associated with a reduced risk of all-cause mortality. Intracranial hemorrhage was uncommon, but the rate was moderately higher among those taking vs those not taking warfarin, however, warfarin therapy was not associated with an increased adjusted risk of non intracranial major hemorrhage.

In our study lack of patients

compliance and withdrawal of warfarin after a period of intake were due to either the high cost of follow-up of INR and being in villages far-away from hospitals or the occurrence of bleeding complications or social problems occurred in 11.5% of patients, but contraindications to warfarin due to high risk of bleeding was observed in only 6% of our cases.

Conclusions

Unfortunately, 76 (38%) patients receive oral anticoagulation regularly, 28 (36.8%) of them (14% of total study) were properly anticoagulated but 124 (62%) patients not receive oral anticoagulation, Prosthetic heart valves, rheumatic heart disease, married were associated with high frequency of warfarin use in our study but hypertension, coronary artery disease, diabetes mellitus, older ages and living in villages were associated with low rates of warfarin use.

Intracranial hemorrhages in patients taking warfarin was 3.1% and thromboembolic complications of AF in our study were 39% in patients not taking warfarin and 11.7% in patients that use oral anticoagulant but INR under controlled level.

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POPULATION BASED EVALUATION OF ANTITHROMBOTIC THERAPY IN PATIENTS WITH ATRIAL FIBRILLATION

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EFFECT OF TAMOXIFEN VERSUS 17 BETA-ESTRADIOL ON SKELETAL MUSCLE PERFORMANCE IN OVARIECTOMIZED RATS

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Abstract

Background: *Estrogen replacement therapy is used to relief post-menopausal symptoms and to improve the physical dysfunction associated with the deficiency of the female sex hormones. The present study was designed to investigate a possible beneficial effect of tamoxifen administration versus estradiol replacement on skeletal muscle contraction (SMC) and its glucose uptake (GU) in ovariectomized rats.* ***Material and Methods:*** *Thirty adult female Sprague-Dawley rats were assigned randomly into five equal groups (n=6): (1) Non-ovariectomized (control) group; (2) Sham-operated (sham) group, in which animals were surgically manipulated without removal of ovaries; (3) Non-treated ovariectomized (OVX) group, in which surgical ovariectomy was done; (4) 17 β-estradiol-treated ovariectomized (OVX+E2) group, in which ovariectomized rats were subcutaneously (s.c.) injected with 17 β-estradiol benzoate (30 μg/kg b.w.), daily for 4 weeks; (5) Tamoxifen-treated ovariectomized (OVX+T) group, in which ovariectomized rats were s.c. injected with tamoxifen (1 mg/kg b.w), daily for 4 weeks. At the end of experiment, phrenic nerve-hemidiaphragm preparation was dissected and used for measurement of the strength of SMC at the onset and after 30 min activity (obtained by both indirect and direct stimulation) and of GU by the muscle during 30 min activity and recovery times.* ***Results:*** *OVX group showed a significant reduction in strength of SMC, and in its GU, when compared with those in control and sham groups. Both*

OVX+E2 and OVX+T groups showed a significant improvement in the strength of SMC and GU versus OVX group. After 30 min activity, the percentage decrease of SMC from the initial level was significantly increased in OVX group compared to control and sham groups indicating more muscle fatigability; values were reduced to normal levels of control and sham rats. In all groups, indirect to direct contraction ratio was insignificantly changed, which excludes the involvement of neuromuscular junction in the observed muscle dysfunction. ***Conclusion:*** *tamoxifen could improve the strength of SMC and delays its fatigue and increase its GU during activity and recovery times in a rat model of surgical ovariectomy. Therefore, tamoxifen could be considered as a potential alternative line of treatment to estradiol replacement therapy in post-menopausal skeletal muscle disturbances in adult humans.*

Keywords: *tamoxifen, 17 β-estradiol, estrogen replacement, ovariectomy, phrenic nerve-hemidiaphragm preparation, skeletal muscle function.*

Introduction

Elderly women have lower functional capacity compared with men [1], including a clear deterioration in muscle performance from early menopause [2]. Given the wide range of activities of endogenous estrogens during the reproductive years and the significantly increased risk of cardiovascular, immune, and skeletal disorders after menopause [3,4], a major effort has been made to develop hormone replacement therapies aimed at providing aging women with the same biological advantages observed before cessation of ovarian functions [5].

Estrogen replacement therapy after menopause improves the health and quality of life for women; estrogen helps to relieve many post-menopausal symptoms, as well as to influence emotional state [6]. There are two isoforms of the estrogen receptors (ER), ER∝ and ERβ [7], whose distribution and density vary depending on the target site. In many tissues, ER∝ has proliferative effects, whereas ERβ has anti-proliferative ones [8].

The continuous administration of female sex hormones was associated with the risk of undesired hyperproliferation in the reproductive tissues[9]. Long-term estrogen replacement therapy in postmenopausal women, who have a uterus, might has the disadvantage of being tissue agonists for endometrium, which increases the incidence of endometrial cancer[10]. Although adding progestin to estrogen can be used to prevent the development of endometrial cancer, this combination may cause some unwanted side effects, as breast cancer, venous thromboembolism, stroke and coronary heart disease[10]. Thus, estrogen replacement therapy is no longer recommended because of its severe side effects[11], and alternative therapies may offer attractive options because they may protect against breast or endometrial cancer, have fewer side effects and still provide health benefits[12], Therefore, an attempt was made to develop compounds agonists in non-reproductive tissues[13].

Selective estrogen receptor modulators (SERMs) are synthetic non-steroidal agents that bind to the ER and produce a change in the biologic activity of the receptor depending on the tissue type[14]. Once a SERM binds to the ER, it causes a change in the shape of the ER that allows recruitment of co-activators, if it is destined to elicit an estrogenic response or co-repressors if its response is anti-estrogenic[15].

Tamoxifen is an example of SERMs, which was originally developed for the treatment of breast cancer[16]. Although tamoxifen is primarily an estrogen receptor antagonist by competing with estrogen in binding to ER[17], it has some actions as an agonist depending on tissue[18]. Tamoxifen binds both ER∝ and ERβ with equivalent affinity[19], however, the ratio of ER∝ to ERβ at a target site may play a role in determining the overall action of a SERM on that tissue [15]. Tamoxifen is easily absorbed by the gut without modification and 98% is bound to albumin after entering the circulation. 4-Hydroxy tamoxifen, and endoxifen are metabolites of tamoxifen formed in the liver and have the same affinity for the ER as estrogen[20]. However, tamoxi-

fen has side effects including hot flushes and vaginal discharges. Thrombo-embolic disorders, as a possible side effect, have also been reported in association with tamoxifen treatment[21].

The present study aimed at investigating the possible beneficial effects of 17 β-estradiol (E2) replacement and, alternatively, treatment with tamoxifen on postmenopausal muscle weakness by an ovariectomized animal model.

Material and Methods

Experimental groups: This study followed a randomized controlled animal experiment design. Thirty adult cycling female Sprague-Dawley rats, weighed between 150-200 g, were obtained from the National Research Center, Cairo, Egypt. Animals were housed in the animal house of Menoufiya Faculty of Medicine with a 12:12-hrs dark/light cycle, and were fed a standard rat chow diet and had free access to tap water ad libitum. Rats were randomly divided into five equal groups (n=6 per group): (1) Non-ovariectomized (control) group, in which animals were left to rest for 1 week, and then they were injected subcutaneously (s.c.) with 1ml/kg of 10% ethanol/90% saline vehicle, daily for 4 weeks; (2) Sham-operated (sham) group, in which animals were exposed to sham operation as described below, got 1 week rest, and then were s.c. injected with 10% ethanol/90% saline vehicle as in control group; (3) Non-treated ovariectomized (OVX) group, in which animals were subjected to surgical ovariectomy according to the method described below, got 1 week rest, and then were injected with 10% ethanol/90% saline vehicle as in the above groups; (4) E2-treated ovariectomized (OVX+E2) group, in which animals, after ovariectomy and 1 week rest, were s.c. injected with 50 μg/kg b.w. of 17β-estradiol benzoate, (Misr Co. Pharm. Ind., Egypt) dissolved in 1ml/kg of 10% ethanol/90% saline vehicle[22], daily for 4 weeks; (5) Tamoxifen-treated ovariectomized (OVX+T) group, in which animals, after ovariectomy and 1 week rest, were s.c. injected with 1mg/kg b.w. of tamoxifen (Nolvadex, Sedico Pharma, Egypt) dissolved in 1ml/kg of 10% ethanol/90% saline vehicle[22], daily for 4 weeks. The

experiment was conducted in accordance with the ethical guidelines for investigations in laboratory animals and was approved by the Ethical Committee of the Faculty of Medicine, Menoufiya University.

Ovariectomy and sham operation: Ovariectomy was performed by the method described by Rogers and Sheriff[23]. Briefly, each rat was laid under light ether anesthesia in supine position and upper and lower limbs were fixed. Lower abdominal region was sterilized and a midline abdominal incision (2 cm) was made, where abdominal viscera were manipulated gently to locate the uterus, fallopian tubes and ovaries. In both sides, the junction between the uterus and each ovary, including oviduct and blood vessels, was circumferentially ligated, followed by an incision just distal to the suture, and ovaries were excised. The muscle wall, subcutaneous tissue and skin were then sutured, and topical sterilization and gentamicin ointment (garamycin, Memphis, Egypt) were applied immediately, and daily for 1 week after operation to help wound healing. Sham-operated rats underwent the same operative procedures, but without ligation or excision of the ovaries. Closure proceeded as above.

At the end of the experiment (5 weeks after ovariectomy), retro-orbital blood samples were obtained from animals, after overnight fasting, through heparinized capillary tubes. Samples were added to EDTA and were centrifuged at 1000 rpm for 15 min for separation of plasma and were stored at $4^{o}C$ to assay plasma estradiol level, as a marker of successful ovarian hormone depletion and replacement [24], by estradiol ELISA (KA0234, Abnova Corporation, Taiwan) according to the manufacturer specifications. The effectiveness of ovariectomy and E2 replacement in this study were proved as plasma E2 levels showed a marked significant drop ($P<0.001$) in OVX (6.9±1.1 pg/ml) and OVX+T (6.3±0.9 pg/ml) rats compared to those of control (59.7±11.2 pg/ml), sham (58.3±10.9 pg/ml) and OVX+E2 (65.8±18.7 pg/ml) animals. The presence of a measurable low amount of E2 in OVX and OVX+T

rats is explained by the capability of the peripheral tissues of converting adrenal-synthesized androgen precursors into estrogen [25].

Phrenic nerve-hemidiaphragm preparation: At the end of the experimental protocol period, animals of all groups were sacrificed by cervical dislocation, and the phrenic nerve-hemidiaphragm preparation was dissected and used for measuring of the strength of skeletal muscle contraction (SMC) and glucose uptake (GU) by the muscle according to a method modified from Bulbring [26]. Briefly, the skin over the chest was opened along the right border of the sternum and the frontal part of the right thoracic wall was removed. The mediastinum behind the sternum was incised and a cut was made just above the frontal insertion of the diaphragm. The frontal part of the left thoracic wall was then removed and the phrenic nerve was exposed. The left abdominal muscles were cut along the costal margin and the last rib was hold with a pair of forceps. A strip of diaphragm was cut out towards the tendinous part of the diaphragm with about 2.5 cm of phrenic nerve attached to it. The preparation had a fanlike shape being 3 mm wide at the tendinous end, while at the costal margin it was about 12 mm wide. The preparation was attached to a standard plastic diaphragm electrode by the costal margin and immersed in a 35 mm diameter and 50 ml capacity organ bath containing Krebs' solution that consists of (in mmol/L) NaCl 118, KCl 4.8, $CaCl.2H_2O$ 2.5, $MgSO_4.7H_2O$ 1.6, KH_2PO_4 1.3, $NaHCO_3$ 35 and glucose 5.5, with pH 7.4. The Krebs solution was pre-warmed to 37°C and aerated with carbogen (95% oxygen and 5% carbon dioxide). The gas bubbles were made to impinge on the tissue to insure adequate oxygenation. The central tendon of the diaphragm was connected by a thread to an isometric transducer (Dynamometer UFI, Pioden Controls), then to 4 channel oscillograph (MK III-S; Narco Bio-System, USA). The nerve was laid over a pair of platinum, and the plastic holder was suitably wired to allow independent direct stimulation of the muscle through platinum electrodes. Each preparation was left in Krebs' solution for 30

min before stimulation to insure stability.

Electrical stimulation: Both direct stimulation of the diaphragm and indirect stimulation through phrenic nerve were done using Dual Impedance Research Stimulator (Harvard, England). The preparation was stimulated with a train of supramaximal tetanic repetitive electrical impulses stimulation (25 volts, 100 Hz) with a frequency of 90/min, and the duration of each train-stimulation was set at 0.1 sec in indirect stimulation and at 0.2 sec in direct stimulation [27]. Initially, the muscle was stimulated directly for half a minute, and then indirectly for 30 min, followed by a direct stimulation again for half a minute. The isometric muscle contraction, in g tension, was recorded at the onset and the end of indirect stimulation as well as during the two short direct stimulations of the muscle. The percentage changes of both direct and indirect contraction after 30 min stimulation from the initial values at the onset of contraction were calculated. The ratio of strength of SMC due to indirect to direct electrical stimulation at the onset of contraction and at its end after 30 min activity time was also calculated.

Estimation of glucose uptake by the muscle: Three samples were collected from the Krebs' solution bathing the phrenic nerve-hemidiaphragm preparation: at the start of experiment, at the end of contraction (after 30 min activity time) and 30 min after the end of contraction (after 30 min recovery time). The glucose level in samples was determined using the enzymatic method of glucose oxidase kits described by Trinder [28], and glucose uptake by the muscle during contraction and recovery periods was calculated in mg/g wet weight.

Statistical analysis: Data were expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA), followed by Fisher multiple comparisons were used to detect level of significance among individual means of all groups. Statistically significant difference was set at $P<0.05$ [29].

Results

Diaphragmatic muscle con-

traction: An insignificant change of the strength of SMC, at the onset and after 30 min activity time, was observed in sham group compared to control animals, whether contraction was induced by indirect or direct electrical stimulation (table 1). The percentage decrease of the strength of SMC at the end of 30 min contraction, obtained by both indirect and direct stimulations, from the corresponding initial value at the onset of contraction, was also insignificantly changed in sham group versus control animals (fig 1).

OVX group showed a significant reduction in the strength of SMC, induced by both indirect and direct electrical stimulations, at the onset of contraction ($P<0.001$) and after 30 min activity time ($P<0.001$), when compared with the corresponding values in control and sham groups. In OVX+E2 group, average values of the strength of SMC, caused by both indirect and direct electrical stimulations, were significantly increased at the onset of contraction ($P<0.001$) and after 30 min activity time ($P<0.001$), when compared with the corresponding values in OVX group; an insignificant variation was observed when values of OVX+E2 group were compared with those in control and sham groups. OVX+T group had values of the strength of SMC, induced by both indirect and direct electrical stimulation, which were significantly elevated both at the onset of contraction ($P<0.001$) and after 30 min activity time ($P<0.001$), when compared with the corresponding values in OVX group, but were still significantly lower than those of control ($P< 0.05$) and sham ($P<0.05$) groups; an insignificant variation between values of the strength of SMC in OVX+T and OVX+E2 groups was observed (table 1).

The percentage decrease of the strength of SMC, obtained by both indirect or direct stimulation, at the end of 30 min activity from that at the onset of contraction was significantly elevated in OVX group ($P<0.001$) versus control and sham groups. In OVX+E2 and OVX+T groups, the percentage decrease was significantly minimized ($P<0.001$), when compared with that of OVX group; a non-significant change in the percent-

age decrease was observed between control, sham, OVX+E2 and OVX+T groups (fig.1).

In all the experimental groups, the ratio of the strength of SMC induced by indirect electrical stimulation to direct one showed an insignificant variation, whether at the onset of contraction or after 30 min activity time (table 2).

Glucose uptake by the diaphragmatic muscle: A non-significant variation in GU by the diaphragmatic muscle of animals of control and sham groups was observed during 30 min contraction and 30 min recovery periods. In OVX group, the average values of GU by the contracting muscles during 30 min activity, as well as by the recovering muscle during the next 30 min, were significantly reduced ($P<0.001$), when compared with the corresponding values in control and sham groups. Both OVX+E2 and OVX+T groups showed a significant increase of GU by the muscle during 30 min contraction and 30 min recovery times ($P<0.001$), when compared with the corresponding values in OVX group, but when compared with each other and with control and sham groups, both OVX+E2 and OVX+T groups showed an insignificant change (fig. 2).

Table 1. The strength of isometric skeletal muscle contraction (g tension) obtained by indirect and direct stimulations.

group		control	sham	OVX	OVX+E2	OVX+T
indirect stimulation	at onset of activity	5.86±0.46^{a}	5.83±0.47^{a}	3.13±0.28^{b}	5.62±0.43^{a,c}	5.27±0.39^{c}
	after 30 min activity	5.14±0.36^{a}	5.11±0.35^{a}	2.56±0.26^{b}	4.96±0.34^{a,c}	4.64±0.33^{c}
direct stimulation	at onset of activity	6.42±0.51^{a}	6.40±0.52^{a}	3.46±0.31^{b}	6.21±0.49^{a,c}	5.78±0.44^{c}
	after 30 min activity	5.67±0.44^{a}	5.64±0.45^{a}	2.82±0.27^{b}	5.48±0.42^{a,c}	5.09±0.36^{c}

Muscles were excited both indirectly and directly by repetitive electrical stimulations (90 impulses/min). control: control group, sham: sham-operated group, OVX: non-treated-ovariectomized group, OVX+E2: 17 β-estradiol-treated ovariectomized group, OVX+T: tamoxifen-treated ovariectomized group. Results are expressed as mean±SD (n=6). Values of the same raw which carry different letters (a-c) are significantly different at $P<0.05$.

Table 2. Ratio of strength of muscle contraction obtained by indirect stimulation to that by direct one.

group	control	sham	OVX	OVX+E2	OVX+T
at onset of activity	0.913±0.068	0.911±0.066	0.908±0.070	0.905±0.061	0.912±0.071
after 30 min activity	0.906±0.059	0.916±0.066	0.907±0.067	0.905±0.061	0.911±0.066

Muscles were excited both indirectly and directly by repetitive electrical stimulations (90 impulses/min). control: control group, sham: sham-operated group, OVX: non-treated-ovariectomized group, OVX+E2: 17 β-estradiol-treated ovariectomized group, OVX+T: tamoxifen-treated ovariectomized group. Results are expressed as mean±SD (n=6). Values of the same raw and column (paired data) show insignificant variations.

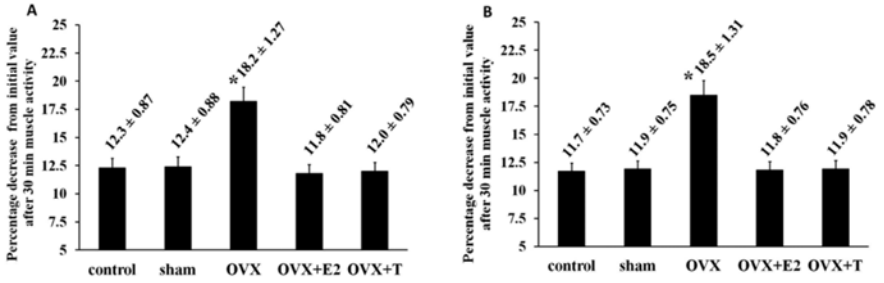

Fig. 1. Percentage decrease of muscle contraction after 30 min activity from the initial value. Percentage decrease obtained by [A] indirect and [B] direct repetitive electrical stimulations (90 impulses/min). control: control group, sham: sham-operated group, OVX: non-treated-ovariectomized group, OVX+E2: 17 β-estradiol-treated ovariectomized group, OVX+T: tamoxifen-treated ovariectomized group. Results are expressed as mean±SD (n=6). The group which carry the mark "*" is significantly different from all other groups at P<0.001.

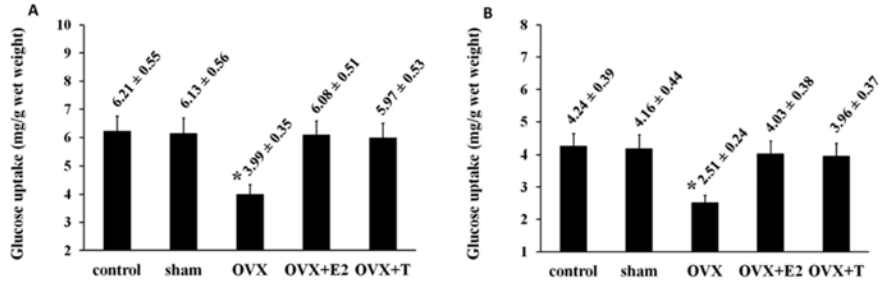

Fig. 2. Glucose uptake by the diaphragmatic muscle. Glucose uptake [A] during 30 min activity time, and [B] during 30 min recovery one. control: control group, sham: sham-operated group, OVX: non-treated-ovariectomized group, OVX+E2: 17 b-estradiol-treated ovariectomized group, OVX+T: tamoxifen-treated ovariectomized group. Results are expressed as mean±SD (n=6). The group which carry the mark "*" is significantly different from all other groups at P<0.001.

Discussion

Decreased serum E2 may occur naturally as a part of the female life process at menopause, or by surgical removal of the ovaries in rat models[30]. The present study showed that ovariectomy in rats resulted in a significant reduction of the strength of the isometric contraction of the diaphragmatic muscle in response to both indirect and direct electrical stimulations, when compared with the corresponding values in normal and sham-operated rats. The percentage decrease in muscle contraction from the initial values, after 30 min activity, was significantly more in ovariectomized rats than in normal and sham-operated ones, which indicates a condition of more muscle fatigability than normal as a consequence of ovariectomy. The data presented here would suggest that ovariectomy may result in adverse consequences on the physiological function of the skeletal muscle. Many studies demonstrated a decrement in force generation and myosin function in muscles that occurs as a result of E2 deficiency in both women and rodents[31-33]. It has been shown that ovariectomy induces a shift from fast to slow myosin heavy chain isoforms in rat skeletal muscles, which results in alterations in their contractile properties[34]. Furthermore, Sitnick et al.[35] reported that ovariectomy resulted in a failed recovery of atrophied muscle mass, and explained that by the need of the presence of ovarian hormones for its regrowth in mature female animals. The changes in hormonal status may be the contributor of the decline in the skeletal muscle function after ovariectomy. It has been reported that the lowered plasma level of E2 in ovariectomized rats with a subsequent histopathological changes in the contractile elements and their properties may be the cause of the observed reduction in the force of muscle contraction [36].

Estradiol replacement to ovariectomized rats resulted in a significant improvement in the strength of the diaphragmatic isometric muscle contractions induced by both indirect and direct electrical stimulations, when compared with the animals subjected to ovariectomy without treatment.

The percentage decrease of the strength of diaphragmatic muscle contraction from the initial value, after 30 min activity, was significantly less in ovariectomized rats treated with E2 than that in non-treated ovariectomized rats; which indicates a less fatigability of skeletal muscles in rats subjected to ovariectomy and received E2 replacement than that in non-treated ovariectomized animals. In consistency with our finding, Moran et al.[31] showed a reduction of about 20% of muscle and myosin functions in ovariectomized mice, and that the losses in force generation at both the whole muscle and molecular levels were completely restored when mice were administered E2 [33]. A meta-analysis on data from about 10,000 postmenopausal women showed that those taking an estrogen-based hormone therapy were stronger than those not taking the treatment[37]. Baltgalvis et al.[38] demonstrated a significantly greater strength in post-menopausal women who were taking estrogen replacement therapy. Moreover, Hertrampf et al. [39] noticed that E2 treatment significantly increased the movement activity of ovariectomized rats. Interestingly, Sotiriadou et al.[40] showed that E2 replacement in ovariectomized rats partially protected the soleus muscle from the injury normally induced by downhill running, and explained their findings by a possible estrogen-induced attenuation of the excitation-contraction coupling failure, a mechanism which may, in part, contribute to the E2 replacement-less muscle fatigability observed in this study. In a trial to explain the mechanism behind the E2-induced improvement of skeletal muscle performance after ovariectomy, Baltgalvis et al.[38] said that ER∝ and ERβ are expressed in skeletal muscle, and only ER∝ is responsive to both acute and chronic changes in circulating E2. Evans et al.[41] explained the effect of estrogen on skeletal muscles by anabolic effects of E2 replacement, mediated by ER present in skeletal muscle. Sipila and Poutamo [1] observed that E2 induces expression of transcription factors in proliferating myoblasts. In fact, the mechanisms of estrogen action are diverse, and include not only actions at ER[42], but also

antioxidant activity[43]. Estrogens have only a weak radical scavenging activity [44], but are able to inhibit oxidative stress markers such as lipid peroxidation[45], and as such, it is possible that E2 itself has a direct effect on the oxidative status of muscle fibers. In addition, Sotiriadou et al.[46] reported that estrogen may have a protective effect on muscle tissue possibly due to its antioxidant and membrane stabilizing properties. Lowe et al. [32] speculated that improving the reduction-oxidation state in muscle fibers by E2, and thus keeping muscle proteins such as myosin free from post-translational oxidative modifications, could contribute to the maintenance of protein structure-function and ultimately strength, based on findings that antioxidant proteins, such as superoxide dismutase and glutathione peroxidase, are regulated through ER by E2 [32].

The main finding of this study is that tamoxifen administration to ovariectomized rats improves the strength of diaphragmatic contraction in response to both indirect and direct electrical stimulation compared to those with no treatment. Treatment of rats with tamoxifen after ovariectomy resulted in a significantly lower percentage decrease in the strength of muscle contraction from the corresponding initial value, after 30 min activity, when compared with the non-treated ovariectomized animals, which reflects less fatigability. Notably, the average values of percentage decrease in tamoxifen-treated ovariectomized animals were statistically insignificant when compared with those of the normal and sham-operated rats, as well as those of ovariectomized rats with E2 replacement therapy. In consistency, Gorzek et al.[47] observed that ovariectomized mice treated with tamoxifen ran distances comparable to those of ovariectomized mice replaced with E2. Although tamoxifen is primarily an ER antagonist by blocking estrogen binding to the ER [48], it has some action as an agonist in certain physiological conditions depending on tissue and tamoxifen concentration [49]. This diverse effect of some SERMs is determined by a complex interaction between factors including ER subtypes, adaptor protein ex-

pression, ligand identity and transcription target sites, which vary with cell type [49]. Tamoxifen has not only an action at ER[42], but also a potential action of scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in vivo[50]. Tamoxifen and its immediate metabolite, 4-hydroxytamoxifen, are effective antioxidants, and they protect smooth muscle cell membrane phospholipids against peroxidation[51]. The benefit of the antioxidant action of tamoxifen in ischemia animal models was highlighted by Wakade et al. [52]. In fact, a balancing oxidative stress in skeletal muscle was found to be crucial for myosin function[53] and overall muscle contractility[54].

In all groups of animals of this study, the ratio of the strength of muscle contraction obtained by indirect electrical stimulation to direct one was insignificantly changed after 30 min of activity from the corresponding initial ratio at the onset of contraction. This finding excludes the neuromuscular junction as the site of the partial muscle fatigue observed in this experiment. It seems that the major contributor to fatigue is the muscle fiber itself and not the nervous system or the neuromuscular junction. Interestingly, the ratios of the strength of muscle contraction induced by indirect stimulation to direct one obtained in non-treated ovariectomized rats and the ovariectomized rats treated with either E2 or tamoxifen were insignificantly changed from those of the control and sham-operated animals, whether at the onset of contraction or after 30 min activity time. The unaltered ratio in the non-treated ovariectomized rats and those treated with E2 or tamoxifen, from control and sham-operated ones, directs the attention to the skeletal muscle, and not the neuromuscular junction, as the primary site of the early dysfunction in post-menopause. In fact, the concept of neuroprotective effects of estrogen remains controversial; while no reports have shown a role of estrogen in the acetylcholine release from neuromuscular junction, an obvious one was demonstrated in nerve fibers to detrusor muscle of urinary bladder [55]. Centrally, es-

trogens play a definite role in the neuroprotection of hippocampus and amygdala of the limbic system, an effect which was linked to their ability to protect mitochondria [56].

In the present work, the glucose uptake by the diaphragmatic muscle during 30 min contraction time and 30 min recovery one was determined. Ovariectomized rats showed a significant reduction in the glucose uptake by the diaphragmatic muscle during both 30 min-contraction and 30 min-recovery times compared to those of normal and sham-operated animals. The decrease in glucose uptake by skeletal muscle after ovariectomy could, at least in part, be explained by estrogen deficiency. Similar result has been reported by D'Eon et al.[57], who observed that glucose uptake during muscle contractions was impaired in ovariectomized rats compared with intact animals.

A marked increase of glucose uptake by the diaphragmatic muscle of ovariectomized rats subjected to E2 replacement versus non-treated ovariectomized animals was observed during both the 30 min muscle activity time and the 30 min recovery one. This finding might be attributed to the estrogen replacement that may improve insulin action brought about by ovarian hormone deficiency [36]. Campbell and Febbraio[58] demonstrated that treatment with E2 can completely restore glucose uptake in skeletal muscle of ovariectomized rats to the control levels. In fact, plasma concentration of E2 may be a determinant factor for insulin activity [59]. In ovariectomized streptozotocin-induced diabetic rats, Ordonez et al.[60] observed that E2 increased basal skeletal muscle glucose uptake by influencing signaling molecules and/or glucose transporter-4 content. Even in the blood-brain barrier, estrogens were found to increase expression of glucose transporter subunits and increase glucose transport[61].

Data of this experiment revealed that tamoxifen improved glucose uptake by the diaphragmatic muscle of ovariectomized rats during both the 30 min activity and 30 min recovery times, when compared with the corre-

sponding values of ovariectomized rats which did not receive any treatment; an effect which could be explained by the ability of tamoxifen to stimulate the ER in tissues, with a subsequent ERβ- and ERβ-mediated signaling pathways, which participate in glucose homeostasis by modulating the expression of genes involved in insulin sensitivity and glucose uptake [62]. In fact, treatment of ovariectomized rats with tamoxifen in this investigation could restore the normal levels of glucose uptake by the diaphragmatic muscle, whether during 30 min contraction time or during 30 min recovery one, an effect on substrate utilization which was definitely reflected on the performance of the muscle of rats.

In conclusion, the present study demonstrates that surgical ovariectomy in rats causes impairment of the skeletal muscle performance, and not of the neurotransmission at the motor end plate. Tamoxifen administration caused an improvement in the strength of skeletal muscle contraction and glucose uptake by the muscle similar to that induced by estradiol replacement therapy, possibly through its ER stimulatory action and antioxidant effects. Putting in mind that tamoxifen has a preventive action on breast cancer [63], and that it is extremely cheap in comparison to estrogen replacement therapy which is an issue in undeveloped countries or in countries with under-funded managed healthcare systems, the results strongly suggest that tamoxifen might be considered as an alternative line of treatment for the management of postmenopausal muscle weakness caused by estrogen deficiency in humans. Further studies are needed to further support these effects, to determine the possible side effects of tamoxifen, and to allow more rational design of any proposed clinical trials.

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EFFECT OF TAMOXIFEN VERSUS 17 BETA-ESTRADIOL ON SKELETAL MUSCLE PERFORMANCE IN OVARIECTOMIZED RATS

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PARTIAL PROTECTIVE EFFECT OF COENZYME Q10 PRE-TREATMENT ON RENAL ISCHEMIA-REPERFUSION INJURY IN ADULT RATS

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Abstract

Background: *Coenzyme Q10 (CoQ10) is essential for electron transport in the mitochondrial respiratory chain and for antioxidant defense. Oxidative stress plays a critical role in renal ischemia-reperfusion (I/R) injury. The purpose of the present study was to explore the effect of early CoQ10 administration on the oxidative insult in renal I/R injury.* ***Material and Methods:*** *Twenty eight adult male albino rats of local strains (150-200 g each) were randomly divided into three groups. (1) Control group (n=10), where normal animals were intraperitoneally (i.p.) injected with olive oil (vehicle), daily for 7 days, and then subjected to sham-operation. (2) Renal I/R injury group (n=8), where animals were injected with vehicle as in the first group, and then exposed to 1 hour of surgical ischemia of the right kidney, followed by 4 hours of reperfusion. (3) CoQ10-pretreated renal I/R injury group (n=10), where animals were i.p. injected with 30 mg/kg body weight of CoQ10 dissolved in olive oil, daily for 7 days, and then exposed to I/R as in the second group. At the end of the experiment, rats were killed by cervical dislocation, and the right kidney was excised and homogenized for measurement of total nitrite/nitrate (NOx) and malondialdehyde (MDA) levels, as well as the activities of the endogenous antioxidant enzymes, glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD).* ***Results:*** *Rats exposed to unilateral renal I/R injury showed a significant elevation of the right kidney homogenate NOx and MDA lev-*

els, and a significant decrease of the GSH-Px, CAT and SOD enzymatic activities, when compared with those of the control group. CoQ10 pre-treatment of the renal I/R rats resulted in an improvement of the oxidative state of the right kidney, as demonstrated by a significant decrease of the NOx and MDA levels, and a significant increase of the GSH-Px, CAT and SOD activities compared to those of rats exposed to renal I/R and did not receive any treatment; however, values of the previously measured parameters in I/R injured rats pretreated with CoQ10 did not return to the normal ones. ***Conclusion:*** *CoQ10 pre-treatment has a partial protective effect against the kidney oxidative stress induced by I/R injury in adult rats.*

Keywords: *Coenzyme Q10, renal ischemia-reperfusion injury, reactive oxygen species, nitrite/nitrate, malondialdehyde, antioxidant enzymes.*

Introduction

Renal ischemia - reperfusion (I/R) injury is the leading cause of acute renal injury with high mortality and morbidity, and it is commonly encountered in clinical situations such as trauma, aortic bypass surgery, hemorrhagic shock, and renal transplantation [1]. In fact, I/R injury leads to the production of excess reactive oxygen species (ROS) that are potentially very reactive molecules and, at high enough concentrations, they cause damage to critical cellular constituents, such as proteins, nucleic acids, carbohydrates, and lipids, resulting in cell necrosis [2]. Therefore, the need for therapeutic modalities to prevent the sequelae of renal I/R injury is quite urgent in order to reduce these hazardous metabolites and to improve the patient's outcome and achieve a good prognosis. Many studies have examined a variety of free radical oxygen scavengers, including the effects of external supplementation of antioxidants [3] and the activities of endogenous enzymatic antioxidant defense system in a kidney I/R injury [4]. Antioxidants have been defined as substances that are able to prevent, delay or remove oxidative damage

to a molecule [5]. Humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, which function interactively and synergistically to neutralize free radicals. These include endogenous antioxidant enzymes that act as a primary defense mechanism by catalyzing free radical quenching reactions, antioxidant coenzymes as coenzyme Q10 (CoQ10), nutrient-derived antioxidants as L-ascorbic acid, α-tocopherol, β-carotene and polyphenols, metal-binding proteins that sequester free iron and copper ions capable of catalyzing oxidative reactions as ceruloplasmin, ferritin and myoglobin, and numerous other antioxidant phytonutrients present in a wide variety of plant foods [6].

CoQ10, also known as ubiquinone (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone), is a naturally occurring quinone that is found in most aerobic organisms from bacteria to mammals, and is located in the hydrophobic domain of the phospholipid bilayer of cellular membranes [7]. CoQ10 can be endogenously synthesized from phenylalanine, acetyl coenzyme A, tyrosine and by seven vitamins (B2, B3, B5, B6, B9, B12 and C); however, food can also supply CoQ10. Primary dietary sources of CoQ10 include oily fish, organ meats and whole grains [8]. Most individuals obtain sufficient amounts of CoQ10 through a balanced diet, but supplementation may be useful for individuals with particular health conditions. The primary biochemical action of CoQ10 is as a cofactor in the electron-transport chain that is involved in the synthesis of adenosine triphosphate (ATP), where it functions as an energy transfer molecule; therefore, the level of CoQ10 is the highest in organs with high rates of metabolism, such as the heart, kidney and liver [9]. Later studies demonstrated the presence of CoQ10 in subcellular fractions other than mitochondria and in plasma [10]. Saini et al. [6] stated that CoQ10 can neutralize free radicals and may reduce or even help to prevent some of the damage they cause. Therefore, the potential use of CoQ10 supplements may help prevent or treat oxidative stress.

The present experimental study was designed, using a rat model of unilateral renal I/R injury, to investigate the potential protective effect of CoQ10 pre-treatment against the associated kidney oxidative stress.

Material and Methods

Experimental animals and groups: This study followed a randomized controlled animal experiment design. Twenty eight adult male albino rats of local strains, each weighed between 100-150 g, were housed with a 12:12-hrs dark/light cycle, and were fed a standard rat chow diet and had free access to tap water ad libitum. Rats were randomly divided into three groups. (1) Normal sham-operated group (control group, n=10), in which animals were injected intraperitoneally (i.p.) with 0.1 ml of olive oil (vehicle), daily for 7 days, and then subjected to sham-operation described below. (2) Renal I/R injury group (I/R group, n=8), in which animals were i.p. injected with the vehicle as in the control group, and then exposed to renal I/R injury according to the method described below. (3) CoQ10-pretreated renal I/R injury group (Q+I/R group, n=10), in which animals were i.p. injected with 30 mg/kg body weight (b.w.) of CoQ10 (MEPACO, Egypt), dissolved in 0.1 ml of olive oil, daily for 7 days[11], and then exposed to renal I/R injury as in I/R group.

Unilateral renal ischemia-reperfusion injury and sham-operation:

Animals of I/R and Q+I/R groups were exposed to unilateral renal ischemia for 1 hour, followed by reperfusion for 4 hours. The model of the renal I/R injury used in this experiment was similar to that described previously by Foglieni et al.[12]. Briefly, rats were initially anesthetized with intramuscular injection of 50 mg/kg b.w. of ketamine hydrochloride (EIPICO, Egypt) followed by a half dose as required during the procedure. The animals did not receive ventilatory support. Body temperature was monitored by a rectal probe inserted into the rectum and was maintained between 37°C and 38°C by a thermal pad and a heating lamp. After a midline laparotomy, the right kidney was iden-

tified and both the right renal artery and vein were clamped with a microsurgical clamp. Ischemia was confirmed by blanching of the kidneys. The incision was temporarily closed during ischemia. After 60 minutes of interruption of the renal blood flow, the incision was re-opened, the clamp was removed and reperfusion was visually verified upon the removal of the clamp. The wound was then closed in two layers with a 4-0 silk suture, and animals were i.p. injected with 0.5 ml of 0.9% NaCl upon completion of the surgery for resuscitation. The animals were allowed to recover with free access to food and water. Reperfusion was performed for 4 hours.

Sham-operated control rats were anesthetized and underwent identical surgical procedures as in animals of the I/R group, but without clamping of the right renal artery and vein.

Preparation of kidney homogenates:

After 4 hours of the reperfusion of the ischemic right kidney or of the end of sham-operation, animals of all groups were killed by cervical dislocation, and the right kidney was dissected out, cut into small pieces and homogenized in ice-cold 50 mM Tris, 1.0 mM EDTA, pH 8.0, with 10 mM 4-(2 aminoethyl) benzenesulfonyl fluoride (AEBSF), 2 mM dithiothreitol, 5 mM leupeptin and 2 mM pepstatin, using an Omni tissue homogenizer (Omni international, Gainesville, VA). The extracts were centrifuged at 14,000 rpm for 30 min at 4°C. The supernatant was stored at -80°C for various assays. Protein concentration was measured by the bicinchoninic acid (BCA) method to correct for differences between preparations [13].

Biochemical Assays:

1. Total Nitrite/nitrate level.

The method for estimation of the total nitrite/nitrate (NOx) amount in right kidney homogenates was done using a commercial assay kit (Oxford Biomed Research, Inc), based on the Griess reaction [14]. NOx level was measured after enzymatic conversion of nitrate to nitrite by nitrate reductase in the presence of NADPH. The oxidation of the coenzyme was monitored by the decrease in absorbance at 340 nm [15]. Results

are expressed in nmol/mg protein.

2. Malondialdehyde level.

Malondialdehyde (MDA) concentration was measured in right kidney tissue homogenates after precipitation of protein with trichloroacetic acid. Thiobarbituric acid (TBA) reacts with MDA to form TBA reactive product, which was measured at 532 nm spectrophotometrically. An MDA solution freshly made by the hydrolysis of 1,1,3,3-tetramethoxy propane was used as standard [16]. The results were expressed as nmol of MDA per mg protein.

3. Glutathione peroxidase enzymatic activity.

The enzymatic activity of glutathione peroxidase (GSH-Px) in the right kidney homogenates was assayed as described by Paglia and Valentine[17], using H_2O_2 and NADPH as substrates. The conversion of NADPH to NADP+ was followed by recording the changes in absorption intensity at 340 nm (Ransel kit, Randox, UK). Results were expressed in U/mg protein, where one unit (U) of GSH-Px activity is defined as the amount of the enzyme required to consume 1 nmol of NADPH per minute per mg protein [17].

4. Catalse enzymatic activity.

The calatase (CAT) enzymatic activity was measured in the right kidney homogenates by the method of Bonaventura et al. [18]. Five µg of proteins from the homogenate were mixed with 2 ml of 7.5 mmol H_2O_2, and a time scan was performed for 10 min at 240 nm at 25°C. Results were expressed in U/g protein, where one unit of CAT activity is defined as the amount of enzyme decomposing 1 µmol of H_2O_2 per minute per mg protein [18].

5. Superoxide dismutase enzymatic activity.

The enzymatic activity of the superoxide dismutase (SOD) in kidney homogenates was assayed following the method developed by Nishikimi et al. [19] and modified by Kakkar et al. [20]. Five µg of protein were mixed with sodium pyrophosphate buffer, phenazine methosulphate (PMT) and nitro blue tetrazolium (NBT). The reaction was started by the addition of NADH. The reaction mixture was incubated for 90 seconds at 30°C.

The reaction was then stopped by the addition of 1 ml of glacial acetic acid. The absorbance of the chromogen formed was measured at 560 nm. Results were expressed in U/mg protein, where one unit of SOD activity is defined as the enzyme concentration required to inhibit chromogen production by 50% in one minute under the assay conditions [20].

Statistical analysis:

Data were expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Fisher multiple comparisons were used to detect significant differences among individual means of all groups. Differences with $P<0.05$ were considered statistically significant [21].

Results

Fig. (1) demonstrates the changes in the NOx and MDA levels in the right kidney homogenates after induction of ischemia for 1 hour and reperfusion for 4 hours, and the effect of pretreatment with CoQ10 before I/R injury. A significant elevation ($P<0.001$) of the NOx and MDA levels was observed in kidney homogenates of I/R group, when compared with the corresponding values in homogenates of control group. Q+I/R group showed a significant decrease ($P<0.001$) of the kidney homogenates NOx and MDA levels, when compared with those of the control group. However, mean values of NOx and MDA levels in Q+I/R group did not return to the normal values, with significant differences ($P<0.001$) from those of the control group.

Fig. (2) demonstrates the effect of 1 hour-ischemia followed by 4 hours-reperfusion of the right kidney on the GSH-Px, CAT and SOD enzymatic activity in its homogenate, as well as the effect of the CoQ10 pre-treatment on the previously mentioned enzymatic activities. I/R group showed a significant reduction ($P<0.001$) in the enzymatic activities of GSH-Px, CAT and SOD, when compared with those of the control group. Q+I/R group showed a significant increase ($P<0.001$) in the GSH-Px, CAT and SOD activities compared to those of I/R group. However, the activities of the antioxidant enzymes mentioned above were

still significantly lower (P<0.001) in CoQ10-pretreated I/R injured rats than those of the control group.

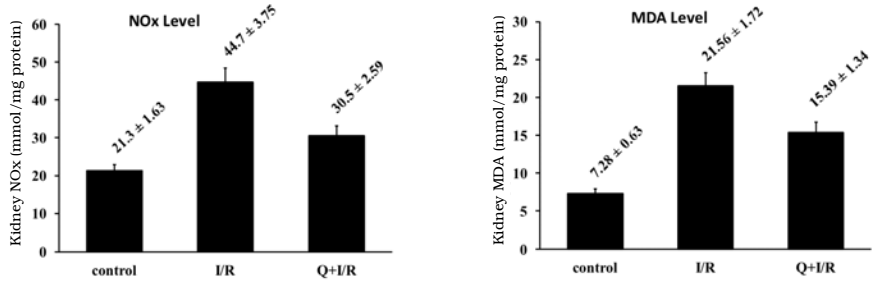

Fig. (1): Kidney homogenate total nitrite/nitrate (NOx) and malondialdehyde (MDA) levels in normal sham-operated (control, n=10), renal ischemia-reperfusion injury (I/R, n=8) and coenzyme Q10-pretreated renal ischemia-reperfusion injury (Q+I/R, n=10) rats. Various columns are significantly different from each other at P< 0.001.

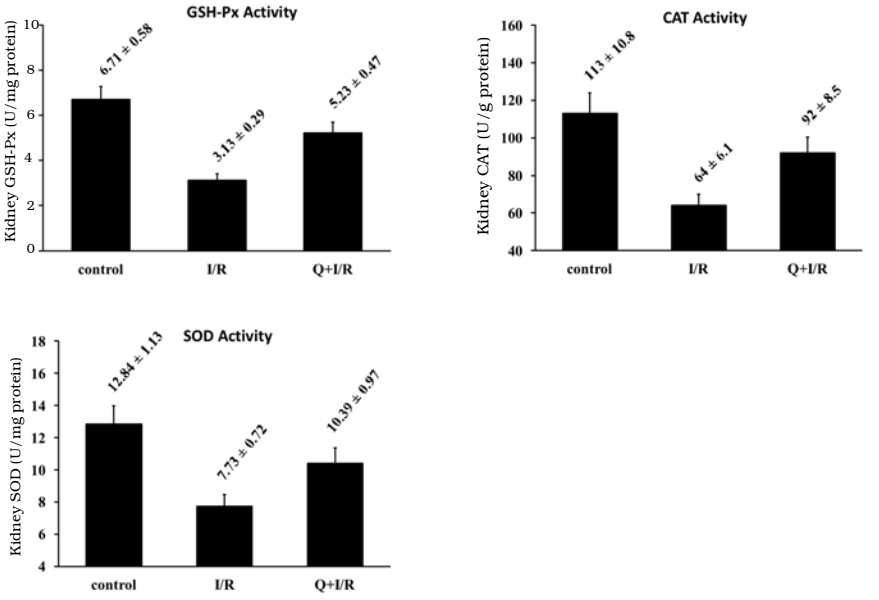

Fig. (2): Kidney homogenate enzymatic activities of glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) in normal sham-operated (control, n=10), renal ischemia-reperfusion injury (I/R, n=8) and coenzyme Q10-pretreated renal ischemia-reperfusion injury (Q+I/R, n=10) rats. Various columns are significantly different from each other at P< 0.001.

Discussion

The data of the present study demonstrate that ischemia of the right kidney of rats for 1 hour, followed by reperfusion for 4 hours caused an elevation of the levels of NOx and MDA, as well as a reduction in the antioxidant enzymatic activities of GSH-Px, CAT and SOD in kidney homogenates, when compared with those of the normal sham-operated animals. Previous studies have demonstrated similar results. Post-ischemic nitrosative damage has been demonstrated in the kidney during the reperfusion phase following ischemia, where superoxide is produced, and it rapidly reacts with NO^- and forms NOx [22]. The same observation has been shown in the heart [23], liver [24], brain [25] and lung [26]. Lipid peroxidation acts as one of the most important sources of oxidative stress, and previous works have already demonstrated evidence indicating its implication in renal I/R injury [27]. MDA content, the index of lipid peroxidation [28], was previously found to increase in I/R injury of the heart [29] and the brain [30].

Our results have shown a decrease of the intracellular antioxidant enzymatic activities in kidney homogenates after I/R injury. The reduction in the activities of the antioxidant enzymes measured in this study is in agreement with many research workers. An earlier study has shown that myocardial mitochondrial Mn-SOD activity was decreased after ischemia [31]. Kizilgun et al. [32] observed that renal I/R resulted in decreased SOD and GSH-Px activities. Testicular damage precipitated by temporary ischemia was associated with oxidative stress with a sudden induction of lipid peroxidation and a concomitant suppression of endogenous antioxidant activities, including those of GSH-Px, catalase and SOD [33]. Moreover, unilateral testicular torsion with subsequent ischemia and excessive ROS generation resulted in depletion of antioxidant enzymes in the contralateral testis [34]. Ischiropoulos et al. [35] observed that NOx reacts with a number of metal-containing enzymes, and can inactivate certain types of the endogenous SOD by nitration of critical residues. On the other hand, Portakal and

Inal-Erden [36] found that GSH-Px, catalase and SOD activities increased during I/R of the liver, as compared with controls. These contradictory results may be explained by different techniques of I/R injury with a resultant different rates of production of ROS, where in the present study, the extent of the possible compensatory mechanisms of the enzymes syntheses to face the oxidative insult was less than the degree of their depletion, with a subsequent final decrease in their activities in kidney homogenates.

The present experiment demonstrates a protective antioxidant effect of CoQ10 administration before induction of renal I/R in rats. CoQ10 pretreatment of rats exposed to I/R injury resulted in an amelioration of the associated oxidative stress in the kidney, as demonstrated by a decrease of NOx and MDA levels, and an increase in the activities of the endogenous antioxidant enzymes GSH-Px, CAT and SOD in kidney homogenates, when compared with those in kidney homogenates of I/R rats that did not receive any treatment. However, the improvement of the oxidative stress was not complete, as the levels of NOx and MDA, and the activities of the antioxidant enzymes measured in kidney homogenates of CoQ10-pretreated rats exposed to renal I/R injury, in this work, did not return to the normal values of the control rats. In fact, as early as 1961, Kaufmann and Garloff [37] suggested that CoQ10, which is a part of the respiratory chains of mitochondria, exerts yet another function in biology by acting as a membrane antioxidant. CoQ10 therapy has also been shown to be protective against kidney I/R-mediated oxidative damage in rats [38]. Moreover, Quinzii et al. [39] showed that partial CoQ10 deficiency causes oxidative stress. The results of Takenaka et al. [40] indicate that CoQ10 administration was accompanied by an increase in the survival rate of ischemic rats, presumably by a protective effect on warm ischemic damage to the rat kidney. In vitro, CoQ10 has been shown to inhibit lipid peroxidation in mitochondrial membranes that have been depleted of α-tocopherol [41]. Furthermore, the reduced form of CoQ10, ubiquinol, was found to be one of

the most potent lipophilic antioxidants in all cell membranes [42]. Ubiquinol inhibits the peroxidation of cell membrane lipids and also that of lipoprotein lipids present in the circulation when they are exposed to oxidizing conditions [10]. Booth et al. [43] suggested that, in the inner mitochondrial membrane, ubiquinol is at least as active as α-tocopherol in removing free radicals because of its similar antioxidant efficiency. In the present work, the increased enzymatic activities of GSH-Px, CAT and SOD in the kidney homogenates of renal I/R injured rats after early CoQ10 administration, compared to non-treated renal I/R injured animals, is in agreement with the observation of Lee et al. [44], and is probably explained by a possible decrease in their depletion as a result of scavenging ROS.

Indeed, CoQ10 therapy has been shown to be protective against many different experimental models of I/R injury. CoQ10 pretreatment protects the myocardium from I/R injury via both antioxidant and bioenergetic pathways[45]. Administered CoQ10 has been shown to protect against acute post-ischemic hepatic injury in experimental animals [46]. The results of Marubayashi et al. [46] are compatible with the assumption that cellular damage caused by hepatic ischemia can be explained by free radical reaction processes during ischemia and, especially, reperfusion and suggest that exogenous CoQ10 functions as an antioxidant with endogenous CoQ10 in lipid peroxidation during reperfusion. A protective effect of CoQ10 against warm ischemia damage in rat pancreatic transplantation has also been observed by Ikeda et al. [47]. Ostrowski [48] detected a beneficial neuroprotective effect of CoQ10 in the cerebral ischemia via a potent antioxidant and free radicals scavenging effects, with a resultant decrease of free radicals concentration. Yokoyama et al. [49] observed that CoQ10 scavenged ROS that was produced in replanted limbs as a result of I/R injury. There is some evidence for antioxidant activity of CoQ10 also in humans. For example, it has been shown that preoperative administration of CoQ10 to patients can increase the tolerance of the

heart to ischemia [50]. In human lenses, CoQ10 was found to ameliorate the oxidative stress, the most common damaging factor responsible for the development of cataract [51].

The mechanisms involved in the antioxidant action of CoQ10 are not completely understood. However, the CoQ10 helps to fight against the free radicals released later during reperfusion probably by two mechanisms, directly by scavenging free radicals produced [52], and indirectly by regenerating the active forms of other antioxidant molecules [53]. In fact, CoQ10 in its reduced form, ubiquinol, is capable of recycling and regenerating other antioxidants such as α-tocopherol and ascorbate [54]. α-tocopherol seems to act as a direct scavenger forming tocopheroxyl radical, whereas ubiquinol reacts with tocopheroxyl radical to regenerate α-tocopherol. Sohal [55] confirmed the sparing/regenerative effect of CoQ10 on α-tocopherol in vivo. In young adult mice, CoQ10 intake effectively augmented the α-tocopherol concentration in tissue homogenates and mitochondria [56]. The sparing effect of the reduced form CoQ10, ubiquinol, on α-tocopherol was also observed by Frei et al. [57], who explained that by a site-specific antioxidant action within the membrane of the two antioxidants.

In conclusion, the present study shows that prophylactic CoQ10 administration appears to attenuate renal I/R injury in rats, as demonstrated by decreasing the levels of the NOx and MDA, and by increasing the activities of the antioxidant enzymes GSH-Px, CAT and SOD. Further studies to explore the possible mechanisms of action behind the partial protective effect of CoQ10 against the oxidative and nitrosative insults associated with I/R injury are still needed.

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PARTIAL PROTECTIVE EFFECT OF COENZYME Q10 PRE-TREATMENT ON RENAL ISCHEMIA-REPERFUSION INJURY IN ADULT RATS

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ROLE OF VITAMIN D AND ADIPOKINES IN OBESITY RELATED TYPE 2 DIABETES MELLITUS

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Abstract

Vit.D has been recently found to be associated with many metabolic disorders as obesity and diabetes.The present work aims to study vit.D and adipokines status in type 2 diabetic females with different body weights and to investigate the probable correlation between serum vit.D level and two of adipokines namely adiponectin and resistin.The study involved 72 females, 36 non diabetic and 36 diabetic, divided into 3 groups according to their body mass index.Biochemical assays for vit.D, resistin, adiponectin, lipid profile and insulin sensitivity markers were done. Vit.D replacement therapy was given to those proven to be vit. D defcient and biochemical assay was repeated. Results: vit. D was deficient in morbid obese non diabetic and morbid obese diabetic females.Adiponectin was significantly reduced in obese and morbid obese non diabetic females and in normal weight diabetic females.Resistin was significantly increased in obese and morbid obese diabetic and non diabetic females and in obese diabetic females.Vit.D replacement therapy improved insulin sensitivity in diabetic females but didn't affect adipokine dysregulation. Conclusion: vit. D deficiency is correlated to morbid obesity and type 2 DM suggesting a possible protective effect for vitamin D in these conditions.

Introduction

Obesity is a major public health problem all over the world,with nearly one-third of

adults are obese.Obesity is a major risk factor for many diseases, mainly cardiovascular disease, cancer and diabetes mellitus [1].

Vit. D has long been known for its effects on calcium metabolism,gut and kidney. However, the discovery of the wide spread distribution of vit. D receptors in pancreas, immune cells, musles, cardiovascular system, nervous system, breast tissue, thyroid gland, parathyroid gland, prostate, endothelial cells and many other tissues revealed the fact that vit. D has a much broader range of actions in the human body[2]. Evidence from recent studies has indicated a role vit. D in mediating many physiologic actions as insulin secretion from β cells of the pancreas and insulin actions on target tissues[3]. A number of possible "novel" pathologic consequences of vit. D deficiency have been identified in recent years, mostly on the basis of associations that have yet to be examined for causation by appropriate intervention studies in humans. These include possible increases in the risk of cancer, ischemic heart disease, chronic kidney disease,autoimmune disease, elevation in blood pressure and diabetes mellitus[4]. Moreover,recent studies suggested a possible role for vit. D in the metabolic dysregulation associated with metabolic syndrome in that vit. D deficiency is suggested to be a cause of common obesity, dyslipidaemia,insulin resistance and hypertension [5]. However, little is known about the correlation between vit. D and adipokines. Therefore, the aim of the present work is to study vit. D and adipokines status in type 2 diabetic females with different body weights and to investigate the probable correlation between serum vit. D level and two of adipokines namely adiponectin and resistin.

Subjects and methods

The study involved 72 Egyptian females aged 30-50 years, the diabetic and obese patients were recruited from those treated in Mansoura university outpatient clinics while the healthy control were recruited from Mansoura university employees and others. Exclusion criteria included ingestion of vit. D or multi-vitamin supplements, anticonvulsants or sys-

temic glucocorticoids, presence of hepatic or renal disease, malabsorptive disorders (Crohn's disease, cystic fibrosis, and celiac disease) and cancer.

The 72 females were divided according to their body mass index (BMI) being types 2 diabetic or not, into 3 main groups as follows, group I included 12 normal weight non diabetic females and 12 normal weight diabetic females, group II included 12 obese non diabetic females and 12 obese diabetic females, group III included 12 morbid obese non diabetic females and 12 morbid obese diabetic females. At the start of the study serum level of vit. D, adiponectin, resistin, insulin, calcium, fasting glucose, lipid profile, creatinine and HBA1C were assayed as described in the commercially used kits for each female. Also insulin resistance was assessed by HOMA (the homeostatic model assessment), according to the equation:

$$\text{HOMA-IR} = \frac{\text{glucose} \times \text{insulin}}{405\ (\text{glucose is given in mg/dl and insulin is given in } \mu\text{U/ml})}.$$

Vit. D deficient females received vit. D replacement therapy in the form of one oral daily tablet containing 1000 IU vit. D for 2 months. Blood samples were collected by the end of this period and the previous parameters were measured again. 25-hydroxy vit. D (25-OH Vit. D) portrays the biological active form of vit. D, which is synthesized in the kidney.It is widely accepted that the measurement of circulating 25-OH vit. D provides better information with respect to patients vit. D status because it is easily measured, has the longest half-life in circulation (approximately 2 or 3 weeks), and the levels of 25- OH vit D correlate with vit. D stores and clinical disease states. According to Immundiagnostik Enzyme - Immuno-Assay kits vit. D is considered sufficiently applied when its serum levels are above 30 n.mol/l and deficiency is considered when its levels are below 30 n.mol/l [6].

Statistical analysis:

Data are expressed as mean value ± SD. Comparisons were carried out using analysis of variance (ANOVA) followed by Tukey's test, using SPSS for Windows Z (15.0 Version). Differences were considered statistically significant when $P < 0.05$. Pearson correla-

tion was used to assess relations between variables.

Results

Table (1) shows that in non diabetic females, TG, fasting insulin, HOMA-IR and resistin were significantly higher in obese non diabetic females compared to normal weight non diabetic females while HDL and adiponectin were significantly low.In morbid obese non diabetic females, cholestrol, TG, LDL, fasting insulin, HOMA-IR, HBA1c and resistin were significantly higher when compared to normal weight non diabetic females, HDL, vit. D and adiponectin were significantly low. Serum levels of cholestrol, TG, LDL, HBA1C and resistin were significantly higher in morbid obese non diabetic females compared to obese non diabetic females but adiponectin was significantly low.

In diabetic females, TG, fasting insulin, HOMA-IR and resistin were significantly higher in obese compared to normal weight females while HDL was significantly low.In morbid obese subgroup, cholestrol, TG, LDL, fasting insulin, HOMA-IR and resistin were significantly higher when compared to normal weight diabetic females while HDL and vit. D were significantly low. Vit. D was significantly low in morbid obese diabetic females compared to obese diabetic females.

Table (2) shows that cholesterol and TG were significantly higher in normal weight and obese diabetic females compared to non diabetic females. However, HDL was significantly low in normal weight, obese and morbid obese diabetic females compared to non diabetic females.

Table (3) shows that fasting glucose, HOMA-IR and HBA1C are significantly higher in normal weight, obese and morbid obese diabetic females compared to non diabetic females. As shown in table (4), adiponectin was significantly lower in normal weight diabetic females compared to normal weight non diabetic females. Vit. D was significantly lower in morbid obese diabetic females than morbid obese non diabetic females. By contrast, resistin was significantly higher in obese diabetic females compared to obese

non diabetic females. Table (5) shows that vit. D was higher after vit. D replacement therapy in both morbid obese diabetic and morbid obese non diabetic females. Fasting glucose, fasting insulin and HOMA-IR were significantly lower after vit. D replacement therapy.

Testing whether vit. D level was correlated or not with different parameters, vit. D was negatively correlated to HOMA-IR in both non diabetic females (figure 1-a) and diabetic females (figure 1-b). Moreover, vit. D was positively correlated to adiponectin in non diabetic females (figure 2-a) and in diabetic females (2-b) but negatively correlated to resistin in diabetic females (figure 2-c).

Table1: Serum levels of lipid profile, vit.D, calcium, adiponectin, resistin, fasting glucose, fasting insulin, HBA1C and HOMA-IR in non diabetic and diabetic females (n=12 for each subgroup).

	Non diabetic women			diabetic women		
	Normal weight	Obese	Morbid obese	Normal weight	Obese	Morbid obese
Cholesterol mg%	186 ±38.5	196.4 ±27.3	252.7 #? ±45.	218.6 ±29.1	237 ±55.8	258# ±37.5
TG mg%	99 ±22.1	178.5* ±43.7	248.9# ? ±54.9	232.2 ±50.7	272.6 * ±42.3	303.5# ±55.6
HDL mg%	61.1 ±16.4	45.33* ±11.1	39.16# ±10.4	47.5 ±13.9	33.5 * ±6.3	30.9 # ±3.3
LDL mg%	104.6 ±21.8	115 ±30.3	165.1#? ±43.8	123.7 ±28.5	148.8 ±58.2	164.1 # ±34.7
Fasting glucose mg%	83.8 ±10.1	85.08 ±15.5	86.5 ±12.5	133.7 ±14.4	138.4 ±20.6	152.9 ±39
Fasting insulin µU/ml	8.9 ±2.6	12.6 * ±4.1	16.42# ±4.8	10.6 ±2.1	16.7 * ±5.9	19.7 # ±6.2
HOMA-IR	1.8 ±0.3	2.7* ±0.75	3.4# ±1	3.5 ±0.5	5.6 * ±1.8	7.6 # ±3.9
HBA1c %	4.5 ±0.8	4.69 ±0.8	5.8 #? ±1.1	6.8 ±1.2	6.7 ±1.4	7.5 ±1.2
Vit. D nmol/l	49 ±18.3	39.5 ±19.6	27.3 # ±12.7	41 ±12.3	33 ±7.6	15.4#? ±3.9
Calcium mg/dl	9.5 ±1	9.3 ±1.3	9.5 ±1.2	9.71 ±1.1	9.1 ±1.2	9.8 ±1.1
Adiponectin mg/l	16.3 ±2.6	10.1* ±1.7	8 # ? ±2.8	9.02 ±2.7	8.6 ±1.4	6.9 ±0.7
Resistin µg/l	13 ±2.9	18.4* ±6.1	26.8 #? ±7.3	13.7 ±2.8	24.5 * ±7.8	29.5 # ±7.4

Table 2: Serum level of cholestrol,TG,HDL and LDL in diabetic versus non diabetic females.

Group 1,normal wt. (n=12 each):								
	Normal wt. non diabetic				Normal wt. diabetic			
	Chol. mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	Chol. mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
Mean +SD	186 +38.5	99.6 +42.18	61.1 +16.4	104.6 +41.8	218.6* +29.1	232.2* +50.7	47.5* +13.9	123.7 +28.5
Group 2,obese (n=12 each):								
	Obese non diabetic				Obese diabetic			
	Chol. mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	Chol. mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
Mean +SD	196.4 +27.36	178.5 +43.71	45.3 +11.18	115 +35.33	237* +55.89	272.6* +42.38	33.58* +6.35	148.8 +58.25
Group 3,morbid obese (n=12 each):								
	Morbid obese non diabetic				Morbid obese diabetic			
	Chol. mg/dl	TG mg/dl	HDL mg/dl	LDL mg/ dl	Chol. mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
M ean +SD	252.7 +45.03	248 +84.93	39.1 +13.47	165 +47.88	258 +37.52	303 +55.66	30.9* +3.36	164.1 +34.78

- All values are expressed as mean+ SD.
- * *P < 0.05 diabetic versus non diabetic (ANOVA followed by Turkey test).*

Table 3: Serum level of fasting glucose,fasting insulin,HBA1c and HOMA-IR in diabetic versus non diabetic females.

Group 1, normal wt. (n=12 each):								
	Normal weight non diabetic				Normal weight diabetic			
	Fasting glucose mg/ dl	Fasting insulin µU/ml	HOMA -IR	HB A1C %	Fasting glucose mg/ dl	Fasting insulin µU/ml	HOMA -IR	HB A1C %
Mean +SD	83.8 +10.12	8.9 +4.01	1.8 +.83	4.5 +.82	115.8 * +17.73	10.68 +4.59	3.05* +1.33	6.8* +1.29
Group 2, obese (n=12 each):								
	Obese non diabetic				Obese diabetic			
	Fasting glucose mg/ dl	Fasting insulin µU/ml	HOMA -IR	HB A1C %	Fasting glucose mg/ dl	Fasting insulin µU/ml	HOMA -IR	HB A1C %
Mean +SD	85 +15.54	12.6 +4.19	2.74 +1.06	4.69 +1.06	118* +32.37	16.7 +5.95	4.91* +2.05	6.74* +1.42
Group 3, morbid obese (n=12 each):								
	Morbid obese non diabetic				Morbid obese diabetic			
	Fasting glucose mg/ dl	Fasting insulin µU/ml	HOMA -IR	HBA1C %	Fasting glucose mg/ dl	Fasting insulin µU/ml	HOMA -IR	HB A1C %
Mean +SD	86.5 +12.5	16.4 +4.8	3.4 +1.01	5.8 +1.12	152.9* +39.01	19.75 +6.27	7.6 * +3.9	7.5 * +1.25

- All values are expressed as mean+ SD.
- * *P < 0.05 diabetic versus non diabetic females (ANOVA followed by Turkey test).*

Table 4: Serum level of vit.D, calcium, adiponectin and resistin in non diabetic versus diabetic females.

Group 1, normal wt. (n=12 each):								
	Normal weight non diabetics				Normal weight diabetics			
	Vit. D nmol/l	Ca++ mg/ dl	Adipo. mg/l	resistin µg/l	Vit. D nmol/l	Ca++ mg/ dl	Adipo. mg/l	Resistin µg/l
Mean +SD	49 +18.3	9.57 +1.05	16.39 +2.66	13.01 +2.93	41 +12.3	9.71 +1.11	9* +2.78	13.75 +2.82
Group 2, obese (n=12 each):								
	Obese non diabetics				Obese diabetics			
	Vit. D nmol/l	Ca++ mg/ dl	Adipo. mg/l	resistin µg/l	Vit. D nmol/l	Ca++ mg/ dl	Adipo. mg/l	Resistin µg/l
Mean +SD	39.5 +19.6	9.3 +1.34	10.14 +1.71	18.4 +6.1	33 +12.5	9.17 +1.25	8.6 +2.4	24.5* +7.89
Group 3, morbid obese (n=12 each):								
	Morbid obese non diabetics				Morbid obese diabetics			
	Vit. D nmol/l	Ca++ mg/ dl	Adipo. mg/l	resistin µg/l	Vit. D nmol/l	Ca++ mg/ dl	Adipo. mg/l	resistin µg/l
Mean +SD	27.3 +12.7	9.5 +1.28	8 +2.8	26.8 + 7.3	15.4* + 5.3	9.8 + 1.14	6.9 + 2.34	29.5 + 7.4

- All values are expressed as mean+ SD.
- * *P < 0.05 diabetic versus non diabetic females (ANOVA followed by Turkey test).*

Table 5: Effect of vit.D replacement on different parameters in morbid obese non diabetic and morbid obese diabetic females.

	Morbid obese non diabetic females		Morbid obese diabetic females	
	Vitamin D deficient females	After vitamin D replacement therapy	Vitamin D deficient females	After vitamin D replacement therapy
Vit. D nmol/l	13.6 ±3.9	50.2* ±10.8	13.5 ±3.7	47.7* ±13.6
Ca++ mg/dl	9.8 ±1.3	10.3 ±0.5	9.7 ±1.3	10 ±.68
Adiponectin. mg/l	7.1 ±1.9	7.42 ±2.5	6.52 ±1.5	6.8 ±1.89
Resistin µg/l	26.2 ±7.1	23.8 ±6.6	30.1 ±8.1	27.2 ±5.65
Cholestrol mg/dl	237.2 ±47.9	235.6 ±41.1	250.4 ±40.1	239.6 ±33.3
TG mg/dl	249.2 ±72.3	230.8 ±55.6	312.5 ±51	286.2 ±43.2
HDL mg/dl	46.2 ±12.6	44 ±12.4	29.6 ±2.8	33.6 ±5.7
LDL mg/dl	140.8 ±38.7	145.4 ± 23.5	163.7 ±32.4	148.7 ±28.8
Fasting glucose mg/dl	92.8 ± 7.1	89 ± 6.7	143.6 ±21	114.7* ±28.1
Fasting insulin µU/ml	16.5 ± 4.3	16.3 ± 3.2	20.2 ±5.8	12.4* ±4.6
HOMA- IR	3.6 ±1	3.4 ±0.82	8.2 ±2.4	3.5 * ±0.9
HB_{A1C} %	6.7 ±.8	5.72 ± 1.2	7.1 ±1	6.5 ±1

Figure (1-a) : Correlation between vit.D and HOMA-IR in non diabetic Females (P =.039, R = -.345). Figure (1-b) correlation between vit.D and HOMA-IR in diabetic females (P = .003 , R = -.479).

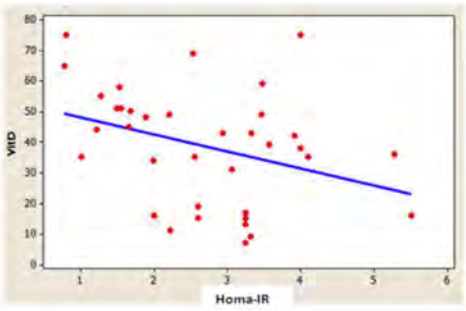


Figure 1-a

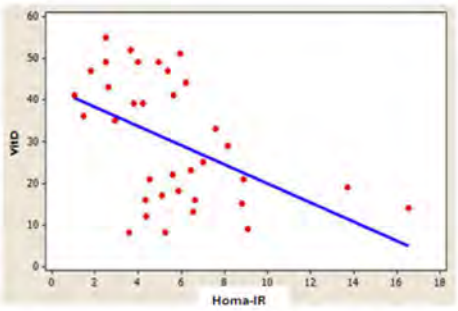
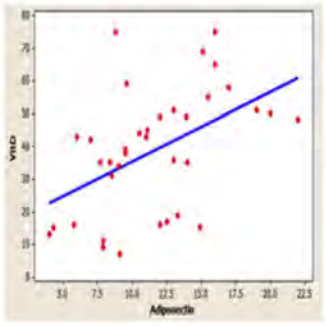
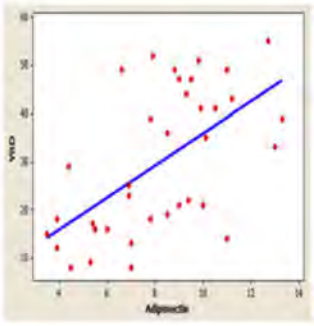


Figure 1-b

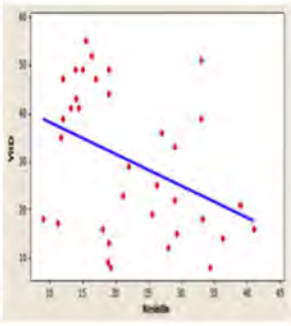
Figure (2-a) : Correlation between vit.D and adiponectin in non diabetic females (P = .003, R = .483).Figure (2-b) correlation between vit.D and adiponectin in diabetic females (P = .000 , R = .590). Figure (2-c) correlation between vit.D and resistin in diabetic females (P = .013, R = -.410).



Adiponectin



Adiponectin



Resistin

Discussion

In the present study, serum level of vit. D was found to be low in both morbid obese non diabetic and morbid obese diabetic females when compared to normal weight or obese females in each group (table 1). Indeed, there is a controversy whether decreased vit. D level in obese persons is a cause or a consequence of obesity. A hypothesis proposed that reduced serum vit. D level is a cause of obesity in that common obesity results from an anomalous adaptation to a cold climate which is induced by a fall in vit. D [5]. A pivotal assumption is that vit. D acts as an ultraviolet (UV) radiation-sensitive sensor which serves to signal changes in sunlight intensity. A fall in UV radiation in the autumn and winter is proposed to be the environmental cue for an acclimatory or adaptive response which enhances winter endurance. The winter response entails an increase in body size by the accumulation of fat mass[5]. According to this hypothesis vit. D deficiency is a potentially significant reason, not only for obesity, but also for hypertension,insulin metabolism and dyslipidemia. On the other hand another study concluded that vit. D deficiency is a consequence of obesity based on the fact that vit. D is fat soluble and thus sequestrated in the larger body pool of fat of obese individuals specially the subcutaneous adipose tissue decreasing its serum levels [7].

Further, vit. D level was found to be greatly reduced in morbid obese diabetic females compared with morbid obese non diabetic females (table 4).Additionally,serum vit.D was negatively correlated with HOMA-IR in diabetic females.Taken together,these results suggest a link between vit.D deficiency and reduced insulin sensitivity, the main hallmark in type 2 DM.

The finding that vit.D was deficient not only in morbid obese diabetic females but also in morbid obese non diabetic raises a question whether the deficiency is linked more to which parameter; morbid obesity or diabetes? Indeed, the small number of patients represents a limitation that should be addressed,as bigger number would determine more precisely the link between

different parameters studied.

Previous studies examined vit.D deficiency-induced insulin resistance suggested that vit.D may have a beneficial effect on insulin action either directly,by stimulating the expression of insulin receptor and thereby enhancing insulin responsiveness for glucose transport, or indirectly via its role in regulating extracellular calcium and ensuring normal calcium influx through cell membranes and adequate intracellular cytosolic calcium pool[8].

The present experiments showed that adiponectin serum level was significantly reduced in obese and morbidly obese non diabetic women women compared to normal weight non diabetic women (Table 1).This negative correlation between adiponectin and obesity has been reported before (9).The mechanism behind concomitant reductions in plasma adiponectin levels with increased visceral fat remains to be clarified.However, one probable mechanism is that decreased adiponectin level is secondary to elevated levels of detrimental adipokines secreted in states of increased adiposity such as TNF- α which inhibits adiponectin secretion[10]. On the other hand, the present study has shown that serum resistin level was increased in obese and morbidly obese women (tables 1), a finding that is in line with previous studies published about the relation between resistin and obesity.Earlier studies showed that increased serum resistin levels and gene expression levels in abdominal depots in states of increased adiposity [11].

The present finding that adiponectin serum level was significantly low in diabetic females while resistin level was elevated is consistent with previous studies[12,13]. The mechanisms by which adiponectin may ameliorate insulin resistance have not been fully elucidated.One proposed mechanism is that adiponectin decreases circulating FFA by increasing fatty acid oxidation by skeletal muscle. This results in decreased triglyceride content in muscle that has been associated with improved insulin sensitivity [14]. On the other hand,

proposed mechanisms for the reduced insulin sensitivity by resistin include reduction in adenosine 5-monophosphate activated protein kinase activity in skeletal muscle,adipose tissue,and liver.In addition,insulin receptor substrate-1 (IRS-1) and IRS-2 protein levels and phosphorylation states,as well as protein kinase B activity, were decreased in hyperresistinemic animal tissues.These alterations decrease tissue insulin sensitivity that results in glucose intolerance, hyperinsulinemia, elevated free fatty acid levels, and hypertriglyceridemia [15].

In the present study, vit.D is positively correlated to adiponectin in both diabetic and non diabetic females and negatively correlated to resistin in diabetic but not in diabetic ones (Figure 2). This supports further the assumption of the relation between vit.D deficiency and the pathogenesis of type 2 DM. These results are consistent with a recent study which indicated a positive correlation between vit.D and adiponectin but with no relation between vit.D and resistin[16]. However, a negative correlation between vit.D and adiponectin has been reported [17].

As indicated in the results section,vit.D was measured in the 72 females included in the study : the percentage of cases with vit.D deficiency was 36% of the total number. However, this ratio increased to be 44% among the diabetic females. Replacement therapy of vit.D continued for 2 months [18] and succeeded to raise serum vit.D levels in the treated females to normal levels (table 5). Vit.D replacement therapy achieved significant reduction in HOMA-IR, insulin levels and blood glucose in morbid obese diabetic females but there was no change in serum insulin, HOMA-IR which remain elevated in morbid obese non diabetic females (table 5). Further, serum level of all other studied variables,namely lipid profile, ABP, calcium, adiponectin and resistin didn't change after vit.D replacement therapy either in morbid obese non diabetic or in morbid obese diabetic females. Absence of an effect of vit.D on these parameters again highlights the need for special design of a study that could follow these

parameters for a longer period of time,specially in light of the fact that patients are recruited from outpatient clinics that makes their compliance to treatment is questionable.An interesting question here is why vit.D replacement therapy improved serum fasting insulin and HOMA-IR in diabetic morbid obese but not in non diabetic morbid obese? further research is needed to clarify this.

Conclusion

There is a possible link between vit.D deficiency and pathogenesis of obesity and type 2 DM. Vit.D level is positively correlated to adiponectin level in diabetic and non diabetic females but negatively correlated to resistin.Measuring serum level of vit.D can be recommended to be done as a routine for morbid obese persons either diabetic or not and vit.D adjuvant therapy can be used in cases of vit.D deficiency. More comprehensive studies are needed to fully clarify the link between vit.D deficiency and dyslipidaemia in morbid obese persons.

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BENHA MEDICAL JOURNAL

ROLE OF VITAMIN D AND ADIPOKINES IN OBESITY RELATED TYPE 2 DIABETES MELLITUS

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INFLAMMATORY MARKERS AND MEAN PLATELET VOLUME IN PATIENTS WITH SLOW CORONARY FLOW AND ITS RELATIONSHIP WITH CLINICAL PRESENTATION

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Abstract

Platelets play an important role in the pathogenesis of atherosclerotic complications by contributing to thrombus formation or plaque rupture. Platelet volume is a marker of platelet activation and function, and is measured using mean platelet volume (MPV). A large amount of data indicated that C-reactive protein (CRP) is a sensitive marker of underlying systemic inflammation. CRP increases among men and women at risk for future cardiovascular events, and the addition of CRP testing to standard lipid screening seems to provide an improved method to determine vascular risk.

Between January 2010 and May 2011 this study was established , included thirty patients with coronary slow flow detected by coronary angiography via Thrombosis In Myocardial Infarction (TIMI) frame count method and fifteen patients with normal coronary arteries were selected as control group.They were attended to cardiology department of Al-hussein , Sayed-Galal and new dameitta university hospitals for cardiac consultation. All patients controls underwent the following: legal consent, Complete history taking, Resting 12 leads ECG, CBC ,fasting blood glucose level, serum triglycerides, LDL, HDL, Mean platelet volume, c-reactive protein(CRP), ESR, interleukin 6 (IL6).at admission and after 6 months of treatment with antiplatelet and antidyslipidemic,Transthoracic Echocardiography and Coronary angiography to diagnose slow coronary flow by TIMI frame count.

Results of this study found that: *interleukin-6 and c-reactive pro-*

tein were increased in patients with coronary slow flow in comparison to control group; mean platelet volume was increased in patients with coronary slow flow in comparison to control group; interleukin 6 and c-reactive protein were decreased after six months of antidyslipidemic drugs(20mg atorvastatin) in comparison to their values at the start; mean platelet volume decreased after six months of antiplatelet(aspirin 150mg) in comparison to their values at the start.

Conclusion: *the results of the present study proved that, 1) increased plasma inflammatory markers including CRP and IL-6 existed in patients with SCF. This suggesting that an inflammation may be a contributor for the development of SCF. In other words, increased concentrations of inflammatory markers in patients with SCF may be an indicator of endothelial activation and inflammation and are likely to be in the causal pathway involving in SCF. Despite the relative good prognosis of patients with SCF, the chronic, frequent nature of the persistent chest uncomfortable can significantly impair quality of life. Therefore, an attention should be paid to this unique phenomenon. However, whether increased inflammatory markers are related to the pathogenesis of SCF in these patients deserved further investigation; 2)-Our findings showed also that MPV is increased in patients with SCF, and SCF patients presenting with USA exhibit significantly increased MPV values, suggesting an altered platelet reactivity and aggregation which require effective anti-platelet therapy in this patient subgroup.*

Keywords: *slow coronary flow, mean platelet volume, inflammatory markers.*

Introduction

Platelets play a role in the pathogenesis of atherosclerotic complications by contributing to thrombus formation or plaque rupture[1]. Larger platelets are hemostatically more active and are a risk factor for developing coronary thrombosis, leading to unstable coronary syndromes[2]. Platelet volume is a marker of platelet activation and function, and is measured using mean platelet volume (MPV). The slow coronary flow (SCF) phenomenon is an angiographic observation characterized by angiographically normal or near-normal coronary arteries with delayed opacification of the distal vasculature[3].

It has been reported that coronary endothelial dysfunction play an important pathogenetic role in patients with SCF. However, the precise mechanisms responsible for this microvascular endothelial dysfunction in patients with SCF are still unknown [4].

Although atherosclerosis has been considered to be multifactorial disease in which genetic, environmental, metabolic factors have been implicated, the gaps remain in our knowledge of the etiopathogenesis of atherosclerosis. There is mounting evidence that inflammation plays an important role in the initiation, development as well as evolution of atherosclerosis, suggesting that atherosclerosis is an inflammatory disease[5]. In addition, a large amount of data indicated that C-reactive protein (CRP) is a sensitive marker of underlying systemic inflammation. CRP increases among men and women at risk for future cardiovascular events, and the addition of CRP testing to standard lipid screening seems to provide an improved method to determine vascular risk. These data, as well as accumulating evidence that CRP may have direct inflammatory effects at the endothelial levels. Besides, among pro-inflammatory cytokines, interleukin-6 (IL-6) is one of the most important factors and has multiple important effects in human pathphysiology[6]. In other studies, the data showed that an enhanced production of IL-6 by monocytes in response to CRP in patients with unstable angina[7]. Moreover, IL-6 is a powerful stimulus for CRP production[8]. Based on those observations, Jian-Jun Li, hypothesis that an inflammatory process may be associated with the development of SCF[9].

Aim of the work

The aim of this study is to evaluate mean platelet volume and some inflammatory markers in patients with slow coronary flow (SCF) and its possible relationship with clinical presentation.

Patients and methods

This study included thirty consecutive patients with coronary slow flow detected by coronary angiography via the Thrombosis in Myocardial Infarction (TIMI) frame count and fifteen patients with normal coronary angiography (as

control group) who were attending the cardiology department of Al Hussein and Elsayed Galal university hospital.

Inclusion criteria: Patients did not receive any medication; patients with coronary artery diseases (lesions<30%) are included in this study.

Exclusion criteria: the following patients were excluded from the study; patients with evidence of obstructive coronary artery diseases (lesions >30%); patients with valvular heart disease; patients with congestive heart failure; patients with echocardiographically proven left ventricular hypertrophy; patients with untreated thyroid disease; patients with sinus node dysfunction or conduction disturbance; patients with estrogen replacement therapy; patients with uncontrolled hypertension; patients with recent major operation (less than 3 months) and finally patients with coronary artery ectesia.

All patients underwent the following: complete history taking; resting 12 leads ECG; CBC, fasting blood glucose level, serum triglycerides, LDL, HDL; mean platelet volume, c-reactive protein (CRP), ESR, interleukin 6 (IL6) before and after 6 months of antiplatelet therapy; transthoracic Echocardiography and finally, coronary angiography to diagnose slow coronary flow by TIMI frame count. All electrocardiograms were performed by the same machine. The papers speed at 25mm/S at standard 10mm/mv. Coronary angiography was performed using the standard Judkin's techniques, and the results were analyzed by at least 2 interventional physicians. Contrast used in angiography of this study is Iopromide (Ultravist-370, and teleberex). Coronary flow rates of all subjects were determined by Thrombosis In Myocardial Infarction frame count (TIMI frame count) because the method is a simple, reproducible, objective and quantitative index of coronary flow velocity .TIMI frame count was determined for each major coronary artery in each patient and control subject according to the method first described by Gibson et al.[10].

EDTA-anticoagulated peripher-

al blood sample were taken after 12-h overnight fast at baseline (before coronary angiography). The plasma was obtained after a centrifugation of 3000 rpm at 4°C for 15 min. The concentrations of CRP were determined using immuno-turbidometry (Beckmann Assay 360). IL-6 was measured with a commercial assay kit (human IL-6, ELISA kit). The assay employs an antibody specific for human interleukin-6 coated on a 96-well plate. Standards, samples and biotinylated anti-human IL-6 are pipetted into the wells and IL-6 present in a sample is captured by the antibody immobilized to the wells and by the biotinylated IL-6 specific detection antibody; after washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed. Following this second step, TMB substrate solution is added to the wells, resulting in color development proportional to the amount of IL-6 bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured 450nm. For laboratory tests, blood samples were collected from the patients after a 12-hr overnight fasting. All routine biochemical tests were carried out on an autoanalyser CBC, fasting blood glucose level, serum triglycerides, LDL, HDL and mean platelet volume.

Statistical analysis of data: The collected data was organized, tabulated and statistically analyzed using statistical package of social science (SPSS) version 16 (SPSS Inc, USA). For qualitative data, frequency and percent distribution were calculated and for comparison between groups, chi square (X2) test was used. For quantitative data, mean, minimum, maximum and standard deviation were calculated and for comparison between two groups, the independent sample (t) test was calculated. For comparison between the same group at two different points of time, paired samples (t) test was calculated. For correlation between two parameters, the spearman's correlation coefficient (r) was calculated; it was mild if $r < 0.3$; moderate if $r \geq 0.3$ and ≤ 0.7, powerful if $r > 7$. For interpretation of results p value ≤ 0.05 was considered significant.

Results

The present study included 45 subjects, 28 of them (62.2%) were males and 17 (37.8%) were females with male to female ratio of 1.65 and there was statistically insignificant difference between study and control groups as regard sex distribution (males represent 63.3% of the study group compared to 60.0% of the control group). As regard age, it ranged from 37 to 65 with a mean of 52.84±7.36 and there was statistically insignificant increase in age of control group in comparison to study group (53.00±8.07 vs 52.76±7.12 age respectively). As regard risk factors, diabetes was reported in 26 cases (57.8%), hypertension reported in 29 cases (64.4%), obesity reported in 15 cases (33.3%) and smoking reported in 21 cases (46.7%) and there was statistically insignificant difference between study and control group as regard risk factors (table 1).

As regard clinical presentation, it was in form of stable angina in 14 cases (31.1%); atypical in 14 cases (31.1%); unstable angina in 15 cases (33.3%) and non ST segment in 2 cases (4.4%) and there was statistically insignificant difference between study and control groups as regard clinical presentation (data not presented). As regard LDL, it ranged from 83 to 170 with a mean of 123.98±23.05 and there was statistically significant increase in study group in comparison to control group (132.43±21.00 vs 107.07±17.24 respectively). In addition, total cholesterol ranged from 73 to 270 with a mean of 189.89±36.39 and there was statistically significant increase in study in comparison to control group (204.13±30.28 vs 161.40±30.95 respectively). On the other hand, there was statistically insignificant difference between study and control groups as regard LDL and triglycerides. As regard fasting blood sugar, it ranged from 70 to 157 with a mean of 108.11±26.98 and there was statistically significant increase in the study in comparison to control group (112.80±27.20 vs 98.73±24.76mg/dl respectively). As regard ESR, it ranged from 7 to 45 with a mean of 20.26±9.0 and there was statistically insignificant increase in the study in comparison to control group (21.86±9.28

vs 17.06±7.74 respectively). On the other hand, CRP ranged from 2.10 to 6.30 with a mean of 4.43±1.16 and there was statistically significant increase of CRP in study group in comparison to control group (4.91±0.93 vs 3.48±1.00 respectively) (table 2).

As regard ECG findings, it was normal in 11 cases (24.4%), flat T wave in 2 cases (4.4%), T wave inversion in 16 cases (35.6%) and ST depression in 16 cases (35.6%) and there was statistically insignificant difference between study and control group as regard ECG findings. As regard echocardiogram findings, it was normal in 17 cases (37.8%); DD grade I in 18 cases (40.0%), DD grade III in 6 cases (13.3%) and RWMA in 4 cases (8.9%), and there was statistically insignificant difference between study and control group as regard Echocardiogram findings (data not presented).

As regard MPV, it ranged from 7.30 from 15.20 with a mean of 11.32±2.13 with statistically significant increase in study group in comparison to control (12.26±1.69 vs 9.44±1.64 respectively). As regard interleukin 6, it ranged from 3.40 to 1000.0 with a mean 114.19±222.7 and there was statistically significant increase in study group in comparison to control group (164.29±259.71 vs 13.98±6.55 respectively) (table 2). In the present study, it was demonstrates that there was statistically significant decrease in MPV, CRP and IL-6 after 6 months in comparison to their values at the start (table 3). In addition, there was statistically significant variance between different clinical presentations as regard LDL and HDL. On the other hand, there was statistically insignificant variance between different clinical presentations as regard TC, TG, fasting blood sugar and ESR (table 4).

In the present work, there was statistically insignificant difference between different clinical presentations as regard ECG findings or Echocardiographic findings. In addition, there was statistically insignificant difference between different clinical presentations as regard MPV at the start, while at 6 months; there was statistically significant variance be-

tween different clinical presentations as regard MPV at 6 months. Furthermore, there was statistically insignificant variance between different clinical presentations as regard CRP at the start and at 6 months. Finally, there was statistically insignificant variance between different clinical presentations as regard IL-6 at the start and at 6 months (data not presented). In the present study, there was proportional (positive), statistically significant correlation between MPV and fasting blood sugar at the start and after 6 months. The same correlation was found between CRP and MPV at the start and at 6 months. The correlation between CRP at 6 months and MPV at 6 months was powerful (table 5)

Table (1): Comparison between study and control group as regard general characteristic and risk factors

	Control group	Study group	Test	P value
Gender Male (n,%)	9 (60.0%)	19(63.3%)	0.047	0.82(NS)
Age (mean±SD)	53.0±8.07	52.76±7.12	0.10	0.92(NS)
Diabetes (n,%)	7(46.7%)	19(63.3%)	1.13	0.28(NS)
Hypertension (n,%)	10(66.7%)	19(63.3%)	0.048	0.82(NS)
Obesity (n,%)	4(26.7%)	11(36.7%)	0.45	0.50(NS)
Smoking (n,%)	6(40.0%)	15(50.0%)	0.40	0.52(NS)

Table (2): Comparison between study and control groups as regard to laboratory findings

	Control group	Study group	Test	P value
LDL	107.07±17.24	132.43±21.0	4.03	<0.001(S)
HDL	51.73±6.20	16.86±8.65	1.93	0.059(NS)
Total cholesterol	161.40±30.95	204.13±30.28	4.42	<0.001(S)
Triglycerides	133.07±28.36	149.23±28.49	1.79	0.079(NS)
Fasting blood sugar	98.73±24.76	112.80±27.20	1.68	0.10(NS)
ESR (mean±SD)	17.06±7.74	21.86±9.28	1.72	0.092(NS)
CRP	3.48±1.0	4.91±0.93	4.72	<0.001(S)
MPV	9.44±1.64	12.26±1.69	5.32	<0.001(S)
IL 6	13.98±6.55	164.29±259.71	2.23	0.031(S)

Table (3): Comparison between values at the start and that at 6 months as regard MPV, CRP and IL 6 in the study group

	At the start	After 6 months	Paired (t)	p
	Mean±S. D	Mean±S. D		
MPV	12.26±1.69	11.35±1.67	4.28	<0.001(S)
CRP	4.91±0.93	4.45±0.82	5.55	<0.001(S)
IL 6	164.29±259.71	75.47±111.81	2.58	0.015(S)

Table (4): Comparison between different clinical presentations as regard

	SA	Atyptical	UA	Non ST	F	p
LDL	134.12±17.90	115.38±21.96	141.75±18.25	138.00±7.07	3.20	0.04(S)
HDL	45.00±8.01	54.50±7.13	42.91±7.82	47.50±3.53	3.96	0.019(S)
Total cholesterol	200.38±25.48	186.50±25.00	219.75±33.00	196.00±5.65	2.34	0.09(NS)
Triglycerides	159.00±33.12	136.88±29.75	148.00±25.21	167.00±5.65	1.09	0.37(NS)
Fasting blood sugar	120.0±23.50	93.50±24.24	116.08±27.47	141.50±3.53	2.75	0.063(NS)
ESR	21.50±11.62	23.12±11.99	22.41±6.28	15.00±0.00	0.40	0.75(NS)

Table (5): Correlation between MPV and other parameters in the study group

	MPV		MPV 6	
	r	P	r	p
LDL	0.35	0.056(NS)	0.31	0.09(NS)
HDL	-0.313	0.093(NS)	-0.368	0.046(NS)
TC	0.318	0.087(NS)	0.19	0.30(NS)
TG	0.28	0.12(NS)	0.23	0.21(NS)
FBS	0.49	0.006(S)	0.57	0.001(S)
ESR	0.346	0.061(NS)	0.129	0.498(NS)
CRP	0.688	0.001(S)	0.55	0.002(S)
CRP 6	0.59	0.001(S)	0.71	0.001(S)
IL 6	0.16	0.20(NS)	0.20	0.28(NS)
IL 6 at 6m	0.084	0.66(NS)	0.17	0.36(NS)

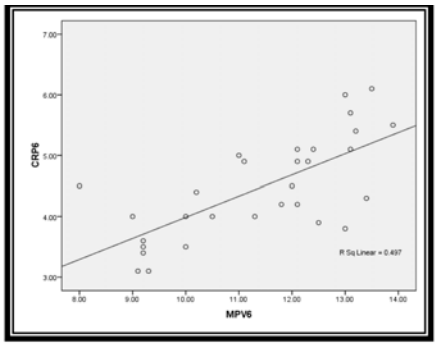

Figure (1): Correlation between MPV at 6 months and CRP at 6 months.

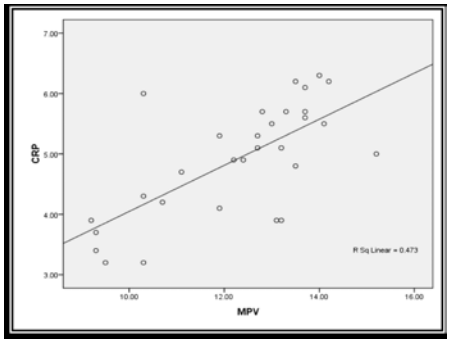

Figure (2): Correlation between MPV at the start and CRP at the start.

Discussion

The aim of this study is to evaluate mean platelet volume and some inflammatory markers in patients with slow coronary flow (SCF) and its possible relationship with clinical presentation. In this study interleukin-6 and c-reactive protein were increased in patients with coronary slow flow in comparison to control group, P value= (0.031 and0.001 respectively),this was in agreement with the study done by Li et al. [9] which included Forty-two patients with SCF detected by coronary angiography via the Thrombosis In Myocardial Infarction (TIMI) frame count method and 30 control subjects with angiographically normal coronary flow The data showed that plasma CRP and IL-6 concentrations were higher in patients with SCF compared with normal control subject P value=0.01

Consistent with this, previous study by Turhan et al.[11] Performed a study to evaluating plasma soluble adhesion molecules; intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin as possible indicators of endothelial activation or inflammation in patients with SCF, but with angiographically proven normal coronary arteries in all three coronary vessels ,Coronary flow rate of all patients and control subjects were documented by TIMI frame count. The results showed that serum ICAM-1, VCAM-1, and E-selectin concentrations of pa-

tients with SCF were found to be significantly higher than those of control subjects with normal coronary flow.

In this study we found that mean platelet volume was increased in patients with coronary slow flow in comparison to control group,P value=0.001. This was in agreement with the study done by Nurkalemet al.[12] who included 50 patients with SCF and otherwise normal coronary arteries were selected for the study group, and 22 patients with normal coronary arteries were selected for the control group, this study proved that MPV is increased in patients with SCF, and SCF patients presenting with USA exhibit significantly increased MPV values, suggesting an altered platelet reactivity and aggregation which may require effective anti-platelet therapy in this patient subgroup. P value= 0.007.

In previous studies, increased MPV was demonstrated in acute myocardial infarction[13], unstable angina pectoris(1) congestive heart failure [14] and coronary artery ectasia[15].

In the present study interleukin 6 and c-reactive protein were decreased after six months of antidyslipidemic drugs (20mg atorvastatin) in comparison to their values at the startup value=0.015. The study done by Li et al.[16] proved that Alternate-day dosing of rosuvastatin could be effective comparable with once-daily dosing of rosuvastatin in Chinese patients in improving not only lipid profile but also inflammatory markers, (interleukin 6 and C - reactive protein). There was another study done by Ye et al.[17] found that short-term (3-12 weeks) treatment with fenofibrate is an effective strategy in the management of elevated CRP concentrations. There was another study done by Li et al. [7] found that statistically significant reduction in CRP after statin treatment in patients with coronary heart disease (CHD) or hyperlipidemia.

In present work, mean platelet volume decreased after six months of antiplatelet in comparison to their values at admission. In addition, there was no significant difference in mean platelet volume as regard clinical presenta-

tion. This results disagreement with the study done by Nurkalem et al. [12] in which there was significant increase in MPV in patients with SCF presenting with unstable angina.In this study we found that there was no significant difference between patients group and control group as regard clinical presentation P value=0.34 this result was in agreement with the study done by Li et al. [9].

In the present study, we found that there was no significant difference in patient group with coronary slow flow and control group as regard systolic function of the left ventricle measured by conventional echocardiography, P value=0.18. But there was significant difference in diastolic function of the left ventricle which were significantly impaired in patients with coronary slow flow compared to the control group by pulsed wave Doppler P value =0.001, this was in agreement with the study done by Seviml et al. [18] which included 22 patients (12 males and 10 females) with coronary slow flow and 22 patients (14 males and 8 females) with normal coronary angiography as control group, which found that no significant difference in systolic function of left ventricle measured by conventional echocardiography between patients group and control group, P value=0.3 ,but that study also found that there was difference in systolic function of the left ventricle measured by tissue Doppler imaging between patients group and control group P value=0.001, this may be due to Tissue Doppler echocardiography is less influenced by several factors such as load condition and PW Doppler mitral inflow velocities. In addition, Seviml et al. [18] found that there were significant difference in diastolic function of the left ventricle by pulsed Doppler and tissue Doppler between patients group and control group.

In the present work, we found that there was no significant difference between patients group and control group as regard resting electrocardiogram, P value=0.23. This was disagreement with the study done by Güne_ et al. [19] this study included 30 patients with coronary slow flow and 30 patients with normal coronary

angiography they found that there were significant changes in resting electrocardiogram in the form of increased P wave duration and P wave dispersion, in patients group which improved after the use of nebivelol for about 3 months.

Results of the present study found that there was no significant difference between patients group and control group as regard ESR P value=0.092 ,This was in agreement with study done by Madak et al.[20] which included 82 patients with angiographically proven normal coronary arteries and slow coronary flow in all three coronary vessels (45 males and 37 females, mean age 59±11 years) and 34 patients with normal coronary arteries and normal coronary flow (19 males and 15 females, mean age 56±10 years) with similar risk profiles they found that Hs-CRP and proBNP are significantly higher in patients with coronary slow flow compared with normal control group but no significant difference in ESR between patients group and control group.

As regard LDL and fasting blood glucose levels, In this study we found that there was significant increase in patients group in comparison to control group P value=0.001. Previous studies (9, 12, 20) proved that, there were no significant differences between patients with coronary slow flow and control group with normal coronary angiography as regard lipid profile, fasting glucose levels, white blood cell and platelet counts.

In short, the results of the present study proved that, 1) increased plasma inflammatory markers including CRP and IL-6 existed in patients with SCF. This suggesting that an inflammation may be a contributor for the development of SCF. In other word, increased concentrations of inflammatory markers in patients with SCF may be an indicator of endothelial activation and inflammation and are likely to be in the causal pathway involving in SCF. Despite the relative good prognosis of patients with SCF, the chronic, frequent nature of the persistent chest uncomfortable can significantly impair quality of life. Therefore, an attention should

be paid to this unique phenomenon. However, whether increased inflammatory markers are related to the pathogenesis of SCF in these patients deserved further investigation; 2)-Our findings showed also that MPV is increased in patients with SCF, and SCF patients presenting with USA exhibit significantly increased MPV values, suggesting an altered platelet reactivity and aggregation which require effective anti-platelet therapy in this patient subgroup.

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INFLAMMATORY MARKERS AND MEAN PLATELET VOLUME IN PATIENTS WITH SLOW CORONARY FLOW AND ITS RELATIONSHIP WITH CLINICAL PRESENTATION

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SACROCOCCYGEAL TERATOMA A RARE DISEASE: CASE REPORT

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Abstract

Sacroccocygeal teratomas are rare congenital tumors that develop early in fetal life. Fetuses with this malformation are at risk of significant perinatal morbidity and mortality. This case report demonstrates the benefits of the early diagnosis and intervention of sacrococcygeal teratoma.

In this case study a fetus was identified with sacrococcygeal teratoma during 33 weeks antenatal scan of the of 11.48x10.97x9.02 cm size. The mother opted for elective caesarean section following counseling but due to pre-term labour at 36 weeks of gestation, the mother underwent emergency caesarean section without any complications. A newborn healthy female was delivered without any fetal complications. The baby was scheduled for enbloc surgical resection of the tumor on the 3rd day of life. Histopathological report did not reveal any malignancy. Neonate had an uneventful recovery. Neonate was followed up with regular follow-ups.

Keywords: *fetal sacroccocygeal teratoma, ultrasonography.*

Introduction

Sacrococcygeal teratomas (SCT) originate from Hensen's node (primitive knot), which is an area of the primitive streak. As the mesoderm proliferates, the primitive streaks may persist moves further caudally where the remnant of Hensen's node descends to the tip of the coccyx or its anterior surface gives rise to a SCT. >90% of SCT is benign[1].

Although generally a rare condition, it is said to be the most common tumor in the newborn period, with a reported incidence of approximately 1 in 35,000-40,000 live births [2-4]. It is a more common tumor in

newborn females,[4] with a male: female ratio of about 1: 3-4[5,6].

The tumors are divided and classified according to their location, type and tumor components according to American Academy of Pediatrics, Surgical Section Classification (table 1)[5].

Case Report

A 31-year-old nulliparous woman Gravida 2 Para 0 Abortion 1, her last menstrual period (LMP) was on 20.02.2011 and the expected date of delivery (EDD) was on 27.11.2011. She presented herself to the antenatal outpatient clinic for regular antenatal checkup at 33 weeks gestation. A year ago, the patient had a missed abortion at 8 weeks of gestation which was followed by dilatation and curettage. Medical and family history was insignificant. There was no family history of birth defects. Relevant hematological and biochemical investigations were all within normal limits. Three dimensional ultrasonography (USG) revealed a single viable fetus with anterior fundal placenta with estimated gestational age of 33 weeks. The measurements were as follow at 33weeks -BPD 8.74cm, OFD 11.51, HC 32.52 cm, AC 30.46 cm and FL 6.38 cm.

USG revealed a mass at the sacrococcygeal region of the fetus. The tumor contained considerable part of solid component and showed vascularity. The fetus was diagnosed to have type II SCT composed of cystic components as well as calcific foci with the 11.48x10.97x9.02 cm size and there was a pre-sacral extension of the cystic component (Figure 1 & 2). The condition was not associated with hydrops or polyhydraminios. The spine, abdomen and pelvic appeared normal. There was no extension of the tumor to the bladder. The fetus did not present any other congenital anomaly. There was no evidence of intrapelvic extension of the mass.

Following the diagnosis, the patient was referred to the pediatric consultant and surgeon. The pregnancy was allowed to continue until fetal viability with regular antenatal checkups. The

patient was closely monitored with USG to determine the size of the mass, amount of liquoramni, placentomegaly, fetal hydrops and fetal wellbeing (lung maturity). Serial scans at weeks 34 and 35 revealed no increase in the size of the mass. By week 36, the patient was advised about the risk of continuing the state of pregnancy following a family counseling for elective caesarean section, but the patient presented to the emergency with pre-term labor and an emergency caesarean was performed without any complications. It was a newborn female of 3.1kg, apgar score of 5 and 8 in 1st and 5th minutes respectively after birth. Placental size and weight were within normal limits (Figure 3).

After that, the baby was transferred to the neonatal intensive care unit and referred to the pediatric surgery department. On observation, the baby was active and healthy looking with a mass in the sacrococcygeal region. The baby passed meconium. On clinical examination the mass showed dilated veins over its surface and a variable consistency. The systemic examinations were normal. There was no intrapelvic extension of the mass.

The newborn was scheduled for surgery on the 3rd day of life. The tumor was sub totally removed by enbloc surgical resection via sacral approach and sent for biopsy and was shown to be benign. The baby had an uneventful postoperative recovery. A follow-up of biochemical profile and physical examination was recommended to determine the developmental milestones of the baby.

Table 1 : American Academy of Pediatrics, Surgical Section Classification[5].

Type	Tumor components
I	Completely external
II	Mostly external with some intrapelvic tumor
III	Mostly intrapelvic with some external tumor
IV	Completely intrapelvic or presacrals

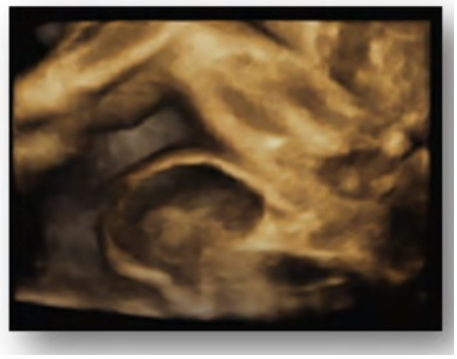

Figure 1

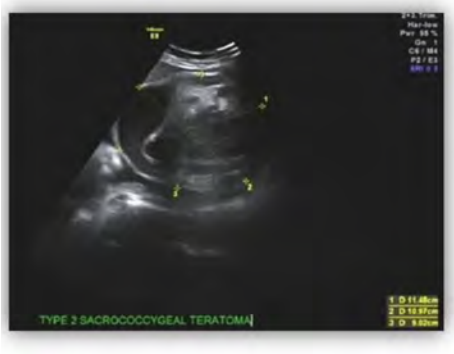

Figure 2

3D ultrasound showing the sacrococcygeal mass

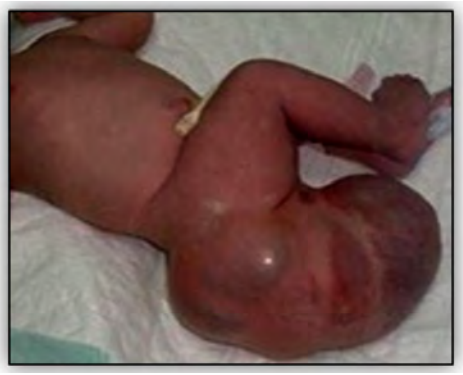

Figure 3. Gross image of the neonate with SCT post caesarean section

Discussion

This is a typical case report of a newborn with SCT diagnosed at week 33 during a routine antenatal scan. Early prenatal USG detection of SCT allows optimal perinatal obstetric and surgical management. Since the presented patient did not have fetal hydrops or cardiac failure signs, the amniotic fluid was within normal ranges, and tumor growing rate was slow. The sacrococcygeal area is the most common extragonadal site for teratoma. They are the rare tumors that develop at the base of the spine by the tailbone(coccyx) although most of these tumors are benign, they grow large, and

surgical intervention is essential. Embryologically, they originate from pluripotent cells and contain three embryologic layers of endoderm, ectoderm and mesoderm. The distribution of these three layers is explained by cessation and disorder in the migration of primordial germ cells. Generally these mature cells do not belong in these places and frequently originate from neurons[7].

In most centers, diagnosis of SCT is made during antenatal period[8]. Early diagnosis will enhance coordinated and well planned management of the patient. A study reported a large series in multiple centers, that the average determination time of SCT is 26 gestational weeks[9]. Usually diagnosis is made with USG.[10] Three-dimensional ultrasound may better define the degree of involvement of the sacrum and pelvic structures for prognostic importance[11].

Sonography can reveal mass at the sacrococcygeal region of the fetus. The tumor contains considerable part of solid component and vascularity. Diagnosis can determine the type of SCT composition and can show the cystic components as well as calcific foci with the size. USG must be followed to observe for further enlargement of SCT and any signs of fetal complications. Prenatal detection can be done by using ultrasound, computed tomography, or MRI in the postnatal period allowing better surgical planning.

Prenatal definition of SCT is important for prenatal management and surgical planning. During vaginal labor, severe dystocia and extremely vascular tumors can cause fetal death due to hemorrhage, caesarean section is recommended for tumors larger than 5 cm[12]. In addition to labor dystocia, tumor rupture can cause massive hemorrhage. Large tumors can cause difficulties even with caesarean sections[13].

Neonates with SCT after excision require long-term follow-up for functional impairment. Surgical trauma, tumor compression or invasion of

nerves contribute to this condition. It is reported that incidence of SCT is approximately 1 in 35,000-40,000 live births[2-4]. Tumor recurrence occurs in 7.5% to 22% of cases[14]. A report suggested that the recurrence rate of SCT in 173 children was 11% within 3 years and was associated with immature and malignant histology and incomplete resection. Postoperative monitoring of serum AFP levels is also essential to detect early tumor recurrence[15].

Early diagnosis of fetal SCT, with routine USG monitoring, is important for prenatal management and surgical planning of SCT[16]. While planning delivery of the fetus, the least traumatic method must be selected and the fetus must be referred to pediatric surgeon as early as possible. During the postoperative period, long-term follow-up is mandatory. Fetal SCT needs to be managed by cooperation and team efforts of the obstetrician, pediatrician and pediatric surgeon.

Conclusion

SCT are relatively uncommon tumors in the neonatal period. With advancement in the less invasive diagnosis and therapeutic techniques, many clinical obstetrician screen for SCT during antenatal period. Early surgical management is mandatory to prevent the spread in case of malignancy. A minimum of three post-operative follow-up is advised to detect the reoccurrence of the case.

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SACROCOCCYGEAL TERATOMA A RARE DISEASE: CASE REPORT

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CORRELATION BETWEEN P WAVE DISPERSION AND MYOCARDIAL FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract

Background: *P wave dispersion because of its relation to the non-homogenous and interrupted conduction of sinus impulses both Intra and inter atrial is a non-invasive indicator that enables the calculation of Atrial fibrillation (AF) risk on the 12 - lead surface ECG and correlates to LV end diastolic pressure.*

Aim: *to study the relationship between P wave dispersion and left ventricular function was investigated in patients presenting with acute anterior myocardial infarction.*

Patients and Methods: *The present study included 50 patients diagnosed as acute anterior myocardial infarction and admitted at coronary care unit of Benha university hospital from January 2011 to September 2011; there mean age was 57±9.4, 88% were male and only 12% were females. All patients were subjected for full history taken included age, sex, risk factors for coronary artery disease (CAD) as diabetes smoking, hypertension. ECG was done for every patients with analysis of P wave to determine maximum P wave dispersion (P max.), minimum P wave dispersion (P min.), and the difference in between P max. and P min. called PWD. Echocardiography was done for all patients to determine LA and LV dimensions, LVEF, and also tissue Doppler performed to determine Em/Am ratio and systolic S wave by pulsed wave tissue Doppler at lateral wall of mitral valve on apical four chamber view.*

Results: *We found that, positive correlation between P wave dispersion and age, LA size, LVEF, tissue Doppler Em/Am ratio, and P wave duration on ECG ($p < 0.001$); also there was increase in only P max. in patients with systolic dysfunction ($P < 0.05$). There is no effect of thrombolytic therapy, Beta Blocker, and ACE inhibitor on P wave dispersion*

and duration (P > 0.05).

Conclusion: *P wave dispersion is simple noninvasive ECG test can used as predictors of occurrence of arrhythmia in patients with AMI.*

Introduction

Diastolic function usually declines before systolic function, and this precedes clinical signs, therefore, diagnosis of diastolic dysfunction is very important for early diagnosis, follow up and treatment [1].

Mitral annulus systolic and diastolic velocities determined by pulsed wave tissue Doppler are relatively preload-independent and reliable variables in evaluating systolic and diastolic left ventricular function [2].

P wave dispersion is related to the non-homogenous and interrupted conduction of sinus impulses intra- and inter atrial; PD is described as a noninvasive indicator of atrial fibrillation risk, which can be calculated easily on a 12-lead surface ECG [3]

Aim of the work

To study the relationship between P wave dispersion and left ventricular systolic and diastolic function in patients with acute anterior myocardial infarction.

Patients and Methods

The study included 50 patients with acute anterior myocardial infarction. Patients were selected from patients attending to coronary care unit of Benha university hospital during the period from January 2011 to September 2011.

Acute anterior myocardial infarction was diagnosed when two or more of the following criteria were present: (according to European society and American college of cardiology committee of definition of myocardial infarction 2000) : Chest pain lasting >30 minutes or ST segment elevation > 2 millimeters in two anterior chest leads or significant elevation in cardiac enzymes.

Patients with arrhythmias or with conduction abnormalities, or cardiogenic shock were excluded.

Methods:

All patients included in the study were subjected to the following:

1- Careful history analysis which include data about age, sex, smoking, hypertension and diabetes.

2- Complete general and local examination of the heart, chest and abdomen.

3- Electrocardiogram:

The P-wave onset was defined as the first atrial deflection from the isoelectric line , and the offset was the return of the atrial signal to baseline[4]. Patients whose measurements could be performed in at least 8 derivations were included in the study. In all patients, derivations were excluded if the beginning or the ending of the P wave could not be clearly identified. Maximum P wave duration (P max) is defined as the longest and minimum P wave duration (P min) is defined as the shortest P wave duration. Leads that showed P min and P max were observed. PWD defined as difference between P max and P min. All the measurements were repeated three times and average values were calculated for each of electrocardiographic parameter. All of the measurements were performed using the same experienced investigators blind to the subject's clinical status.

The 12-lead surface electrocardiogram will be calculated manually, with paper speed at 25 mm/s and 10mv/cm standardization, the measurements are performed manually in all 12 leads by using a 0.5 mm scale precision ruler, and magnifying lens. This method has been found to be more precise than the standard caliper method for QT interval [3].

4- Echocardiogram:

All patients will be evaluated by two-dimensional, M-mode, pulsed wave Doppler and pulsed wave tissue Doppler within hours of myocardial infarction. EF% will be calculated from apical two and apical four chamber views using modified Simpson's method, LA size, FS% will be determined [5].

Trans mitral Doppler parame-

ters will be analyzed; peak early (E) and late (A) trans-mitral filling velocities, the ratio of early to late peak velocities (E/A), deceleration time of (E) will be determined [6].

Pulsed wave tissue Doppler echocardiography will be performed with a 2 mm sample volume placed at the lateral corner of the mitral annulus from the apical four chamber view, Early (Em) and late diastolic (Am), and peak systolic (Sm) mitral annular velocities will be recorded. the ratio of (Em) to (Am) (Em/Am) and E/Em, which is an index for predicting elevated left ventricular filling pressure will be calculated [7].

Statistical Analysis

Data was analyzed using SPSS (Statistical Package for Social Sciences) version 15. Qualitative data was presented as number and percent. Quantitative data was tested for normality by Kolmogrov-Smirnov test. Normally distributed data was presented as mean ± SD. Student t-test was used to compare between two groups. Regression curve was done. $P < 0.05$ was considered to be statistically significant.[8]

Results

The present study included 50 patients with acute anterior myocardial infarction, their mean age was 57.5 ± 9.4 years, 88% are males and 12% are females, fifty percent were diabetic, 58% were hypertensive, 36% were smokers and 50% had abnormal lipid profile.

Systolic dysfunction (EF<50%) was present in 27 (54%) of patients, one patient had sever (2%), 16 (32%) with moderate systolic dysfunction and 10 (20%) had mild systolic dysfunction.

Diastolic dysfunction was present in 39 (78%) of patients, 15 patients (30%) had grade I, 18 patients (36%) had grade II and 6 patients (12%) had grade III diastolic dysfunction.

Thirty-nine patients (78%) received streptokinase, 32 patients (64%) received beta blocker and 46 patients (92%) received ACE inhibitor.

Fig. (1): shows that, age is the only parameter that correlates positively and significantly with

P wave dispersion.

Table (1),(2),(3) shows no significant effect of thrombolytic therapy, patients received Beta blocker or ACE inhibitor on P wave duration and dispersion.

There is insignificant increase in P wave duration and dispersion with increase in severity of systolic dysfunction (P > 0.05). Table (4)

Table (5): shows, there is insignificant increase in P wave duration and dispersion in patients with diastolic dysfunction compared to those without (P > 0.05).

In comparing p wave dispersion and different echocardiography parameters, we found that significant increase in p wave dispersion parameters with impaired LVEF, increased LA size (p value < 0.01, and <0.001 respectively) {Table (6), Fig.(2), (3)}, also there is significant increase in p wave dispersion in correlation with increased left ventricular filling pressure (Am/Em ratio and E/Em indices) (p value <0.001, <0.05). Table (6) Fig. (5), (6).

Table (1): Comparison of P wave duration and dispersion between patients received and those not received thrombolytic therapy.

	Non thrombolysis group (n=11)	Thrombolysis group (n=39)	P
P max.	114.27±11.22	116.95±15.2	>0.05
P min.	57.55±14.64	57.62±11.03	>0.05
PWD	56.73±13.52	59.59±16.41	>0.05

Table (2): Relation between beta blocker therapy and (P) wave parameters.

	Non beta blocker group (n=18)	Beta blocker group (n=32)	P
P max.	123.06±13.92	122.59±13.39	> 0.05
P min.	59.17±14.06	56.72±10.38	>0.05
PWD	54.44±14.49	55.88±15.78	>0.05

Table.(3): Relation between ACE inhibitor therapy and (P) wave parameters.

	Non ACE. inhibitor group (n=4)	ACE. inhibitor group (n=46)	P value
P max.	120.50±9.00	116.00±14.76	>0.05
P min.	66.75±10.28	56.80±11.63	>0.05
PWD	53.75±13.15	59.41±15.99	>0.05

Table.(4): correlation between P wave dispersion and systolic dysfunction.

	Mild systolic dysfunction (n=10) (mean ±SD)	Moderate (n=16) (mean ±SD)	Sever (n=1) (mean ±SD)	P value
P max. (msc)	114±8.2	122.5±13.1	130.0±0.0	>0.05
P min. (msc)	54±5.2	61.1±12.2	65±0.0	>0.05
PWD (msc)	61±4.1	61.4±17.2	65±0.0	>0.05

Table.(5): Comparison of P-wave duration and dispersion in patients with and without diastolic dysfunction.

	Patients without diastolic dysfunction (n=11) (mean ±SD)	**Patients with diastolic dysfunction (n=39)** (mean ±SD)	**P value**
P max. (msc)	111.6±11.9	117.7±14.9	>0.05
P min. (msc)	57.3±13.3	57.7±11.5	>0.05
PWD (msc)	54.4±16.6	60.3±15.5	>0.05

Table (6): Correlation between echocardiographic data and P wave dispersion:

	PWD	
	r	**P**
EF	**-0.451**	**< 0.01****
Left atrium size	**0.688**	**< 0.001*****
E/A ratio	**0.639**	**< 0.001*****
Sm	**0.720**	**>0.05**
EM	**-0.089**	**>0.05**
Em/Am	**0.489**	**< 0.001*****
E/Em	**0.317**	**<0.05***

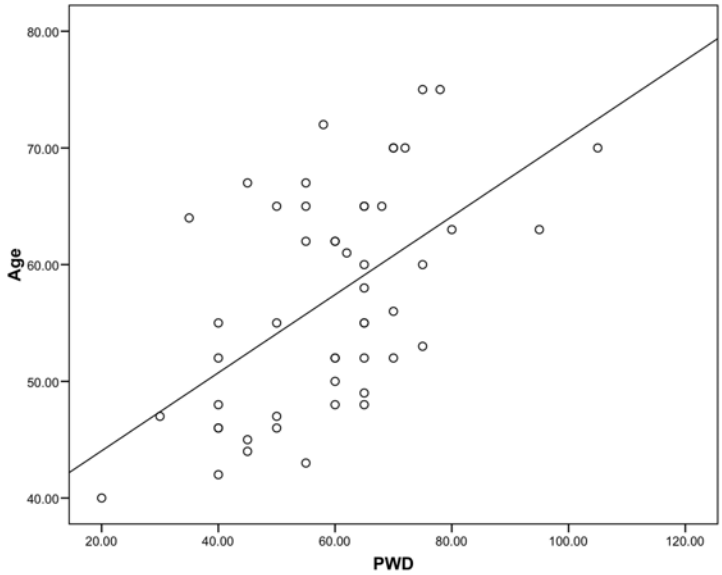

Fig. (1) show correlation between P wave dispersion and age

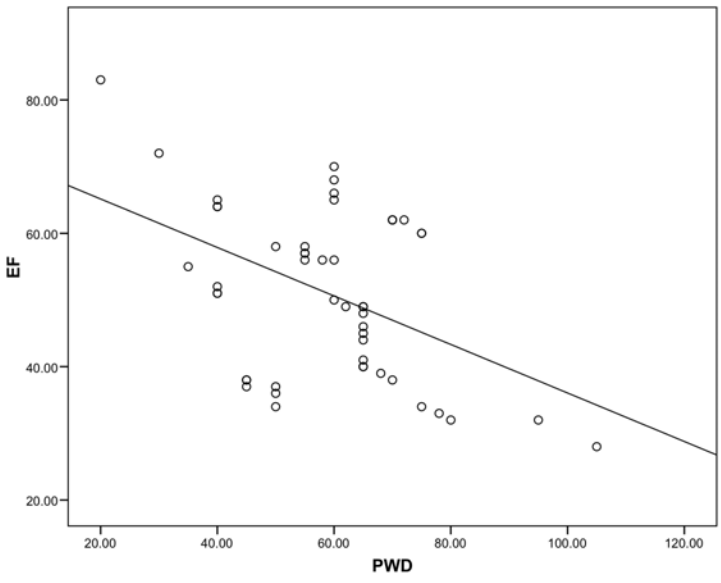

Fig. (2) : correlation between p wave dispersion and LVEF.

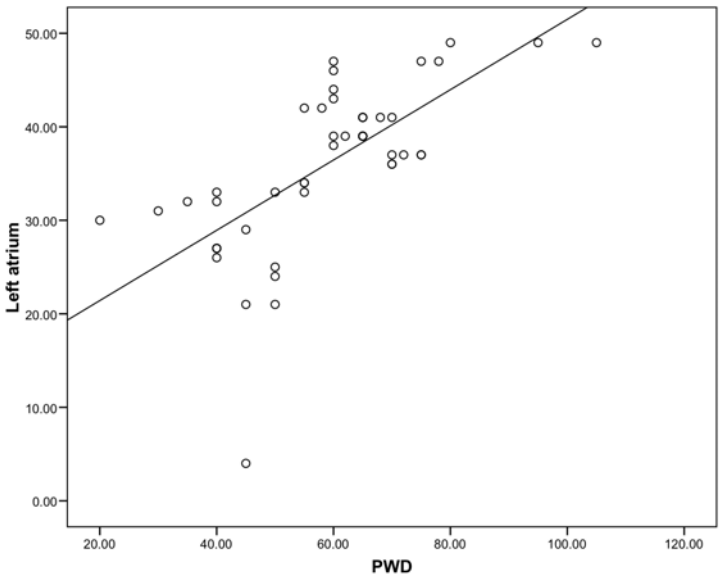

Fig. (3) : Correlation between p wave dispersion and LA size.

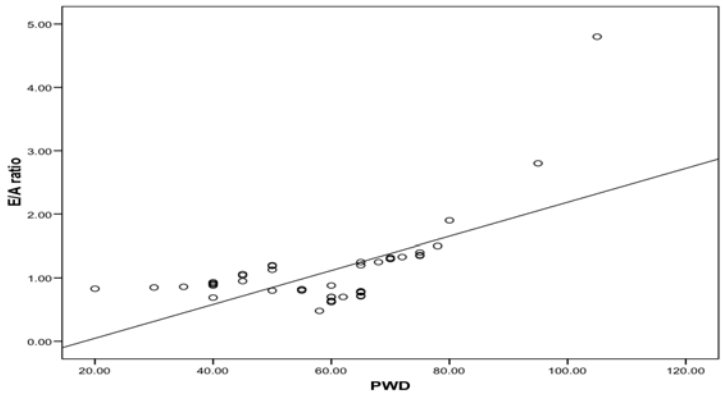

Fig.(4) : Correlation between p wave dispersion and diastolic E/A ratio.

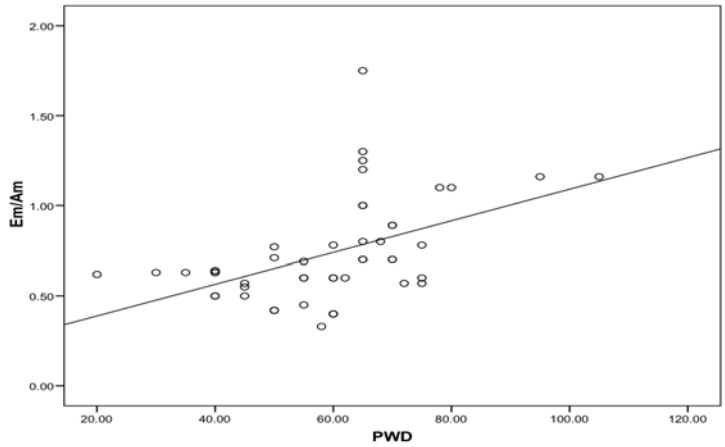

Fig. (5) : Correlation between p wave dispersion and Em /Am ratio

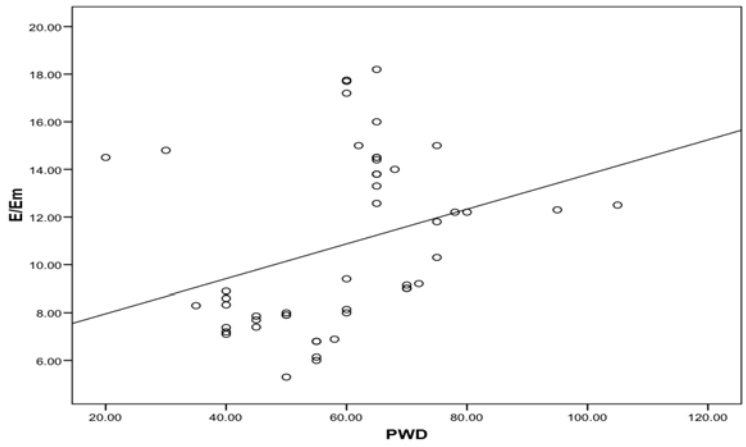

Fig (6) : Correlation between p wave dispersion and E/Em.

Discussion

Patients with acute myocardial infarction, frequently have both left ventricular (LV) systolic and diastolic dysfunction, although both isolated systolic and diastolic LV dysfunction can be identified [1].

There is a growing recognition that LV dysfunction is associated with marked alternation in the electro physiologic properties of the myocardium which is the precursor of the cardiac conduction and rhythm abnormalities [9].

Several non-invasive electrocardiographic (ECG) indicators have been investigated to predict the occurrence of arrhythmia in patients with left ventricular dysfunction.[10]

In the present study, the relationship between P wave dispersion and left ventricular function was investigated in patients presenting with acute myocardial infarction.

In the present study, there is increase in the mean values of P wave maximum (119.63msc vs 112.52msc) and P wave dispersion (61.41 msc vs 56.09msc) in patients with systolic dysfunction compared to patients without LV systolic dysfunction.

In addition, the present study shows that the P wave duration and P wave dispersion increase along with the severity of LV systolic dysfunction.

Correlation studies in the current study have demonstrated significant positive correlation between LV ejection fraction and P wave dispersion ($r = 0.30$, $p = 0.03$).

These results demonstrate the close association of P wave dispersion and left ventricular systolic dysfunction and are in agreement with the results of other previous studies such as that of.[11]

Yilmaz et al (2004). study is the first study in literature to demonstrate a direct correlation between P wave dispersion and tissue Doppler echocardiographic parameters of systolic LV function in patients with acute myocardial infarction. [10]

Additionally, Yilmaz et al. study demonstrated that worsening of LV systolic function parameters was related to the increase of P wave dispersion.[10]

Huseyin (2005) reported that there is a significant relationship between P wave dispersion and LV ejection fraction.[11]

In the current study, there is insignificant increase in P wave dispersion in patients with compared to patients without LV diastolic dysfunction (60.3 +_15.5msc vs. 54.4 _+16.6 msc, p >0.05).

In the present study, there is a strong positive correlation of P wave dispersion and different parameters of left ventricular diastolic dysfunction: E/A ratio (r=-0.714, p <0.001), and E/Em (r=0.3, p<0.05).

As the ratio between E and Em has shown to be a reliable index of left ventricular filling pressure , it can concluded from these results that the increase in left ventricular filling pressure may be reflected on the surface ECG as an increase in P wave dispersion.[7]

Ilknur et al. (2010) studied the relationship between P wave dispersion and diastolic dysfunction in patients with significant and insignificant coronary artery disease and reported similar results as the present study.[12]

They concluded that P wave dispersion is a non-invasive marker for left ventricular end diastolic pressure; they stated that P wave dispersion is another alternative for assessment of left ventricular diastolic dysfunction in coronary artery disease. They failed to show any significant change in P wave dispersion with the three different stages of diastolic dysfunction and related this failure to the small studies groups.

kato (2003) compared hypertensive patients with LV diastolic dysfunction with hypertensive patients without LV diastolic dysfunction and found P wave dispersion to be higher in LV diastolic dysfunction patients.[13]

Yilmaz et al. (2005) reported that there was a statistically sig-

nificant positive correlation between the E/Em and P wave dispersion suggesting that the increase of LV filling pressure is associated with increase in P wave dispersion, they concluded that the E/Em ratio early after acute myocardial infarction can be used for predicting elevated LV end diastolic pressure and occurrence of atrial fibrillation.(10)

In the present work, there is significant strong positive correlation between left atrial dimension and P wave dispersion (r= 0.677, p<0.001).

Similarly, Ilknur et al. (2010) showed that P wave dispersion is significantly correlated to left atrial volume and dimension in all studied population whether having significant or non-significant coronary artery disease.(12)

However Dilaveris et al (2000), has been stated that left atrial diameter is not an important predictor for atrial fibrillation and that P wave duration is unrelated to left atrial diameter.(3)

The cause of difference between the present study and previous studies may be due to small sample size (in the present study), different population studied, different clinical sitting (hypertension vs, acute myocardial infarction vs. coronary artery disease) and the different methods used to calculate P wave dispersion.

In the current study, among the different clinical variables (age, sex, coronary risk factors, heart rate and blood pressure), only the age was found to be strongly correlated with P wave dispersion (r =0.689, p<0.001).

In contrary, Huseyin et al. (2005) found that, there is insignificant relationship between P wave dispersion and these variables including age.(11)

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CORRELATION BETWEEN P WAVE DISPERSION AND MYOCARDIAL FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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OUTBREAKS OF FOOD POISONING DUE TO BACTERIAL CONTAMINATION AT FAST FOOD RESTAURANTS AROUND AL HARAM AL NABAWI - AL MADINAH AL MONOWARAH

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Abstract

Food poisoning represents a widespread international health problem. It can be attributed to contamination of food and drinking water by bacteria, parasites, viruses, toxins or chemicals. The massive proliferation of fast food restaurants in Saudi Arabia, serving people at Haj and Umrah have resulted in a corresponding increase in fast food consumption. Thus, the aim of this study was to assess the incidence of food poisoning outbreaks and to determine the types of bacterial contamination in fast food restaurants in Al-Madina Al-Monowarah. Several food samples were collected from 30 fast food restaurants around Al Haram Al Nabawi in Al Madinah Al Monowarah. Culture methods were used for detection of S. aureus, Salmonella, E. coli, etc. The results revealed two large outbreaks of food poisoning in Al Madina Al-Monowarah. It was found that the majority of the fast food samples and their wrappings were contaminated by 9 different types of bacteria. The most common organisms detected were staphylococcus aureus (44.7%) followed by coagulase negative staphylococci (36%). Levels of bacterial contamination were unacceptable for human consumption and should be avoided to prevent outbreaks of food poisoning. Food handling with bare hands and poor hygiene during catering represent risk factors for food contamination. Cleanliness and safe hygienic environment are recommended to reduce the risk of food borne illness.

Keywords: *food poisoning, bacterial contamination, fast food.*

Introduction

Food-borne illness or its synonymous food poisoning represents a widespread international health problem and a significant source of human morbidity and mortality in addition to economic losses.[1]

The global incidence of food-borne illnesses is difficult to estimate, but it has been reported that about 1.8 million people died from diarrheal diseases in 2005. A great proportion of these cases can be attributed to contamination of food and drinking water by a wide variety of bacteria, parasites, viruses, toxins or chemicals. Food poisoning is characterized by short incubation period, acute illness and clinical symptoms of gastro-intestinal disturbances.[2-4]

It has been reported that there are five primary factors contributing to food poisoning. They include improper cooking procedures; temperature abuse during storage; lack of hygiene and sanitation by food handlers; cross-contamination between raw and fresh ready-to-eat foods; and acquiring food from unsafe sources.[5] On the other hand, food poisoning outbreaks are defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food[6]. They are common in many countries despite the dramatic improvement of food safety, educational efforts and exploratory microbiology.[1,2]

The massive proliferation of fast food restaurants in Saudi Arabia, serving people at Haj and Umrah have resulted in a corresponding increase in fast food consumption. Thus, the aim of this study was to assess the prevalence of food-borne illnesses, the incidence of outbreaks of food poisoning and types of bacterial food contamination in fast food restaurants in Al-Madina Al-Monowarah.

Material and Methods

1) A retrospective investigational study:

This was done to identify the incidence of food borne illness and to determine the extent of food poisoning outbreaks in Saudi Arabia.

2) Questionnaire:

It was issued by the investigator towards the degree of sanitary care of the restaurant concerning cleanliness of the floor and food containers. Special emphasis was directed towards the safety measures taken by food handlers during food handling as regards wearing gloves, masks, overall and overheads. This was done after taking approval from Ministry of Health.

3) Sampling :

• Collection of outer wrappings of food, outer surface of the sandwiches and food they contained was done in 30 fast food restaurants around Al Haram Al Nabawi in Al Madinah Al Monowarah. Different types of food were included such as shawarma, eggs, shashqua, bean tamiah, salad and potatoes.

- The whole sandwiches were inserted into a sterile polyethylene bag. The samples were transported within 1 hour to the laboratory in a cool-box.
- After taking informed consent, fingernail and hand swabs were collected from food handlers and put into an ethylene oxide sterilized bag. Aseptic techniques were maintained during media preparation, collection, transportation and analysis of samples. Examination was carried out on the same day of samples collection. [7]

4) Bacteriological cultures:

Culture methods were used for detection of S. aureus, Coagulase negative staphylococci, Salmonella, Coliforms, E. coli, Shigella shiga, Enterococcus feaccalis, Bacillus spp., Bacillus cereus and Pseudomonous aeuroginosa.[8] All foodstuff samples were diluted ten folds in sterile saline and peptone water media. Then, they were incubated aerobically at 35º C for 24-48 hours to clarify the colonies then they were plated and subcultured onto the following media:

1. Blood Agar: for selective isolation of haemolytic micro-organisms.
2. MacConkey Agar (Mac): selective isolation of S. aureus.
3. Mannitol salt Agar (MSA) : selective isolation of S. aureus.
4. Salmonella Shigella Agar (S.S Agar).
5. Xylose lysine deoxycholate

(XLD) Agar: for isolation of salmonella and shigella.

6. Thiosulphate citrate bile salt sucrose Agar (TCBS): selective isolation of vibrio cholera.
7. Cysteine Lactose electrolyte deficient (CLED) Agar: for isolation of urinary tract organisms.
8. Nutrient Agar: for growth of non-fastidious organisms.
9. Malt Agar: for the cultivation of fungi Aspergillus niger, Candida ablicans, Pencillium roquefortii and Trichophyton mentagraphytes.
10. Diagnostic sensitivity test (DST) Agar : used as an antibiotic sensitivity testing medium.

Representative colonies were Gram stained to differentiate between Gram-positive and Gram-negative bacteria by using the standard techniques and Analytical Profile Index (API) system (Bio Merieux, France). [9]

5) Statistical analysis:

It was carried out with Microsoft Excel program. Descriptive statistics regarding all the variables were given as mean ± SD. Differences between groups were analyzed by "comparing more than two proportions" test. Differences between the values were analyzed by Chi-Square test. P value ≤ 0.05 was considered statistically significant.

Results

Table (1) illustrated the socio-demographic data and overall incidence of food borne illness in Saudi Arabia during the period from 1990-2001. It was found that the total number is 10.173 cases. In Al Madina Al Monowarah, 928 cases of food-borne illness were detected. All the recorded cases were sporadic and not considered as an outbreak of food poisoning.

More recent reports (in the last ten years) demonstrated that there are only two large outbreaks of food poisoning related to mass catering occurred in 2003 and 2011 in Al-Madina Al-Monowarah.

Table (2) demonstrated abundance of bacterial loads in the collected samples.

Frequency and percentages of positive samples contaminated by different bacterial species in food substances and their wrappings are shown in table 3.

Figure (1) shows the frequency of using protective overalls, overheads and masks among food handlers in fast food restaurants.

The differences between bacterial isolate percentages in food handlers in relation to wearing masks and gloves were found statistically significant as seen in figures 2 and 3 respectively.

Table (1): Sociodemographic data and overall incidence of food borne illness in different regions in Saudi Arabia (1990-2001).

Studied Regions	Number	Sociodemographic data of poisoned cases									
		Age (years)				Gender			Nationality		
		< 7	7-18	> 18	Not Registered	Male	Female	Not Registered	Saudi	Other	Not Registered
Middle region	4914				4914			4914			4914
Eastern region	1071	128	50	622	271	569	502		528	543	
Western region	2476	1155	53	65	1203	1352	1124		1672	804	
Al Madina-Al Monowarah	928	421	8	250	249	502	426		579	349	
Asir	587				587			587			587
North region	197	80			117	86	111				197
Total number	10173	1754	111	973	7224	2509	2163	5501	2779	1696	5698

Table (2): Abundance of bacterial loads in the collected samples .

Bacteria	Abundance (Colony forming units: CFU/ml)																	
	<10			$10^1 - 10^2$			$10^2 - 10^3$			$10^3 - 10^4$			$10^4 - 10^5$			> 10^5		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Staphylococcus Aureus	----	----	1	----	----	1	-----	2	2	1	5	11	2	13	12	1	8	10
Coagulase -ve Staphylococci	----	----	1	----	----	1	----	2	3	1	4	5	5	7	9	3	5	8
Bacillus spp.	----	-----	-----	-----	1	2	-----	1	1	-----	1	2	----	1	3	----	3	---
Bacillus cereus	----	-----	-----	-----	1	1	-----	1	1	-----	1	3	----	1	3	----	1	2
Escherichia coli	----	-----	-----	1	-----	1	2	1	3	1	----	1	1	2	3	----	---	---
Enterococcus feaccalis	----	-----	-----	-----	-----	-----	1	1	1	-----	----	1	1	----	2	___	---	---
Salmonella	----	-----	-----	-----	-----	1	-----	-----	-----	-----	----	1	----	----	---	-----	----	----
Shigella Shiga	----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----	----	1	----	----	-----	-----	----
Pseudomonous aeuroginosa	----	-----	-----	-----	-----	-----	-----	-----	1	-----	----	----	----	----	----	-----	-----	----

S1: swapping from outer wrapping of food; S2 swapping from outer surface of the sandwiches; S3:swapping from food content

Table (3): Frequency and percentages of positive samples contaminated by different bacterial species.

Bacterial Species	No. of positive samples (total number=150)	(%)
Staphylococcus aureus	67	44.7%
Coagulase-negative staphylococci	54	36%
Bacillus spp.	14	9.3%
Bacillus cereus	13	8.7%
Escherichia coli	12	8%
Enterococcus feaccalis	7	4.7%
Salmonella	3	2%
Shigella shiga	1	0.67%
Pseudomonous aeuroginosa	1	0.67%

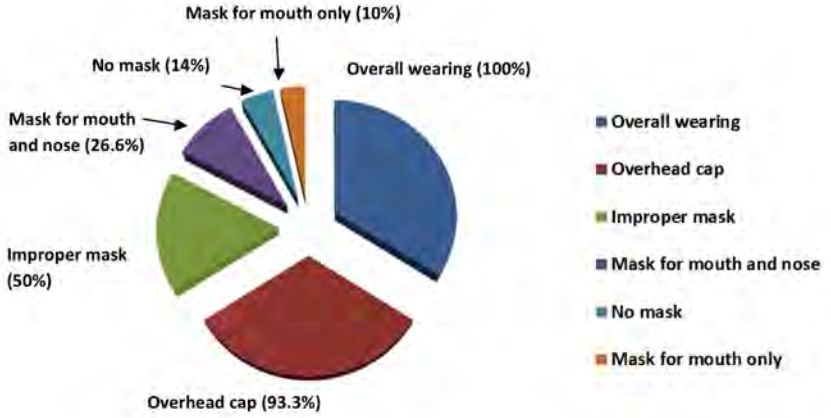

Figure (1): A pie chart showing percentages of using protective overalls, overheads and masks among food handlers in fast food restaurants.

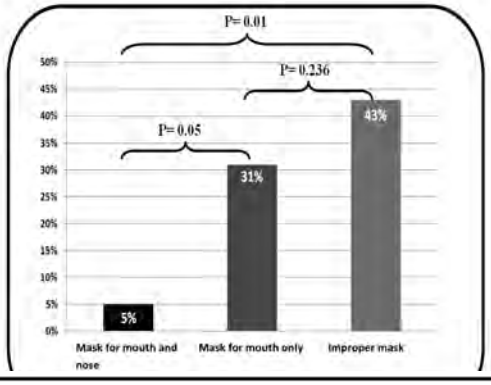

Figure (2): Bacterial isolate percentages in relation to wearing masks.

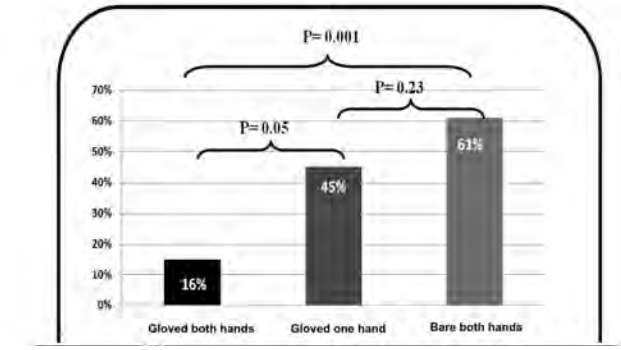

Figure (3): Bacterial isolate percentages in relation to wearing gloves.

Discussion

Food-borne disease outbreaks and acute infectious diseases of the gastrointestinal tract are problems of great importance in haj and Umrah due to large numbers of populations consuming food at fast food restaurants widely spreading around El-Haram El-Nabawi. Hence, the aim of this study was to assess the incidence of food borne illness in Saudi Arabia and to determine the causes and types of bacterial food contamination in fast food restaurants in Al-Madina Al-Monowarah.

In the present work, an overall statistical report indicated an increased incidence of food-borne illnesses in many areas of Saudi Arabia during the period from 1990-2001. In the last ten years, two large outbreaks of food poisoning were reported in Al Madina Al-Monowarah. The first outbreak was in 2003 where 492 Iranian pilgrims were complaining of vomiting (95%), abdominal pain (78.7%), nausea (75.4%) and diarrhea (27%) after mass catering. Most of the patients were treated at the emergency departments and recovered from symptoms within 24 hours while thirteen cases (6.3%) were admitted to the hospital. Four food items were eaten: rice, chicken, bread and yoghurt. Staphylococcus aureus was isolated from the stool cultures of 13 workers and from the nasal and throat swabs of two responsible for food preparation. It was also isolated from the vomitus of

10 cases and food remnant specimens. [10]

Asao et al.[11] stated that staphylococcal food poisoning is one of the most common foodborne diseases in the world. It is caused by ingestion of staphylococcal enterotoxin (SE) produced in food by certain strains of staphylococcus aureus.[12,13] The intoxication is characterized by sudden onset of nausea, vomiting, abdominal cramps and diarrhea which usually appear within 1 to 6 hours. The symptoms generally last from 24 to 48 hours and the mortality rate is very low or nil. [14].

A 2nd outbreak happened in 2011 where a total of 81 Bangladeshi pilgrims were taken to several hospitals in Al-Madina Al-Monowarah suffering from manifestations of food poisoning after eating a meal at an unlicensed caterer. They received their medical treatment and recovered from symptoms within 24 hours. The occurrence of these outbreaks urged the Ministry of Health (MOH) to recommend the investigations of the causes of food poisoning and assessment of bacterial contamination in different food stuffs with the necessity of more awareness and strict control measures on food safety. [15]

The results of the present work indicated that 44.7% of the fast food samples and their wrappings were contaminated by 9 different types of bacteria and each sample was harboring 1-4 bacterial species. The most common organisms detected were staphylococcus aureus (44.7%) followed by coagulase negative staphylococci (36%). In addition, bacillus spp. (9.3%), bacillus cereus (8.7%) and salmonella (2%) were isolated from the food samples. However, no cases of human infection of salmonella were reported in the area at the time of the study.

Likewise, Isara et al.[4] reported a high prevalence of food contamination in the fast food restaurants in Nigeria (37.5%) with staphylococcus aureus and bacillus cereus the most commonly detected bacteria. Mensah et al. [16] found nearly similar results in Ghana where street foods were heavily

contaminated with micro-organisms. Unacceptable levels of bacterial contamination were detected in 356 (69.7%) specimens "salads, macaroni, fufu, omo tuo and red pepper": 172 had Enterobacteriaceae (33.7%), 163 contained Staphylococcus aureus (31.9%) and Bacillus cereus was detected in 28 samples (5.5%). Shigella sonnei and Escherichia coli were isolated from macaroni, rice, and tomato stew.

In contrast to the present findings, a lower prevalence of bacterial contamination was reported by Oh et al.[17] in Korea where S. aureus was detected in just 8.6% of ready-to-eat tested food samples (n = 3332). Moreover, in Thailand, S. aureus concentration $>10^2$ CFU/g was detected among 10.8% of food samples[18] which may be attributed to more strict safety measures on food handling.

The present work revealed the presence of Escherichia coli and Enterococcus faecalis (8% and 4.7% respectively) in food samples and their wrappings. Soomro et al. [19] stated that Escherichia coli can cause severe diarrhea and vomiting. Furthermore, the detection of these pathogens is a reliable indicator of faecal contamination of water and food and points to the high bacterial contamination of the hands of food handlers during work periods with lack of enough hygiene and improper glove use. [20]

The present study revealed that all the examined foodstuffs contained different bacterial loads ranging from 10^2 to more than 10^5 colony forming units (CFU) / ml. Hence, the analyzed food items have unacceptable microbiological quality according to the published microbiological guidelines. These foodstuffs have potential risk for human consumption as there are chances for bacterium's multiplication during the time of food poisoning particularly if these foods are kept at room temperature for a long time in tropical climate. [21]

According to the US Food and Drug Administration, 10^5 CFU/g S. aureus is capable of causing staphylococcal intoxication[22]. The present results showed that the number of S. aureus popula-

tion is within the required dose to cause food poisoning and may be responsible for the detected outbreaks.

Additionally, all the examined food samples (shawarma, eggs, shashqua, bean tamiah, salad and potatoes) whether fresh, heated or stored were contaminated. More or less similar, Schmid et al. [23] reported that outbreaks of staphylococcal food poisoning are usually associated with protein-rich foods. Food contamination usually occurs during catering with extensive manual handling, often in combination with inadequate heating and/or inappropriate storage of food. [24]

Regarding the analysis of hand swabs, a high bacterial isolate percentage was detected in samples from bare hands (61%) while it was 16% in those samples from gloved hands. A highly significant difference was found between bare and gloved hands ($p < 0.05$). The present findings showed that all the studied food items were contaminated which may be explained by the fact that these foods require direct hand contact and thus could be contaminated during preparation by the bare hands of the food handlers.

Asao et al. [11] reported that 57.5% of food handlers harbored S. aureus and they are the most frequent source of contamination of food in staphylococcal food poisoning mainly due to lack of proper hygienic measures during food preparation. The rather high isolation rates of S. aureus and coagulase-negative staphylococci from the hand swabs in this study could be explained by the fact that these organisms are found in the permanent flora of the skin. Moreover, S. aureus primarily inhabits the nasopharyngeal region and hair of humans. [25, 26] Loeto et al. stated that food can be easily contaminated if food handlers sneeze or cough during food preparation, and if they do not wash their hands properly after making contact with their nose or after using the rest room. [27]

Conclusions

This study has shown that the majority of fast food in the Al-Madina Al Monawarrha was contaminated with several pathogenic

bacteria with unacceptable and unsafe levels for human consumption as they could lead to outbreaks of food-borne illnesses. They also indicate poor hygiene level and lack of cleanliness in food preparation practices.

Recommendations:

It is recommended to apply good food hygiene as it greatly reduces the risk of transmission of food-borne disease via infected food handlers or through cross-contamination. Training on proper hand washing and wearing gloves in both hands with adequate mask during food handling in the fast food restaurants should be improved.

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BENHA MEDICAL JOURNAL

OUTBREAKS OF FOOD POISONING DUE TO BACTERIAL CONTAMINATION AT FAST FOOD RESTAURANTS AROUND AL HARAM AL NABAWI - AL MADINAH AL MONOWARAH

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PROSPECTIVE COMPARISON OF TRANSIENT ELASTOGRAPHY AND LIVER BIOPSY FOR THE ASSESSMENT OF FIBROSIS IN CHRONIC HEPATITIS C BEFORE AND AFTER INTERFERON THERAPY

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Abstract

Background: *Transient elastography (fibroscan) is a rapid, non invasive and reproducible method allowing liver stiffness measurements (LSM) to evaluate liver fibrosis. The aim of this study: to evaluate the accuracy of fibroscan in assessment of liver fibrosis in patients with chronic hepatitis C in comparison with liver biopsy (LB). Also, to assess the impact of treatment with pegylated interferon and ribavirin on the liver histology.*

Methods: *This prospective study enrolled 134 patients with chronic hepatitis C (genotype 4). All patients underwent LB, LSM (Fibrsocan, Echosens, Paris, France) and routine laboratory investigations. Liver fibrosis was assessed by Metavir scoring system with scale range from 0 to 4 by 2 experienced pathologists. Also steatosis and necroinflammatory activity were reported. The accuracy of fibroscan was assessed by measurement of the areas under the receiver operating characteristics (ROC) curve, sensitivity and specificity. After treatment with combination therapy of pegylated interferon alpha 2a weekly and ribavirin for 48 weeks. Twenty patients achieved sustained virological response (SVR) were reassessed by post-treatment liver biopsy after 1 year of discontinuation of treatment.*

Results: *Fibroscan values ranged from 2.4 to 75.4 kilopascals. For F ≥ 2, the cut off was 7.8 kpa with AUROC of 0.82, sensitivity of 67% and specificity of 91%. For F ≥ 3, the cutoff was 11.9 kpa with AUROC of*

0.91, sensitivity of 86% and specificity of 85%. For F = 4, the cut off was 19.3 kpa with AUROC of 0.96 sensitivity of 95% and specificity of 92%. Liver stiffness measurement by fibroscan was correlated significantly with Metavir fiborsis stages (P = 0.000) but not with the degree of necro-inflammatory activity or steatosis (P = 0.311, P = 0.384 respectively). Twenty patients achieved SVR, reassessed after interferon therapy and there was progression in fibrosis score in 3 patients (15%), stationary course in 7 patients (35%) and improvement in 10 patients (50%) but this didn't reach statistical significance (P = 0.334) while significant decrease in the degree of necroinflammatory activity was reported (P = 0.000).

Conclusion: *Fibroscan is a simple, non invasive and acceptable method for assessing liver fibrosis. Combination of pegylated interferon and ribavirin could improve histological activity grade after one year of the end of treatment in chronic hepatitis C patients.*

Introduction

Hepatic fibrosis is a wound healing response characterized by accumulation of extracellular matrix (ECM) that follows chronic liver disease. The ECM components in fibrotic liver are similar regardless of the underlying cause. Activation of hepatic stellate cells is the central event in hepatic fibrosis these persiniusoidal cells orchestrate an array of changes including degradation of the normal ECM of liver, deposition of scar molecules, vascular and organ contraction and release of cytokines [1].

The prognosis and management of chronic liver diseases after depend strongly on the degree of liver fibrosis. This is particularly true of chronic hepatitis C virus infection[2]. Until recently, liver biopsy (LB) examination was the only way of evaluating liver fibrosis[3]. However, LB examination is invasive and painful and can have life threatening LB examination can lead to treatment delays, and LB examination is difficult to repeat in poorly, symptomatic subjects. The accuracy of LB examination for assessing fibrosis also has been questioned because of sampling errors and intra- and interobserver variability that may lead

to over or understaging of fibrosis [4].

So, there is a need for accurate noninvasive methods of measuring the degree of liver fibrosis proposed approaches include physical examination, routine biochemical and hematological tests such as aspartate transaminase to platelets ratio index (APRI) [5] and more recently transient elastography (fibroscan) [6].

Transient elastography (fibroscan) is a novel, rapid, noninvasive and reproducible method for measuring liver stiffness. Briefly, an ultrasound transducer probe is mounted on the axis of a vibrator; vibrations of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissue. Pulse-echo ultrasound acquisitions are used to follow the propagation of the shear wave and to measure its velocity, which is related directly to tissue stiffness. The stiffer the tissue, the faster the shear wave propagates. Preliminary reports suggest that fibroscan accurately predicts hepatic fibrosis in patients with chronic hepatitis C [7].

Aim of the study:

The aim of this study to compare the diagnostic performance of fibroscan with that of liver biopsy and routine biochemical and hematological test (the aspartate transaminase to platelets ratio index APRI) in the assessment of fibrosis in chronic hepatitis C. Also we aimed to estimate the degree of improvement in fibrosis scores after successful antiviral treatment of chronic hepatitis C.

Patients and Methods

The study was conducted on 134 chronic HCV patients diagnosed by seropositivite HCV antibodies and HCV RNA by PCR. Patients were selected from Mansoura International Hospital for assessment prior to interferon therapy as a part of the national program for combating viral hepatitis (HCV). All patients were subjected to full history taking, clinical examination and investigation in the form of liver biopsy (ultrasound guided), liver stiffness measurement using fibroscan and

other laboratory tests. Nine patients were excluded because biopsy specimens length < 1.5 cm or contain < 10 portal tracts.

The patients were included according to the following criteria:

Inclusion criteria:

1) Age > 18 years and < 60 years.
2) Compensated liver disease (total serum bilirubin < 1.5 mg/dl, INR < 1.5, serum albumin > 3.5 gm/dl).
3) Acceptable hematological indices (hemoglobin ≥12 g/dl, White blood cell count (WBC) ≥4,000/mm^3, Neutrophil count ≥2,000/mm^3, Platelets count ≥100,000/mm^3).
4) Signed written informed consent for this study and willing to be treated and to adhere to treatment requirements.

Exclusion criteria:

- Liver biopsy contraindications: [international normalized ratio INR > 1.6, platelets < 100,000/mm^3].
- Liver diseases other than HCV (as metabolic, storage, immune liver diseases).
- Decompensated liver disease.
- Pregnancy or breast feeding.
- Active seizures which require medication.
- Immunologically mediated diseases (ulcerative colitis, Crohn's disease, ITP, SLE, autoimmune hemolytic anemia, scleroderma, severe psoriasis, rheumatoid arthritis).
- Organ transplants.
- Substance abuse.
- Patients with BMI > 30.
- Severe pre-existing psychiatric conditions.
- HBsAg positive.

Patients were subjected to the following:

1- History taking and full clinical assessment.
2- Complete blood picture especially (HB, RBCs, platelets, absolute neutrophilic count).
3- Liver function tests such as (AST, ALT, Total serum bilirubin [direct and indirect bilirubin], serum albumin, serum alkaline phosphatase, prothrombin time and inter-

national normalized ratio [INR]).

4- Renal function tests such as (S.creatinine, Bl. urea).

5- B-HCG for all females in the child bearing period.

6- HBsAg was routinely checked for diagnosis of chronic HBV infection.

7- Antischistosomal antibodies were tested.

8- Alpha-fetoprotein.

9- TSH.

10- ANA (antinuclear antibodies).

11- ECG, fundus examination.

12- Abdominal ultrasound was performed to evaluate liver condition including (size, surface, texture).

13- Liver stiffness measurement using the transient elastography (fibroscan) [8].

- It was performed on the same day of liver biopsy using the fibroscan device (Echosense, Paris, France) which consist of 5MHz ultrasound transducer probe mounted on the axis of a vibrator.
- Vibration of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissues.
- Velocity is directly related to tissue stiffness, the stiffer the tissue, the faster the shear wave propagates.
- The patient was lying in the dorsal decubitus with the right arm in maximal abduction. The tip of the transducer was covered with a drop of gel and placed on the skin, between the rib bones at the level of right lobe of the liver.
- Under the control of TM and A-mode, the operator located a liver portion at least 6 cm thick and free of large vascular structures, when the target area had been located, the operator pressed the probe button to commerce. Stiffness is measured on a cylinder of hepatic tissue of 1 am diameter and 2 to 4 cm of length.
- The median value of ten successful acquisitions expressed in kilopascal (kpa) and was kept as representative of liver stiffness meas-

urement. Only procedures with 10 validated measurements and a success rate of at least 60% are considered reliable.

14- Ultrasound guided liver biopsy: liver biopsy was performed using a semi-automatic true cut needle (16G). All biopsy specimens were analyzed independently by 2 experienced pathologists blinded to the results of fibroscan and APRI. Liver biopsy specimens were fixed in formalin and embedded in paraffin. Liver biopsies than contain less than 10 portal tracts (except for cirrhosis) were excluded from histological analysis. Liver fibrosis and necro inflammatory activity evaluated semiquantitatively according to the METAVIR scoring system. Also, steatosis, hemosidrosis and bilharzial ova were assessed.

Fibrosis was staged on a 0-4 scale as follows (METAVIR Score):

F0, No fibrosis.
F1, Portal fibrosis without septa.
F2, Portal fibrosis with rare septa.
F3, Numerous septa without cirrhosis.
F4, Cirrhosis.

Activity (A) was graded as follows (METAVIR Score):

A0, No histologic necroinflammatory activity.
A1, Mild activity.
A2, Moderate activity.
A3, Severe activity.

Patients eligible to interferon based therapy were received a pegylated interferon alpha 2a 180 microgram or pegylated interferon alpha 2b 1.5 mcg/kg weekly and an average dose of ribavirin about 15 mg/kg/d for 48 weeks. Follow up of those patients were done by quantitative PCR for HCV RNA at 12^{th} week and qualitative PCR for HCV RNA at 24^{th}, 48^{th}, 72^{nd} weeks. Patients who achieved sustained virological response (negative PCR for HCV RNA after 6 month of discontinuation of treatment) followed up for another 6 months. Re biopsy was done to these patients who achieved SVR and agreed to assess liver fibrosis after treatment.

Results

Table (1) shows that according to metavir scoring system, 53.6% of patients represent F0, F1 group, 26.4% of patients represent F2 group, 11.2% of patients represent F3 group and 8.8% of patients represent F4 group.

Table (2) shows that according to metavir scoring system, 33.6% of patients were A1, 58.4% of patients were A2 and 8% of patients were A3.

Table (5) shows APRI and fibroscan values in the studied patients, the mean APRI was 0.34 and mean of fibroscan was 11.16.

Table (6) shows the mean values of fibroscan for F0, F1, F2, F3, F4 stages were 3.7, 6.4, 9.1, 14.3, 43.7 respectively and there was statistical significance between different stages.

Table (7) shows that the mean values of fibroscan for A1, A2, A3 grade were 9.4, 12, 11.9 respectively and there was no statistical significance between different grades.

Table (8) shows that the mean values of fibroscan for patients with steatosis and patients without steatosis were 10.6 and 11.8 respectively and there was no statistical significance between the two groups.

Table (10) and Figure (5) show that our new fibroscan cut off level for detection of significant fibrosis F ≥2 was 7.85 (kPa) with sensitivity 67%, specificity 91%, positive predictive value (PPV) 88% and negative predictive value 73%.

Table (11) and Figure (6) show that our new fibroscan cut off level for detection of F ≥3 groups was 11.9 (kPa) with sensitivity 86%, specificity 85%, positive predictive value (PPV) 85% and negative predictive value (NPV) 86%.

Table (12) and Figure (7) show that our new fibroscan cut off level for detection of cirrhosis F = 4 was 21.3 kPa with sensitivity 95%, specificity 92%, positive predictive value (PPV) 92% and negative predictive value 95%.

Table (13) shows that the mean

values of APRI for F0, F1, F2, F3, F4 stages were 0.20, 0.30, 0.34, 0.43 and 0.51 respectively and there was statistical significance between different stages.

Table (14) shows that the mean values of APRI for A1, A2, A3 grades were 0.36, 0.33, 0.35 respectively and there were no statistical significance between different grades.

Table (15) shows that there was regression in fibrosis stages in some cases after interferon therapy but this does not reach statistical significance.

Table (16) shows that there was significant decrease in activity grades after interferon therapy.

Table (17) shows significant difference between age groups as regard SVR which was higher in 30-39y group and no significant difference between hemosedrosis and steatosis as regard SVR.

Table (1): **Fibrosis stages in liver biopsy according to Metavir scoring system before treatment.**

Fibrosis	No	%
0	9	7.2%
1	58	46.4%
2	33	26.4%
3	14	11.2%
4	11	8.8%
Total	125	100%

Table (2): Activity grades in liver biopsy according to Metavir scoring system before treatment.

Activity	No	%
1	42	33.6%
2	73	58.4%
3	10	8%
Total	125	100%

Table (3): Steatosis in liver biopsy before treatment.

Steatosis	No	%
Positive	29	23.2%
Negative	96	76.8%
Total	125	100%

Table (4): Hemosidrosis in liver biopsy before treatment.

Hemosidrosis	No	%
Positive	4	3.2%
Negative	121	96.8%
Total	125	100%

Table (5): APRI and fibroscan in the studied patients.

	Median	Range
Fibroscan	7.6	2.50 – 75
(APRI) AST/PLATLET	0.29	0.08 – 1.62

Table (6): Relationship between liver stiffness measured by fibroscan and fibrosis stages in liver biopsy according to Metavir scoring system in different stages.

	N	Mean ± SD	F	P value
0	9	3.70 ± 0.79	149.649	0.000
1	58	6.49 ± 1.44		
2	33	9.18 ± 1.67		
3	11	14.36 ± 3.52		
4	14	43.79 ± 15.44		

Table (7): Activity grades in liver biopsy according to Metavir scoring system in relation to fibroscan in different grades.

	N	Mean ± SD	F	P value
1	42	9.49 ± 10.77	0.661	0.518
2	73	12.02 ± 12.26		
3	10	11.91 ± 9.11		

Table (8): Steatosis in liver biopsy in relation to fibroscan.

	Steatosis	No	Mean ± SD	t	P value
Fibroscan	Positive	29	9.52 ± 10.64	0.874	0.384
	Negative	96	11.66 ± 11.80		

Table (9): Our fibroscan score cut off level for F ≥ 1 group according to ROC Curve.

	F ≥ 1 F0 versus F1, 2, 3, 4
Optimal cut off (Kps)	4.75
Sensitivity	88
Specificity	79
Accuracy	87
Predictive value of +ve result	82
Predictive value of –ve result	85

Table (10): Our fibroscan score cut off level for F ≥ 2 group according to ROC Curve.

	F ≥ 2 F0, 1 versus F2, 3, 4
Optimal cut off (Kps)	7.85
Sensitivity	67
Specificity	91
Accuracy	79
Predictive value of +ve result	88
Predictive value of –ve result	73

Table (11): Our fibroscan score cut off level for F ≥ 3 group according to ROC Curve.

	F ≥ 3 F0, 1, 2 versus F 3, 4
Optimal cut off (Kps)	11.9
Sensitivity	86
Specificity	85
Accuracy	85
Predictive value of +ve result	85
Predictive value of –ve result	86

Table (12): Our fibroscan score cut off level for F ≥ 4 group according to ROC Curve.

	F ≥ 4 F0, 1, 2, 3 versus F4
Optimal cut off (Kps)	21.35
Sensitivity	95
Specificity	92
Accuracy	94
Predictive value of +ve result	92
Predictive value of –ve result	95

Table (13): APRI in relation to fibrosis stages in liver biopsy according to Metavir scoring system in different groups.

	N	**Mean ± SD**	**F**	**P value**
0	9	0.21 ± 0.07	3.316	0.003
1	58	0.31 ± 0.28		
2	33	0.34 ± 0.19		
3	11	0.43 ± 0.14		
4	14	0.51 ± 0.21		

Table (14): APRI in relation to activity grades in liver biopsy according to Metavir scoring system in different groups.

	N	Mean ± SD	F	P value
1	42	0.36 ± 0.32	0.266	0.767
2	73	0.33 ± 0.19		
3	10	0.35 ± 0.19		

Table (15): Comparison between fibrosis stages in liver biopsy before and after interferon therapy.

Before therapy / **After therapy**	**2**		**3**		**4**		**Total**	
	No	%	No	%	No	%	No	%
2	5	25	5	25	2	10	12	60
3	3	15	1	5	3	15	7	35
4	0	0	0	0	1	5	1	5
Total	8	40	6	30	6	30	20	100
Chi-square	4.573							
P value	0.334							

Table (16): Comparison between activity grades in liver biopsy before and after interferon therapy.

Before therapy / **After therapy**	**1**		**2**		**3**		**Total**	
	No	%	No	%	No	%	No	%
1	2	10	0	0	0	0	2	10
2	1	5	13	65	2	10	16	80
3	0	0	0	0	2	10	2	10
Total	3	15	13	65	4	20	20	100
Chi-square	21.250							
P value	0.000							

Table (17): **Sustained virological response (SVR) in relation to age groups, hemosedrosis and steatosis.**

	SVR –ve (n = 52)	SVR +ve (n = 53)	P value
20-29y	**9 (17.3%)**	**18 (34%)**	**0.015**
30-39y	**17 (32.7%)**	**24 (45.3%)**	
40-49y	**22 (42.3%)**	**10 (18.9%)**	
50-60y	**4 (7.7%)**	**1 (1.9%)**	
Hemosedrosis			**0.317**
+ve	**1 (1.9%)**	**3 (5.7%)**	
–ve	**51 (98.1%)**	**50 (94.3%)**	
Steatosis			**0.205**
+ve	**13 (25%)**	**8 (15.1%)**	
–ve	**39 (75%)**	**45 (84.9%)**	

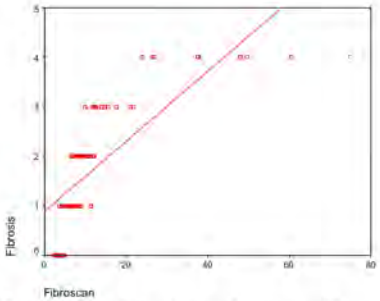

Figure (1): Correlation between fibroscan and fibrosis stages in liver biopsy according to Metavir scoring system.

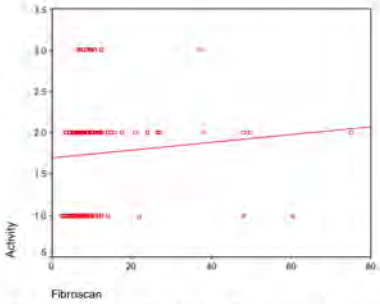

Figure (2): Correlation between fibroscan and activity grades in liver biopsy according to Metavir scoring system.

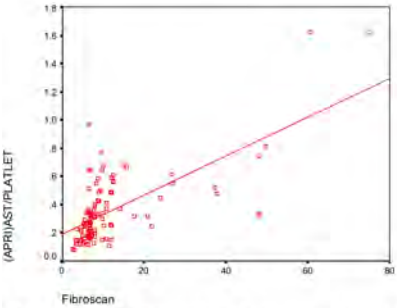

Figure (3): Correlation between fibroscan and APRI (r = 0.245, P=0.004).

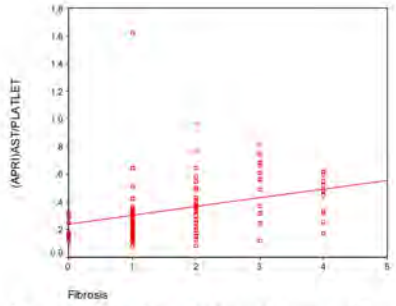

Figure (4): Correlation between fibrosis stages in liver biopsy according to Metavir scoring system and APRI (r = 0.279, P = 0.002).

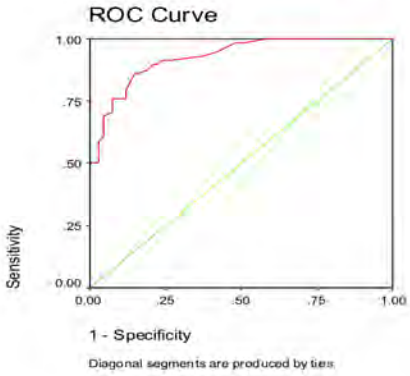

Figure (5): ROC Curve to detect our fibroscan cut off level for F ≥ 2 group.

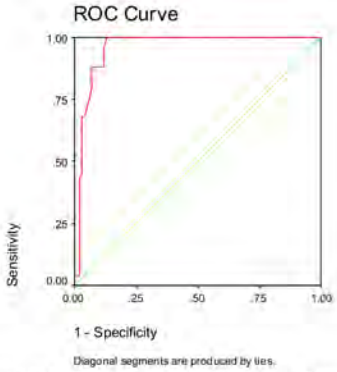

Figure (6): ROC Curve to detect our fibroscan cut off level for F ≥ 3 group.

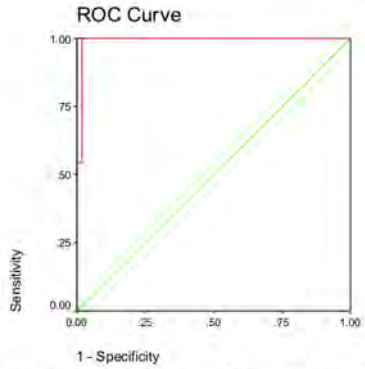

Figure (7): ROC Curve to detect our fibroscan cut off level for F ≥ 4 group.

Discussion

The development of liver fibrosis is the underlying pathogenic phenomenon that leads to most clinical complications in patients with chronic hepatitis C virus (HCV) infection [9].

Liver biopsy is currently "The gold standard" for assessing hepatic fibrosis. It is an invasive and painful procedure, however, with rare but potentially life threatening complications. In addition, the accuracy of liver biopsy in assessing fibrosis may be questioned because of sampling error and inter-observer variability. Thus, there is a need to develop and validate non invasive tests that can accurately reflect the full spectrum of hepatic fibrosis [10].

Ideally, a non invasive marker of liver fibrosis should be liver specific, easy to perform, reliable and inexpensive. It should in addition to be accurate not only for the staging of fibrosis, but also for the monitoring of disease progression and antiviral therapy efficacy. A variety of methods including the measurement of liver stiffness using transient elastography (TE) and serum markers as APRI (the aspartate transaminase to platelets ratio index), have been proposed for the non invasive assessment of hepatic fibrosis [8].

In the present study and as regarding the results of pathology 9 patients were F0, 58 patients were F1, 33 patients were F2, 14 patients were F3 and 11 patients

were F4 as assessed by Metavir score and the mean of liver stiffness measurements were 3.7, 6.4, 9.1, 14.3 and 43.7 kpa respectively.

We found a significant positive correlation between liver stiffness measurement (Fibroscan) and fibrosis stages in patients with chronic hepatitis C (r = 0.77, P = 0.000). This observation is consistent because stiffness of tissues largely depends on their molecular building blocks (collagen) and on the microscopic structural organization of these blocks (septa) [11]. But there was no significant correlation between liver stiffness measurement (Fibroscan) and the degree of necroinflammatory activity or steatosis (P = 0.311, P = 0.384 respectively). These results agreed with Foucher et al. [12] who found that correlation of liver stiffness was not affected by steatosis or activity grade but there was significant correlation between liver stiffness measurements and fibrosis stages assessed by liver biopsy.

The current study concluded that the cut off level of significant fibrosis F ≥ 2 assessed by the Metavir score as regard fibroscan was (7.85) kpa with AUROC of (82%). Sensitivity of (67%) and specificity of (91%). Castera et al. [8] reported cut off level of 7.1 kpa for detection of significant fibrosis. This small difference may be attributed to the number of patients in this group (134 versus 183 patients in Castera study) and fat distribution in patients. Also our study was done on Egyptian patients where HCV genotype 4 is prevalent but Castera studied other types.

In this study, the cut off was 11.9 kpa with AUROC of (91%) sensitivity of (86%) and specificity of (85%) for patients group F ≥ 3. Also, we reported a cut off level of 19.3 kpa for detection of cirrhosis with AUROC of (96%), sensitivity of (95%) and specificity of (92%). But, Castera et al.[8] reported a cut off level of 12.5 kpa for detection of cirrhosis. So the increase in our cut off value for F4 may be due to schistosmal coinfection in our patients. Also the prevalence of cirrhosis varied in both studies (11% versus 25% in Castera study).

Gomez-Domingues et al. [13] reported that the cut off for F4 is 15.6 kpa in patients with primary biliary cirrhosis while Marcellin et al. [14] said that the cut off for F4 is 18.2 kpa in patient with hepatitis B virus. Many studies reported different cut off values of liver stiffness measurement for the diagnosis of cirrhosis. This difference may be explained as the aetiologies of liver diseases in these studies were different.

This study showed that the results of detection of F0, F1 group and F4 group is better than F2, F3 group and this agreed with Foucher et al.[12]. They reported that the best results of transient elastography at differentiating cirrhosis versus non cirrhosis. So fibroscan can be used for diagnosis or exclusion of liver cirrhosis.

Our study reported that there was significant positive correlation between APRI and both fibroscan values and different fibrosis stages in liver biopsy according to Metavir scoring system (P = 0.004, P = 0.002 respectively). Also this study concluded that APRI has a high positive predictive value to identify patients with significant fibrosis and a high negative predictive value to Rule out cirrhosis and this agreed with Wai et al.[5] who showed that it is possible to predict the presence or absence of significant fibrosis in 51% of patients and to predict the presence or absence of cirrhosis in 81% of patients, but not agreed with Lieber et al.[15] and Shiha[16]. It may be attributed to that their studies or patients with alcoholic liver disease and chronic hepatitis B virus respectively.

In this study, 20 patients were assessed to study the impact of treatment and histological outcome after interferon therapy. We found that there was significant decrease in activity grades according to Metavir scoring system after interferon therapy. Also there was regression in fibrosis stages in some cases after interferon therapy but this does not reach statistical significance and this may be due to the number of patients and short term follow up of those patients. The decrease in fibrosis stages in those patients support the idea about reversibility of hepatic fibrosis.

This is supported by Issa et al.[17] who showed that in clinical circumstances where an effective treatment for the underlying insult is available, remodeling of the scar tissue can occur and a return towards architectural normality has been documented even in advanced fibrosis. This has been most clearly documented in autoimmune liver disease. Also, in haemochromatotic patients after vensection and in patients with hepatitis B and C after successful interferon therapy.

Regression of fibrosis studied by Bateller and Brenners[18] which showed that the hepatic mRNA content of TIMPS dropped greatly in livers which concided with the most rapid phase of collagen degradation by collagenase. Another important observation was that there was prominent apoptosis of activated HSC during recovery.

Conclusion

Non invasive methods such as transient elastography (Fibroscan) and APRI are easy and quick clinical non invasive methods for assessment of fibrosis. Results are available immediately and they are accurate methods for prediction of significant fibrosis, detection of cirrhosis and in the follow up of patients during and after receiving antiviral treatment. Also non invasive methods can be helpful in patients refuse biopsy and in patients with relative contraindication to liver biopsy (such as hemophilia or anticoagulation therapy in patients who are at high risk of developing thrombotic events if treatment is interrupted). But this non invasive methods have limitations. First, these tests generally have not been applied to the evaluation of other potentially contributing disease processes such as non alcoholic steatohepatitis. Second, the degree of hepatic inflammation is not assessed by these tests. Third, the utility of these tests has not been evaluated in selected populations such as dialysis patients, HIV patients persons and liver transplant recipients. Fourth, fibroscan is not helpful in obese patients as the fatty thoracic belt attenuates elastic waves making liver stiffness measurement impossible. Combination of pegylated interferon and ribavirin could improve histologi-

cal activity grade but not fibrotic stage after treatment.

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PROSPECTIVE COMPARISON OF TRANSIENT ELASTOGRAPHY AND LIVER BIOPSY FOR THE ASSESSMENT OF FIBROSIS IN CHRONIC HEPATITIS C BEFORE AND AFTER INTERFERON THERAPY

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EXPRESSION OF TENASCIN DURING PRENATAL DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM IN ALBINO RATS

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Abstract

Background: *Tenascin is an extracellular matrix (ECM) glycoprotein abundantly expressed in neural and non-neural tissues. In the developing central nervous system (CNS) of rodents, Tenascin is expressed in specific spatial and temporal patterns in different brain regions. This expression must be of importance during major developmental processes such as cell proliferation, cell migration and axonal guidance, as well as synaptic plasticity.*

Aim of the work: *The present study was designed to detect the expression of tenascin in CNS of the rat embryos to study its possible role in the development of CNS.*

Material and Methods: *Seventy (70) Albino rat embryos (obtained from twenty (20) albino rat females) were included in the present study. Matting of male and female rats were allowed and Females were checked for the presence of vaginal plugs each morning; which represents the day 0 of pregnancy (E0). The embryos of pregnant female rats were obtained after scarifying female rats at different periods of pregnancy as the following:* ***Group1:*** *at E8;* ***Group 2:*** *at E10;* ***Group 3:*** *at E12;* ***Group 4:*** *at E14;* ***Group 5:*** *at E16;* ***Group 6:*** *at E18;* ***Group 7:*** *at E20. Each group consisted of 10 embryos. All rats were taken as a whole mount, then they were put into paraffin blocks to be sectioned for immunohistochemical staining. Longitudinal sections were incubated with primary antibody; tenascin antibody. These sections were incubated with biotinylated anti-mouse secondary antibody followed by the avidin-biotin complex (ABC) method. Finally, the sections were visualized*

with 3, 3-diaminobenzidine (DAB). The slides were studied using light microscopy and subjected to morphometric and Statistical analysis.

Results of the present study revealed the following: *Tenascin was recognized in ventricular zones with the age of E10, 12, and 14 days; the maximal increase was in E14. In subventricular zone, tenascin expressed only in E14 and E16 days with the level increased in E16 more than E14; In cerebral cortex, tenascin was expressed at E16, 18 and 20 days. The maximal increase was in E20 . In corpus striatum, it was expressed in E14 and increased in E16. In hippocampus, it was expressed in E18 and increased in E20 ; In spinal cord, tenascin was recognized in E16, 18 and 20 days. The maximal expression was in E20 .*

Conclusion: *The present study revealed that, the expression of tenascin in the development of CNS of albino rat embryos. The expression of tenascin was different in different areas of CNS and increased with the increase of age due to proliferation and migration of cells during development.*

Keywords: *tenascin expression, prenatal development, central nervous system*

Introduction

Most cells do not float freely in the body but are linked to each other and to the surrounding environment, extracellular matrix (ECM). The ECM is secreted by the resident cells and is composed of supporting molecules (collagens and protoglycans) as well as extracellular linkage molecules. The shape of cell, their capacity for motility and their polarity are determined by how the cell interacts with its neighboring cells and extracellular matrix. These interactions are known as cell to cell and cell to matrix interaction [1].

Maris et al.[2] mentioned that the extracellular matrix plays a number of central roles in many biological processes, such as proliferation, angiogenesis and migration which all are achieved through its principal elements, tenascin and thrombospondin. Tenascin is an extracellular matrix glycoprotein abundantly expressed in neural and non-neural tissues. In the developing central

nervous system (CNS) of rodents, tenascin is expressed in specific spatial and temporal patterns in different brain regions. This expression must be of importance during major developmental processes such as cell proliferation, cell migration and axonal guidance, as well as synaptic plasticity [3].

Jones and Jones [4] mentioned that the extracellular matrix proteins, tenascin (TN) family of glycoproteins (TN-C, TN-R, TN-W, TN-X, and TN-Y) display highly restricted and dynamic patterns of expression in the embryo, particularly during neural development, skeletogenesis, and vasculogenesis. These molecules are reexpressed in the adult during normal processes such as wound healing, nerve regeneration, and tissue involution, and in pathological states including vascular disease, tumorigenesis, and metastasis.

Tenascin R (TN-R), a member of the tenascin gene family, is highly expressed in perineuronal nets of inhibitory interneurons. Tenascin R expression is being started by corpus striatum, at late embryonic stages. TNR is also expressed by oligodendrocytes, immature astrocytes in the somatosensory cortex and at nodes of Ranvier in the central nervous system [1].

Tenascin is believed to be important for several cellular processes including adhesion, migration, and proliferation of cells at different regions. Tenascin may play its important roles in nervous tissue histogenesis, possibly by modulating signal transduction across the plasma membrane. Also, tenascin is able to play a number of morphoregulatory roles during the processes of development and tissue remodeling as well as in disease [2].

The objective of this study is to study the expression of tenascin in the development of central nervous system of the albino rat embryos at different ages.

Materials and Methods

Thirty (30) adult albino rats (20 females and 10 males) were brought from animal house, Cairo Faculty of Medicine. Rats were housed in standard individual

maternity cages under controlled temperature and humidity with free access to water and food. Matting of male and female rats were allowed and females were checked for the presence of vaginal plugs each morning; which represents the day 0 of pregnancy (E0). Seventy embryos of pregnant female rats were obtained after scarifying female rats at different periods of pregnancy and were divided into 7 groups. **Group1:** at E8; **Group 2:** at E10; **Group 3:** at E12; **Group 4:** at E14; **Group 5:** at E16; **Group 6:** at E18; Group 7: at E20. Each group consisted of 10 embryos, the embryos are taken as a whole mount after extraversion of the internal organs of abdomen and thorax and then they were put into paraffin blocks to be sectioned for immunohistochemical staining. Longitudinal sections were incubated with primary tenascin antibody. These sections were incubated with biotinylated anti-mouse secondary antibody followed by the avidin-biotin complex (ABC) method. Finally, the sections were visualized with 3, 3-diaminobenzidine (DAB). The slides were studied using light microscopy and subjected to morphometrical and Statistical analysis. Anesthesia and sectioning were done according to method described by[5]. Sections were stained with hematoxylin and Eosin[6] and immunostained by the indirect immunohistochemistry using avidin-biotin complex (ABC)[7].

Statistical analysis: The densities of tenascin expression in different parts of the central nervous system in all studied age groups were organized, tabulated and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 16, running on IBM compatible computer with Windows 7 operating system. For quantitative data, mean and standard deviation (SD) were calculated and for comparison between two means, the student (t) test was used. For comparison between more than two means, the one way analysis of variance (ANOVA) tests was used. For interpretation of results, the p value ≤ 0.05 was considered significant.

Results

Hematoxylin and eosin stained sections: Light microscopic examination of parasagittal

sections of albino rat embryo aged (E18 day), showed cerebral cortex (CC), thalamus (TH), corpus striatum (CS), midbrain (MB), pons (PO), medulla (M) cerebellum and spinal cord (SC) (figure 1).

Immunostained sections: Extracellular matrix tenascin expression was assessed morphologically in different parts of central nervous system, ventricular zone, sub-ventricular zone, sub-plate zone, preplate zone, cortical plate zone, marginal zone ,corpus striatum, hippocampus and spinal cord. The intensity differed from one age group to another.

i- Brain: in the present study, tenascin expression could not be detected before E10. The expression, started at E10 and gradually increased with the age. Expression of tenascin at E10 was limited to the ventricular zone (fig. 2). At E12, expression of tenascin was increased (fig.3) and started to invade subventricular zone at E14 (fig. 4). Then, it gradually increased till it completely covered the subventricular zone at E16 (fig. 5). Regarding the cerebral cortex, tenascin started to appear at E16 by limited expression at the preplate zone (fig. 6). At E18, the cortex appeared divided into 3 zones: sub-plate, cortical plate and marginal zones, with unequal expression at the three zones, being most expressed in cortical plate more than subplate than marginal zones (fig. 7). Finally at E20, the cortex appeared as single zone (cortical zone) and it showed intense expression of tenascin (Fig.8). Regarding subcortical region, tenascin expression could be detected in corpus striatum at E14, and increased in intensity in E16 (figs.9, 10). In addition, tenascin expression could be detected in hippocampus at E18, which increased in intensity till E20 (Figs. 11, 12).

ii- Spinal Cord: tenascin expression could be detected at the dorsal plate of the spinal cord at E16 (Fig. 13), and started to appear in the ventral plate of spinal cord at E18. At E20, tenascin was equally expressed in both plates (Fig. 14).

Morphometric assessment of tenascin expression in studied age groups: The densities of tenascin expression were assessed

in different parts of the central nervous system in all studied age groups. The mean values of tenascin densities were calculated and compared with each other. The mean percentage values of density of tenascin in the ventricular zone were 5.30±1.16; 7.20±1.14; 9.50±1.08 in groups 2, 3 and 4 in sucdcession. In subventricular zone, the mean values were 15.20±1.32, 19.30±1.77 in groups 4 and 5 respectively. In cerebral cortex, it was 34.10±2.60, 38.80±3.49 and 53.90±10.26 in groups 5, 6 and 7 in succession. The mean values of density of tenascin in corpus striatum were 16.100±1.79, 20.80±2.74 in groups 4 and 5 respectively. In hippocampus, the mean values of density of tenascin were 23.60±3.60, 28.10±4.07 in groups 6 and 7 respectively. In spinal cord, the mean values of density of tenascin were 5.40±0.96, 7.40±0.96 and 9.60±1.43 in groups 5, 6 and 7 in succession. In the ventral root of spinal cord the mean values were 5.30±0.95, 2.80±1.14 and 4.70±1.25 in groups 5, 6 and 7 in succession and in the dorsal root of spinal cord the mean values were 4.60±0.97, 4.90±1.37 in groups 6 and 7 respectively. In ventricular zone, there was statistically significant increase of tenascin density in group 4 (E14) when compared to groups 3 (E12) and group 2 (E10) .In sub ventricular zone and corpus striatum, there was significant increase of tenascin density in group 5 (E16) in comparison to group 4 (E14). In cerebral cortex, there was significant increase of tenascin density in group 7 (E20) in comparison to groups 6 (E18) and group 5 (E16)(table1).In hippocampus, there was significant increase of tenascin density in group 7 (E20) in comparison to group 6 (E18) (table 1).

In group 4 (E14), there was significant increase of tenascin density in corpus striatum in comparison to sub ventricular and ventricular zones. On the other hand, there was significant increase of tenascin density in sub ventricular zone in comparison to ventricular zone. In group 5 (E16), there was significant increase of tenascin density in cerebral cortex when compared to subventricular zone, corpus striatum and spinal cord. In addition, there was signif-

icant increase of tenascin density in subventricular zone and corpus striatum when each compared to the spinal cord. In group 6 (E18), there was significant increase of tenascin density in cerebral cortex when compared to either spinal cord or hippocampus. In addition, there was significant increase in hippocampus when compared to spinal cord. In group 7 (E20), there was significant increase in cerebral cortex when compared to spinal cord and hippocampus. In addition, there was significant increase in hippocampus when compared to the spinal cord. In spinal cord, there was significant increase of tenascin density in group 7 (E20) in comparison to groups 6 (E18) and 5 (E16). Also, there was statistically significant increase of tenascin density in the dorsal plate of spinal cord in group 5 (E16) in comparison to group 6 (E18) and no significant difference in the dorsal plate between group 5 (E16) and group 7 (E20). In the ventral plate of spinal cord there was significant increase of tenascin density in group 6 (E16) in comparison to groups 5 (E16) and 7(E20) in addition there was significant increase of tenascin density In the ventral plate in group 7 (E20) in comparison to group 5 (E16). When compared tenascin density of dorsal plate with ventral plate in group 5 (E16), there was significant increase of tenascin density in dorsal plate, while in group 6 (E18), there was significant increase of tenascin density in ventral plate. In group 7 (E20), there was no significant difference between dorsal and ventral plates (table 2).

Table (1): The mean percentage values of density of tenascin in different areas of central nervous system in different groups of albino rat embryos.

Variables	Groups (mean±SD)						F*-t	P
	group2 (E10)	group3(E12)	group4(E14)	group5(E16)	group 6(E18)	group7(E20)		
V	5.30±1.16	7.20±1.14	9.50±1.08	-	-	-	F* = 282.67	0.000(S)
SUB V	-	-	15.20±1.32	19.30±1.77	-	-	t = 5.88	0.000(S)
CC	-	-	-	34.10±2.60	38.80±3.49	53.90±10.26	F* = 279.69	0.000(S)
CS	-	-	16.10±1.79	20.80±2.74	-	-	t = 4.54	0.000(S)
H	-	-	-	-	23.60 ±3.60	28.10±4.07	t = 2.62	0.02 (S)
SC	-	-	-	5.40±0.96	7.40±0.96	9.60±1.43	F* = 283.66	0.000(S)
DP	-	-	-	4.60±0.97	1.20±0.75	4.90±1.37	F* = 128.61	0.000(S)
VP	-	-	-	2.80±1.14	5.30±0.95	4.70±1.25	F* = 98.14	0.000(S)

*= ANOVA TEST V=ventricular zone CC=cerebral cortex SUB V= sub ventricular zone
S = significant CS= corpus striatum H=Hippocampus VH=ventral plate
SC=spinal cord DP=dorsal plate P < 0.05 Significant

Table (2): comparison of tenascin density of different areas of central nervous system in each group of albino rat embryos.

GROUPS	VARIABLES						F*	P
	V	SUB V	CC	CS	H	SC		
group 4 (E14)	9.50±10.8	15.20±1.32	-	16.10±1.79	-	-	62.87	0.000 S
Group 5 (E16)	-	19.30±1.77	34.0±2.60	20.80±2.74	-	5.40±0.96	298.3	0.000 S
Group 6 (E18)	-	-	38.80±3.49	-	23.60 ±3.60	7.40±0.96	283.71	0.000 S
Group 7 (E20)			53.90±10.26		28.10±4.07	9.60±1.43	119.89	0.000 S

S = significant *= ANOVA TEST; V = ventricular; SUB V = subventricular;
CC = cerebral cortex; H = Hippocampus; SC: spinal cord

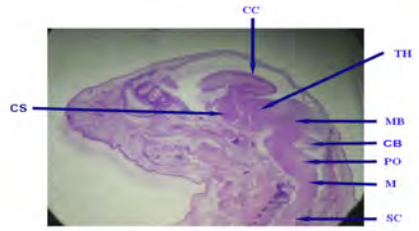

Figure (1): A photomicrograph of parasagittal section of the brain and spinal cord of albino rat embryo aging (E18), showing cerebral cortex (CC), thalamus (TH), corpus striatum (CS), midbrain (MB), pons (PO), medulla (M) , cerebellum (CB) and spinal cord (SC).

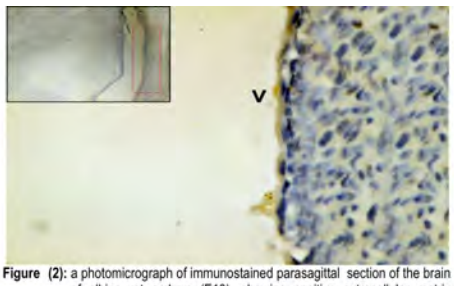

Figure (2): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E10), showing positive extracellular matrix tenascin expression in the ventricular zone (X 1000) (inset 100)

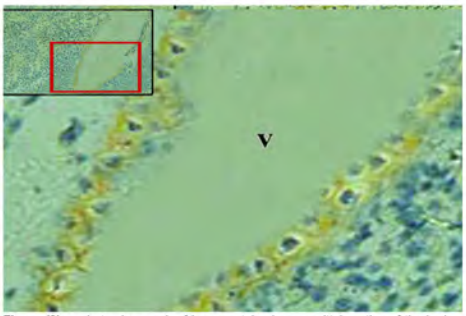

Figure (3): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E12), showing an increase of positive extracellular matrix tenascin expression in the ventriclular zone. (X 400) (inset 100)

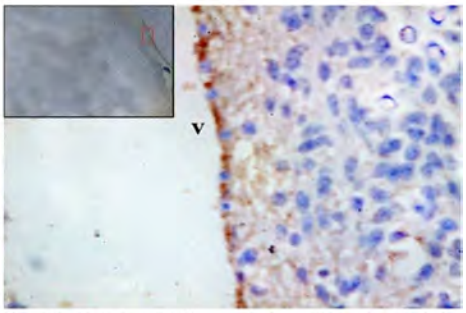

Figure (4): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E14), showing relative an increase of positive extracellular matrix tenascin expression in the ventricular and subventricular zone (X 1000). (inset 100)

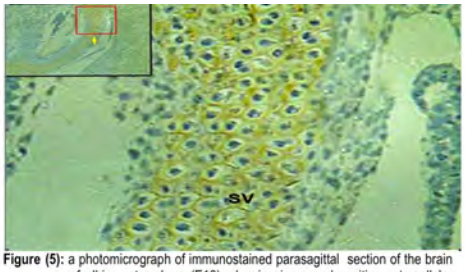

Figure (5): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E16), showing increased positive extracellular matrix tenascin expression in the sub ventricular zone (X 1000) (inset 100)

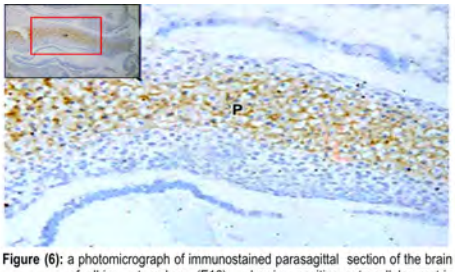

Figure (6): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E16), showing positive extracellular matrix tenascin expression in the preplate zone of the cerebral cortex (X 400) (inset 100)

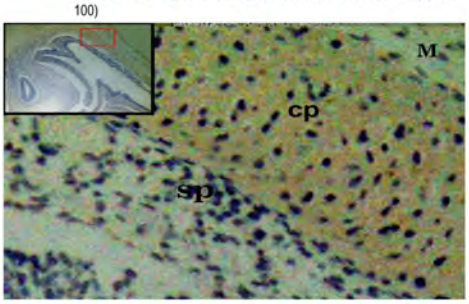

Figure (7): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E18), showing positive extracellular matrix tenascin expression in the sub plate, cortical plate and marginal zone of the cerebral cortex (X1000) (inset 100)

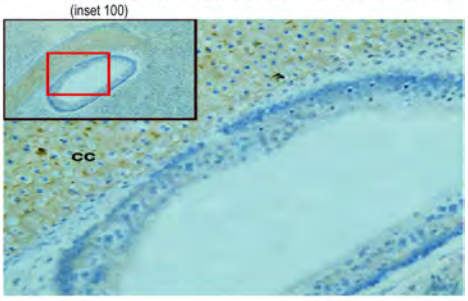

Figure (8): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E20), showing positive extracellular matrix tenascin expression of the whole cerebral cortex (X 400) (inset 100)

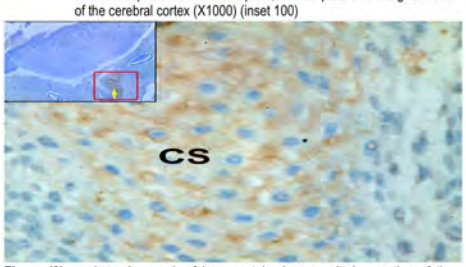

Figure (9): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E14), showing positive extracellular matrix tenascin expression in the corpus striatum (X 1000) (inset 100)

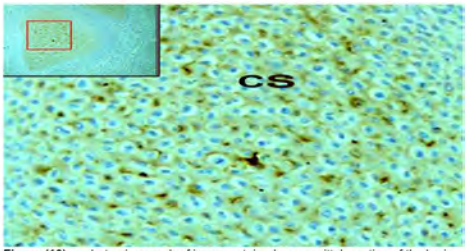

Figure (10): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E16), showing increased positive extracellular matrix tenascin expression in the corpus striatum (X 1000) (inset 100)

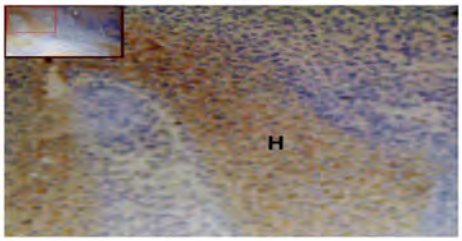

Figure (11): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E18), showing positive extracellular matrix tenascin expression in the c-shaped hippocampus (X 400) (inset 100)

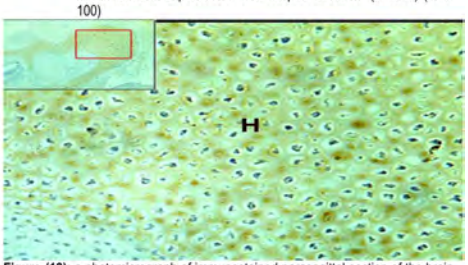

Figure (12): a photomicrograph of immunostained parasagittal section of the brain of albino rat embryo (E20), showing increased positive extracellular matrix tenascin expression in hippocampus (X 1000) (inset 100)

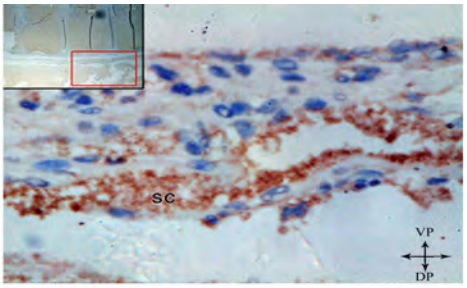

Figure (13): a photomicrograph of immunostained parasagittal section of the spinal cord of albino rat embryo (E16), showing positive tenascin expression in the dorsal and ventral plates of the spinal cord (X 1000) (inset 100)

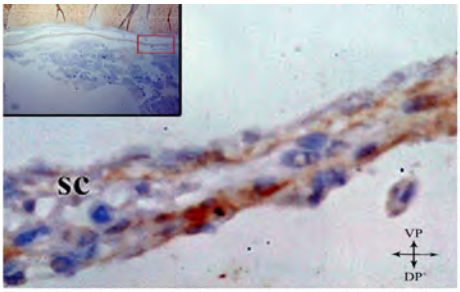

Figure (14): a photomicrograph of immunostained parasagittal section of the spinal cord of albino rat embryo (E18), showing positive tenascin expression in the ventral plate of the spinal cord (X 400) (inset 100)

Discussion

Tenascins are a family of glycoproteins found in the ECM of many tissues. In vertebrates, the tenascin family consists of only four members named tenascin-C, tenascin-X, tenascin-R and tenascin-W [8]. The first member of the tenascin family, known as tenascin-C (TN-C), was discovered independently almost 30 years ago by several groups which simultaneously investigated different aspects of cell, developmental, and tumor biology [9]. This new protein, tenascin attracted interest because of its selective expression in developing and pathological tissues. The expression of Tenascin showed oncofetal predominance at epithelial-mesenchymal interaction with prominent expression seen during embryogenesis and tumorigenesis [10].

In the present study, tenascin upregulated in ventricular zones with the age of E10, E12, and E14; the maximal increase was in E14. These results agreed with[11] who reported that, tenascin is prominently expressed during forebrain development in the ventricular and subventricular zone where neural stem cells reside. In accordance with these results, Tucker et al.[12] reported that, restricted expression of TN has been reported in the developing chicken central nervous system. They suggested that TN is a component of the Reissner's fibre, a thread-like strand of extracellular matrix that is produced by floor plate cells and extends the length of brain ventricles into the

central canal of the spinal cord.

In the present work, tenascin expressed in the subventricular zone only at the age of E14 and E16 and the level of expression was increased in E16 more than E14. In agreement with this study, Garcion et al. [13] reported that, tenascin is highly expressed in the subventricular zone as, a result of migration of cells from ventricular zone.

The present study showed limited tenascin expression in the preplate zone at E16. Then at E18 the cortex appeared divided into three zones, sub-plate, cortical plate and marginal zones, being most expressed in cortical plate zone. Then the cortex appeared as single zone at E20. The maximal increase of tenascin expression was in (E20). In agreement with this study, Allan et al. [14] found that, during embryonic period tenascin expression appeared first in preplate zone, then, sub-plate, cortical plate and marginal zone then distributed through the whole cortex. Tenascin is expressed from the onset of cortical development in radial glial cells which not only provide scaffolding for migrating neurons but also participate in the generation of both neuronal and glial cells [15].

As corticogenesis proceeds, TN appears in the subplate and marginal zones and becomes widely expressed in the cortex, from where it gradually disappears by the end of the second week. The temporal pattern of protein expression coincides with periods of generation and migration of diverse cortical cells, establishment of synaptic connections, myelination and rapid functional tissue maturation [3].

On the other hand, Yuasa [16] reported that, expression of tenascin is particularly prominent during neuronal differentiation and migration in the cerebral cortex. These results agreed with that of the present study.

In contradiction to results of the present study, Derr et al. [17] reported that, during early brain development, when cortical neurons migrate, no expression has been reported so far. In addition, it was reported that, in the cere-

bral cortex, TN may not be involved in neuronal migration or axonal growth, since it does not appear until after cortical layers and axonal pathways are established [18].

In this study, tenascin was expressed in corpus striatum at the age of E14 and E16, and increased in E16 more than E14. In many cases, tenascin, immunoreactivity is located in a discrete fashion, and one remarkable feature of the resulting patterns is the development of a boundary like appearance, which correlates with the functional subdivision of neuroanatomical systems. Thus, tenascin has been found in the so-called barrel boundaries of the somato-sensory cortex and at the interface between compartments in the developing nigro-striatal pathway [19].

In agreement with this work, Gunnar et al. [1] reported that, tenascin is highly expressed in perineuronal nets of inhibitory interneurons, including those in the striatum, which start to express tenascin at late embryonic stages.

In the present research, tenascin was expressed in hippocampus at the age of E18 and E20, and increased in E20 more than E18. In accordance with the present results, Alison et al. [20] reported that, the expression of tenascin by oligodendrocytes and small interneurons in the hippocampus and cerebellum is highly regulated during development of these regions. Erickson and Bourdon [21] added that this complex glycoprotein displays both adhesive and anti-adhesive properties that contribute to the formation and maintenance of synapse. In addition, the extracellular matrix (ECM) molecule tenascin has attracted interest due to its restricted and rapidly changing pattern of expression during embryogenesis. Tenascin is found especially at sites of mesenchyme-epithelium interaction in many developing organs.

In the study of spinal cord, tenascin expression was appeared at E16, E18 and E20. The maximal expression was in E20. Tenascin expression started at the dorsal plate of spinal cord then ventral plate after that the expres-

sion was equal in the two plates. In agreement with the present result Bartsch et al. [22] stated that, tenascin is an oligodendrocyte-derived molecule; it is maximally expressed during the time of active myelination, and it has been found to be associated with the surface of myelinating oligodendrocytes, neurons, and type 2 astrocytes, it appeared at the dorsal part where proliferation of precursor cells occur then migrated with the cell to other parts of cord. Jones and Jones [4] reported that, this expression must be of importance during major developmental processes such as cell proliferation, cell migration and axonal guidance, as well as synaptic plasticity in the adult organism.

The main findings of the present study showed that, tenascin were upregulated with age according temporal and spatial expression. These results indicate that, tenascin may play a pivotal role in CNS development. These results are in accordance with several previous reports, as it was reported that, TN- has so far been found exclusively expressed in the developing and adult CNS by oligodendrocytes and small subsets of neurons (mainly interneurons and motoneurons), such as the small inhibitory inter- neurons of the cerebellar cortex (stellate and basket cells), motoneurons in the spinal cord and the brain, and the horizontal cells of the retina [23]. In agreement with results of the present work, Pesheva and Probstmeier [24] reported that, TN-R protein and mRNA are amply present in the presumptive white matter within the pathways of oligodendrocyte precursor migration and during the phase of active myelination when the protein becomes abundant in the white matter of different CNS regions. After myelination have ceased, both mRNA and proteins levels are downregulated to lower adult values when TN-R appears predominantly associated with the surface of oligodendrocytes, myelinated axons and the nodes of Ranvier. With neuronal maturation (i.e. during the first two to three postnatal weeks in mammals), TN-R has been shown to accumulate in perineuronal nets of interneurons and motoneurons, often in a colocalization with TN-C.

Conclusion

The present study revealed that, the expression of tenascin in the development of CNS of albino rat embryos. The expression of tenascin was different in different areas of CNS and increased with the increase of age due to proliferation and migration of cells during development.

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EXPRESSION OF TENASCIN DURING PRENATAL DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM IN ALBINO RATS

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PROPHYLACTIC IMPACT OF CURCUMIN ON ADRIAMYCIN-INDUCED NEPHROTOXICITY : HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY ON ALBINO RAT

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Abstract

Objectives: *The present experimental study was designed to evaluate the impact of prophylactic curcumin (CUR) on chronic adriamycin (ADR) exposure-induced structural renal alterations.*

Materials & Methods: *The study comprised 30 normal healthy growing male albino rats divided into 3 groups: Control group given distilled water, ADR group injected once intraperitoneally by ADR in a dose of 50 mg/kg, 5 rats were sacrificed every 2 weeks after injection (ADR-2, ADR-4 & ADR-6) and CUR group included 15 rats received 200 mg/kg body weight of curcumin in 1% gum acacia, orally for 7 days before intraperitoneal injection of ADR in dose of 50 mg/kg and daily thereafter throughout the study and 5 rats were sacrificed every 2 weeks after injection (CUR-2, CUR-4 & CUR-6). Kidney tissue was obtained for histological studies using hematoxylin and eosin (Hx & E) and Masson's trichrome stains. Immunohistochemical and morphometric study to determine golmerular area cortical and medullary connective tissue percentage and the count of PCNA +ve endothelial, mesangial and interstitial nuclei.*

Results: *There was time-course progression of harmful effect of ADR on renal structure manifested as minimal congested renal glomeruli and interstitial congestion in ADR-2 specimens progressed in ADR-4 specimens to congested glomeruli with narrowing extending to partial obliteration of Bowman's space but in ADR-6 group, there were multiple shrunken and congested renal glomeruli with vacuolation of cytoplasm of the lining cells of the proximal tubules and intense mononuclear cellular infiltration. Minimal glomerular and interstitial congestion with*

preserved Bowman's space and no obvious cellular infiltration was reported in CUR-2 and CUR-4 groups, and CUR-6 rats showed picture comparable to control animals apart from mild congestion and occasional shrunken glomeruli with preserved Bowman's space and minimal cellular infiltration. In Masson's Trichrome stained sections, ADR-4 specimens showed focal dense fibrous bands that become dense in ADR-6 group, while few thin bands between the proximal tubules were detected in CUR-6 specimen. Multiple PCNA +ve rounded nuclei of mesangial cells were detected among the +ve flattened endothelial nuclei in glomeruli recruiting obliterated Bowman's space in ADR-4 group and in the shrunken glomeruli of ADR-6 specimens, while were occasional in CUR-6 group. Areas of PCNA +ve nuclei were detected between the proximal tubules were detected in ADR-4 groups and detected in wider areas in ADR-6 group, while group CUR-6 showed smaller areas of +ve flattened nuclei than in ADR-6. Mean glomerular area was significantly larger and smaller in groups ADR-4 and ADR-6 compared to groups CUR-4 and CUR-6, respectively. Both cortical and medullary connective tissue percentage were significantly higher in ADR-4 and ADR-6 groups compared to control group and compared to CUR-4 group both cortical and medullary connective tissue areas were significantly higher in ADR-4 group. Compared to control, mean PCNA positive nuclei of endothelial cells was significantly lower in ADR-6 animals, but mean PCNA positive nuclei of mesangial cells was significantly higher in ADR-4 and ADR-6. Mean positive PCNA count in nuclei of interstitial cells of ADR-6 animals was significantly higher compared to ADR-4 and CUR-6 animals, no positive PCNA nuclei were detected in interstitial cells in other groups.

Conclusion: *It could be concluded that prophylactic administration of curcumin could ameliorate the nephrotoxic effects of chronic treatment with adriamycin and was recommended in the tried dose for patients assigned to adriamycin treatment.*

Introduction

Adriamycin (ADR), a quinone-containing anthracycline cytotoxic antineoplastic drug, is used in the treatment of various solid tumors. It works by interfering with the function of DNA in rapidly dividing cells. Cancer cells divide particularly rapidly, and doxorubicin can cause them to die. However,

certain types of normal body cells also divide rapidly; doxorubicin damages them as well. This leads to a variety of possible side effects, including hair loss, digestive problems, reduced immunity, excessive bruising or bleeding, anemia, mouth sores and male infertility. The efficacy of adriamycin continues to be challenged by significant toxicities including nephrotoxicity[1].

In human renal disease, sustained heavy proteinuria is usually accompanied by rapid loss of renal function. Rats that are given adriamycin provide an experimental model of the association between proteinuria and loss of function, as described by Bertani et al.,[2]. These animals develop heavy proteinuria within a few weeks after adriamycin administration. Protein excretion rates remain high thereafter, and the GFR declines gradually,[3]. Various studies showed that the appearance of proteinuria is associated with injury to the glomerular visceral epithelial cells and that the subsequent decline in GFR is associated with both glomerular sclerosis and widespread tubulointerstitial injury[4].

Adriamycin associated nephrotic syndrome is characterized by heavy proteinuria, albuminuria, hypoalbuminaemia and hyperlipidaemia, [5]. Several lines of evidence suggest reactive oxygen species (ROS) as the principal mediator in the development of nephrosis caused by ADR. The hypothesis was proposed, that if ADR nephrotoxicity is related to free radical formation and lipid peroxidation then antioxidant therapy may protect ADR toxicity in kidney[6].

Agapito et al., [7] rreported a significant increase in lipid peroxidation products in brain, heart, and kidney tissues after a single administration of adriamycin, which was attenuated by pre- and post-treatment with a low pharmacological dose of melatonin and concluded that oxidative damage induced by adriamycin can be reduced by low pharmacological doses of melatonin. Sayed et al., (8) found Propionyl-l-carnitine, a naturally occurring compound that has been considered for the treatment of many forms of cardi-

omyopathies completely protects against ADR-induced inhibition of mitochondrial beta-oxidation of long-chain fatty acids; induces a powerful antioxidant defense mechanism against ADR-induced lipid peroxidation of cardiac membranes without effect on the antitumour activity of ADR.

Curcumin, the yellow curry pigment isolated from turmeric (Curcuma longa) is its active principle. Curcumin represents a class of anti-inflammatory antioxidants reported to be a potent inhibitor of reactive oxygen species formation and modulates hydrogen peroxide-induced peroxidation of microsomal membrane lipids and DNA damage,[9]. Curcumin has been also shown to provide protection against oxidative stress in a renal cell line,[10] and has been reported to reduce ischaemic renal injury,[11]. Therefore, the present study was undertaken to evaluate the protective effects of curcumin against ADR nephrotoxicity.

The present experimental study was designed to evaluate the impact of prophylactic curcumin on chronic adriamycin exposure-induced structural renal alterations.

Materials & Methods

The study comprised 30 normal healthy growing male albino rats, weighing 200-400 gm. Rats were purchased from the laboratories of Ministry of Agriculture, and kept under standard conditions, temperature 20oC, humidity 60% and 12-hs day/night cycle, and maintained on standard diet and free water supply till the start of study regimens. The animals were divided into 3 groups, each in a separate cage:

1. Control group included 6 rats given distilled water. One rat was sacrificed at the same time with rats of each experimental.
2. ADR group included 15 rats injected once intraperitoneally by ADR in a dose of 50 mg/kg, (2). Five rats were sacrificed every 2 weeks after injection (ADR-2, ADR-4 & ADR-6).
3. Curcumin (CUR) group included 15 rats received 200 mg/kg body weight of curcumin in 1% gum acacia, orally for 7 days before intraperitoneal injection of ADR in dose of 50 mg/kg and daily thereafter throughout

the study. Five rats were sacrificed every 2 weeks after injection (CUR-2, CUR-4 & CUR-6).

Rats were sacrificed by decapitation and kidney tissue was cut into 2 pieces, one fixed in 85% cold alcohol for histological studies and the other in 10% buffered formalin for immunohistochemical study:

a. Histological studies: included Hx & E and Masson's trichrome stain, [12].
b. Immunohistochemical stu-dy: using streptavidin-biotin peroxidase method, monoclonal antibody against proliferating cell nuclear antigen (PCNA) (DACO Cooperation) was performed. Tonsil sections served as positive control, [13].
c. Morphometric study: using Leica Qwin 500 LTD image analysis system, the area of renal glomeruli in Hx & E stained sections, the count of PCNA +ve endothelial and mesangial nuclei were measured in 10 low power fields (LPF) using interactive measurements menu. The area% of connective tissue fibres in cortex and medulla and the area of PCNA +ve interstitial nuclei were measured in 10 LPF using binary mode,[14].

Statistical analysis

Morphometric results were presented as mean±SD, ranges and ratios. Statistical analysis was conducted using Wilcoxon (Z-test) test for unrelated data using SPSS (Version 10, 2002). P value of <0.05 was considered significant.

Results

A) Histological results

Histological examination of the kidney specimens of the control group showed normal architecture of renal glomeruli, and proximal and distal tubules were detected in the renal cortex, (Fig. 1). High magnification of renal glomeruli showed normal endothelial cells, mesangial cells Bowman's space and glomerular blood capillaries, (Fig. 2).

In ADR group, there was time-course progression of harmful effect of ADR on renal structure manifested as the presence of minimal congested renal glomeruli and interstitial congestion in ADR-2 specimens progressed in ADR-4 specimens to congested glomeruli

with narrowing extending to partial obliteration of Bowman's space, (Fig. 3) with some mononuclear cellular infiltration, (Fig. 4). High magnification examination showed Malpighian corpuscles surrounded by proximal tubules with proliferated mesangial cells in addition to some infiltrating mononuclear cells between the proximal convoluted tubules, (Fig. 5). In ADR-6 group, there were multiple shrunken and congested renal glomeruli with vacuolation of cytoplasm of the lining cells of the proximal tubules, (Fig. 6) and intense mononuclear cellular infiltration, (Fig. 7).

Prophylactic curcumin highly ameliorated the renal toxic effect of ADR manifested as minimal glomerular and interstitial congestion in groups CUR-2 and CUR-4, (Fig. 8) groups with preserved Bowman's space and no obvious cellular infiltration. Such protective effect continued till 6-weeks, renal specimens obtained from CUR-6 rats showed picture comparable to control animals apart from mild congestion and occasional shrunken glomeruli but still with preserved Bowman's space and minimal cellular infiltration (Fig. 9).

Control specimens showed normal structure of the collecting tubules and no obvious changes were reported in ADR-2 and CUR-2 specimens from control specimens. However, obvious congestion with fibrotic infiltration was recorded in ADR-4 specimens and became dense in ADR-6 specimens, (Fig. 10) with vacuolated cytoplasm of some of the lining cells of some collecting tubules and completely vacuolated cytoplasm with flattening of nuclei of other tubules, (Fig. 11). Only minimal congestion was recorded in CUR-4 specimens and in CUR-6 specimens few fibroblasts were detected between the collecting tubules with minimal vacuolation of some lining cells of some tubules (Fig. 12).

In Masson's Trichrome stained sections; minimal connective tissue fibres were detected between the proximal convoluted tubules of the renal cortex in control, (Fig. 13) ADR-2, CUR-2 and CUR-4 groups. ADR-4 specimens showed focal dense fibrous bands that become dense in ADR-6 group, (Figs.

14). On contrary, few thin bands between the proximal tubules were detected in CUR-6 specimen, (Fig. 15). The renal medulla revealed minimal fibers between the collecting tubules in control, ADR-2, CUR-2 and CUR-4 groups. ADR-6 group showed multiple reticula of dense fibrous bands besides scattered connective tissue fibres among the collecting tubules, (Figs. 16). On the other hand, in CUR-6 group a single reticulum of fibrous bands was observed in the different fields examined, (Fig. 17).

B)Immunohistochemical results:

Few PCNA +ve rounded nuclei of mesangial cells appeared among more numerous +ve flattened nuclei of endothelial cells of glomerular capillaries in control, (Fig. 18), ADR-2, CUR-2 and CUR-4 groups, (Fig. 19), while in CUR-6 group occasional +ve rounded nuclei and some +ve flattened nuclei were detected, (Fig. 20). In group ADR-4 multiple PCNA +ve rounded nuclei of mesangial cells were detected among the +ve flattened endothelial nuclei in glomeruli recruiting obliterated Bowman's space, (Fig. 21), while in ADR-6 group multiple +ve rounded and few +ve flattened nuclei in the shrunken glomeruli, (Fig. 22).

Similarly, PCNA +ve immunostaining of few nuclei lining some of the proximal and distal tubule in the renal cortex and some of the collecting tubules in the renal medulla of control rats, (Fig. 23). In ADR-4 group, areas of PCNA +ve nuclei were detected between the proximal tubules, (Fig. 24) and ADR-6 group showed wider areas of PCNA +ve flattened nuclei between the renal tubules, (Fig. 25), while group CUR-6, (Fig. 26) showed smaller areas of +ve flattened nuclei than in ADR-6.

C) Morphometric results :

Compared to control group, mean glomerular area was significantly larger in groups ADR-2 and ADR-4 (Fig. 27) and CUR-4 and non-significantly in group CUR-2, while was significantly smaller in group ADR-6 and non-significantly smaller in group CUR-6. Mean glomerular area was significantly larger and smaller in groups ADR-4 and ADR-6 compared to groups CUR-4 and CUR-6, respectively, with non-signifi-

cant difference between groups ADR-2 and CUR-2, (Fig. 28).

Both cortical (Fig. 29) and medullary (Fig. 30) connective tissue areas were significantly higher in ADR-4 and ADR-6 groups, but non-significantly higher in other groups compared to control group. Compared to CUR-4 group both cortical and medullary connective tissue areas were significantly higher in ADR-4 group, while the difference was significant in cortical but was non-significant in medullary area in ADR-6 compared to CUR-6 group,(Table 1, Fig. 31 & 32).

Mean PCNA positive nuclei of endothelial cells was significantly lower in ADR-6 animals compared to control and other studied groups with non-significant difference between other studied groups compared to control and in-between, (Fig. 33). On contrary, mean PCNA positive nuclei of mesangial cells was significantly higher in ADR-4 and ADR-6 animals compared to control and other studied groups with significantly higher number in ADR-6 animals compared to ADR-4 animals. Also, the mean number of PCNA positive nuclei of mesangial cells was significantly higher in CUR-4 animals compared to control and CUR-2 and CUR-6 groups, with non-significantly different number between other groups, (Fig. 34). No positive PCNA nuclei were detected in interstitial cells in control, ADR-2, CUR-2 and CUR-4 groups; however, mean positive PCNA count in nuclei of interstitial cells of ADR-6 animals (Fig. 35) was significantly higher compared to number recorded in ADR-4 and CUR-6 animals, (Table 2 Fig. 36).

Table (1): Morphometric measures reported in studied groups.

	Mean glomerular area			Connective tissue area (Cortical)			Medulla connective tissue area		
	Area	Statistical analysis		Area (%)	Statistical analysis		Area (%)	Statistical analysis	
		t	p		t	p		t	p
Control	5.9±0.25			3.16±0.51			1.56±0.4		
ADR-2	6.2±0.41	1.087	$p_1>0.05$	3.7±0.3	2.529	$p_1>0.05$	1.7±0.3	0.775	$p_1>0.05$
ADR-4	5.98±0.25	0.560	$p_1>0.05$	5.68±0.4	7.418	$p_1=0.002$	2.02±0.3	2.875	$p_1=0.045$
ADR-6	2.66±0.32	15.885	$p_1<0.001$	7.72±0.78	16.585	$p_1<0.001$	4.1±0.9	4.486	$p_1=0.011$
CUR-2	6.02±0.25	0.802 2.706	$p_1>0.05$ $p_2>0.05$	3.4±0.3	2.058 2.372	$p_1>0.05$ $p_2>0.05$	1.64±0.5	0.279 0.179	$p_1>0.05$ $p_2>0.05$
CUR-4	6.04±0.27	1.360 3.746	$p_1>0.05$ $p_2>0.05$	3.78±0.73	1.634 4.890	$p_1>0.05$ $p_2=0.008$	1.8±0.2	1.672 1.622	$p_1>0.05$ $p_2>0.05$
CUR-6	5.54±0.23	3.497 6.226	$p_1=0.025$ $p_2<0.001$	3.9±0.4	2.266 10.831	$p_1>0.05$ $p_2<0.001$	1.92±0.5	1.318 4.455	$p_1>0.05$ $p_2=0.011$

Data are presented as mean±SD. p_1: significance versus control group p_2: significance versus ADR group

Table (2): Positive PCNA count in nuclei of examined specimen in studied groups.

	Endothelial cells			Mesangial cells			Interstitial		
	Count	Statistical analysis		Count	Statistical analysis		Count	Statistical analysis	
		t	p		t	p		t	p
Control	5.9±0.25			1.42±0.31			0		
ADR-2	6.18±0.41	1.087	$p_1>0.05$	1.58±0.29	0.758	$p_1>0.05$	0		
ADR-4	5.98±0.25	0.560	$p_1>0.05$	8.3±1.27	12.745	$p_1<0.001$	1994±92		
ADR-6	2.66±0.32	15.885	$p_1<0.001$	4.4±0.41	14.304	$p_1<0.001$	5412±795		
CUR-2	6.02±0.25	0.802 0.695	$p_1>0.05$ $p_2>0.05$	1.64±0.27	0.990 1.008	$p_1>0.05$ $p_2>0.05$	0		
CUR-4	6.04±0.27	1.205 0.872	$p_1>0.05$ $p_2>0.05$	2.56±0.43	3.967 9.778	$p_1=0.017$ $p_2=0.001$	0		
CUR-6	5.54±0.23	0.785 1.124	$p_1>0.05$ $p_2>0.05$	1.52±0.23	0.609 14.697	$p_1>0.05$ $p_2<0.001$	1772.2±284	14.82	$p_2<0.001$

Data are presented as mean±SD p_1: significance versus control group p_2: significance versus ADR group

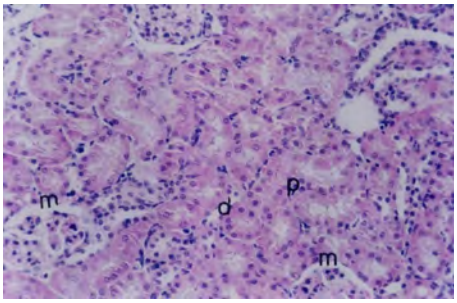


Fig. 1 : Control group specimen showing Malpighian renal corpuscles (m), proximal tubules (P) and distal tubules (d) (Hx & E; X 200).

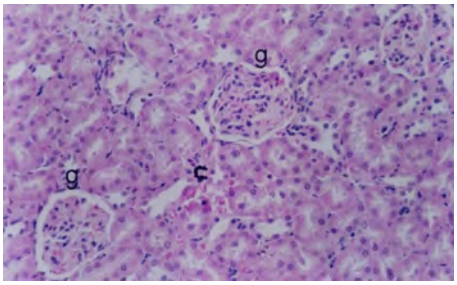


Fig. 3 : ADR-4 group specimen showing congested renal glomeruli (g) and interstitial congestion (C) with partial narrowing and obliteration of Bowman's space (Hx & E; X 200).

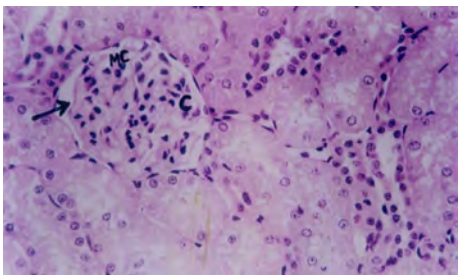


Fig. 2 : High magnification of a Malpighian corpuscle in control group showing Bowman's space (arrow), mesangial cells (MC) and glomerular blood capillaries (C) (Hx & E; X 400).

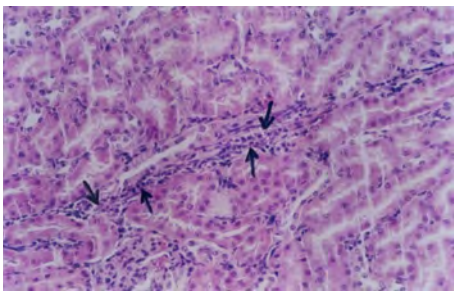


Fig. 4 : ADR-4 group specimen showing some infiltrating mononuclear cells (arrows) (X 200).

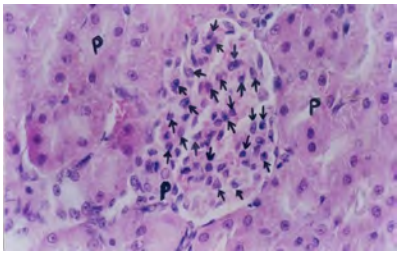


Fig. 5 : ADR-4 group specimen showing high magnification of a Malpighian corpuscle surrounded by proximal tubules (P) with proliferated mesangial cells (arrows) (Hx & E; X400).

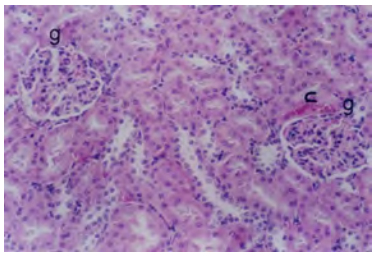


Fig. 8 : CUR-4 group specimen showing minimally congested glomeruli (g) and minimal interstitial congestion (C) are observed, (Hx & E; x200).

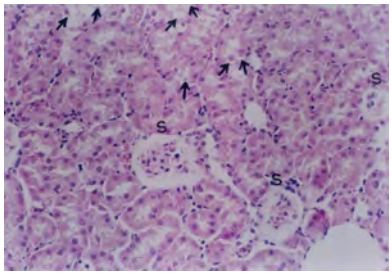


Fig. 6 : ADR-6 group specimen showing multiple shrunken and congested glomeruli (s) and vacuolated cells lining the proximal tubules (arrows), (Hx & E; x200).

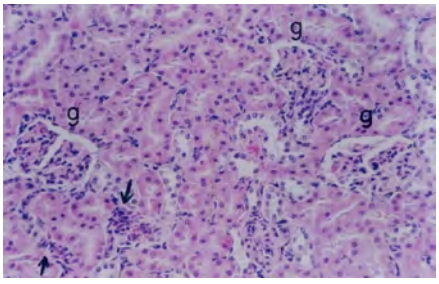


Fig. 9 : CUR-6 group specimen showing glomeruli (g) comparable to control with mild congestion with preserved Bowman's space and less infiltration (arrows), (Hx & E; x200).

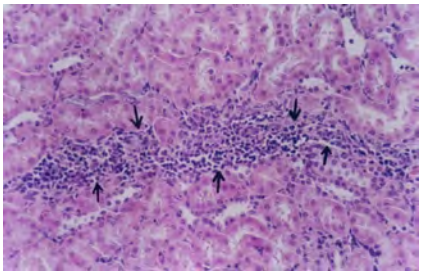


Fig. 7 : ADR-6 group specimen showing intense mononuclear infiltration (arrows)(Hx & E;x200).

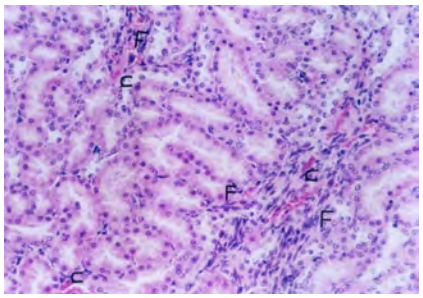


Fig.10 : ADR-6 group specimen showing obvious congestion (C) and multiple fibroblasts (F) between the collecting tubules, (Hx & E; x200).

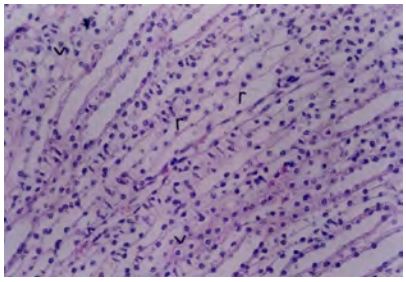


Fig.11: ADR-6 group specimen showing vacuoles in the cytoplasm (V) of lining cells of some collecting tubules. Other tubules show total vacuolations of cytoplasm and flattening of nuclei (r). (Hx & E; x200).

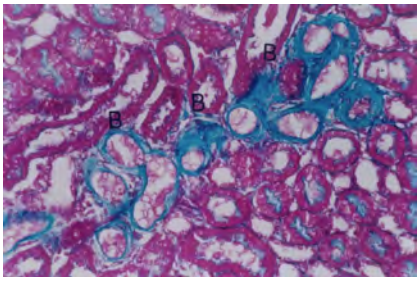


Fig.14 : ADR-6 group specimen showing few thin fibrous bands (B) between proximal tubules (Masson's Trichrome, x100).

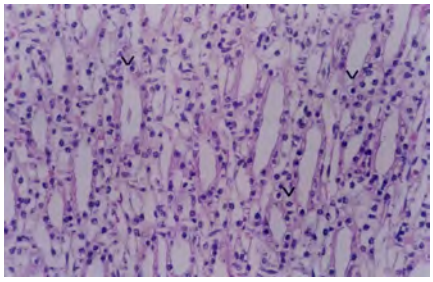


Fig.12: CUR-6 group specimen showing some collecting tubules with minimal vacuolations (V) of the cytoplasm (Hx&E;x200).

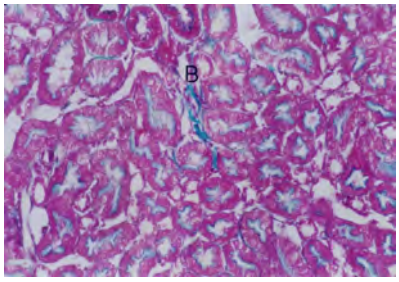


Fig. 15: CUR-6 group specimen showing few thin fibrous bands (B) between proximal tubules (Masson's Trichrome, x100).

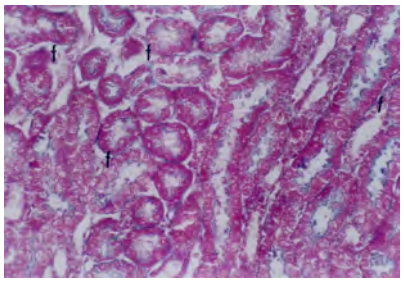


Fig.13: Control group showing minimal connective tissue fibres (f) between proximal tubules (Masson's Trichrome, x100).

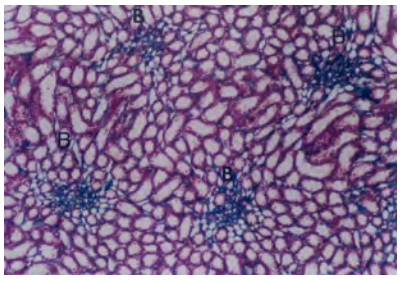


Fig.16 : ADR-6 group specimen showing multiple reticula of dense fibrous bands (B) among the collecting tubules (Masson's Trichrome, x100).

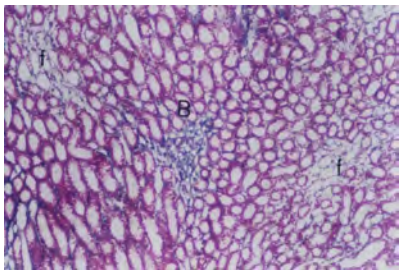


Fig.17: CUR-6 group specimen showing a reticulum of less dense fibrous bands (B) and minimal fibres (f) (Masson's Trichrome, x100).

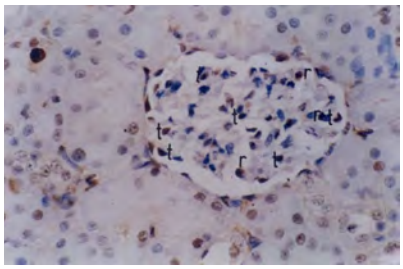


Fig. 20 : PNCA immunostaining of CUR-6 group specimen showing some +ve flattened nuclei (t) and three + ve rounded nuclei (r), (x200).

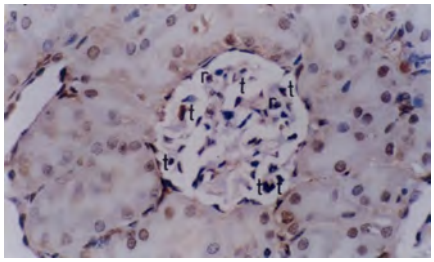


Fig.18: PNCA immunostaining of control group specimen showing some + ve flattened nuclei (t) of endothelial cells lining blood capillaries and two + ve rounded nuclei (r) of mesangial cells.

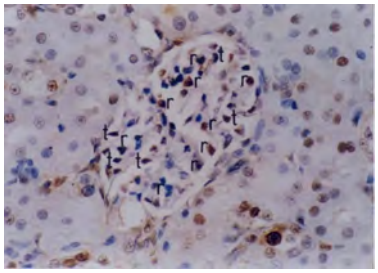


Fig. 21: PNCA immunostaining of ADR-4 group specimen showing some +ve flattened nuclei (t) and multiple + ve rounded nuclei (r), (x200).

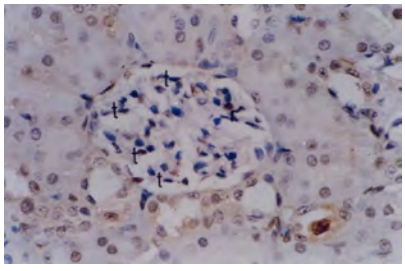


Fig.19:PNCA immunostaining of CUR-4 group specimen showing few +ve flattened nuclei (t) and one + ve rounded nuclei (r), (x200).

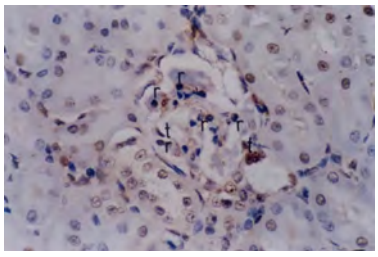


Fig.22:PNCA immunostaining of ADR-6 group specimen showing two +ve flattened nuclei (t) and multiple +ve rounded nuclei (r) in a shrunken glomerulus, (x200).

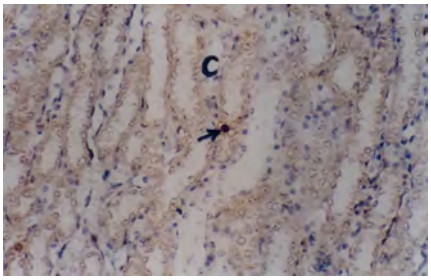


Fig.23: PNCA immunostaining of control group specimen showing a +ve nucleus (arrows) lining a collecting tubule (C) (x200).

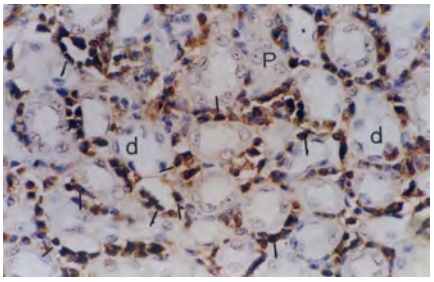


Fig.25: High magnification of PNCA immunostaining of ADR-6 group specimen showing multiple +ve flattened nuclei (arrows) between the proximal (P) and distal (d) tubules (x400).

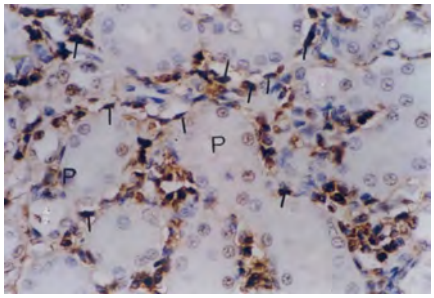


Fig.24: High magnification of PNCA immunostaining of ADR-4 group specimen showing multiple +ve flattened nuclei (arrows) between the proximal (P) and distal (d) tubules (x400).

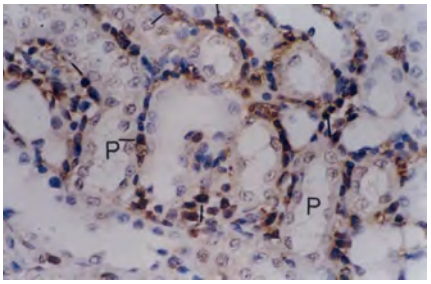


Fig. 26 : High magnification of PNCA immunostaining of CUR-6 group specimen showing multiple +ve flattened nuclei (arrows) between the proximal tubules (x400).

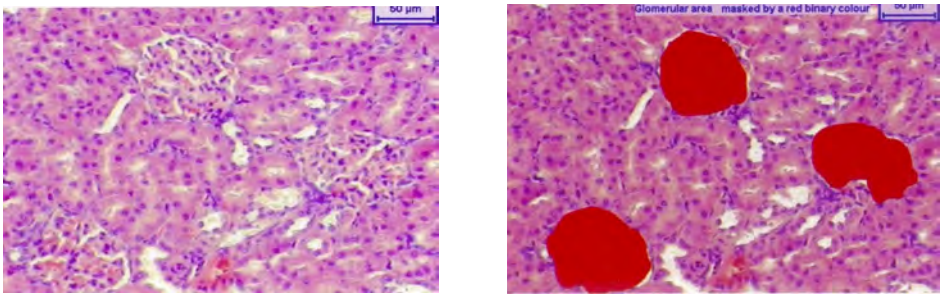


Fig. 27 : Showing glomerular area in ADR-4 group.

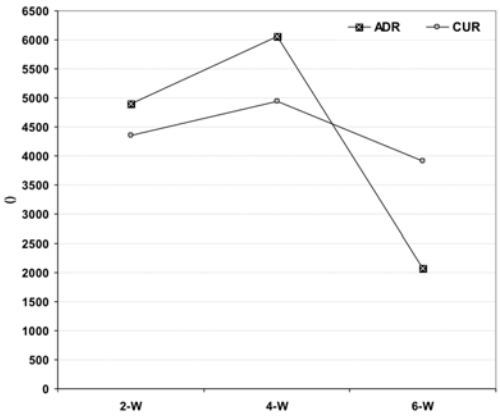


Fig. (28): Time-couse changes of glomerular surface area in studied groups

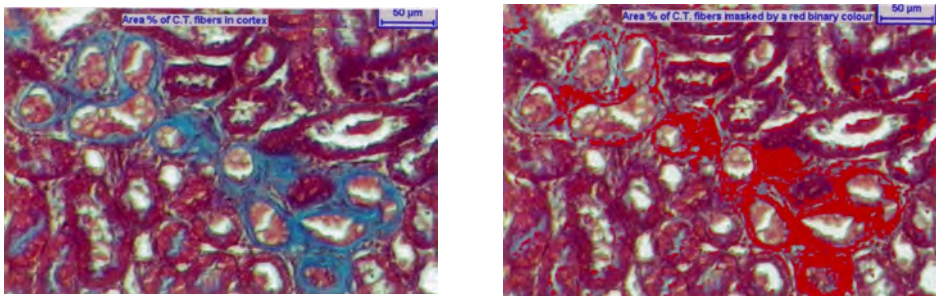


Fig. 27 : Showing area percentage of connective tissue fibers in renal cortex in ADR-6 group .

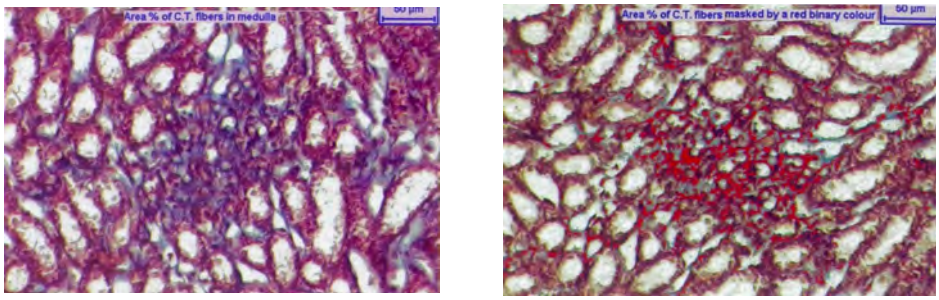


Fig. 30 : Showing area percentage of connective tissue fibers in renal medulla in ADR-6 group.

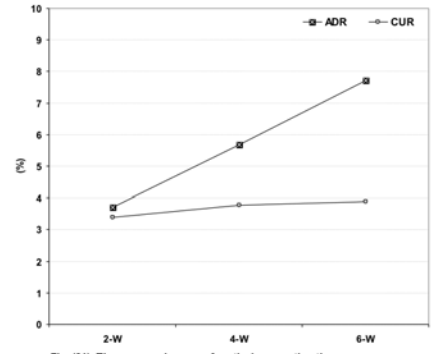


Fig. (31): Time-couse changes of cortical connective tissue area in studied groups

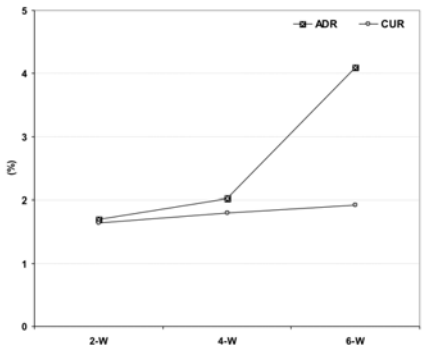


Fig. (32): Time-couse changes of medullary connective tissue area in studied groups

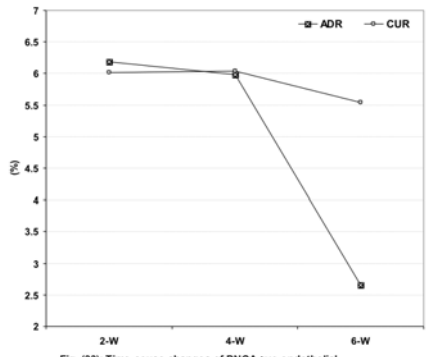


Fig. (33): Time-couse changes of PNCA +ve endothelial cells nuclei in studied groups

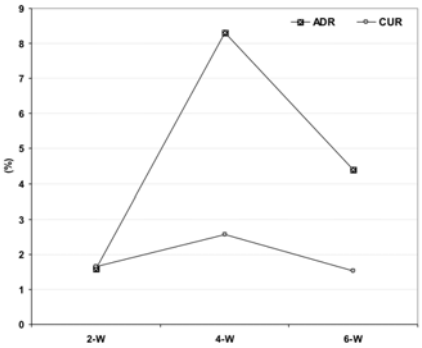
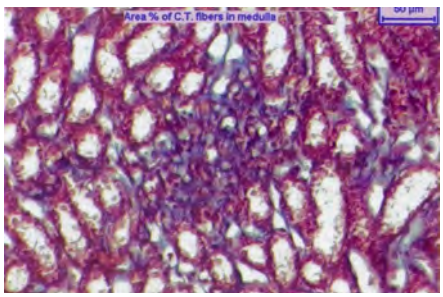


Fig. (34): Time-couse changes of PNCA +ve mesangial cells nuclei in studied groups



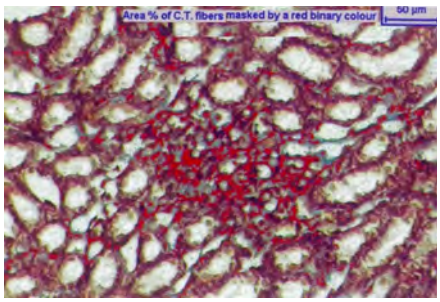


Fig. 35 : Showing count of PNCA positive interstitial nuclei in ADR-6 group.

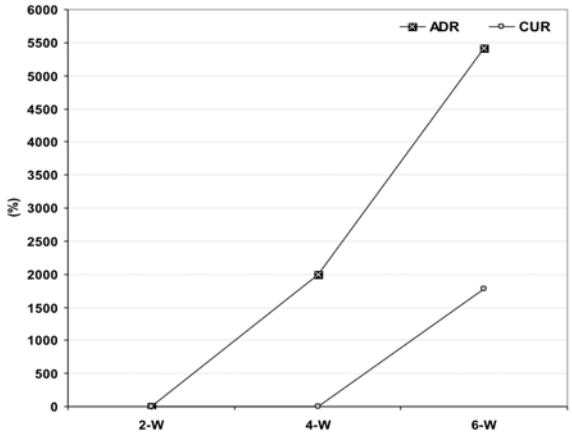


Fig. (36): Time-couse changes of PNCA +ve interstitial cells nuclei in studied groups

Discussion

Anti-cancer agent adriamycin has demonstrated high anti-tumor efficacy. However, its use in chemotherapy has been limited largely due to its diverse toxicities, including renal toxicity, such as nephrotic syndrome with proteinuria. Podocyte injury leads to glomeruli proteinuria, [15]. Moreover, adriamycin exposure affect vascular function in a distinct fashion in adriamycin-induced nephrotic syndrome, [16].

The obtained results of the present study illustrated the deleterious toxic effect of ADR on renal tissue that was manifested as glomerular congestion, proliferation of mesangial cells resulting in partial to total obliteration of Bowman's space, multiple congested shrunken glomeruli, mononuclear cellular infiltration and vacuolation of the cytoplasm of the lining cells of proximal and collecting tubules and in addition, some of the collecting tubules revealed total vacuolation of the cytoplasm of the lining cells with flattening of the nuclei. These findings agreed with Kelly et al.,[17] stated that interstitial infiltration with a variety of inflammatory cell subtypes is a prominent feature of human and experimental focal segmental glomerulosclerosis and correlates well with the degree of renal insufficiency. Bricio et al., [18] reported

that CD4 + and CD8+cells are prominent components of interstitial inflammation in murine ADR nephropathy. Amore et al., [19] hypothesized that CD4 + cells do not mediate renal injury, but have a protective effect and CD4+ cell depletion aggravated histologic and functional damage in ADR nephropathy. Rangan et al., (20) recorded an increase in CD8 cells in the renal interstitium associating CD4+ cell depletion and lysis of native cells by cytotoxic CD8 + T-cells appears to be an important cause of organ dysfunction in many situations. Gorgun et al.,[21], observed vacuoles in the cytoplasm of renal tubular cells between the nuclei and the cellular membranes, on electron microscopic examination. Wang et al.,[22] attributed the pathogenesis of ADR nephrosis to cellular infiltration and in their experimental animal study, reported that partial macrophage depletion starting before but not after ADR exposure protected against the initiation of renal injury as regards both renal function and structure.

These Structural changes showed time-dependency and were minimal within 2-weeks of exposure and progressed to severe after 6-weeks of exposure. Glomerular affection, started at 2-weeks, preceded tubular affection that was evident in 6-weeks specimens. These findings suggest that tubular affection may be secondary to glomerular affection and go in hand with previously reported in literature; Bertani et al., [2,23] found tubule injury in adriamycin nephrosis is considered to be secondary to glomerular injury and proteinuria and obstruction by casts is one mechanism by which proteinuria could injure tubules, these casts have been localized mostly to the distal nephron. Various studies tried to explore the mechanisms of such sequence of injury; Okada et al.,[24] found that reducing urine concentration with a V2 receptor antagonist would limit cast formation, lessened proteinuria and consequent tubule injury in adriamycin nephrotic rats. On the other hand, Sugiura et al.,[25] found that a forced increase in water intake reduces both glomerular and tubulointerstitial injury in rats subjected to renal ablation. Javaid et al.,[3], found increased water intake and

urine excretion of approximately the same magnitude induced by V2 blockade in nephrotic rats had no beneficial effect. Haas et al.,[26] suggested that adriamycin associated proteinuria is toxic for the tubular cells and antiproteinuric therapy protects the remaining tubular cells by decreasing hypercatabolism.

In Masson's trichrome sections focal cortical fibrous bands appeared in ADR-4 and became diffuse in ADR-6 groups, in addition to fibrous reticula in the renal medulla that appeared multiple in ADR-6 group. These findings indicated intiation and continuation of nephron sclerosing changes induced by continued exposure to ADR. In support of these findings, the reported multiple shrunken and congested renal glomeruli in Hx & Eosin section and the significantly reduced glomurluar area and significantly higher mean area percentage of cortical and medullary connective tissue fibres observed in ADR-6 group.

These results agreed with that reported by Nakayama et al., [27] who reported a greater fibronectin score in the cortical tubulointerstitium in animals treated with ADR. Jovanovic et al., [28] reported that chronic experimental exposure to ADR induced syndrome nephroticum with glomerular sclerosis, tubular dilatation and interstitial fibrosis developed which led to chronic renal failure. Tamaki et al., [29] attributed these sclerotic changes to increased CD8 + cells infiltration which secrete transforming growth factor-β; one of the most potent stimulator of collagen and fibronetin production in fibroblasts such attribution could be supported by the progressively increased cellular infiltration reported in examined specimens in the current study and such cellular infiltration preceded appearance of sclerosis manifested as the reported shrunken glomeruli in ADR-6 specimens. Wang et al.,[30] referred the term glomerulosclerosis to increase in the percentage of relative mesangial area; this definition goes in hand with the reported significant increase of the mean glomerular area ADR-4 group versus other groups. Deman et al.,[31] stated that mesangial cells injury stimulates

cytokines production by macrophages that induce proliferation such explaination is supported by the significant increase in the mean count of PCNA +ve mesangial nuclei in ADR-4 group compared to other group.

The ameliorating effect of prophylactic curcumin was evident and illustrated in non-significant histological and immunohistochemical alterations observed in CUR-2 and CUR-4 specimens compared to control specimens and the significant decrease of such alterations in CUR-6 specimens compared to ADR-6 specimens. In support of such protective effect, morphometeric studies showed non-significant changes in mean glomerular area, mean percentage of cortical and medullary connective tissue in CUR groups compared to control group.

These findings could be attributed to the potent anti-oxidative and anti-proliferative effects of CUR and go in hand with that previously reported in literature; Gaedeke et al.,[32] tried to determine the antifibrotic properties of CUR in glomerular fibrosis and reported that CUR potently induced mesangial cell heme oxygenase-1 expression in vitro and up-regulated glomerular heme oxygenase-1 expression in nephritic animals in vivo and CUR treatment led to a significant, dose-dependent reduction of markers of fibrosis particularly plasminogen activator inhibitor-1, transforming growth factor-beta, fibronectin, periodic acid-Schiff staining and concluded that CUR has antifibrotic effects in glomerular disease, which are mediated through an induction of heme oxygenase-1.

Sharma et al., [33] examined the effect of curcumin on renal function and oxidative stress in streptozotocin-induced diabetic rats and found chronic treatment with curcumin significantly attenuated both renal dysfunction and oxidative stress in diabetic rats. Farombi & Ekor, [34] investigated the effect of curcumin on gentamicin-induced-renal oxidative damage in rats and found CUR significantly attenuated the gentamicin-induced increases in both plasma and kidney malondialdehyde and lipid hydroperoxide formation and normalized renal

function parameters. Eybl et al., [35] examined the antioxidative effect of curcumin pre-treatment on cadmium-induced oxidative damage and cadmium distribution in an experimental model in mice and found CUR oral pre-treatment completely prevented the cadmium-induced lipid peroxidation and inhibition of glutathione peroxidase activity but did not affect cadmium distribution in the tissues.

Kuhad et al., [36] evaluated the protective effect of CUR on cisplatin-induced acute renal failure and found CUR treatment significantly and dose-dependently restored renal function, reduced lipid peroxidation, and enhanced the levels of reduced glutathione and activities of superoxide dismutase and catalase and attributed this protective effect of CUR on cisplatin-induced experimental nephrotoxicity to its direct anti-inflammatory and strong antioxidant profile.

It could be concluded that prophylactic administration of curcumin could ameliorate the nephrotoxic effects of chronic treatment with adriamycin and was recommended in the tried dose for patients assigned to adriamycin treatment.

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PROPHYLACTIC IMPACT OF CURCUMIN ON ADRIAMYCIN-INDUCED NEPHROTOXICITY : HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY ON ALBINO RAT

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SINGLE INCISION TRANSPERINEAL REPAIR OF SIMPLE AND RECURRENT RECTOVAGINAL FISTULA WITH VITAL BULBOCAVERNOSUS MUSCLE FLAP

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Abstract

Background : *Rectovaginal fistulas (RVFs) are abnormal epithelial-lined connections between the rectum and vagina. RVFs may be simple or complex. Current study analyzed the outcome of single incision transperineal repair of simple rectovaginal fistula with vital bulbocavernosus muscle flap.*

Methods: *A total of 11 consecutive patients with simple and recurrent RVFs were included and assigned to single incision transperineal repair with vital bulbocavernosus muscle flap. Postoperatively, patients were followed up by outpatient clinic or telephone interviews with specific questionnaires to collect the information about status of fecal control, flatus or fecal leakage from vagina, and quality of personal and social life for sex months. Functional results were evaluated after 6 months by anal manometry and a questionnaire reflecting the symptomatic results, WIS and the FSFI.*

Results: *Mean hospital stay was 10.37±1.12 days. Mean follow up period was 8.81±2.56 months. The results of mean anal pressure measurements postoperatively showed no significant differences compared with preoperative measurements. The results of pre- and postoperative Wexner incontinence scores and female sexual function indices showed high significant postoperative improvements in all patients (P =0.001). By the time of the last follow-up, there was no recurrence of RVF, and all patients reported normal fecal continence and returned to a normal life.*

Conclusion: *Though the rectovaginal fistula is troublesome to the surgeons, it could be cured by using our procedure. It seems that this technique is both simple and effective, giving excellent anatomical and functional results without the need for a protecting stoma.*

Introduction

Rectovaginal fistulas (RVFs) are abnormal epithelial-lined connections between the rectum and vagina, being uncommon accounting for 5% of anal rectal fistulas [(1)]. They can be quite bothersome to both the patient and the surgeon due to their irritating and embarrassing symptoms and high failure rate after repair. RVFs can be the result of congenital malformations or acquired etiologies. Acquired Rectovaginal fistulas may be caused by prolonged labor with necrosis of the rectovaginal septum, obstetric injury with a third- or fourth-degree perineal tear or episiotomy[(2)]. Cryptoglandular anorectal abscesses and Bartholin gland infections may spontaneously drain causing a low rectovaginal fistula[(3)]. Inflammatory bowel disease, diverticular disease, tuberculosis, lymphogranuloma venereum, radiation therapy and malignancy have also been reported[(4)].

RVFs are classified on the basis of location, size, and etiology into simple and complex fistulas, which affects the treatment plan and prognosis (5, 6). Simple RVFs consist of small or medium, low or mid-zonal fistulas secondary to infection or trauma[(7)]. RVFs are considered complex if they are large, high, recurrent or caused by inflammatory bowel disease. Small-sized fistulas are less than 0.5 cm in diameter, medium-sized fistulas are 0.5-2.5 cm, and large-sized fistulas exceed 2.5 cm. Low and mid-zonal RVFs are located between the lower third of the rectum and the lower half of the vagina while high fistulas occur between the middle third of the rectum and the posterior vaginal fornix [(8, 9)]. Low fistulas generally have healthy, well-vascularized surrounding tissue, which can be repaired with local perianal techniques [(7)].

To provide the best chance of successful repair, healthy, well-vascularized tissue needs to be introduced after resection of diseased tissue[(8,9)]. In simple RVFs, rectal advancement flaps

are the most popular transanal procedure among colorectal surgeons[10,11]. Two newer approaches are introduced, one approach involves the use of a bio- prosthetic fistula plug made from porcine intestinal submucosa (Anal Fistula Plug, Cook Surgical Inc., Bloomington, IN)[12], the second recently popularized surgical treatment is coined LIFT (ligation of intersphincteric fistula tract)[13]. Bulbocavernosus flap is a versatile flap for repair of simple vaginal fistulae. Anteriorly based flap is good for urogenital fistulae and posteriorly based flap for RVFs [10].

In complex rectovaginal fistulas, abdominal resection procedures or tissue interposition techniques are used. Tissue interposition using a perineal approach include a labial fat pad, bulbocavernous muscle, and pedicled muscle flaps (rectus, sartorius, and gluteal muscles), as well as bioprosthetic materials [14].

In our research, a single incision transperineal repair with left posteriorly based bulbocavernosus muscle flap, through the same incision, is applied on 11 cases of simple rectovaginal fistula.

Patients and Methods

The potential study population comprised 16 patients with rectovaginal fistulae referred to our General and Colorectal Surgery Unit, Mansoura University Hospital in the period from January 2010 to January 2012 with one or more of the following symptoms: passage of flatus, liquid or solid stool per vagina, a malodorous vaginal discharge, recurrent vaginitis and or dyspareunia. Patients with Cohn'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 11 patients fulfilled the study criteria. A detailed informed consent was obtained from all patients after approval from local ethics committee.

All patients were evaluated preoperatively by clinical interview including Wexner Incontinence Score (WIS) [15] and Female Sexual Fuctional Index (FSFI)[16]. 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).

Patients were given a mechanical bowel preparation the day before surgery and an enema of sorbitol was administrated on the morning of the operation. The patient was placed in the lithotomy position under general anesthesia. Antibiotics in the form of third generation cephalosporin and 500 mg of Metronidazole (Baxter, Deerfield, IL, USA) were given.

Submucosal lidocain 5% in adrenalized saline 1:200,000 was injected around the fistula at the vaginal and rectal sides. Surgical excision of the fistula, without the use of diathermy, using scalpel number 15 was done. Biopsies of rectal mucosa and the fistula margin were also obtained for pathological evaluation to exclude an underlying active IBD or malignancy. A transverse perineal incision was done with elevation of the posterior vaginal wall till the cervix uteri. Dissection of the internal sphincter fibers away from the external sphincter fibers was done allowing a tension-free rectal closure at the fistulous site (Fig. 1). Closure of the fistulous opening at the rectal side was done using Vicryl 3/0 sutures (Vicryl; Ethicon, Somerville, NJ, USA). This is followed by suturing the rectovaginal septum to the internal sphincteric fibers using Vicryl 3/0 sutures (Fig. 2). The bulbocavernous muscle flap was harvested from the left side in all patients at its anterior part, preserving the postero-external vascular pedicle (Fig. 3), through the same incision and sutured across the rectum to its fellow on the other side (Fig. 4), using 2/0 Vicryl sutures. Advancement of the vaginal flap at the fistula site and sutured to the perineal skin using Vicryl 3/0 sutures (Fig. 5). No suction drain was used. No covering stoma was done. Postoperatively, 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. Meanwhile, patients abstained from vaginal intercourse for three months.

Postoperatively, patients were followed up by outpatient clinic or telephone interview with specific questionnaires to collect the information about status of fecal control, flatus or fecal leakage from vagina, and quality of personal and social life. 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, WIS and the FSFI.

Success was defined clinically by the absence of any vaginal passage of feces, flatus or mucous discharge.

Statistical analysis:

The statistical analysis of the data in this study was performed using the SPSS version 10 under windows XP. The tests used were the arithmetic mean value (average) and standard deviation, Student's t test (a P value <0.05 was considered significant).

Results

The mean age of the patients was 31.26 ± 3.927 years (range 26-40). The etiologies of RVFs are shown in Table 1. Two of the eleven patients underwent two trials for RVF surgical repair. All patients had symptoms of passage of flatus, liquid or solid stool through the vagina and or signs of vaginitis. Openings of the fistula were all located above the anal sphincter and varied between 5 and 25 mm in diameter. The preoperative MARP, MASP and rectoanal inhibitory reflex were normal in all patients.

Symptoms occurred after the surgery included discomfort in the anus in all patients, tenesmus without diarrhea in three patients, and feeling of incomplete defecation in two patients. Mean hospital stay was 10.36 ± 1.12 days (range 9-12). All the symptoms spontaneously disappeared in 2 weeks after surgery. Wound swollenness was seen in three patients on the third postoperative day, which was caused by hematoma in one patient and infection in two patients. However, there was no incision disruption and all surgical wounds were healed in 21 days after surgery.

No postoperative mortality was seen.

Mean follow up was 8.81 ± 2.56 months (range 6-14). Patients were followed up by clinic or telephone interview with specific questionnaires to collect the information about status of fecal control, flatus or fecal leakage from vagina, and quality of personal and social life. 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 both the WIS and FSFI. Two patients complained of mild dyspareunia, which requires no further surgical management.

The results of mean anal pressure measurements postoperatively showed no significant differences compared with preoperative measurements (Tables 2). The results of pre- and postoperative Wexner incontinence scores showed high significant postoperative improvements in all patients (p=0.001) (Table 3). The results of pre- and postoperative female sexual function indices showed high significant postoperative improvements in all patients (p =0.001) (Table 3). By the time of the last follow-up, there was no recurrence of RVF, and all patients reported normal fecal continence and returned to a normal life.

Table 1: Patient characteristics and follow-up data of 11 patients.

Patient's number	Age (years)	Multiparous	Etiology	Stoma	Previous repair	Follow up (months)
1	29	yes	Prolonged labor	no	no	7
2	32	yes	Iatrogenic injury during rectocele repair	no	no	9
3	28	yes	Iatrogenic injury during episiotomy	no	no	6
4	33	yes	Prolonged labor	no	no	9
5	27	yes	Iatrogenic injury during rectocele repair	no	no	6
6	34	yes	Prolonged labor	no	no	9
7	40	yes	Prolonged labor	no	no	6
8	32	yes	Iatrogenic injury during episiotomy	no	no	10
9	33	yes	Failed previous RVF repair	no	2	14
10	26	no	Failed previous RVF repair	no	2	12
11	30	yes	Prolonged labor	no	no	9

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

	Preoperative	Postoperative	*p* Value
MARP	62.45±2.20	62.81±2.13	Non-significant
MASP	121.45±3.45	121.36±2.83	Non-significant

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

Table 3: Comparison between preoperative and postoperative Wexner incontinence score and FSFI changes in our patients:

	Preoperative	Postoperative	*p* Value
WIS	20	0.91±1.45	0.0001
FSFI	13.07±2.33	27.08±2.50	0.0001

WIS = Wexner Incontinence Score. FSFI = female sexual function indices.

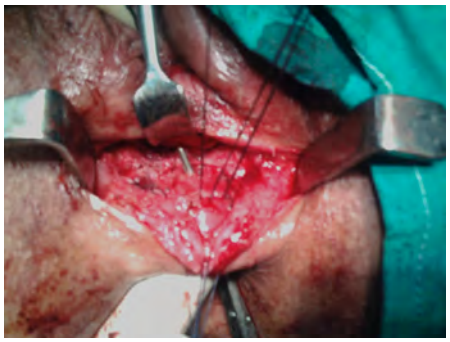

Fig.1: Dissection of internal sphincter fibres from external sphincter fibres.

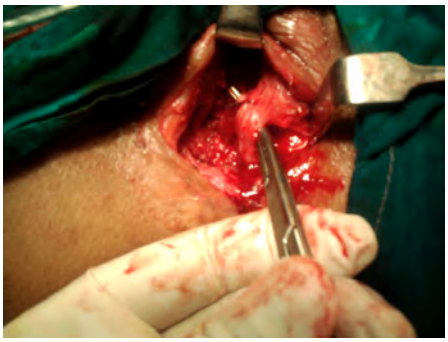

Fig.3: Bulbocavernosus flap preparing.

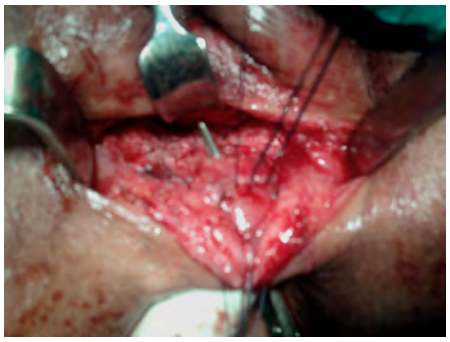

Fig.2: Suturing of internal sphincter fibres to rectovaginal septum.

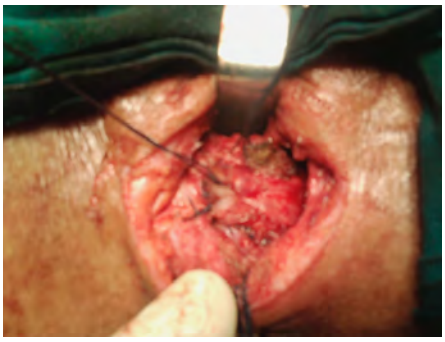

Fig.4: Bulbocavernosus flap suturing to its fellow.

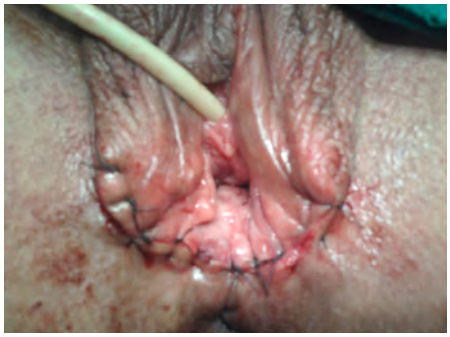

Fig.5: Vaginal advancement flap.

Discussion

Fistulas between the rectum and vagina are generally debilitating and often resistant to repeated repair procedures. The management of rectovaginal fistulas depends on the size, location and cause of the fistula, anal sphincter function, overall health of the patient, as well as the skill and judgment of the surgeon [2,5,6].

Various surgical procedures have been suggested for the repair of these fistulas, including fecal diversion, primary repair, endorectal advancement flap, transvaginal repair, coloanal sleeve anastomosis, and transposition flaps [17, 18]. The relative rarity of this type of fistula makes prospective studies and randomized controlled trials difficult to carry out.

Careful preoperative assessment of the fistula, surrounding tissue, anal sphincter and exclusion of associated disease are essential. Timing of repair is also of importance. The chances of success are increased if the surrounding tissue is in optimal condition, i.e. not inflamed or infected. A rest period of three to 6 months is suggested by some authors [19].

More importantly, the underlying disorders such as IBD or tumor should also be diagnosed at the same time because these conditions can lead to repeated failure of a correct surgical procedure. Therefore, biopsy of the mucosa from the rectum or the margin of the fistula, pelvic CT, and colonoscopy should be considered mandatory prior to surgical repair [2, 3].

Noble first described the use of a sliding flap for repair of a RVF in 1902 and in 1983, Farkas and Gingold described the first case of rectal advancement flap (RAF) for RVF in Crohn's disease [20]. The advantages of the flap procedure are: absence of a perineal wound and of keyhole deformity, no worsening of incontinence, no aggravation of patients' symptoms in case of failure and the presence of a stoma is not mandatory [21].

The reported success rate for RAF technique to repair rectovaginal fistulas in different series ranges between 29 percent in a mixed group of patients with both simple and complex fistulas after varying numbers of previous repair attempts to as high as 88 percent in patients with simple fistulas who had an advancement flap as their primary procedure[18]. The success rate decreases with repeated attempts at repair. Successful repair correlates with the number of previous repairs, i.e. none, 88% success; one, 85% success; two, 55% success [22].

A RAF is preferred whenever easily feasible because the flap is created on the high-pressure anorectal side of the fistula rather than from the low-pressure vaginal side [23].

In our study, transperineal combined rectal and vaginal advancement flaps with intervening vital bulbocavernosus muscle flap was accomplished. Its use is based on the hypothesis that the interposition of tissue between the sutures lines will result in enhanced blood supply to the devascularized epithelium, obliteration of dead space and the interruption of sutures lines along the length of the multilayer closure. Furthermore the flap interposition prevents vaginal stenosis.

Certain basic principles should be followed, including excision of the epithelialized tract, complete closure of the rectal opening, inversion of the rectal edges, adequate tissue mobilization, hemostasis, and tension free multilayer closure.

As the described fistulas were located in the lower-half of the vagina, we decided together with the

patients at their request, not to perform a protecting stoma. If the fistula is located in the upper-third of the vagina it may be prudent or even essential, to divert the fecal contents by means of a temporary ileostomy or colostomy.

Rates of healing reported after other procedures appear to be lower. The Musset technique obtained good initial results in 87-100% of patients but with the risk of anal incontinence, owing to sphincterotomy required for this procedure[8]. Rectal advancement flap alone, which avoids direct anal sphincter damage, resulted in a healing rate in 80% of patients[12]. Repairing fistulas in patients with Crohn's disease has a lower success rate ranging from 50% to 70[13]. We have successfully cured all of nine patients without any significant morbidity. Neither fecal incontinence nor recurrence was found during the time of follow-up. Two patients experienced mild dyspareunia, for whom further treatment was not needed.

Some reasons contributed to this success. The etiology of fistulas in our patients was relatively simple, which may contribute to the excellent outcome in these patients. Interposing a health and well-vascularized tissue avoids direct apposition of two suture lines and introduces well-vascularized tissue to the area. Other reasons are the surgeons' understanding about the anatomy of rectum, anus, and pelvic floor, and the operators' skill in meticulous dissections, without diathermy, for protecting the blood vessel of the pedicle.

Conclusion

Though the rectovaginal fistula is troublesome to the surgeons, it could be cured by using our procedure. Despite the small patients' cohort in this series, it seems that this technique is both simple and effective, giving excellent anatomical and functional results without the need for a protecting stoma.

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GLIOGENESIS OF THE OPTIC NERVE IN THE RAT DURING EARLY POSTNATAL PERIOD

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Abstract

Oligodendrocytes are the end product of a cell lineage which has to undergo a complex and precisely timed program of proliferation, migration, differentiation to finally produce the insulating sheath of axons, myelin. The present study is designed to trace the changes in densities and distribution of oligodendrocytes in both chiasmal and retinal ends of the optic nerve during the period of brain growth spurt which is the first two postnatal weeks in rats, corresponding to the third trimester in human. To achieve this goal, immunohistochemical staining of the optic nerves from rats at 4 age groups at postnatal days 2, 5, 10 and 15 each group contained 10 rats. The antibody used in the present study is directed against myelin basic protein to detect oligodendrocyte. And by comparing olgodendrocytes densities in both chiasmal and retinal ends we could trace the path of migration. The present study could detect significant increase in the density of oligodendrocytes with aging with considerable difference in density between both ends up to the end of second week of postnatal life denoting persistence of active gliogenesis during that period of life with an active migration toward the retinal end.

Key words: *Optic nerve, oligodendrocyte, myelin basic protein, Gliogenesis.*

Introduction

Myelination has evolved in vertebrates to electrically insulate axons to promote rapid, energy efficient action potential propagation which facilitates rapid saltatory conduction along their length[1], ensures their long-term viability[15] and contributes to functional regulation of nervous system plasticity[7]. In the central nervous system (CNS), the production of multilamellar myelin sheaths is performed by oligoden-

drocytes (OLs)[6]. Oligodendrocytes are the end product of a cell lineage which has to undergo a complex and precisely timed program of proliferation, migration, differentiation to finally produce the insulating sheath of axons, myelin[23]. Oligodendrocytes precursor cells (OPCs) migrate out of germinal zones in the brain and spinal cord. When the cells reach their final destination, they become postmitotic, extend processes, and begin to synthesize the components of myelin as extensions of their plasma membrane. In most mammals this occurs relatively late in CNS development during late embryonic and early postnatal life, after neurons and astrocytes are formed[11]. Regarding rat optic nerve, precursor cells enter the nerve before birth and colonize the nerve in a chiasmal-to-retinal gradient during the first postnatal week[21]. Differentiated oligodendrocytes are first detected 4 days after birth (P4) in the chiasmal end of the optic nerve, and populate the nerve in a chiasmal-to-retinal gradient between P4and P7[4]. Myelination is first detected around P6 and myelination progresses in a retinal-to-chiasmal gradient[20].

The present study is designed to trace the process of migration of OPCs in an "in vivo" manner by applying the antibody myelin basic protien (MBP) as a marker for mature (myelinating) oligodendrocytes during the early postnatal period. And comparing their densities in chiasmal and retinal ends at four postnatal days during the brain growth spurt, a period characterized by cellular proliferation and differentiation, neuronal and glial migration, axonal growth, and synaptogenesis, occurs during the third trimester of pregnancy in humans and in the first two weeks after birth in rats[13].

Material and Methods

Animals:

All experiments were performed in accordance with the guide for the care and use of laboratory animals[16].

Ten pregnant Wistar rats derived from Animal Care Center Mansoura University, Mansoura, Egypt, kept in sanitary cages with free access to chow and water.

Pregnant rats were checked at 9:00 and 18:00 h daily for delivery of pups. Day of delivery was counted as postnatal day 0 (P0). Four Groups of pups at ages of postnatal days 2, 5, and 10 and 15 (P2, P5, P10 and P15) were used each group contained 10 pups chosen from different mothers in a random manner. The first two postnatal weeks was selected because it has already demonstrated that this is the time when the phase of rapid postnatal development of the optic nerve is completed[5].

Fixation and sectioning:

Under deep phenobarbital anesthesia (100mg/Kg b. wt), Wistar rat pups were perfused transcardially with saline containing heparin (2 U/ml), and subsequently with a fixative consisting of 4% paraformaldehyde in phosphate buffered saline (PBS).

The whole length of the optic nerves from pups was carefully dissected out, avoiding traction on the nerves. Immediately put into the same fixative over night at 4˚C. After overnight cryoprotection in 20% sucrose in PBS each optic nerve was mounted in OCT compound. Transverse sections of ~ 10 µm from optic nerves at chiasmal and retinal ends were obtained using cryostat (Lieka co. Tokyo Japan)

Immunohistochemistry:

The method of immunostaining applied in this study is the indirect immunohistochemistry using avidin-biotin complex (ABC)[14]. Briefly; antigen retrieval was done using enzymatic proteolysis via 0.1% Trypsin in Tris buffer at pH 7.6. Endogenous perioxidase activity was quenched by incubating the specimens in 3% H_2O_2 in PBS at room temperature for 10 minutes. Permiabelization was achieved using 0.25% of triton X100 diluted in PBS (PBS-T) for 10 minutes at room temperature. Further, it was used in dilution of antibodies and wash.

Sections were then incubated with primary antibody. The sections were then incubated with biotinylated anti-mouse secondary antibody followed by the avidin-biotin complex (ABC) method (Vector, CA, USA). Finally, the sections were visualized

with 3, 3-diaminobenzidine (DAB) (Dojin, Japan).

Primary antibodies:

Anti-MBP (SMI 94), Mouse monoclonal antibody against myelin basic protein (MBP) recognizing mature oligodendrocyte and myelin sheath (Sternberger Monoclonals inc., Maryland, USA).

The optimal concentrations of the antibody were determined by trying different concentrations and choosing the optimum one with least background staining. The following concentration was used (1: 2000).

The specimens were incubated with the primary antibody in a well-sealed humid chamber overnight at 4˚C.

Quantification of oligodendrogyes Densities

Oligodendrocyte cell density was quantified by counting MBP-labeled cell bodies in optic nerves at the chiasmal and retinal ends from test and control rats using a 40 x objective lens, a rectile (10 x 10 grid), and Zeiss Axiophot microscope. The number of MBP-labeled cells per grid area was determined and the total area analyzed in each section was calculated. For each portion, total areas and MBP+cells numbers were calculated and expressed as cells/ mm^2 of tissue.

Statistical analysis:

The data were expressed as mean ± standard error of mean (SEM). The significance of differences between results was determined by using Student t-test and one-way analysis of variance (ANOVA). In case of significant F ratio Post Hook Turkey test for multiple comparisons was done at $p<0.01$ level of significance.

Statistical studies were done using SPSS software package “version 16”.

Results

Assessment of the density and morphology of mylinating oligodendrocye:

Mature oligodendrocytes were labeled by antibodies detecting the myelin basic protein (MBP) which produced by the myelinating oligodendrocutes and constitute 35% of the total myelin sheath protein.

MBP+ cells were counted at postnatal day 2, 5, 10 and 15 in both the chiasmal and retinal ends. The number was significantly reduced in retinal end at all examined age groups (p value <0.01) except at age of P15 where the difference in densities between both ends was insignificant Table (1) and Fig. (5).

The morphology of the MBP+ cells was changed with aging during the first two post natal weeks as it appeared polygonal with short non branching processes in P2 rats (Fig. 1). At P5 the cells acquired longer processes which were more abundant and more branched at the chiasmal ends more than in retinal ends (Fig. 2). At P10 myelinated axons started to appear especially at the retinal end (Fig. 3). At P15 rats the fields appeared to be fully occupied by myelinated axons, in the chiasmal end transverse and longitudinal oriented myelinated fibers and rows of MBP+cells seen in between them (Fig. 4).

Table 1: Comparison of numerical density between the chiasmal and retinal ends of the optic nerves in different age group (Number of +ve cells/mm^2 ± Standard deviation).

Age	Chiasmal end	Retinal end	P value
P2	20±1.75	0.8±1.25	≤0.01
P5	176±26.02	112±25.54	≤0.01
P10	355±19.24	286±24.21	≤0.01
P15	653±98.65	635±55.25	≥0.01

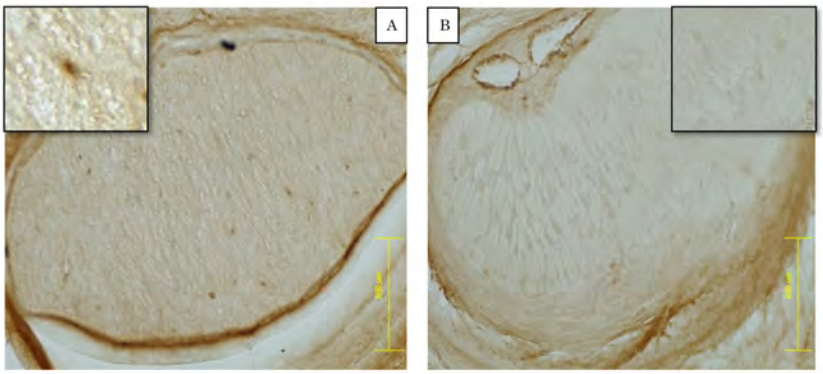

Fig. 1: Transverse sections of the optic nerves at P2 rats Immuno-stained with MBP antibody (A) Chiasmal end, (B) Retinal end. +ve cells start to appear in a very small number in chiasmal end but not in the retinal end. The cells appeared with short thin processes. (X 400 & Inset, x1000 oil immersion).

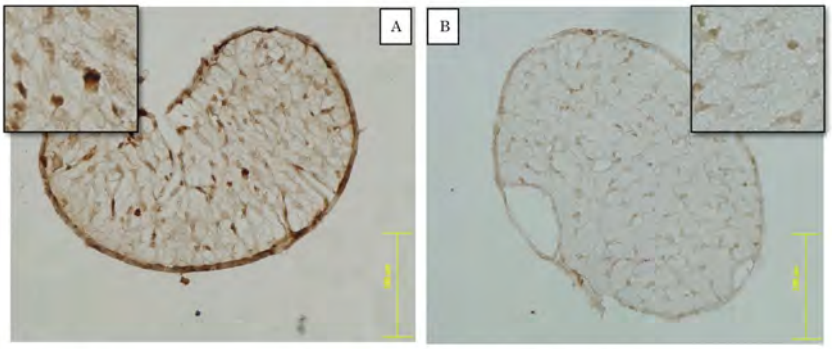

Fig. 2 : Transverse section of the optic nerve at P5 rats immuno-stained with MBP antibody; (A) chiasmal end, (B) Retinal end. The number of positive cells is increased compared to previous ages, the cells acquired longer processes and appeared completely mature but no myelinated axons could be identified. (X 200 & Inset, x1000 oil immersion).

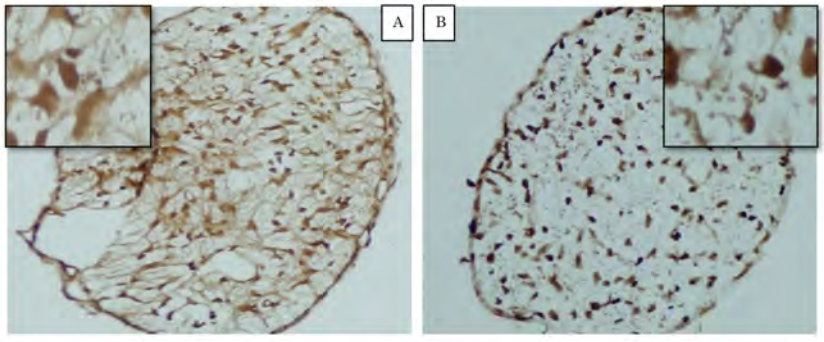

Fig. 3 : Transverse section of the optic nerve at P10 rats immuno-stained with MBP antibody; (A) chiasmal end, (B) Retinal end. The number of positive cells is increased compared to previous ages; the cells acquired longer processes and appeared completely mature. A number of myelinated axons could be identified; the number of myelinated axons is increased in retinal end compared with that in chiasmal end. (X 200 & Inset, x1000 oil immersion).

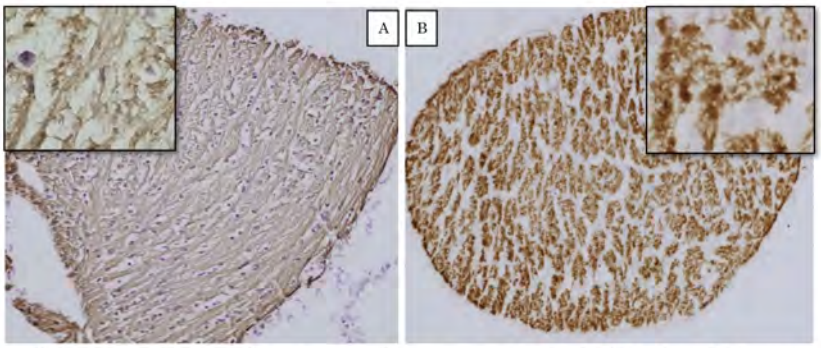

Fig. 4: Transverse section of the optic nerve at P15 rats immuno-stained with MBP antibody; (A) chiasmal end, (B) Retinal end. The number of positive cells is increased compared to previous ages; the cells acquired longer processes and appeared completely mature. The number of myelinated axons is abundant in both ends some fibers are oriented longitudinally in the chiasmal end with a rows of mature MBP+ oligodendrocytes in-between. (X 200 & Inset, x1000 oil immersion).

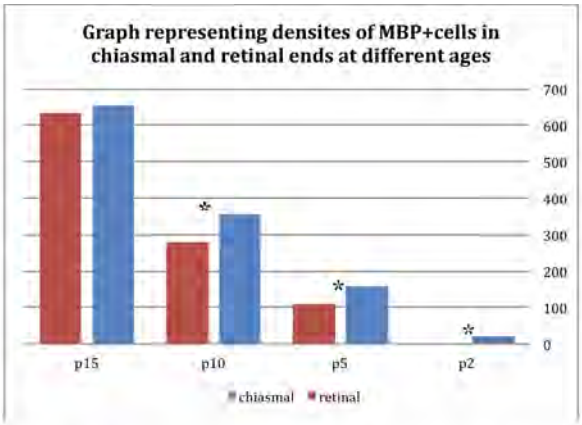

Fig. 5: Graph representing difference in densities of MBP+cells between chiasmal and retinal ends (* = significant P value ≤ 0.01).

Discussion

The present study is designed to trace the migration and differentiation of OPCs "in vivo" by applying specific antibody directed against the final stage in the course of OPCs differentiation into mature oligodendrocytes. And compare the densities at the retinal end of optic nerve with that at the chiasmal end. In this respect, the optic nerve was chosen as it offers several advantages. First, it is one of the simplest parts the CNS, it contains only two main cell types-astrocytes and oligodendrocytes in addition to the axons of the retinal ganglion cells[3]; second, the majority of precursor expansion and all of oligodendrocyte differentiation and myelination occur postnatally and can be easily assayed[22].

The study of the oligodendrocyte lineage was facilitated by the identification of the oligodendrocyte precursor using the A2B5 antibody that recognizes a surface ganglioside[18]. Although this antibody was first used to label neurons and is notoriously difficult to use in vivo, when used in vitro, it clearly identifies a small, round, process-bearing cell that will become an oligodendrocyte under most conditions. These cells were originally identified in cultures derived from optic nerve but are present throughout the CNS and spinal cord[10]. Initial studies of the oligodendrocyte precursor cells (OPCs) in vitro found that in serum-free medium, the OP will quickly differentiate and express myelin-associated proteins. When gown in 10% - 20% serum or when treated with growth factors, such as bone morphogenetic protein (BMP), the OP will continue to express A2B5 and also express glial fibrillary acidic protein (GFAP), a major internal filament associated astrocytes. Raff and coworkers termed these novel cells “type 2 astrocytes” to distinguish them from the classic type of astrocytes (“type 1 astrocytes”). They called the OP cells “O-2A” to credit their bipotential nature[18]. Several other antibodies are now known to identify the OPCs; one detects the alpha subunit of the receptor for platelet-derived growth factor (PDGFRα) a potent mitogen, survival and differentiation factor for oligodendrocytes[12]. Another OPC antibody detects chondroitin

sulphate proteoglycan NG2 and appears slightly after PDGFR?. Both antibodies co-localize both in vitro and in vivo and disappear at differentiation[11]. As OPCs begin to differentiate, they express a sulfated surface antigen known as POA (pro-oligodendroblast antigen) that is recognized by the antibodies O4. Expression of these antigens coincides with the extension of numerous branching processes and a cessation of migration[2]. The proolgodendroblast is known to represent a transition stage that is characterized by continued proliferation but altered response to mitogens when compared to A2B5 +/O4-[9]. As oligodendrocytes differentiate, they begin to synthesize the structural proteins of myelin. Each of these is expressed at specific time in the development of the cell on both the RNA and protein level. The first to appear is 2',3'-cyclic nucleotide 3'-phosphohydrolase, a basic myelin protein that form 4% of all myelin[19]. More abundant myelin proteins, proteolipid protein (PLP), constitutes with its alternatively spliced form, DM20 about 50% of the protein mass of myelin and myelin basic protein (MBP), constitute 30%-40% of CNS myelin protein, appear several days after galactocerebrocide in cultured cells[11]. Several other minor components of myelin have begun to be characterized. For example, myelin-associated glycoprotein (MAG) constitutes 1% of myelin protein and is involved in neuron-glial interaction[8].

In the present work, MBP antibody was used to label mature oligodendrocytes which could be detected at the chiasmal end of the optic nerves derived from P2 rats while no positive cells could be detected at the retinal ends of that age. This finding is inconsistent with earlier reports of Small et al. (1987) who could not trace MBP+cells at ages earlier than P4 rat in an "in vitro" study[21]. Other reports identified MBP+cells at the chiasmal end at earlier ages[11] who could detect MBP+ cells at the chiasmal end as early as the first day of postnatal life (P1). With further aging MBP+ve cells could be identified in both ends of the optic nerve but with significant difference in density being much reduced in the retinal ends

compared with that in the chiasmal ends up to age of P15 where no significant difference could be elicited, a finding which was confirmed by earlier reports[21,20&4]. Regarding the morphology, the MBP+ve cells appeared less mature in early postnatal ages, being less ramified with shorter processes at P2 and P5 ages compared to more extensively ramified with longer branching processes in more mature cells at later ages (P10 and P15). Maturation of oligodendrocytes is accompanied with morphological changes. As they begin to extend a complex meshwork of processes with the goal of contacting multiple axons. Once axoglial contact has been established, oligodendrocytes produce large amounts of specialized membrane (myelin) that form multiple wraps around the contacted axons. The final stages of myelination involve the expulsion of cytoplasm from the membrane wraps to form compact myelin. In the current study, the number of myelinated axons at the retinal end increased compared with that in the chiasmal end at P10 and P15. This finding is consistent with previous reports using electron microscopic morphometric analysis[20&4]. The molecular events underlying oligodendrocyte proliferation, survival, and maturation are poorly understood, yet knowledge of these processes is of great value for the development of therapeutics for demyelinating diseases like MS[17].

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GLIOGENESIS OF THE OPTIC NERVE IN THE RAT DURING EARLY POSTNATAL PERIOD

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EFFECT OF INTERACTION BETWEEN NUTRITIONAL ELEMENTS CALCIUM AND MANGANESE ON BONE MINERAL DENSITY (BMD)

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Abstract

The material for the present study included 24 patients with post – menopausal osteoporosis selected from Rheumatology and Rehabilitation out patient clinic Mansoura university hospital with age range (40 – 68 years) Bone mineral density (BMD) was determined using Dual energy X-ray absorptiometry technique (DEXA). T score (hip) was (-2.5). 8 healthy volunteers with matched age and sex served as (control group). Serum calcium, manganese, alkaline phosphatase and 24 hours urinary excretion of both calcium and phosphorous were determined for all patients and volumteers. Both serum calcium and manganese concentrations were found to be significantly decreased (P< 0.05) among patients with post. Menopausal osteoporosis mean while serum alkaline phosphatase and 24 hours urinary excretion of both calcium and phosphorous were significaully increased (P= < 0.05). Our results were discussed in light of previous literature and we concluded that manganese deficiency may lead to osteoporosis, probably through diminished bone calcium fixation, decreased intestinal calcium, absorption, and increased urinary clearance of both calcium and phosphorous. Manganese and calcium dietary supplements are recommended particularly for elderly post-menopausal women.

Introduction

The role of inadequate calcium intake on acceleration of osteoporosis is well Known. The daily requirement of calcium remains under debate some authors suggest

value of 1200 mg for peri-menopausal women aged 35-50 years, and 1500 mg for post menopausal women[1] also manganese is one of several trace elements, that are necessary for bone health, taking a combination of Ca, Zinc copper and manganese (Mn) reduce bone loss in post menopausal women[2] in one set of studies concerning interaction between nutritional elements supplemental calcium was found to decrease slightly manganese bioavailability[3] Mean while calcium deficiency increased manganese absorption[4] Recent studies have led researchers[5][6] to suggest that it may be time to change the hypothesis of osteoporosis as being a result of calcium deficiency. The researchers suggest that manganese which has a role in fixation of calcium in bones could be the real culprit[6] lack of manganese was almost as if the glue that sticks calcium to bones is missing, the bones can't absorp and fix calcium and the body excretes excess of Ca in urine. The primary aim of the present study was to investigate, the effect of interaction between nutritional elements calcium and manganese on bone mineral density (BMD) a second objective was to validate the new hypothesis suggesting that manganese deficiency may be the primary cause of osteoporosis.

Material and Methods

The material for the present study included 24 ♀ patients with post menopausal osteoporosis selected from Rheumatology and Rehabilitation,out patient clinic, Mansoura University Hospital with age range (40 - 68 years) bone mineral density (BMD) was determined using dual energy x-ray obsorptiometry (DEXA) value of T score(hip) was(-2.5). 8 healthy volunteers with matched age and sex served as (control group).

All patients and volunteers (control group) were subjected to the following study :

1) Determination of both serum calcium and serum alkaline phosphatase according to the methods desribed by Tietz NW [7]
2) Serum manganese concentration was determined using Perkin Elmer atomic absorption spectrophotometry [8]
3) 24 hours urine calcium

and phosphorous excretion were determined according to the methods described by Rockwell[9] Chernecky and Berger [10]

Results

1- Both serum calcium and manganese concentrations were found to be significantly decreased (P = < 0.05) among ♀ patients with post Menopausal osteoporosis (table I , II).

2- serum alkaline phosphatase was found to be significantly increased (P = < 0.05) among patients with post menopausal osteoporosis ompared with healthy volunteers (control group) table III.

3- 24 hours urinary excretion of both calcium and phosphorous were found to be significantly increased (P = < 0.05) among post. Menopausal osteoporotic patients compared with control group (table (IV).

4- Serum manganese concentration was found to be significantly lower (P = < 0.05) among elderly post menopausal osteoporotic patients with age > 60 years compared with middle aged patients < 50 years table (V).

Table I : Serum calcium level in post menopausal women versus control group .

post menopausal women serum calcium M± SD	control group serum calcium M± SD	P value
7.8 mg/dl ± 0.12	9.6 mg/dl ± 0.25	<.05

Table II : Serum manganese level in post - menopausal women versus control group .

post menopausal women serum manganese M± SD	control group serum manganese M± SD	P value
0.022 µg/dl ± 0.012	0.049 µg/dl ± 0.011	< 0.05

Table III : Serum alkaline phosphatase in post - menopausal women versus control group.

post menopausal women serum alkaline phosphatase M± SD	control group M± SD	P value
144 ± 13.5 u/L	85 ± 6.12 u/L	(< 0.05)

Table IV : 24 hours urinary calcium and phosphorous excretion in elderly post menopausal women versus control group

24 hour urine excretion	Post- menopausal women M± SD	control group M± SD	P value
Ca/ mg/24h	312 ± 16.5	143.6 ± 25.5	(<0.05)
P gm/day	1.5 ± 0.11	0.61 ± 0.12	(<0.05)

Table V : Serum manganese level in elderly post menopausal women (> 60 years) versus middle aged women (<50 years)

elderly post menopausal women age > 60 (16 patients)	Middle aged patients age < 50 (8 patients)	P value
Serum Mn level M± SD 0.019 ± 0.001 µg/dL	Mn level M± SD 0.024 ± 0.002 µg/dL	< 0.05

Discussion

In the present study serum manganese (Mn) concentration was found to be significantly decreased among ♀ patients with post - menopausal osteoporosis similar finding was previously reported in several studies[11][12]. Women with osteoporosis have been found to have not only decreased plasma or serum level of Mn but also an enhanced plasma response to an oral dose of Mn, suggesting they have lower Mn status, than women without osteoporosis[3]. Manganese is one of several trace elements, including vanadium and boron, that are necessary for bone health, taking a combination of Ca, Zinc, Copper and Mn reduce bone loss in post - menopausal women, also low levels of manganese in the body can contribute to bone mal formation and weakness [2] Mn deficiency may also decrease plasma oestrogen level[14] and aggrevate osteoprosis.

In the present study serum Mn

concentration was found to be significantly lower among elderly post- menopausal osteoporotic women with age > 60 years compared with middle aged patients < 50 years. Elderly osteoporotic women commonly absorps less Mn due to both lost appetite and inadequate dietary intake also consumption of Mn is increased in elderly, since manganese is component of antioxidant enzyme super - oxide dismutase which helps to fight against free radicals which damage cell membrane and DNA and contribute to ageing[13].

Also brain consumption of Mn is increased with age which exhaust manganese reserves since brain function decline with age and Mn helps to sustain brain work, which uses (25%) of our energy intake when at rest [6].

In the present study serum calcium concentration was found to be significantly decreased among ♀ patients with post - menopausal osteoporosis. the role of prolonged inadequate calcium intake on the acceleration of osteoporosis is well known. One of the most important uses of calcium is to prevent and treat osteoporosis. Hypocalcaemia observed in our patients was assocciated with concomitant increase in 24 hours urinary execration of both Ca and phosphorous. Hypocalcaemia in our patients may also be related to possible multiple interactions between (Ca) and (Mn). Calcium, phosphorous and manganese work closely together in the body for this reason you may need more manganese if you are getting more calcium, calcium was reported to accelerate the uptake of manganese, and manganese retard the uptake of Ca by brain Mitochondria [13].

Recently researchers[5][6] suggest that manganese is necessary for ca absorption and fixation in bones, manganese deficiency means not only decreased intestinal absorption of Ca, but also Ca dose not stick to bones, easyly released from bones, and excreted in urine. Hypocalcaemia in our patients was associated with concomitant elevation of serum alkaline phosphatase. Hypocalcaemia trigger parathyroid hormone release, which increases urinary phosphate

clearance, mobilize Ca from bone, decrease bone mineral density activate osteoblasic activity and increase release of serum alkaline phosphatase our results were discussed in light of previous literature and we concluded that manganese deficiency may lead to osteoporosis probably through diminished bone calcium fixation and increased urinary clearance of both calcium and phosphate. Mn and Ca dietary supplements are recommended particularly for elderly post - menopausal women.

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ROLE OF EXERCISE REHABILITATION IN RHEUMATOID ARTHRITIS PATIENTS

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Abstract

Objective: *The aim of the present work was to evaluate the effect of exercise rehabilitation on general health, Pain, fatigue, disease activity score (DAS-28) and quality of life in patients with rheumatoid arthritis (RA).*

Subjects and Methods: *forty RA patients were divided into two groups: group A: 20 patients who introduced into exercise rehabilitation program and group B: 20 patients who are not introduced into the exercise rehabilitation program and served as a control group. All patients were subjected to full history taking, thorough clinical examination with evaluation at both the beginning and after twenty weeks of the training program. Assessment included general health, Pain, fatigue, assessment of disease activity using Disease Activity Score (DAS-28), quadriceps strength, grip strength and assessment of quality of life using the Euro health-related quality of life (QoL) questionnaire.*

Results: *there were no statistical significant differences in all items of QoL between both groups at the entrance to the study. Statistical significant difference between both groups was observed at the end of the study as regards morning stiffness, general health, pain, fatigue, grip strength, quadriceps strength, disease activity and all items of QoL.*

Conclusions: *This study supports the role of exercise rehabilitation in the management of patients with RA. We have found significant improvement in general health, pain, fatigue, DAS-28 and quality of life in*

patients with rheumatoid arthritis. Beneficial effects of exercise training were evident in RA patients who introduced into exercise rehabilitation program compared with patients who were not introduced into exercise rehabilitation program.

Keywords: *Rheumatoid arthritis; Pain; Exercise; Fatigue; Health-related quality of life; Disease activity.*

Introduction

Rheumatoid arthritis is a chronic, systemic, autoimmune, inflammatory condition that is associated with a reduced life expectancy, patients with RA have restricted range of motion of joints, joints pain, reduced physical functioning and higher mortality rate[1]. In addition to the direct effects of RA on joints, this disease is characterized by a loss of muscle mass[2].

RA is most prevalent in individuals aged 40 years or older[3]. Although outcomes of the disease have improved with modern approaches to drug treatment, the disease is still a progressive one with long-term joint damage and disability[4].

Low physical activity is an important and reversible characteristic of RA, it has been demonstrated that RA patients do less exercise than the healthy population; more than 80% of RA patients are physically inactive in some countries, whilst in the UK it is believed that approximately 68% of RA patients are physically inactive[5]. The extreme physical inactivity of RA patients' becomes a vicious circle in terms of health and disease progression, thus it has become apparent that encouraging physical activity is an important and essential part of the overall treatment of RA[4].

Habitual physical activity is decreased in RA patients due to joint pain, restricted mobility, fatigue, reduced muscle mass, strength, and endurance, and these will result in decreased physical function and quality of life[6] Low physical activity increases the risk for RA patients to develop cardiovascular disease, osteoporosis, insulin resistance, and obesity[7].

Patients with RA have pain and stiffness as the most limiting factors of their illness[8] and report lower health-related quality of life (QOL) than healthy individuals [9], a relationship that is even more pronounced for RA patients who do not participate in regular physical activity [10]. In the last few decades, research illustrating the importance of physical activity for patients with RA has begun to accumulate. Several treatment trials have demonstrated that physical activity has positive impacts upon pain, disease activity, and functional status [11].

The aim of the present work was to evaluate the effect of exercise rehabilitation on general health, Pain, fatigue, DAS-28 and quality of life in patients with RA.

Subjects and Methods

The present study included forty RA patients diagnosed according to the American College of Rheumatology/European League Against Rheumatism Collaborative Initiative[12], who were attending the outpatients clinic of the Rheumatology and Rehabilitation Department of Benha University Hospitals, they were divided into two groups:

Group A : 20 patients who introduced into exercise rehabilitation program, their mean ages were 45.74±9.23 years and the mean disease duration was 11.46±5.67 years.

Group B: 20 patients who are not introduced into the exercise rehabilitation program and served as a control group. Their mean ages was 43.76±8.23 years and the mean disease duration was 10.56±6.39 years.

Eclusion Criteria: treatment with >10 mg glucocorticoid per day, noncompliance to disease modifying anti-rheumatic drugs (DMARD) treatment, disease activity score 28 (DAS-28) more than 5.1 in the past 3 months and/or global functional status in RA class III or IV[13], cardiovascular events, i.e. transitory ischemic attack, myocardial infarction, hypertension, congestive heart failure, stroke, major diseases which may prevent exercise (cardiac failure or late phase renal or pulmonary diseases), high-grade mechanical or degenerative disorders, other

rheumatological diseases or orthopedic or neurological disorder of walking were excluded from the study.

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

All Patients Were Subjected to the Following:

- Full history taking and thorough clinical examination with stress on:
- Body mass index (BMI) calculated by dividing body weight in kilograms by the square of body height in meters.
- Evaluation at both the beginning and at the end of the study (i.e. after twenty weeks of the training program).

Assessment:

- **Echocardiography:** to exclude RA organ manifestations, each patient had an echocardiography assessment before and at the end of the study.
- Blood pressure tested before and after each session.
- General health perception rated on a 10cm visual analogue scale (VAS) from 'Totally fine' (=0) to 'Worst imaginable health' (=10)[14].
- Perceived pain rated on a 10 cm VAS from 'No pain' (= 0) to 'Maximal pain' (=10)[15].
- Fatigue rated on a 10 cm VAS from 'No fatigue' (=0) to 'Maximal fatigue' (=10)[16-17].
- Disease Activity Score (DAS-28) measures disease activity based on erythrocyte sedimentation rate, number of swollen and tender joints and self-reported general health perception (VAS 0-10 cm scale)[18]. The DAS28 is scored 0-10 with scores below 3.2 indicating low disease activity and those up to 5.1 as moderate disease activity.
- **Quadriceps strength (QdS):** The force of the maximum voluntary contraction of the knee extensors was measured. The subject

sat upright in an adjustable straight-backed chair with the lower leg dependent and the knee flexed to 90. A cuff was placed round the leg just proximal to the malleolus and attached horizontally to an electrical strain gauge with a digital readout. Quadriceps strength was recorded from the mean of three voluntary contractions separated by 1 minute interval and expressed in Newtonmeter.

- Grip strength: The force of the maximum voluntary contraction of the hand was measured using handheld dynamometer. The test was done three times and the mean was calculated.

- Assessment of quality of life:

For assessing quality of life the EuroQoL questionnaire was used[19]. The QoL questionnaire is a standardized and valuing health related quality of life. The QoL instrument measures five domains. For each modality there are three options. Each of the five domains measured had points from 1 to 3, hence the best performance of the QOL is 15 degrees and the worst performance of the QoL is 5 pints. The score of each patient was recorded and the mean score of each group was calculated for both groups at initial presentation and at the end of the study programs.

- Laboratory investigations: serum concentrations of the following parameters were measured: complete blood count, ESR, CRP, RF, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), creatinine, and ALP.

Training program [20]

Combined strength and endurance training program: The training programme was designed to improve muscle strength, flexibility, endurance and balance. Twenty subjects participated in a 20 weeks combined strength training and endurance training program undertaken in two sessions, on non-consecutive days of the week. A brief warm-up period required 10 min of stretching exercises before each training session began.

Strength training (ST)-During the first 2 weeks of the training program, patients began with

resistance levels they could do easily and increased gradually, the weight was kept to a minimal level so that the patients could learn the exercise techniques and allow muscles to adapt to the training, as well as to prevent muscle soreness and joint pain. From the third week, the objective of the training was to achieve strengthening, so two sets of exercises per muscle group were undertaken each week.

The ST program consisted of a series of exercises for major muscle groups. Resistance and intensity of exercise were designed after individual evaluation of each patient and modified according to pain, the available range of movement and fatigue. Regular breaks and relaxation sessions were allowed. Each session was preceded by a 'warm-up' and followed by a 'cool-down'.

Endurance training (ET)- Systematic ET was performed on a cycle ergometer. During the first 4 weeks, ET participants trained for 15 min per session, two times per week. From the fifth week, exercise sessions were increased by 5 min every 4 weeks. Heart rate (HR) was monitored continuously throughout the training period.

Control group

The patients in the control group were instructed to perform stretching exercises twice a week, without using additional resistance, to maintain their joint mobility. They were free to continue their recreational physical activity with the exception of ST or ET.

Statistical analysis:

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

Results

- Baseline characteristics of the studied groups were represented in (table 1).

- There were no statistical significant differences in all items of QoL between both groups at the entrance to the study (table 2).

- Statistical significant difference between both groups was observed at the end of the study as regards morning stiffness, general health, pain, fatigue, grip strength, quadriceps strength and disease activity (table 3).

- Comparison of QoL between both groups showed statistical significant improvement in all items of QoL at the end the study period (table 4).

Table (1): Baseline characteristics of group A (no=20) and group B (no=20)

Variable	Group A	Group B	t	P
Age (years)	45.74±9.23	43.76±8.23	0.72	P>0.05
Sex (female/male)	16/4	15/5	-	-
Disease duration (yrs)	11.46±5.67	10.56±6.39	0.47	P>0.05
BMI (Kg/m2)	27.93±6.35	28.83±6.48	0.44	P>0.05
Morning stiffness (min)	18.34±7.45	17.85±6.93	0.21	P>0.05
General health (VAS)	7.34±3.54	6.45±3.12	0.84	P>0.05
Pain (VAS)	6.39±2.87	6.23±3.12	0.17	P>0.05
Fatigue (VAS)	5.78±3.71	6.37±3.43	0.52	P>0.05
Grip strength	14.23±1.43	14.31±1.49	0.17	P>0.05
Quadriceps strength (QdS) (kg)	42.45±2.34	42.31±2.27	0.19	P>0.05
No. of tender joints	11.43±6.63	11.6±6.59	0.08	P>0.05
No. of swollen joints	6.65±3.69	6.53±3.61	0.1	P>0.05
DAS-28	3.54±1.45	3.47±1.48	0.15	P>0.05

P>0.05 non significant

Table (2): Quality of life (QoL) assessment in both groups at entrance to the study

Variable	Group A	Group B	T	P
Mobility	1.32±0.61	1.48±0.59	0.84	P>0.05
Self care	2.11±0.41	2.27±0.34	1.34	P>0.05
Usual activities	1.56±0.41	1.54±0.43	0.15	P>0.05
Pain/discomfort	1.44±0.43	1.51±0.52	0.46	P>0.05
Anxiety/depression	1.63±0.57	1.58±0.73	0.24	P>0.05

P>0.05 non significant

Table (3): Comparison of characteristics of group A (no=20) and group B (no=20) after the study period

Variable	Group A	Group B	T	P
Morning stiffness (min)	13.31±4.37	17.13±6.37	2.21	P< 0.05
General health (VAS)	4.86±2.48	6.53±2.41	2.15	P< 0.05
Pain (VAS)	3.78±1.84	6.26±3.53	2.79	P< 0.05
Fatigue (VAS)	3.53±2.56	5.73±3.51	2.26	P< 0.05
Grip strength	16.36±1.35	14.24±1.57	4.58	P< 0.05
Quadriceps strength (QdS) (kg	48.45±3.56	42.56±2.34	6.1	P< 0.05
No. of tender joints	7.46±4.36	10.34±4.49	2.05	P< 0.05
No. of swollen joints	3.15±1.34	6.42±3.37	4.03	P< 0.05
DAS-28	2.38±1.05	3.35±1.43	2.44	P< 0.05

P< 0.05 significant

Table (4): Comparison of quality of life (QoL) between both groups after the study period.

Variable	Group A	Group B	T	P
Mobility	2.41±0.45	1.52±0.61	5.25	P< 0.05
Self care	2.78±0.48	2.31±0.45	3.19	P< 0.05
Usual activities	2.23±0.62	1.67±0.53	3.07	P< 0.05
Pain/discomfort	2.29±0.58	1.59±0.68	3.5	P< 0.05
Anxiety/depression	2.14±0.87	1.63±0.56	2.2	P< 0.05

P< 0.05 significant

Discussion

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, causing progressive damage to the musculoskeletal system and loss of function [21]. Joints pain is typically caused by inflammation and in advanced cases can relate to joint destruction, further limiting the patients' physical activities[22]. Consequently, patients with RA suffer from muscle loss and reduced muscle strength[23], these factors contribute to the loss of physical function and quality of life[6].

The aim of the present work was to evaluate the effect of exercise rehabilitation on general health, Pain, fatigue, DAS-28 and quality of life in patients with rheumatoid arthritis.

Cooney et al. 2011[4], reported that exercise is fundamentally

beneficial for RA patients, it results in improved cardiorespiratory fitness and cardiovascular health, increased muscle mass, reduced adiposity (including attenuated trunk fat), improved strength, and physical functioning, all achieved without exacerbation of disease activity or joint damage. Exercise training in stable RA is effective in increasing aerobic capacity, muscle strength, joint mobility, and physical function with no detrimental effect on disease activity in patients with controlled [24] or active RA[25].

Exercise is an effective treatment for various chronic musculoskeletal pain disorders[26]. Several mechanisms are suggested to play a role in exercise-induced endogenous analgesia (EA), including release of endogenous opioids and growth factors[27], and activation of (supra) spinal nociceptive inhibitory mechanisms orchestrated by the brain[28]. These mechanisms are related to cardiovascular changes (i.e. increase in heart rate and blood pressure) during exercise, patients with hypertension show reduced pain sensitivity[29]. The interaction can be explained by similar brain stem nuclei, neurotransmitters (e.g. monoamines) and peptides (e.g. opioids)[30]. The exercise-induced blood pressure activates arterial baroreceptors, resulting in increased supraspinal inhibition sensitivity[29] and stimulation of brain centers involved in pain modulation[31].

Patients with RA usually suffer from disability, severe pain, joint stiffness, and fatigue which impair physical function[32]. However, exercise has been shown to significantly improve some or all of these symptoms, especially function as well as psychological well-being[24, 33,34].

In the present study there were statistical significant differences between RA patients who introduced into the rehabilitation program and those who were not introduced into the rehabilitation program after the study period as regards pain and disease activity. This finding was in accordance with those of Strasser and his collegues 2011[35], who found that disease activity, the number of

clinically active joints and pain were found to be reduced after 6 months of combined strength and endurance training in RA patients and they reported that the typical patient with RA is non-trained as regards fitness and strength and no negative outcomes for function and disease activity was observed with dynamic exercise training. They also found improvement in general health and functional ability in those patients after exercise training.

Previous studies that assessed exercise and the radiologic progression of the small joints found that exercise, even long-term ST, is safe for the joints of hands and feet [36-39].

Fatigue limits overall function in RA patients, it is frequently experienced in RA with 42% of RA patients experiencing severe fatigue[40]. Often patients report fatigue as one of the most annoying symptoms[41]. Fatigue can be described as a subjective experience, a feeling of "extreme, persistent tiredness, weakness or exhaustion which can be both mental and physical" [42].

In the present study there was statistical significant difference as regards fatigue between RA patients and controls after the study period. This finding was also concluded by Neill et al 2006[34], who reported that both aerobic and resistance exercise interventions reduce RA fatigue in their review on fatigue in RA patients.

Physical activity improves QOL among patients with RA, this may be explained, at least in part, by improvements in patients' perceived control over their disease, where the achievement of treatment goals was shown to predict increased feelings of control over illness and increased QOL[43].

In the present study there was statistical significant difference in all items of QoL between RA patients who introduced into the rehabilitation program and those who does not introduced into the rehabilitation program after the study period. This result was in accordance with those of Baillet et al. 2009[44], who found improvement in disease activity and QoL in RA patients after 4-weeks of dynamic exercise programme.

Cooney et al. 2011, reported that the importance for inclusion of exercise training in the treatment of RA is now clear and proven, exercise in general seems to improve overall function in RA without any proven detrimental effects to disease activity, thus all RA patients should be encouraged to include some form of exercise training as part of their routine care.

Conclusion

This study supports the role of exercise rehabilitation in the management of patients with RA. We have found significant improvement in general health, pain, fatigue, DAS-28 and quality of life in patients with rheumatoid arthritis. Beneficial effects of exercise training were evident in RA patients who introduced into exercise rehabilitation program compared with patients who were not introduced into exercise rehabilitation program. More research is required for the optimal period and types of exercises.

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ROLE OF EXERCISE REHABILITATION IN RHEUMATOID ARTHRITIS PATIENTS

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EFFECT OF PEGYLATED INTERFERON AND RIBAVIRIN THERAPY IN HCV RELATED NEPHROPATHY

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Abstract

Background: *chronic hepatitis C infection is a major health problem, where it causes a major burden of illness for the patients and community, however nephropathy is a common extra hepatic manifestation for this diseases whether associated with cryoglobinemia or not; moreover, the standard treatment of chronic HCV infection now is the combined peg-interferon and ribavirin.*

Aim of the work: *This study was conducted to estimate the prevalence of HCV-related nephropathy and cryoglobinemia in our setting and its response to the standard treatment of chronic hepatitis C namely (pegylated interferon and ribavirin).*

Patient and method: *eighty-four patients with chronic hepatitis C genotype 4 who were eligible for therapy with pegylated interferon and ribavirin were tested for nephropathy (presence of proteinuria and/or heamaturia) and presence of cryoglobinemia before treatment, after 12 weeks, at end of treatment(48 weeks), and 6 months after end of treatment.*

Data analysis: *The collected data was statistically managed by SPSS software version 17. Chi-square (?2) was used to test statistically significant difference among the qualitative variables and student t test in quantitative variables. The significance level was accepted if the p value ≤ 0.05*

Results: *the prevalence of nephropathy before treatment was 38.1% and prevalence of cryoglobinemia was 34.5% where both decreased segnifecantly to 16.7%. However nephropathy with cryoglobinemia more significantly responded to treatment while those with nephropathy and*

no cryoglobinema showed insignificant decrease from 7.1% to only 6%. As regard the virological response EVR was 67.8%, but SVR was 47.6%.

Conclusion: *HCV-related nephropathy associated with cryoglobinemia responds significantly to standard therapy for HCV genotype 4 with pegylated interferon and ribavirin.*

Introduction

Hepatitis C virus (HCV) infection is a major public health problem with an estimated number between 130 and 170 million people infected worldwide and accounts for over 1 million deaths as a result of cirrhosis and liver cancer[1,3]. Hepatitis C virus genotype 4 (HCV-G4) is prevalent in the Middle East and Africa and has spread to several regions in Europe [4]. Membranoproliferative glomerulonephritis is thought to be the most common extra hepatic HCV infection, either cryoglobulinemic or noncryoglobulinemic[5,6]. An association between HCV infection and focal segmental glomerulosclerosis (FSGS) and membranous nephropathy has also been reported[7,8]. Other forms of kidney disease have been previously linked to chronic HCV infection, including acute exudative and proliferative glomerulonephritis and immunoglobulin M nephropathy [9]. Although in patients with HCV infection and liver disease, renal involvement may be mild or clinically silent [10], a study showed that patients with HCV and age < 70 years have a two folds increase in risk for developing end-stage renal disease (ESRD) when compared with patients without HCV infection[11]. The principal clinical manifestations of glomerular disease in HCV-infected patients are the presence of proteinuria and microscopic hematuria with or without impaired kidney function[12]. While the standard treatment of HCV now is the combined pegylated-interferon and ribavirin, Of relevance is the fact that both interferon (IFN)[13,14] and ribavirin (RBV) [15,16] are excreted by the kidney and their clearances are impaired in patients with kidney failure[17,18]. Consequently, both drugs should have their doses adjusted according to the degree of kidney function. However No dose adjustment is

needed for patients with GFR > 60 ml/min [19].

Aim of the work

This study was conducted to estimate the prevalence of HCV-related nephropathy and cryoglobinemia in our setting and its response to the standard treatment of chronic hepatitis C (namely pegylated-interferon and ribavirin).

Subjects:

This was a prospective study conducted in King Fahd hospital in AlMadina Almonawara - KSA between July 2008 and December 2011; our subjects were 84 consecutive eligible patients who attended the gastroenterology and hepatology clinic with chronic hepatitis C genotype 4 and fulfilled the criteria for interferon therapy. Exclusion criteria were the presence of diabetes, hypertension, concurrent hepatitis B or human immunodeficiency virus infections, autoimmune hepatitis, hemochromatosis, or Wilson's disease; active alcohol consumption; antiviral or corticosteroid treatment; chronic renal disease or history of dialysis; collagen vascular disease, kidney calculus, chronic pyelonephritis, and reflux nephropathy, or any confounding factor for proteinuria (such as fever, exercise, and dehydration).

Methods:

Before starting the standard therapy for the patients, Blood samples were collected after an overnight fast. Serum creatinine was measured by means of the modified kinetic rate Jaffe method; and a random spot urine sample was obtained from each participant using a clean-catch technique and sterile containers, and the sample was analyzed on frozen nonhematuric specimens. Urine protein was measured in a 24-hour urine sample, and proteinuria was defined as a urine protein level higher than 500 mg (Dipstick, Biosystem, Germany). Urine also was tested microscopically for detection of haematuria where clinical nephropathy was defined as proteinuria and/ or microscopic haematuria; finally Cryogluobbenemia was measured in all patients and all these tests were repeated at the time of investigations for the early virological response (EVR) after 3 monthhs,

at the end of treatment after 48 weeks and after 6 months of the end of treatment at the time of detecting the sustained virological response (SVR). Our 84 patients received 180 αg of peginterferon α-2a (Pegasys®; Hoffmann-LaRoche, Basel, Switzerland) subcutaneously once weekly and ribavirin (Copegus®; Hoffmann-La Roche, Basel, Switzerland) at a daily oral dose of 1000 mg (body weight < 75 kg) or 1200 mg (body weight > 75 mg) for 48 wk. and all the patient had the standard protocol for interferon therapy follow up.

Results

Out of 84 patients with chronic hepatitis C, who were fulfilled the criteria for the standard therapy with peginterferon α-2a and ribavirin, 56 (61.9%) were females while 32 (38.1%) were males. The mean age for the patients were 41.2 (ranging 21-48 years). Blood urea, serum creatinin and glomerular filtration rate were normal in all patients (table 1), however, clinical nephropathy was evident in 32 patients (38.1%), out of them 26 patients (81.3%) had cryoglobulinemia and only 6 patients (18.7%) have nephropathy without cryoglobulinemia (table 2). After 3 months of starting the therapy EVR was achieved in 57 patients out of 84 (67.8%), while cure of nephropathy was achieved in 2 patients out of 32 (6.3%) both of them had cryoglobinemia and showed EVR while there was no improvement for those with nephropathy without cryoglobinemia. at the end of treatment the virological response was achieved in 43 patients (51.2%), and nephropathy cured in 21 out of 32 patients with nephropathy (65.6%), after 6 months of treatment SVR achieved in 40 patients (47.6%), while cure of nephropathy persist in 18 patients (56.2%) (Figures 2, 3). Cryoglobinemia was found in 29 patients out of 84 (34.5%) before treatment, however the prevalence were 33.3% (28 patients), 14.3% (12 patients), and 16.7% (14 patients) after 3 months of treatment, end of treatment and 6 months after treatment respectively.

Patients with nephropathy and cryoglobinemia decline significantly from 31% (26 patients) before treatment to 28.6% (24

patients) after 3 months of therapy and finally became 10.7% (9 patients) after 6 month of end of treatment, while those with nephropathy without cryoglobinemia changed from 7.1% (6 patients) before treatment to only 5.9% (5 patients) at end of treatment and persists the same 6 months after treatment which was statistically insignificant (figure 1).

Table (1): Liver and renal function tests of the stuverdy sample (n= 84).

	Range	Mean ± SD
ALT	13.0 -139.0 u/l	49.4±26.9
AST	13.0-135.0 u/l	50.6±26.9
Total protein	5.1-9.6 g/dl	7.0±0.7
Serum albumin	1.7-6.0 g/dl	4.0±0.9
Total bilirubin	0.1-3.1 mg/dl	1.1±0.9
INR	1.0-2.3	1.3±0.3
urea	22-51 mg/dl	33±7
creatinine	0.6-1.4 mg/dl	1.1±0.2

Table (2): Prevalence of nephropathy with and without cryoglobinemia during treatment course in relation to total nephropathy.

	Pre treatment		At EVR		At SVR		P
	No.	%	No.	%	No.	%	value
Nephropathy With cryo.	26	81.3	24	75	9	28.1	0.001
Nephropathy Without cryo.	6	18.7	6	18,7	5	15.6	NS
Total	32	100%	30	93.7	14	43.7	0.001

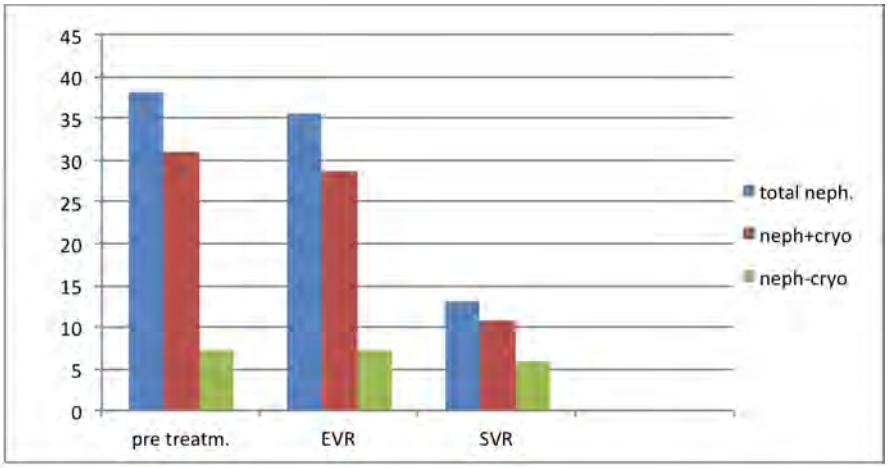

Figure (1): prevalence of total nephropathy, nephropathy with and witho cryoglobinemia during treatment course in the study sample.

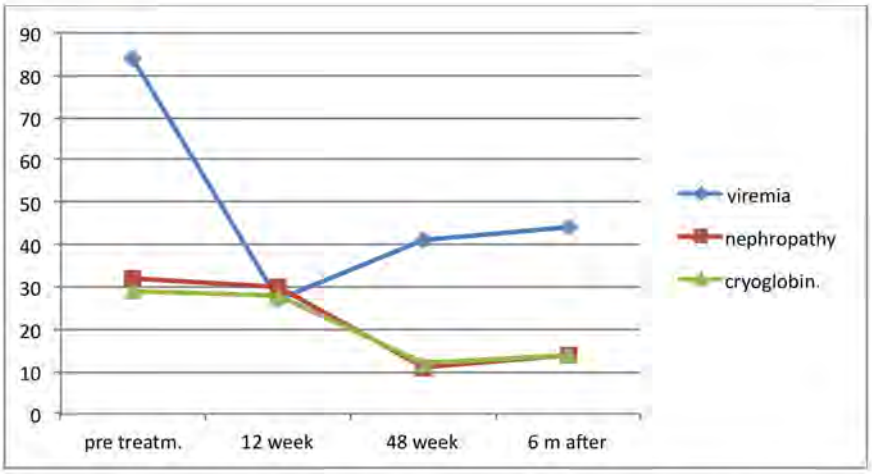

Figure (2): number of patients with viraemia, nephropathy and cryoglobinemia duri treatment course.

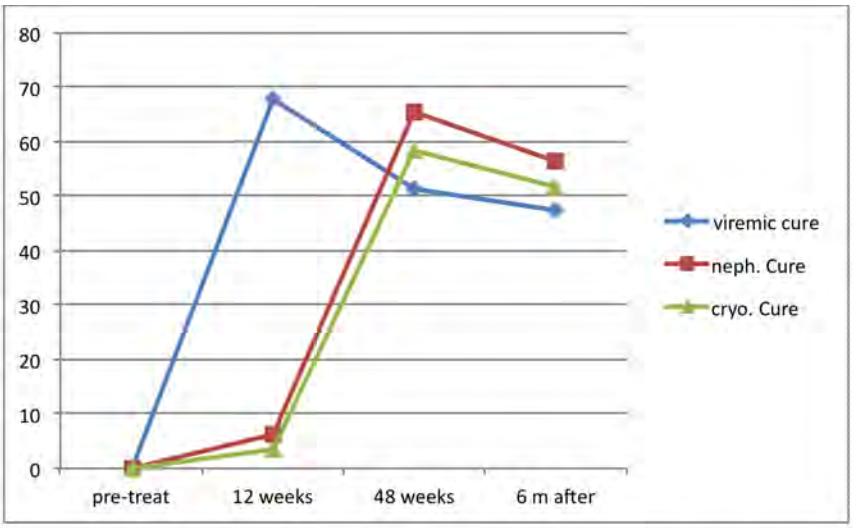

Figure (3): Cure rate for viraemia, nephropathy and cryoglobinemia through treatment course.

Discussion

There is no doubt that chronic hepatitis C, nephropathy and cryoglobinemia are interrelated subjects. Although the mechanisms that cause renal damage are not fully elucidated[20], it seems that the formation of immune complexes [HCVantigens, anti-HCV IgG antibody and rheumatoid factor (RF) IgM] contribute to the insitu tissue destruction owing to the deposition of virus nuclear protein and immunoglobulins in the glomerular capillary wall, renal interstitium and tubules[21]. While Cryoglobulins are cold-insoluble immune complexes containing IgM rheumatoid factor, polyclonal IgG, HCV RNA and complement. Deposition of cryoglobulins on the vascular endothelium of organs such as skin, nerves and kidneys can induce a vasculitis that clinically manifests as palpable purpura, arthritis, neuropathy or glomerulonephritis[22].

However the prevalence of nephropathy and cryoglobinemia in patients with chronic hepatitis C showed significant variations between studies; in our study nephropathy was found to be 38.1% which is matched with a

study by Derball et al, who reported a prevalence of 39.7% in all his study sample with chronic hepatitis C being higher in diabetics compared to non diabetics (49.6% versus 37.6%) [23]; nevertheless other studies shows variations between 4% up to 50%[24,27]. The same discrepancy can be applied to the prevalence of cryoglobinemia; while in our study it was 34.5% which is not far from the study by Diana et al where it was 37.5% [24], and an important misanalysis which reported a mean prevalence of cryoglobinemia to be 44% [28]; however other studies shows variations from 25% up to 100%. These variations can be explained on the bases of the differences for the study sample as regard viral genotype, the duration of illness, the type and degree of renal pathology, presence of other co-morbid condition specially diabetes and the sample size[24,26,28,29]. More over the prevalence of nephropathy with cryoglobinema in our study was more than those with nephropathy without cryoglobinemia [81.3% versus 18.7%], and this obvious relationship was reported in many other studies[30,32], as most of HCV-related nephropathy is due to cryoglobinemia while 20% to 30% of positive HCV cryoglobinemic patients have clinically renal diseases. As regard the treatment and according to the guidelines Treatment regimens and their doses will vary with the severity of the kidney disease. No dose adjustment is needed for patients with GFR > 60 ml/min [19]. This recommendation is based on the results of three large randomized trials that were completed in IFN-naive patients with normal kidney function [33,35]. The efficacy and safety of combined IFN and RBV therapy is not affected in patients with a GFR > 50 ml/min/ 1.73 m^2. As such, the results reported in patients with normal kidney function treated with peg-IFN and RBV should apply to kidney disease stages 1 and 2. So we apply these recommendations for our patients as all of them having normal renal functions. SVR was achieved in 47.6% of our patients which is reasonable in genotype 4, normal kidney function with or without cryoglobinemia and using the standard therapy and go with other studies [36,37]. Moreover, we get a significant improvement

in nephropathy after completion of the treatment and 6 months after treatment, and this was more marked in those with cryoglobenemia more than those without cryoglobinemia; and when looking to cure curves through treatment course, both of nephropathy and cryoglobinemia were positively correlated and always preceded by a significant viremic cure; and though other studies showed a significant improvement in proteinuria and other kidney diseases due to HCV infection, better outcomes have been achieved by combined use of IFN with RBV[38-41], and peg-IFN with RBV[42-46]. For instance, Sabry et al.[41] treated 20 patients with IFN for 3 months, and in case of no-response addition of RBV 15 mg/kg for 12 months, Proteinuria was markedly reduced while serum creatinine was unchanged. Similarly, Bruchfeld et al.[38] treated 7 patients with IFN- 2 b 3 MU (n = 4) or 1.5 MU (n = 1) 3times weekly or peg-IFN- 2 b 50 g once a week (n = 2) + RBV 200-800 mg daily for 24 weeks to 15 months and showed SVR in 71.4% (5 patients). Proteinuria was markedly reduced, hematuria resolved in 6 of 7 patients and GFR improved in 3 patients and was stable in others. Results were stable at 24 months of follow- up. While, Alric et al. [43] treated 18 patients with IFN-2b (3 MU 3 times weekly, (n = 14) or peg-IFN- 2 b (1.5 g/kg per week, n = 4) and RBV 600-1,000 mg/day for 6-24 months. SVR occurred in 66.7% of the patients, and only patients with SVR showed reduction of proteinuria. These differences can be explained as a result of different kidney pathology and the renal function, as most of these studies showed significant glomerulonephritis in renal biopsy. Nevertheless, those patients with nephropathy without cryoglobinemia in our study, did not show a reliable improvement which may reflect a persistent and irreversible direct pathological effect of HCV, or presence of other intrinsic renal cause which need renal biopsy for better diagnosis.

Conclusion

HCV-related nephropathy and cryoglobinemia are prevalent conditions and significantly improved in response to peg-interferon and ribavirin in patients with normal renal functions, on the contrary,

nephropathy without cryoglobinemia poorly respond to this standard therapy and need further studies.

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EFFECT OF PEGYLATED INTERFERON AND RIBAVIRIN THERAPY IN HCV RELATED NEPHROPATHY

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CONCOMITANT THREE- DIMENSIONAL CONFORMAL BOOST TO GROSS TUMOR VOLUME IN CONCURRENT CHEMORADIATION FOR PATIENTS WITH UNRESECTABLE STAGE III NON- SMALL CELL LUNG CANCER

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Abstract

Purpose: *We evaluated the efficacy and toxicity of synchronous three- dimensional conformal boost to the gross tumor volume (GTV) versus conventional fractionation in concurrent chemoradiation for patients with locally advanced non- small cell lung cancer (LA-NSCLC).* ***Patients and Methods:*** *Thirty patients (25 males and 5 females, median age 61 years) with stage IIIA and IIIB NSCLC (no pleural effusion and no supraclavicular nodal involvement), Eastern Cooperative Oncology Group performance score of 0-2, were assigned to the two treatment arms; in the concomitant boost arm (group A): included 15 patients (13 males and 2 females, median age 62 years, range 26-76 years), who have received a dose of 1.8 Gy to the planning target volume (PTV) with concomitant boost of 0.6 Gy to the GTV, with a total dose of 60 Gy to the GTV and 45 Gy to PTV in 25 fractions during 5 weeks by using 3-D conformal radiotherapy, the conventional arm (group B): included 15 patients (12 males and 3 females, median age 60 years, range 28-74 years), who have received, a dose of 1.8 Gy in 25 fractions to PTV then another 10 Fractions to GTV with a total dose of 63 Gy during 7 weeks. Both arms received concurrent chemotherapy consisted of cisplatin 50 mg/m^2 days 1, 8, 29, 36 with gemcitabine 1000 mg/m^2 days 1, 8, 29, 36, a total of 6 cycles were given during and after irradiation. All patients were monitored for hematological, esophageal and lung toxicities.* ***Results:*** *The tumor response was evaluated 4 weeks after the end of radiotherapy where the overall response (OR) was seen in*

12 patients (80%) in group A versus 8 patients (53.3%) in group B; complete response (CR) was achieved in (13% vs. 7%), (P- value 0.08). The only significance was recorded in relation to TNM staging as OR in patients with stage IIIA was 90% in group A vs. 50% in group B (P-value < 0.05). Overall toxicity was acceptable as two patients (13%) in group A vs. one (7%) in group B had grade 3 esophagitis (P- value >0.05). ***Conclusion:*** *The addition of daily 3- D conformal concomitant boost in concurrent chemoradiation is feasible and well tolerated in patients with LA -NSCLC as compared to concurrent conventional chemoradiation.*

Key words: *Locally advanced Non- small cell lung cancer, Chemoradiation, Three- dimensional conformal radiotherapy, concomitant boost.*

Introduction

Non small cell lung cancer (NSCLC) is the most frequent type of primary lung malignancy. More than one- third of patients are stage III at presentation who is not candidate for surgical resection with very low cure rate[1]. But during the last two decades, the use of chemoradiotherapy (CRT) has improved the outcomes for those patients with locally advanced non-small cell lung cancer (LA-NSCLC)[2].

The concurrent chemoradiotherapy (cCRT) improved median survival time and 5-year survival rate compared with sequential chemotherapy and radiotherapy as improvement in loco-regional control can still play a role in prolonging patient survival [3].

The NSCLC is a rapidly growing tumor and its potential doubling time is estimated to be 5-7 days [4]. Cancer cells can proliferate even more rapidly during the later stage of RT, which is known as "accelerated repopulation" and is a cause of radiation failure [5].

Short course of radiation which still retains a high dose would produce a much stronger effect in killing tumor cells, because it will provide less chance for tumor cells to proliferate[6]. Therefore, an accelerated fractionation schedule using synchronous 3-D conformal boost to the GTV in the setting of concurrent chemoradiation (cCRT) for patients with LA-NSCLC was

designed; which delivers a slightly greater daily dose to the GTV than in conventional fractionation to shorten overall treatment time and overcome accelerated repopulation [7].

Based on this knowledge, we designed this study is to evaluate the efficacy and toxicity of concurrent chemoradiation with addition of daily 3-D conformal boost as compared to conventional fractionation for patients with LA-NSCLC.

Patients and Methods

Eligibility:

We entered 30 patients who met the following eligible criteria: informed consent; histologically or cytologically proven stage IIIA and IIIB NSCLC according to AJCC[21] excluding patients with pleural effusion and supraclavicular nodal involvement; age of 18 years or older; No prior chemotherapy, thoracic radiotherapy or thoracic surgery; Eastern Cooperative Oncology Group (ECOG) performance score (PS) of 0 to 2; Adequate bone marrow (absolute neutrophil count 1,500/μL, platelets 100,000/μL, and hemoglobin 10 g/dL at least), renal function (serum creatinine 2 mg/dL or calculated creatinine clearance 60 mL/min at least), and hepatic function (bilirubin less than 1.5 mg/dL and AST/ALT less than twice the upper limit of normal.

Patient assessment:

All patients had pretreatment evaluation, including complete medical history and physical examination; assessment of performance status; complete blood count (CBC), liver functions test (LFT) and kidney function test (KFT). Adequate pulmonary function test. Radiological studies were routinely done including chest roentgenography, thoracic and upper abdomen computed tomography scan (CT), pelvi-abdominal ultrasonography, a brain magnetic resonance imaging (MRI) and a bone scan.

Treatment Schedule:

All patients in the study were treated definitively using concurrent chemoradiation. Patients were randomly stratified into two groups; Group A: fifteen patients who received concurrent chemoradiation using 3-D conformal RT

with addition of daily 3-D conformal boost to the gross tumor volume (GTV). Group B: Other fifteen patients who treated with concurrent chemoradiation using conventionally fractionated radiation using 3-D conformal RT without conformal boost.

Radiotherapy plan:

Patients were treated in the supine position and immobilized, lying comfortably with arms above the head using an arm pole or with hands behind the head and with the head on a low sponge, patients set up was done by reference to tattoos on the skin and bony landmarks, usually the sternum and mid-axillary lines. CT based planning was performed and the patient was CT scanned at 5 mm intervals in the treatment position, the radiotherapy targets were defined in accordance with International Commission on Radiation Units and Measurements (ICRU 2001). Pulmonary lesions were delineated with the "lung window" setting and lymph nodes were outlined using "mediastinal window" settings.

The GTV encompassed all detectable tumors and lymph nodes 1 cm or greater in short-axis diameter observed on CT. The clinical target volume (CTV) included the GTV plus a 1 cm margin to account for microscopic tumor extension. Ipsilateral hilum was commonly included in the CTV. The planning target volume (PTV) included the CTV plus a 5-15 mm margin.

Contouring of normal organs at risk (OARs; esophagus, spinal cord, lung and heart) was carried out.

The 3D conformal RT was used for all patients involving a minimum of three coplanar isocentric fields designed to adequately cover target volumes and minimize doses to the OAR. At least one of these fields was designed to spare spinal cord to avoid radiation myelitis. Treatments were delivered by using linear accelerators with a 6-15 MeV; Large-field thoracic irradiation was used encompassing the gross and microscopic regions of spread which includes all abnormal regions on radiology as well as mediastinum, hilum and ipsilateral supraclavicular

fossae (only for upper lobe lesions).

The radiotherapy dose and fractionation for group (A) was 1.8 Gy daily to the PTV with a synchronous boost of 0.6 Gy to the GTV to bring its daily dose to 2.4 Gy/fraction. Total cumulative doses were 60 Gy to the GTV and 45 Gy to the PTV in 25 fractions over 5 weeks.

For group (B); a dose of 1.8 Gy was delivered daily to the PTV in 25 fractions over 5 weeks reaching 45 Gy followed by a boost of 18 Gy to the GTV in 10 fractions over 2 weeks.

The total dose to spinal cord should not exceed 45 Gy with a daily dose of 1.8 Gy or less, the volume of both lungs that receive more than 20 Gy should not exceed 35% of total lung, the volume of esophagus that receives more than 55 Gy should not exceed 30%, and total dose to the whole heart should not exceed 36 Gy.

Chemotherapy

Began on day 1 concurrently with initiation of radiation and was given as follows; Vigorous intravenous hydration was given immediately before and during each infusion (at least 0.5 L of saline with 125 mg of mannitol). Premedication with antiemetics was used including combination of steroid, serotonin 5-HT3 receptor antagonist (Ondansetron), and dopamine antagonists (Domperidone and Metoclopramide).

Concurrent chemotherapy regimen was the same in both groups and consisted of Cisplatin 50 mg/m^2 days 1, 8, 29, and 36 with gemcitabine 1000 mg/m^2 days 1, 8, 29 and 36. A total of 6 cycles of chemotherapy were given, during and after the course of irradiation, with 3 weeks interval between the cycles provided full clinical and hematological recovery.

Post-irradiation chemotherapy consisted of Cisplatin 75 mg/m^2 day 1 with gemcitabine 1000 mg/m^2 day 1and 8 every 3 weeks up to another four cycles. During the course of irradiation, complete blood count was performed twice weekly, electrolytes; kidney and liver profiles were performed before each cycle of chemotherapy.

Dose modifications of chemotherapy, blood transfusions or blood products, antibiotics, antiemetic, and GM-CSF were allowed when needed.

Response and toxicity criteria

CT chest was done 2 weeks after completion of the third cycle of chemotherapy to evaluate the response and those showing complete or partial response or stable disease proceeded to complete the 6 cycles of chemotherapy.

Response and toxicity were evaluated according to WHO criteria [8]. A complete response (CR) was defined as the disappearance of all known disease as determined by two observations not less than 4 weeks apart. A partial response (PR) was defined as more than 50% decrease in total tumor size of the lesions that have been measured. There should be no appearance of new lesions or progression of any lesions. A stable disease (SD) was defined as 50% decrease in total tumor size cannot be established. A progressive disease (PD) was defined as at least 25% increase in the size of measurable lesions or the appearance of new lesions.

Acute toxicity was defined as occurring within 90 days from starting treatment; so assessment was done weekly during treatment and thereafter including complete blood count, kidney and liver functions; chest x- ray. Chest CT was done if the lesion was not measurable on the chest x- ray; Esophagitis less than grade II-III, a neutrophil count of 1500/ mm^3 or more, a platelet count 100,000/mm^3 or more, a hemoglobin of 10 gm. /dl or more and no signs of organ toxicity were required for continuing treatment; otherwise, chemotherapy and radiotherapy were postponed until recovery.

Statistical methods

Data were checked, entered and analyzed by using SPSS version 15. Data were expressed as mean ± standard deviation for quantitative variables; number and percentage for qualitative variables. T-test or chi-square (X^2) was used when appropriate. P value < 0.05 was considered statistically significant.

Results

Patient characteristics:

A total of thirty patients were enrolled in this study between January 2008 and December 2009.

Their characteristics are listed in table (1). Median age was 61 years (range, 26-76 years), and 25 patients (83%) were men. Twenty one patients (70%) had stage IIIA, nine patients (30%) had stage IIIB, Twenty three patients (77%) had weight loss less than 10%, twenty three patients (77%) had Eastern Cooperative Oncology Group performance score of 0 or 1, nineteen patients (64%) had histological type of squamous cell carcinoma and nine (30%) had adenocarcinoma. Primary tumor was localized to: left upper lobe in (13%), left lower lobe in (24%), right upper lobe in (13%), right middle lobe in (27%), and right lower lobe in (23%). Nodal stages of the patients were as follow: N1 (33%), N2 (47%), and N3 (20%).

Response :

All patients completed their course of concurrent conformal chemoradiation either with concomitant boost in period of 5 weeks as in group A or without concomitant boost in period of 7 weeks as in group B.

Initial tumor response was evaluated 4 weeks after the end of radiotherapy where overall response (OR) was seen in 12 patients (80%) in group A versus 8 patients (53.3%) in group B but with no significant difference (P-value; 0.08). Tow cases (13%) had complete response (CR) versus only one (7%), ten (67%) had partial response (PR) versus 7 (47%), 2 (13%) had stable disease (SD) versus 5 (33%) and one patient (7%) had progressive disease (PD) versus two patients (13%), in group A and B respectively (p- value; 0.08). (Table 2).

The only significance was recorded in relation to TNM staging as OR in patients with stage IIIA was 90% in group A versus 50% in group B (P- value; < 0.05).

Treatment related toxicity:

The hematological toxicities after concurrent chemoirradiation were mild in both groups, as ten patients (67%) in each

group had grade I-2 anemia and thrombocytopenia and 9 patients (60%) had grade 1-2 neutropenia in each group, and 3 patients in both groups needed whole blood transfusion and 6 patients in each group needed GM-CSF injections until hematological recovery, with insignificant difference between the two groups (P > 0.05), as regard esophageal toxicity thirteen patients (87%) had grade 1-2 esophagitis in group A versus 12 (80%) in group B, and no one had grade 4 esophagitis in both groups, meanwhile the lung toxicity grade 1-2 was recorded in thirteen patients (87%) in group A versus fourteen (93%) in group B (P > 0.05). So the treatment schedule was tolerable by most of our patients with insignificant morbidity. (Table 3).

Table (1): Patient characteristics.

Characteristics	Group A cCRT (15 patients) with concomitant boost	Group B cCRT (15patients) without concomitant boost	P value
Age in years			
Median	62	60	0.99
Range	26 -76	28 -74	
Sex			
Male	13	12	1.0
Female	2	3	
Stage			
IIIA	11	10	0.73
IIIB	4	5	
PS			
0-1	12	11	0.9
2	3	4	0.73
Weight loss < 10%	4	3	0.7
Histology			
Squamous cell	10	9	0.75
Adenocarcinoma	4	5	0.73
Large cell	1	1	1.0
Nodal stage			
N1	6	4	0.82
N2	7	7	1.0
N3	2	4	0.84
Tumor Location			
LU lobe	2	2	1.0
LL lobe	4	2	0.66
RU lobe	2	3	0.87
RM lobe	3	5	0.67
RL lobe	4	3	0.95

Abbreviation: cCRT; concurrent conformal chemoradiotherapy, PS; performance score, LU; left upper, LL; left lower, RU; right upper, RM; right middle, RL; right lower

Table (2): Response Rate.

	Group A with concomitant boost		Group B Without concomitant boost		P value
	No	%	No	%	
OR	12	80	8	53.3	0.08
CR	2	13	1	7	
PR	10	67	7	47	
SD	2	13	5	33	
PD	1	7	2	13	

Table (3): The incidence of acute toxicity.

	Group A with concomitant boost				Group B without concomitant boost				P value
Toxicity	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4	
Anemia	3	7	3	2	4	6	4	1	>0.05
Neutropenia	4	5	5	1	5	4	4	2	>0.05
Thrombocytopenia	4	6	4	1	5	5	3	2	>0.05
Esophagitis	5	8	2	0	6	6	3	0	>0.05
Pneumonitis	7	6	2	0	8	6	1	0	>0.05

Discussion

Chemoradiotherapy is considered the standard of care for many subsets of stage III disease, which conceptually can be considered a two-compartment model, locoregional compartment in the chest and a distant compartment harboring potential micrometastasis. So we must eradicate the disease from both compartments to achieve cure [9].

Tumor cell proliferation during conventionally fractionated radiotherapy can negatively influence the treatment outcome in patients with unresectable NSCLC.

Accelerated and hyperfractionated radiotherapy may therefore have an advantage over conventionally fractionated radiotherapy. Moreover, earlier studies suggested improved survival with

addition of cisplatin-based chemotherapy [10].

In our study, the age range for both groups was between 26 - 76 years with a mean age of 61 years which was similar to that reported in the study by Stupp et al[11], but slightly lower than the mean age of 66 years reported by Uitterhoever et al [12]. The male to female ratio was 4.7:1 in our study which was close to that stated by Semrau et al [13] that had a male to female ratio of 4.5:1. Performance status is one of the most important prognostic factors regarding selection of treatment modality and expected response rate. Available evidence suggested that patients with poorer performance status have higher rates of serious adverse effects[14], our study included patients with performance status of 0 to 1 similar to that reported by Kwan et al [7] to stand concomitant chemoradiotherapy. Fifty percent of patients in our study were presented with squamous cell carcinoma, 30% with adenocarcinoma and 20% with large cell carcinoma, while 69% of patients were presented with squamous cell carcinoma, 12% with adenocarcinoma and 19% with undifferentiated carcinoma in the study reported by Mustafa et al[15]. Seventy percent of our patients had stage IIIA and 30% had stage IIIB at presentation, that nearly similar to patients included in the study carried out by Meydan et al[16].

Weight loss <10% was present in 23% of patients which was close to what reported by Kwan et al [7] (27%) but lower than reported by Meydan et al [16] (58%). The primary tumor, in our study, was localized to the right middle lobe in 27%, right lower lobe in 23% and left lower lobe in 20%, while the site of the primary tumor, reported by Mustafa et al[15], was 42% in left upper lobe, 38% in right upper lobe, and 12.5% in left lower lobe.

The overall response was 80% in patients received concurrent conformal chemoradiation with concomitant boost as the dose per fraction increased to 2.4 Gy in 25 fractions to GTV reaching for total dose of 60 Gy versus 53.3% in group (B) that received 63 Gy, 1.8 Gy per fraction in 35 fractions

with insignificant difference between both groups. The overall response in group (A) was comparable to those found in the study underwent by Meydan et al[16] in which the overall response was 75% and also comparable to the results of Sureyya et al[17] in which the overall response was 73%. The overall response in group (A) was lower than that reported by Kwan et al[7] in which the overall response was 93%. The overall response in group (B) was also slightly lower to than that reported by Mustafa et al[15] in which the overall response was 58%.

The hematological toxicity profile in group (A) was mild and similar to that of group (B). 60% of patients developed grade I-II neutropenia and 40% of patients needed GM-CSF injections until hematological recovery, 20% of patients in both groups needed whole blood transfusion.

The toxicity profile of both groups were better than those stated by Hirose et al.[18] who treated patients with locally advanced NSCLC with conventionally fractionated radiotherapy in combination with cisplatin plus vinorelbine where 84.6% had grade III to IV neutropenia, 3.8% of patients had grade III to IV thrombocytopenia and 61.5% of patients had grade III to IV anemia. As regards to the incidence of treatment related esophagitis, most of the patients in both groups experienced moderate esophagitis within the fourth and fifth weeks of radiation and resolved spontaneously two to four weeks after ending the course of radiotherapy. The results were similar to that reported by Kaplan et al[19] where moderate esophagitis was noted in 89.4% of patients.

Although there are limits to draw conclusions from this study with small sample size, several explanations for the favorable outcome of our study are possible, as follows. First, exclusion of patients with supraclavicular nodal involvement who have poor outcomes because they tend to develop distant metastases and it is common site of local failure, and also the prognostic significance of stage IIIB with involved supraclavicular LN was similar to

that of stage IV[20]. Second, there was relatively larger squamous cell population than none- squamous cell population (64% vs. 36%) as squamous carcinoma tend to respond better to accelerated radiotherapy but related OR had insignificant difference. Third, the chemotherapy protocol of cisplatin and gemcitabine was effective and tolerable by most of patients. Last, the use of synchronous GTV boost achieved better local control.

Conclusion

In conclusion, our results revealed that concurrent chemoradiation with addition of daily 3-D conformal boost are feasible and well tolerated by patients with locally advanced NSCLC giving satisfactory results. In addition to the potential radiobiological gain other advantages were gained as single daily treatment was convenient for patients despite multiple daily treatments, shorter course; 5 weeks than conventional; 7 weeks consuming time and cost.

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CONCOMITANT THREE-DIMENSIONAL CONFORMAL BOOST TO GROSS TUMOR VOLUME IN CONCURRENT CHEMORADIATION FOR PATIENTS WITH UNRESECTABLE STAGE III NON-SMALL CELL LUNG CANCER

Khaled A. Mansour MD, Muhammad A. Badawy MD and Maher Abdul Hameid MD

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nanogram (s)	ng	cubic millimeter	cmm
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